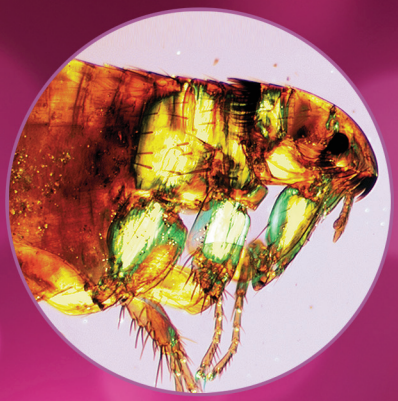
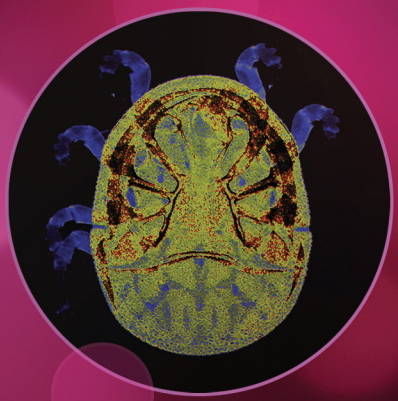
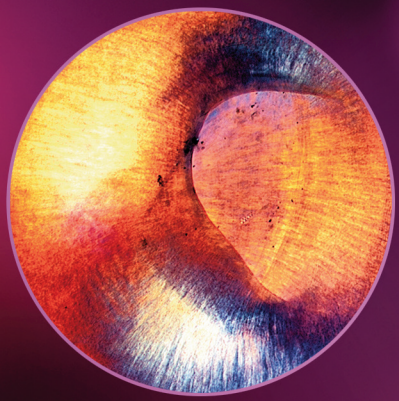
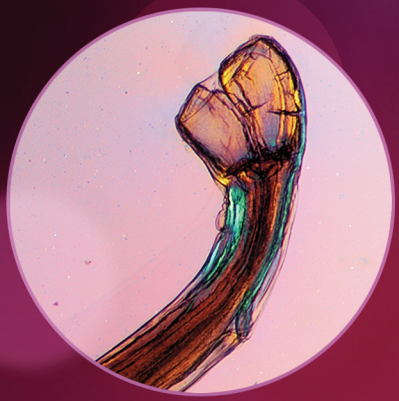
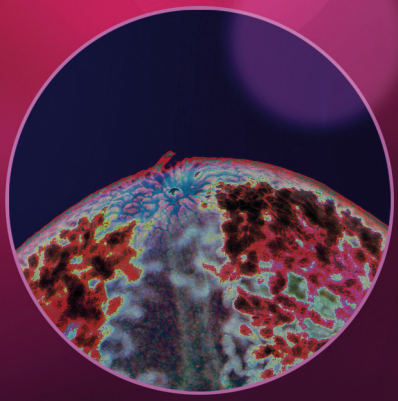
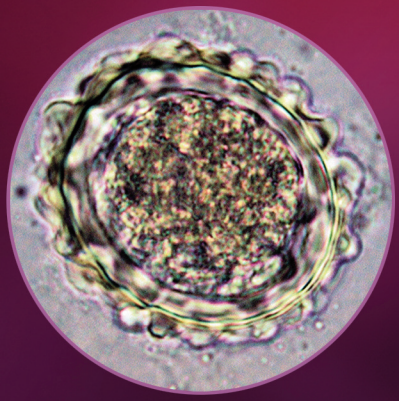
Alan Gunn and Sarah J. Pitt

Parasitology

An Integrated Approach



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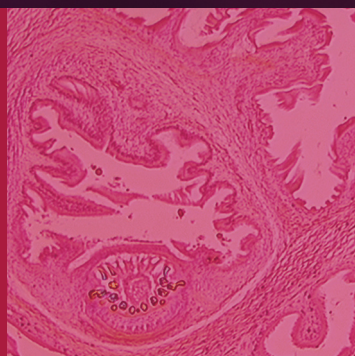

Parasitology

Parasitology

An Integrated Approach

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None of us truly live alone

Contents

Preface

Protozoan and helminth parasites are fascinating organisms and examples of their parasitism are found in a broad range of hosts, including plants, invertebrates and vertebrates. The interaction between a parasite and its host is complex and dynamic. Therefore we think that studying parasitology is a useful tool for appreciating a range of concepts in biological sciences including growth and reproduction, biochemistry, immunology and pathology.

Parasites do not live within their hosts in isolation. We feel that it is instructive to recognise how an individual organism might interact with other members of the same species, other species of protozoa and helminths and other classes of microorganism within a particular host. The effects on the host of harbouring a particular species of parasite are influenced by a range of host factors, including genetic constitution, immune status, and behaviour. Also, for parasites of humans in particular, consideration of social, religious, and cultural factors is often necessary. We have therefore called this book *Parasitology: An Integrated Approach* to emphasise how parasites influence, and are influenced by, a complex web of interacting factors.

We have divided the book into conventional chapters but because we wish to show how topics are inter-related, the reader will find certain subjects are picked up, put down, discussed in more detail elsewhere, and then returned to in a later chapter. This is also a good way of learning since it is better to take in bite-sized chunks of information and return to them frequently rather than attempting to grasp all aspects of a topic in a single sitting. We first introduce the concept of parasitism and the terms used by parasitologists to describe parasite lifestyles (Chapter 1). We then provide three chapters (Chapters 2–4) in which we introduce some of the 'key players', explain their basic biology and how they interact with one another. We have not included many diagrams of parasite life cycles as there is an excellent online resource available at the DPDx – CDC Parasitology Diagnostic Web Site (http//:dpd.cdc.gov/dpdx).

There follows a chapter on parasite transmission (Chapter 5) in which we consider, among other topics, not only how parasites exploit other animals as vectors and intermediate hosts but also how they manipulate their host's behaviour to increase their chances of transmission. We provide separate chapters on immunology (Chapter 6) and pathology (Chapter 7) but in truth it is virtually impossible to separate these topics because they are so inter-dependent. Chapter 8 is designed as a counterbalance to the bad press that parasites receive. Parasites not only can be used for the treatment of medical conditions but also may (in small doses) actually be good for us. Before one can begin to study parasites, one needs to be able to find them and count them. Even if the host is dead, this is not necessarily as simple as it sounds. Correct parasite diagnosis is essential before treatment can be given and to determine the necessity for or success of a control programme. We therefore devote Chapter 9 to parasite diagnosis that encompasses techniques ranging from straightforward light microscopy to advanced molecular biology. Finally, the book ends with Chapter 10 on treatment and control in which we again emphasise how these topics are informed by advances in medicine, genomics, and economics.

Figure 0.1 One can form a close relationship with a tapeworm! Source: © Serre, C. (1984) Stuffing. Methuen London Ltd, London, UK

This book is designed mainly for undergraduate students of biological sciences, biomedical sciences, medicine and veterinary sciences who need to know about protozoan and helminth parasites and understand how they affect their hosts. It would also be useful for postgraduate students who need background information about parasites to support their research and for members of any other professional group who need an insight into the subject for their work.

In the spirit of integration, we have provided web-based support material via the publisher's website. This includes the numerous photographs of parasites that we could not include in the book without increasing its size and cost. There are also more extensive questions based on each chapter, and a number of project ideas that do not require access to complex laboratory facilities or use laboratory animals such as mice, rats, and rabbits.

Alan Gunn and Sarah J. Pitt, 2011

1
Animal associations

1.1 Introduction

In this introductory chapter we will introduce the concept of parasitism as a lifestyle and explain why it is such a difficult term to define. We shall also introduce some of the terms that are commonly used by parasitologists. Like all branches of science, parasitology has a number of associated specialist terms such as 'intermediate host', 'definitive host' and 'zoonosis' that need to be understood before it is possible to make sense of the literature. We will explain why the study of parasites is so important and why parasites are likely to remain a problem for many decades to come. We will end by introducing the study of taxonomy because this will help inform the chapters on specific groups of organisms as well as the chapters on diagnosis, treatment, and control. Taxonomy is nowadays something of a Cinderella subject among biologists but it cannot be ignored because scientists must agree on the names things are to be called if they are to communicate with one another.

1.2 Animal associations

All animals are in constant interaction with other organisms. These interactions can be divided into two basic types: *intra-specific interactions* and *inter-specific interactions*.

Intra-specific interactions are those that occur between organisms of the same species. They range between relatively loose associations such as those between members of a flock of sheep, to highly complex interactions such as those seen in colonial invertebrates (e.g. Bryozoans and some of the Cnidaria (jellyfish and sea anemones)). For example, the adult (medusa) stage of certain jellyfish may appear to be a single organism but it is actually composed of colonies of genetically identical but polymorphic individuals. These colonies divide labour between themselves in a similar manner to that of organ systems within a non-colonial organism, for example, some colonies are specialised for reproduction while others are specialised for feeding.

Inter-specific interactions are those that take place between different species of organism (Figure 1.1). As with intra-specific interactions, the degree of association can vary between being extremely loose to highly complex. Odum (1959) classified these interactions on the basis of their effect on population growth using the codes '+' = positive effect, '−' = negative effect, and '0' = no effect. This leads to six possible combinations (00, 0−, 0+, etc.) and these too can be broken down into further subdivisions (Toft *et al*., 1993). Some authors also include a consideration of the direction and extent of any physiological and biochemical interactions between the two

Parasitology: An Integrated Approach, First Edition. Alan Gunn and Sarah J. Pitt.

Figure 1.1 Different species will occasionally co-operate for mutual benefit

organisms. A wide range of terms have been suggested in an attempt to compartmentalise these interactions (e.g. phoresis, mutualism, predation) but these are merely convenient tags and they cannot be defined absolutely. This is because the variety of organism interactions is extremely broad and even within a single interaction there are a host of variables such as the relative health of the two organisms that determine the consequences of the interaction for them both. It is therefore not surprising that there is a multiplicity of definitions in the scientific literature and it is not unusual for two authors to arrive at two different terms for the same type of interaction between species. In this section, we will discuss symbiosis, commensalism, phoresis, mutualism and finally parasitism, with some examples of each.

1.2.1 Symbiosis

The term symbiosis is usually translated as 'living together' and is derived from the Greek *syn* meaning 'with' and *biosis* meaning 'life'. It was originally used in 1879 by Heinrich Anton de Barry to define a relationship of 'any two organisms living in close association, commonly one living in or on the body of the other'. According to this original definition, symbiosis covers an extremely wide range of relationships. Some authors state that both organisms in a symbiotic relationship benefit from the association (i.e. it is [++]) although this is clearly a much more restrictive definition and it is more appropriately referred to as mutualism. However, some authors state that symbiosis and mutualism are synonymous – this only adds to the confusion. For the purposes of this book we will keep to de Barry's original definition.

Symbionts Strictly speaking, a 'symbiont' is any organism involved in a symbiotic relationship. However, the vast majority of scientists tend to restrict the term to an organism that lives within

or upon another organism and provides it with some form of benefit – usually nutritional. The association is therefore referred to as a host: symbiont relationship and the majority of symbionts are microorganisms such as bacteria, algae or protozoa. Where the symbiont occurs within the body of its host, it is referred to as an endosymbiont, while those attached to the outside are referred to as ectosymbionts. Two types of endosymbiont are recognised: primary endosymbionts (or 'p-endosymbionts') and secondary endosymbionts. Primary endosymbionts form obligate relationships with their host and are the product of many millions of years of co-evolution. They are usually contained within specialised cells and are transferred vertically from mother to offspring. As a consequence, they undergo co-speciation with their host and form very close host-specific relationships. By contrast, secondary endosymbionts are thought to be the product of more recent host: symbiont associations and, in the case of insects, the symbionts are contained within the haemolymph (blood) rather than specialised cells or organs. Secondary endosymbionts tend to be transmitted horizontally and therefore do not show the same close host: symbiont relationship. It is not known how endosymbionts begin their association with their hosts but some authors suggest that they arise from pathogens that attenuated over time. The suggestion that a parasite–host relationship tends to start off acrimoniously and then mellow with time was once widespread in the literature, but while this may sometimes occur, it is not a foregone conclusion.

The importance of symbionts to blood-feeding organisms Although blood contains proteins, sugars and lipids as well as a variety of micronutrients and minerals, it lacks the complete range of substances most organisms require to sustain life and to reproduce. Consequently, many of the animals which derive most or all of their nutrition from feeding on blood (haematophagy) have evolved symbiotic relationships with a variety of bacteria that provide the missing substances, such as the B group of vitamins. The need for supplementary nutrients is particularly acute in blood-sucking lice (sub-order Anoplura) because they have lost the ability to lyse (break up) red blood cells and therefore many nutrients will remain locked within these cells. In many cases, the bacteria are held within special cells called mycetocytes that are grouped together to form an organ called a mycetome. Although these terms appear to indicate the involvement of fungi, they originate from a time when scientists did not distinguish between the presence of yeasts and bacteria within cells. Many scientists continue to use the term 'mycetocyte' regardless of the nature of the symbiont but others use the term 'bacteriocyte' where it is known that the cells harbour only bacteria. In blood-feeding leeches belonging to the order Rhynchobdellida (there is a popular misconception that all leeches feed on blood; many of them are actually predatory), mycetomes are found surrounding or connected to the oesophagus. Mycetomes are not found in all blood-feeding leeches and in the medicinal leech, *Hirudo medicinalis*, the symbiotic bacteria are found within the lumen of the gut (Graf *et al*., 2006). The bacteria present in *Hirudo medicinalis* have been identified as *Aeromonas veronii* (earlier work on leeches often refers to it as *Aeromonas hydrophila*), a species of bacteria that has been associated with a number of other blood-feeding organisms. *Aeromonas veronii* has also been reported as causing wound infections in humans and inducing septicaemia and gastroenteritis. (Graf, 1999). Leeches are extremely useful in modern medicine, particularly to aid wound drainage following plastic surgery, but one of the risks associated with their application is that the patient acquires an *Aeromonas* infection. These infections are often trivial but they can become serious and lead to the formation of an abscess or cellulitis (e.g. Snower *et al*., 1989). This is a difficult problem to solve because the symbiotic bacteria are essential for the leeches.

Box 1.1 The role of symbionts in the life of tsetse flies and their transmission of trypanosome parasites

Tsetse flies, like most other blood-feeding organisms, harbour bacterial symbionts that facilitate the breakdown of the blood meal and provide essential nutrients to the fly. In the case of tsetse flies, these are principally B group vitamins, vitamin H (Biotin), folic acid and pantothenic acid and in the absence of the symbionts, the adult female fly is unable to reproduce. Tsetse flies have at least three different symbionts that are found within certain gut epithelial cells and these are passed on from the female fly to her larvae as they develop in her uterus. Of these symbionts, *Sodalis glossinidius* is thought to be the most important in influencing the establishment of trypanosomes in the tsetse fly. Tsetse flies have an effective immune system that protects them from invading micro-organisms. This includes the production of lectins that attach to and kill the invading organisms and toxic reactive oxygen species such as superoxide and hydrogen radicals (Macleod *et al*., 2007). However, *Sodalis glossinidius* releases N-acetylglucosamine which interferes with the activity of the lectins and scavenges reactive oxygen species, thereby allowing the trypanosomes to establish. It is possible that there are differences between strains of *Sodalis glossinidius* in the production of N-acetylglucosamine and this may be (to a greater or lesser extent) the reason why there are differences in the susceptibility of tsetse flies to infection with trypanosomes.

In nymphs and adult males of the human body louse, (*Pediculus humanus*; sub-order Anoplura) intracellular symbionts are found within a mycetome that is sometimes referred to as the 'stomach disc'. This mycetome is located on the ventral side of the mid-gut but unlike the leeches mentioned above, there is no actual connection between the mycetome and the lumen of the gut (Sasaki-Fukatsu *et al*., 2006; Perotti *et al*., 2008). In adult female lice, the bacteria re-locate to the oviducts and the developing eggs. This is in keeping with the observation that primary endosymbionts are transmitted within the eggs (i.e. transovarially) to the offspring. The bacteria associated with *Pediculus humanus* have been identified as belonging to the gamma (γ) proteobacteria and have been given the name *Riesia pediculicola*. Interestingly, molecular phylogenetic analysis is unable to distinguish between the symbiotic bacteria isolated from human head lice (*Pediculus humanus capitis*) and human body lice (*Pediculus humanus humanus*). This adds support to phylogenetic analysis of the lice themselves (Light *et al*., 2008) that indicates that although head lice and body lice occupy different ecological niches and body lice tend to lay their eggs on clothing while head lice attach their eggs to hair shafts, they are two morphotypes of the same species rather than two separate species. One suggestion is that the body lice evolved from head lice relatively recently in human evolution following the common practice of wearing clothing. The association between *Riesia* and *Pediculus* is estimated to be between 12.95 and 25 million years old, which makes it one of the youngest host: primary endosymbiont relationships so far recorded (Allen *et al*., 2009). In common with other primary endosymbionts, *Riesia* has undergone a reduction in genome complexity and lost genes: this is because it has come to rely on its host for the provision of many nutrients, protection from the environment and protection from predators. In addition, because its transmission is via the eggs of its host, each louse symbiont population is in

reproductive isolation and unable to undergo recombination with other strains of *Riesia* in other lice. This has led to the suggestion that *Riesia* will lack the capacity to develop rapid resistance mechanisms to antibiotics, and because the *Riesia* is essential for the lice, killing the symbiont would result in host mortality (Perotti *et al*., 2008).

1.2.2 Commensalism

The term 'commensalism' is derived from the Latin *commensalis* and means 'at the same table together'. Most definitions indicate that one species benefits from the association and the other is unharmed (0+). The concept of 'harm' within any definition leads to complications because it may be difficult to measure and depends upon the circumstances. Similarly, a 'benefit' may not be immediately apparent and it is possible that some of the associations that are commonly cited as commensal involve a degree of benefit to both parties (++) albeit they may not benefit to the same extent. A commensal association may be 'facultative', in which both species are able to live independently of one another, or 'obligatory', in which one of the associates must live in association with its partner. For example, in many of the warmer parts of the world, the cattle egret (*Bulbulcus ibis*) is often observed riding on the back of cattle and big game from which it swoops down periodically to capture lizards and insects that are disturbed as its ride moves through the undergrowth. The egret is perfectly capable of living apart from cattle but it benefits from its mobile 'vantage point-cum-beater'. The egrets are not thought to remove many ectoparasites from the cattle and they get their Arabic name *Abu Qerdan* 'father of ticks' from the large number of ticks associated with their nesting colonies. The cattle, therefore, appear to gain little from the relationship although it is likely that the egret acts as an early warning system of the approach of predators. African Cape Buffalo (*Synceros caffer*) have a good sense of smell but notoriously poor eyesight and are therefore vulnerable to predators approaching from downwind. The red-billed oxpecker (*Buphagus erythrorhynchus*) is sometimes said to have a similar commensal relationship with cattle but this is almost certainly not the case. Unlike cattle egrets, the red-billed oxpecker has an obligatory relationship with cattle and big game, and far from removing ticks, it feeds primarily on scabs and wound tissue pecked from their host. This can delay wound healing and thereby make the affected animal vulnerable to infections and infestations with blowfly larvae (Weeks, 2000).

The amoeba, *Entamoeba coli* (not to be confused with the gastrointestinal bacterium *Escherichia coli* which is also abbreviated to *E. coli*) is a common commensal that lives within the human large intestine. Unlike its highly pathogenic cousin, *Entamoeba histolytica*, *Entamoeba coli* does not invade the gut mucosa or consume red blood cells and it feeds on bacteria and gut contents. *Entamoeba coli* is of little interest *per se*, but due to its morphological similarity to *Entamoeba histolytica*, it is important to be able to distinguish between the two species in faecal samples.

1.2.3 Phoresis

This association is usually described as one in which one species provides shelter, support or transport for another organism of a different species. This interaction may be temporary or

permanent. For example, apart from the first instar, the larvae and pupae of the blackfly *Simulium neavei* attach themselves to the outer surface of freshwater crabs. The larvae feed by filtering out phytoplankton and detritus from the water and the crabs act as a suitable firm yet mobile substrate on which to attach. An appreciation of this association is important because adult *Simulium neavei* are important vectors of the *Onchorcera volvulus* – the nematode that causes the disease 'river blindness' (see Chapter 3).

1.2.4 Mutualism

Mutualistic (from Latin, *mutuus* meaning 'reciprocal') relationships are those in which both species benefit from the association in terms of their growth and survival (++). Some authors further restrict the definition to one in which neither of the partners in the association is capable of living on their own, while others are less prescriptive. The association between *Wolbachia* bacteria and the filarial nematode, *Onchocerca volvulus*, is clearly mutualistic. The bacteria are confined to the cells of the reproductive tissues and hypodermis of the female worms. The *Wolbachia* provide metabolites which are demonstrably essential to the worms. If the bacteria are removed, for example, by exposure to the antibiotic tetracycline, the worms are unable to establish themselves in their host and grow and, in the case of adult worms, the female is rendered infertile (Taylor and Hoerauf, 1999). The bacteria are therefore a potential target for the chemotherapy of filarial nematode infections.

Whether or not the relationship between the Cnidarian *Hydra viridis* and its algal partner *Chlorella* should be considered mutualistic depends upon the strictness of one's definition. *Hydra viridis* are capable of growing and reproducing in the absence of their algal partner but there is some debate in the literature whether the strains/species of *Chlorella* associated with *Hydra viridis* can survive independently. The algae live within vacuoles in the endodermal cells of the *Hydra* and thereby impart the *Hydra*'s characteristic green coloration. Whether this provides camouflage that is any way beneficial is not known. When the *Hydra* reproduces by budding, its algal partner is passed on to the offspring; the algae are not essential to the budding process but *Hydra viridis* seldom undergoes sexual reproduction if the algae are absent. Experiments in which the algae are removed from the *Hydra* by exposure to high light intensities (Habetha *et al*., 2003) indicate that the nature of the relationship varies depending upon the environmental conditions. Like other *Hydra* species, *Hydra viridis* obtains its food by capturing prey on tentacles that are armed with nematocysts, while the alga carries out photosynthesis and releases the sugars maltose and glucose-6-phosphate that can potentially be used by *Hydra viridis*. If there is suitable illumination and plenty of prey for the *Hydra*, the growth of *Hydra viridis* with and without algae is similar. This indicates that the sugars released by the algae have little importance for the *Hydra*. If, however, there is illumination but no food for the *Hydra*, then those *Hydra* lacking algae die after a few weeks, while those containing algae reduce in size but are able to survive for at least three months and will feed again if presented with food. Therefore, if *Hydra viridis* is starved, then the symbiotic algae play an important role in its survival. By contrast, if *Hydra viridis* are kept in the dark but with plenty of prey available, those lacking algae grow much better than those containing them. Furthermore, the algal population declines by about 60% although they are not lost entirely and the *Hydra viridis* remain pale green. This indicates that under these conditions, the algae must be receiving nutrients from the *Hydra* to such an extent that the nature of the relationship has changed from mutualism to one akin to parasitism.

1.2.5 Parasitism

Parasitism is a surprisingly difficult term to define and there are numerous explanations in the literature. For the purposes of this book, the following definition has been used: 'Parasitism is a close relationship in which one organism, the parasite, is dependent on another organism, the host, feeding at its expense during the whole or part of its life (− +).' It is frequently a highly specific relationship that always involves a degree of metabolic dependence of the parasite upon its host and often, though not always, results in measurable harm to the host. The association is usually prolonged and although it may ultimately result in the death of the host, this is not usually the case. It is therefore distinct from predation in which a predator usually kills and consumes its prey within a short period of time. However, owing to the complexities of animal relationships, there are always 'grey areas' in which any definition starts to become unstuck. This is particularly apparent in the case of blood-feeding. Mosquitoes and tsetse flies would not be considered parasites because they only feed for a few seconds or minutes before departing; in contrast, hookworms and crab lice would be considered parasitic, because they are permanently associated with their host. Blood-feeding leeches, however, are free-living organisms that remain attached to their host for several hours while taking a blood meal; some authors consider them to be parasites while others define their feeding as a type of predation.

Box 1.2 From welcome guest to villain: the derivation of the term 'parasite'

The word 'parasite' is derived from the Greek '*para*' meaning 'beside' and '*sitos*' meaning 'food'. In Ancient Greece, the term 'parasite' had religious connotations and nothing to do with infectious organisms. According to a stone tablet in the temple of Heracles (Hercules) in Cynosarges, the priest was required to make monthly sacrifices in the presence of 'parasites' who were to be drawn from men of mixed descent. Refusal to act as a parasite would result in being charged with committing an offence. (Cynosarges was an area near to the city walls of Athens. In addition to the temple, there was also a gymnasium and it was where the Cynic philosophers gave classes.) Subsequently, the word was debased and came to mean someone who shared one's food in return for providing amusement and flattery. The '*parasitus ridiculus*' was a popular character in Greek and early Roman comedies and they even had joke books to help them should they run out of witticisms. The greed of the parasite was a constant source of fun for dramatists and he was often given crude nicknames such as 'little brush – because he swept the table clean'. Double entendres were as popular over 2000 years ago as they are today and the Latin for little brush '*peniculus*' is also a diminutive for penis (Maltby, 1999).

Some organisms are obligate parasites and at a particular stage in their life cycle they have to live as parasites of their host while others are facultative parasites and can develop as parasites or free-living organisms depending upon the circumstances they find themselves in. For example, the larvae of the warble fly *Hypoderma bovis* have to develop as parasites of cattle and are therefore obligate parasites. By contrast, the larvae of the blowfly *Lucilia sericata* are facultative parasites because they are able to develop as parasites should the eggs be laid upon a live sheep or as free-living detritivores if the eggs are laid on a dead sheep.

As mentioned above, some organisms, such as the human body louse *Pediculus humanus*, are parasitic at all stages of their life cycle, while others are only parasitic at one or more stages. For example, the blood fluke *Schistosoma haematobium* is a parasite of humans during its adult stage and of snails during two of its larval stages but it also has two non-feeding free-living stages. The act of being a parasite is therefore 'stage-specific'. Some estimates suggest that as many as 50% of all known species are parasites at some point in their life cycle. However, this estimate is subject to the caveat that there is some debate about what constitutes a species, especially among the prokaryotes. The number of species is also reflected in the interests of biologists in different groups of animals. For example, insects have been studied intensively for over 200 years and this is probably at least partly the reason why they are said to account for 72% of all known species. In one order alone, the 'species-rich' order Hymenoptera (bees, wasps), approximately 100,000 species are classed as parasitoids. By contrast, mites and nematodes have proved much less popular and the diversity of their parasitic species is probably vastly underestimated. Nevertheless, parasitism is a remarkably common lifestyle and parasites (and their hosts) have been described from all the major groups of living organisms including the Archaea, Bacteria, Fungi, Plantae, Protozoa, invertebrates and vertebrates. There is some debate as to whether viruses should be considered to be parasitic organisms. At one level, this would appear to be self-evident since viruses are incapable of maintaining themselves or reproducing except when within their host cell. However, being composed of complex organic molecules and having the capacity to evolve is not necessarily synonymous with being a living entity, especially when those attributes are dependent upon existing within a host cell. The arguments against viruses being alive are discussed in detail by Moreira and López-Garcia (2009). In this book, we will mainly consider parasitic helminths (flatworms and nematodes), arthropods and protozoa. The relationships between parasites in these groups and their hosts have been extensively studied and some of them have a major impact on our health and that of our domestic animals.

1.2.6 Intra-specific parasites

Although most parasitic relationships involve two different species of animals, it is not unknown for intra-specific parasitism to take place. This is most often associated with adaptations to sexual reproduction in which the male attaches to the female and becomes dependent upon her for the provision of nutrients. For example, in certain deep-sea angler fish belonging to the suborder Ceratioidea, the larval fish develop in the upper 30 metres of sea water and then gradually descend to deeper regions as they metamorphose into adults. The adolescent males have a very different morphology to the females: they are much smaller, they have larger eyes and in some species they develop a large nasal organ that is presumably involved in their search for females. Furthermore, the males cease feeding and rely upon reserves laid down in their liver during the larval period to fuel their swimming. Upon finding a suitable female, the male grasps onto her using special tooth-like bones that develop at the tips of his jaws (his actual teeth degenerate during metamorphosis). Once he has attached, the male grows (although he remains much smaller than his consort) and his testes mature. His skin and blood vessels fuse with hers at the site of attachment and he remains attached for the rest of his life and draws all his nourishment from her. Some authors suggest that the male must find a virgin female but although most females carry only a single male, there are records of females with three or more males attached to them. This is presumably an adaptation to life in the deep-sea regions in which the opportunity to locate suitable mates is limited. It does,

however, beg the question of how sexual selection takes place because it is unusual in nature for a female to mate with just one male for life, especially if that male is the first one to turn up. This type of relationship is not found in all ceratioid anglerfish; in some species the males are facultative parasites rather than obligate ones as described in the above scenario, while in other species the males are free-living, capable of capturing their own food, and form only temporary attachments to females. Molecular evidence suggests that the development of the parasitic males is a variable phenomenon among anglerfish and has evolved and subsequently become lost on several occasions (Shedlock *et al*., 2003; Pietsch, 2005).

1.2.7 Parasitoids

The term parasitoid is restricted to certain parasitic insects whose hosts are almost exclusively other insects – although a few species attack certain crustacea, spiders, millipedes, centipedes and earthworms. Some parasites cause mortality and may even depend on the death of their host to effect transmission to the next stage of their life cycle, but host death is not inevitable. By contrast, parasitoids slowly consume their host's tissues over a period of time so that the host remains alive until the parasitoid has completed its development. At this point the host dies either through the loss of vital tissues or through the parasitoid physically eating its way out of its host. Parasitoids are all parasitic during their larval stage and the adult insect is free-living and feeds on nectar, pollen or is predatory, depending upon the species. Parasitoids can develop as endoparasites within their host or as ectoparasites attached to the outside but with their mouthparts buried deep within the host's body. The larva has only the one host in or on which it develops and those that are endoparasites tend to exhibit the most host specificity. This lifestyle is therefore distinct from those insects such as warble flies (e.g. *Hypoderma bovis*) and bot flies (e.g. *Gasterophilus intestinalis*) which exhibit a more 'traditional' parasitic way of life that does not inevitably result in the death of the host. Many of the order Hymenoptera (bees, ants, wasps) are parasitoids and it is also a common lifestyle among the Diptera (true flies) but it is absent or very rare among the other orders. By contrast, most of the insect orders are hosts to parasitoids. Hyperparasitism is also common in which a parasitoid parasitises another species of parasitoid. Parasitoids are effective for the control of agricultural pests, particularly within closed environments such as greenhouses. However, they have had limited success as control agents for parasites, their vectors, or intermediate hosts.

The parasitoid lifecycle typically begins with the adult female locating a suitable host and either injecting one or more eggs into the host or attaching them to the outer surface. Sometimes she also injects a toxin that temporarily or permanently disables her victim. The host is chosen on the basis of its stage of development which may be anywhere from the egg to the adult stage.

Box 1.3 Parasitoid: virus interactions

A number of endoparasitic wasps belonging to the families Icheumonidae and Braconidae have a fascinating relationship with certain polydnaviruses. The viruses replicate within the calyx cells of the wasps' ovaries and are secreted into the oviducts. When a wasp injects her eggs into a suitable host, usually a caterpillar, the virus is transmitted as well. The viruses are unable to

replicate within the caterpillar but they can invade several cell types within which they integrate into the genome and cause the expression of substances that facilitate the establishment of the parasitoid. For example, one of the main immune responses that insects express in response to an invader is encapsulation (see Chapter 6). Encapsulation first depends upon the invader being recognised, and then a co-ordinated response occurs, during the course of which the invader is surrounded by amoeboid-like cells present in the haemolymph and then killed through the production of toxic chemicals and/or lack of oxygen, or the invader is physically isolated and therefore unable to damage the host. Wasp eggs that are implanted into suitable hosts without the virus are quickly encapsulated and killed. It is thought that the virus may cause the caterpillar to express protein tyrosine phosphatases and thereby interfere with the encapsulation process. Protein tyrosine phosphatases dephosphorylate the tyrosine residues of a number of regulatory proteins and are therefore closely involved in the regulation of signal transduction. Altering the levels of regulatory proteins makes it impossible for the host to develop an effective immune response and the parasitoid egg is able to develop unmolested. The viruses also have other effects on the parasitoid's host including preventing its further development once it reaches the stage at which the parasitoid is to emerge. The polydnaviruses therefore have a mutualist-like relationship with the parasitoid within which they replicate. They are vertically transmitted as an endogenous 'provirus' that is integrated into the wasp genome but has a pathogenic relationship with the parasitoid's host, within which it is unable to replicate (Webb *et al*., 2006). Not all wasp parasitoids have relationships with viruses but they are still capable of causing similar disruption to the host immune response and host development through the injection of toxins. This has led some authors to suggest that the polydnaviruses found in the Ichneumonidae and Braconidae may have evolved from wasp genes. Many workers, however, think that the two wasp families, probably independently, evolved relationships with existing viruses (Dupas *et al*., 2008; Espagne *et al*., 2004).

1.2.8 The concept of harm

The term 'harm' is often used when describing interactions between organisms but is particularly pertinent to the description of parasitism. Unfortunately, harm is a difficult term to define and is not always easy to measure. For example, parasites are usually much smaller than their host and a single parasite may have such a minor impact that it cannot be measured in terms of its effect on the physiology and well-being of the host. By contrast, a large number of the same parasite could lead to serious illness or even death. Similarly, a low parasite burden may have little impact upon a healthy, well-nourished adult host but the same number of parasites infecting an unhealthy, starving young host may prove fatal. A common analogy is that a single glass of water will not harm you and may even do you good, but the rapid consumption of a thousand glasses of water would kill you. Does that mean that water is beneficial or poisonous? Clearly, it can be both and, likewise, harm is dependent upon the context in which it is being considered. It is therefore not a good idea to make the ability to record measurable harm a prerequisite for the classification of the relationship between two organisms. Indeed, it is now recognised that, in certain instances, low levels of parasitic infection may actually be beneficial to the well-being of the host (Weinstock *et al*., 2004). Nevertheless, many parasites have the capacity to cause morbidity, that is, a diseased

state, and some may cause mortality (death). The possible beneficial consequences of low parasite burdens will be discussed in more detail in Chapter 8.

The morbidity that parasite infections induce is often reflected in a reduction in the host's fitness as measured in terms of its growth or reproductive output. This is often attributed to the direct pathogenic effect of the parasite, such as through the loss of blood and the destruction of tissues or competition for resources (e.g. gut parasites feeding on nutrients in the intestine). However, in reality, the situation is far more complicated than this. Although a functional immune system is crucial to an organism being able to protect itself against pathogens, they are energetically costly and these costs often have to be traded off against other physiological processes. Ilmonen *et al*. (2000) demonstrated this by injecting one group of breeding female pied flycatchers (*Ficedula hypoleuca*) with a diphtheria-tetanus vaccine and a control group with a saline solution. The vaccine was not pathogenic and did not induce an infection but it did cause the activation of the birds' immune system. They found that the birds injected with the vaccine exhibited a lower feeding effort, invested less in self-maintenance and had a lower reproductive output, as determined by fledgling quality and number. The authors therefore concluded that the energetic consequences of activating the immune system can be sufficient to reduce the host's breeding success.

1.3 Parasite hosts

'Parasite host' is the term used to define the organism on or in which the parasite attaches and from which it derives its nutrition. The host is usually not related taxonomically to the parasite although this is not always the case (see intra-specific parasites). Most parasites are highly host-specific and only infect one host species or a group of closely related species. This is due to the complex adaptations the parasite is required to evolve in order to identify, invade and survive within their host. For example, the nematode *Ascaris suum* is primarily a parasite of pigs while *Ascaris lumbricoides* is primarily a parasite of humans. A few parasite species, however, are able to exploit a wide range of hosts. For example, the protozoan parasite *Toxoplasma gondii* is capable of infecting, growing and asexually reproducing in virtually all warm-blooded vertebrates although sexual reproduction only takes place within the small intestine of cats.

1.3.1 Protozoa and helminths as hosts

Parasites can be infected by viruses although there is limited published information on how these affect their biology. Viruses have been identified in many parasitic protozoa, such as *Entamoeba histolytica* (Mattern *et al*., 1974) and *Giardia lamblia* (Wang and Wang, 1986), and it would be surprising if they were not common in helminth parasites. Parasites are also infected by prokaryotic (e.g. bacteria) and eukaryotic (e.g. fungi, and protozoa) parasites. Those parasites that infect other parasites are known as hyperparasites. For example, the microsporidian *Nosema helminthorum* is parasitic on the tapeworm *Moniezia expansa* that lives within the small intestine of sheep and goats (Canning and Gunn, 1984). The infective cysts of *Nosema helminthorum* must therefore first be consumed by a sheep and then come into contact with and penetrate the tegument (tapeworms lack a gut of their own) of the tapeworm. Within the tapeworm, *Nosema helminthorum* reproduces and causes numerous raised opaque bleb-like patches but is not thought to be especially

pathogenic. Related microsporidia affect a range of other platyhelminth parasites (Canning, 1975) but there are remarkably few reports of them infecting parasitic nematodes (e.g. Kudo and Hetherington, 1922). The discovery of microsproridia infecting the free-living nematode *Caenorhabditis elegans* (Hodgkin and Partridge, 2008; Troemel *et al*., 2008) has opened up the potential of developing a laboratory model for studying both nematode immunity and the biology of microsporidia. This is because *Caenorhabditis elegans* is a commonly used model organism whose full genome has been sequenced. Microsporidia cause a number of pathogenic infections in humans and domestic animals and a simple laboratory model would prove extremely useful, for example, in the development of drug treatments.

1.3.2 Classes of hosts for parasites

Hosts can be divided into classes, depending upon the role they play in the parasite's life cycle. The 'definitive' (or final) host is the one in, or on, which the parasite reaches maturity and undergoes sexual reproduction, while the 'intermediate' host is the one in which the parasite undergoes its developmental stage(s). There may be just one or several intermediate hosts and the parasite may or may not undergo asexual reproduction during this time but it cannot develop into an adult or reproduce sexually. In this way, a parasite can exploit its hosts to maximum effect by combining the reproductive power of asexual reproduction in the larval stage with the advantages of sexual reproduction during the adult stage.

Parasites are able to concentrate more of their energies on reproduction than free-living animals would, since they do not have to worry about food, shelter and fluctuations in environmental conditions. This is important because the chances of any offspring locating and establishing themselves within a suitable host are very low. The completion of the parasite's life cycle is sometimes dependent upon the death of the intermediate host, leading to consumption of the larval form by the definitive host. In this situation, the parasite is often very pathogenic in its intermediate host but has relatively minor effects on the definitive host. The intermediate host is not always killed or consumed by the definitive host. For example, after undergoing asexual reproduction in the snail intermediate host, the cercariae of the liver fluke *Fasciola hepatica* physically and chemically bore their way out and swim off to transform into metacercaria attached to aquatic vegetation. The snail survives the damage to its tissues and the lifecycle is completed when the metacercaria are consumed by the sheep definitive host.

A paratenic host is one that a parasite invades and is able to survive within but where it is unable to undergo further development. Paratenic hosts are not usually essential for a parasite to complete its life cycle although they may provide a useful bridge between the infective stage/intermediate host and definitive host. For example, the definitive hosts of the nematode *Capillaria hepatica* are primarily rodents, though a range of other mammals, including humans, can be infected. Human infections are rare, but potentially fatal. The adult worms reside in the liver and the eggs they produce remain there until the definitive host dies or is consumed by a predator. Although the definitive host is infected by ingesting the eggs, these do not embryonate while in the liver and therefore consuming egg-infested liver does not lead to an infection. Instead, the eggs pass through the gut then pass out with the faeces and ultimately embryonate to the infective stage in the soil. Alternatively, if the definitive host dies naturally, the liver decomposes and the eggs reach the soil where they embryonate. Infective embryonated eggs of *Capillaria hepatica* have been described from earthworms, which would ingest them while feeding on soil and detritus, and as many

rodents will consume earthworms, it is thought that the worms act as a paratenic host facilitating the transfer of the infection to the definitive host.

1.4 The co-evolution of parasites and their hosts

Evolution can be defined as a change in gene frequency between generations, but in order for this to occur, three criteria need to be met. First, there must be genetic variation within the population. If the population is genetically homogeneous, then variation can only occur sporadically through random mutation. The second criterion is that the variation must be heritable: if the variation cannot be passed on to offspring, then it will be lost regardless of the benefits it imparts. The third and final criterion is that the variation must influence the probability of leaving reproductively viable offspring. If the variation imparts benefits, then the organism possessing it would be expected to leave more offspring; however, unless these are reproductively viable, the variation would be quickly lost from the gene pool. Parasites live in close association with their hosts and the two organisms will co-evolve. The nature of the host: parasite relationship may therefore change with time. For example, provided the above three criteria are met, the host can be expected to evolve resistance/susceptibility factors depending upon the pressure exerted by the parasite. Although ever greater resistance to infection may appear to be 'ideal', this is unlikely to arise if the energetic cost impacts on the ability to leave viable offspring. At the same time, the parasite can be expected to evolve virulence/avirulence factors.

Box 1.4 Parasites in the fossil record

Most parasites lack the sort of hard structural features that would ensure their preservation in the fossil record. It is therefore impossible to be sure whether parasitism has always been such a common 'lifestyle'. Conway Morris (1981) suggested that a survey of the commensals, symbionts and parasites of those organisms that have remained apparently unchanged for millions of years (the so-called 'living fossils') might reveal unusual organisms and provide insights into animal associations but an in-depth study still needs to be done. For example, horseshoe crabs (Phylum Chelicerata, Subclass Merostomata) have existed almost unchanged for hundreds of millions of years. There is little published information on their parasites although flatworms of the family Bdellouridae are found only in association with horseshoe crabs (Lauer and Fried, 1977).

Copepod ectoparasites that are morphologically similar to those in existence today have been identified attached to fossil teleost fish dating to the Lower Cretaceous (Cressey and Boxshall, 1989). Evidence of nematode parasites is largely restricted to those infecting insects that became trapped in amber (Poinar, 1984). Helminth eggs can be identified in coprolites (fossilised faeces) but while there have been extensive studies on animal and human faeces found in archaeological sites (Sianto *et al*., 2009), there is less data on coprolites dating back millions of years. As with any faecal analysis, one has to remember that the presence of an organism within faeces may not be a consequence of parasitism but result from passage through the gut following accidental consumption (e.g. eggs of a parasite of another animal) or invasion of faeces after it was deposited (e.g. eggs of a detritivore). Where animals were preserved in special circumstances, such

as being rapidly mummified under desiccating conditions or frozen in tundra, it is possible to identify soft-bodied parasites with greater accuracy. For example, nematodes and botfly larvae can be identified from woolly mammoths that died thousands of years ago on the Siberian tundra (e.g. Grunin, 1973; Kosintsev *et al*., 2010).

Sometimes the presence of parasites is inferred from the pathology they cause (e.g. Lukševics *et al*., 2009). For example, the pearls found in mussels and oysters often form as a consequence of infection by trematode parasites. Pearls thought have been caused by trematode parasites have been identified in fossil mussels dating back to the Triassic era (250–200 million years ago) (Newton, 1908; Brown, 1940). Dinosaurs almost certainly had their full complement of parasites although their evidence is sadly lacking from the fossil record. However, marks found on the bones of the dinosaur *Tyranosaurus rex* are thought to resemble the pathology caused by the protozoan parasite of birds *Trichomonas gallinae* (Wolff *et al*., 2009). Poinar and Poinar (2008) have even suggested that parasites were a major factor in the ultimate extinction of the dinosaurs.

1.4.1 Evolutionary relationships between host and parasite

It is often stated that the longer a parasite and its host have been living in association, the less pathology is caused. This is based on the reasoning that if the parasite kills its host, then it will effectively 'commit suicide' because it will have destroyed its food supply. Consequently, over time, it is to be expected that the parasite will become less harmful to its host – that is, it becomes less virulent. However, this assumption has been questioned because a pathogen's virulence is often a reflection of its reproductive success. For example, let us consider two hypothetical strains of the same nematode species, A and B, that lives in the gut of sheep. Strain A is highly virulent and causes the death of the sheep while strain B is relatively benign and seldom causes any mortality. At first glance, one might expect that strain B would leave more offspring because its host lives for longer. However, if virulence was linked to the nematode's reproductive output and the eggs were released at a time when they were likely to infect new hosts, then it would be strain A that would bequeath more of its genes to subsequent generations. Consequently, the proportion of strain A in the nematode population would be expected to increase with time and there would be constant selection for ever increasing virulence. The sheep and the parasites may eventually be driven to extinction by these changes but no animal alive, including humans, gives any indication of being able to plan wisely for the future.

The hypothetical scenario described above naturally begs the question of, if this is true, why does life still exist today? This is because, on this basis, parasites and other pathogens should have killed everything off many millions of years ago. The answer is that the scenario is too simplistic and all host: parasite or pathogen relationships involve a complex array of competing factors. Consequently, the evolutionary end-point of any relationship is very case-dependent. Sometimes the parasite becomes more virulent, and sometimes its virulence attenuates to an intermediate level but one cannot assume that the natural end-point is a mutually beneficial form of mutualism. Indeed, the relationship between a parasite and its host is often likened to a 'co-evolutionary arms race' in which the parasites attempt to acquire more resources from the host in order to produce their offspring while the host evolves mechanisms for reducing its losses and eliminating the

parasite. This has given rise to the ecological theory known as the Red Queen's Race. The name is derived from the Red Queen in Lewis Carroll's *Alice Through the Looking Glass* who says, 'Now, here, you see, it takes all the running you can do, to keep in the same place' (Ladle, 1992).

Parasites and other pathogens are generally smaller than their hosts and reproduce faster. Consequently, they might be expected to win any arms race because of their ability to select for adaptations that overcome any new measure the host is able to generate that limits the parasite's acquisition of host metabolites. However, hosts that are comparatively long-lived usually have sophisticated immune systems that are able to identify and kill or neutralise new parasite variants. The host is therefore not a constant environment for the parasite. Parasite virulence is also affected by the mode of transmission. Horizontally transmitted parasites, especially those with a wide host range, can 'afford' to be highly virulent because there are lots of potential hosts and if one or more of them dies, it has no direct consequences. However, when the parasite is vertically transmitted (e.g. via the eggs of its host or across the placenta), there is a direct link between the effect of the parasite on its host and its own reproductive success. For example, if the parasite is so virulent that it kills the female host before it can reproduce, then the virulent parasite's genes will not be transmitted. Similarly, if it kills the host's eggs while they are *in utero* or reduces the number of host eggs that are produced or survive to become adults and reproduce themselves, then the parasite is compromising its own reproduction. It is therefore to be expected that, as a general rule (there will always be exceptions), vertically transmitted parasites should be less pathogenic than those that are transmitted horizontally. There is some support for this hypothesis. For example, Tompkins *et al*., (1996) found that two ectoparasites of swifts – a louse and fly – that are vertically transmitted, had no effect on nestling growth or fledgling success even when the numbers of these parasites was artificially increased or the birds were stressed. Similarly, in feral pigeons, Clayton and Tompkins (1995) observed that a vertically transmitted louse had little impact on the birds' health but horizontally transmitted ectoparasitic mites caused so much distress that the birds' reproductive success was reduced to zero.

1.4.2 Parasites and the evolution of sexual reproduction

Sex has fascinated biologists (among others) for generations. In particular, from a logical point of view, sexual reproduction does not make sense because of what is referred to as the two-fold cost of sex. First, the males, which usually constitute in the region of 50% of the population, serve only to inseminate the females and do not reproduce themselves. Furthermore, a great deal of time and effort is often employed in searching for a mate and mating can itself be an energetically expensive and potentially dangerous process. By contrast, in an asexually reproducing organism, 100% of the population is able to reproduce. Consequently an asexually reproducing population is theoretically able to grow faster and respond to any changes in the environment (e.g. increased food supply) faster than one that reproduces sexually. The other 'cost' of sexual reproduction is that the gametes are haploid and the process of recombination at meiosis means that an individual is only able to pass on 50% of its genes to each of its offspring. Consequently, useful genes and gene combinations could be lost in the process of generating new genetic variants. Despite these problems, and several others, the vast majority of organisms undertake sexual reproduction and therefore it must have some major advantage(s).

There are several theories why so many organisms reproduce sexually and these are admirably evaluated by Sherratt and Wilkinson, (2009). One of the most popular theories is that of Hamilton

et al. (1990) who suggested that sexual reproduction has arisen as a mechanism by which organisms can limit the problems of parasitic infections. As we have seen, parasites can potentially reproduce faster than their hosts and therefore they will evolve to overcome the most common combination of host resistance alleles. As a consequence, hosts with rarer resistance alleles will then be at a competitive advantage and ultimately one of these will become the most common resistance allele combination in the host population. And so the arms race will continue with the parasites adapting to the most common resistance allele combination and the host generating new allele combinations. The process of recombination ensures that (provided the initial gene pool is sufficiently diverse) there will be a constant supply of novel resistance alleles. Furthermore, a resistance allele combination to which parasites have adapted need not be lost from the population because it may prove useful again in the future. By contrast, in an asexually reproducing organism, the offspring will have the same resistance allele combinations as their parents and once parasites have overcome these, then the whole population is vulnerable to disease.

If sexual reproduction has arisen as means of reducing the depredations of parasites, then one would expect it to be common where parasites are abundant and challenge is frequent. By contrast, asexual reproduction would be expected to be favoured where parasites were absent or the level of challenge reduced. Although there are several instances of exactly this in the literature, they remain remarkably few. The best-known example is that of the snail *Potampyrgus antipodarum* that originated in New Zealand and has since spread to many parts of the world. It exists as sexually reproducing populations, asexually-reproducing populations, and mixed sexually- and asexually-reproducing populations. Positive correlations have been described between the extent of parasitism by parasitic flatworms and the frequency of sexual reproduction in the snails. Sexual reproduction is rare where flatworm parasite challenge is low and, conversely, it is common where the parasite challenge is high (Lively, 1987; Lively and Dybdahl, 2000). Another commonly cited example is that of minnow populations living in Mexico (Lively *et al*., 1990). These minnows exist as both asexually-reproducing and sexually-reproducing populations, but those reproducing sexually tend to have lower parasite burdens (except where inbreeding has resulted in reduced genetic diversity). However, it is becoming increasingly accepted that although parasitic infections may be a major factor in the evolution and maintenance of sexual reproduction, there are probably many other factors also involved.

Most multicellular parasites reproduce sexually themselves, although some combine it with asexually-reproducing larval stages (e.g. schistosomes), and even some parasitic protozoa (e.g. trypanosomes) exhibit something akin to sexual reproduction. This would suggest that even endoparasites living in protected environments such as the gut or bloodstream of another animal remain vulnerable to infections. It certainly shows a need to adapt within the host to factors such as the immune response and (in mammalian hosts) drug treatments.

1.5 Parasitism as a ‘lifestyle’: advantages and limitations

Provided one can get away with it, stealing is easier than having to make something oneself or to earn enough money to purchase it. It should therefore come as no surprise that many organisms have adopted a parasitic lifestyle to some extent. If one takes the view that the main purpose of any organism’s existence is to ensure that as many of its genes as possible are transferred into the next generation, then all organisms should maximise their reproductive output. However, producing

offspring is energetically costly and these costs have to be traded off against other activities such as finding food and then digesting and absorbing it, finding a mate, protecting oneself against predators and the environment. By living upon or within a host, a parasite can reduce many of these costs and thereby devote more of their time and energy to reproduction. Most parasites stay in association with their host for the duration of a life cycle stage and therefore, having located and infected their host, the need for sensory apparatus and locomotion are reduced because the parasite has access to a guaranteed food source. This guarantee also means that the parasite does not have to extract as much energy as possible from each 'unit of resource'. Instead, it can afford to be wasteful and many parasites have reduced metabolic pathways. Furthermore, there is no need to lay down metabolic reserves beyond those required for the next life cycle stage. Parasites rarely need well-developed food gathering apparatus and in some cases, such as the tapeworms, they have dispensed with a mouth and gut altogether, relying on nutrients being absorbed across the body wall.

1.5.1 Main advantages of a parasitic lifestyle

Because parasites live within or upon their host, they have a reduced need to maintain body surfaces and behaviours that protect them from desiccation, heat, cold, because this is done by the host. Similarly, the parasite is to a large extent protected from predators and pathogens, which would have to overcome the host's immune system before locating the parasite. Even ectoparasites are protected to some extent because hosts are not always able to distinguish between a predator that is attempting to take a bite out of them from one that is intent on removing a flea or louse.

A parasite will be transported wherever the host goes and therefore the limits of its dispersal depend upon the dispersal powers of its host, coupled with whatever other special needs the parasite has to complete its life cycle (e.g. the presence of a suitable vector or environmental conditions). Consequently, a parasite does not have to devote energy to dispersal.

If the benefits are so enormous, this therefore begs the questions why there are not more highly specialised parasites and why parasitism tends to be extremely common among some groups of organisms but rare among others. For example, there are comparatively few parasitic higher plants, Lepidoptera or vertebrates.

1.5.2 Main limitations of a parasitic life style

Any would-be parasite must first overcome the putative host's immune defences and adapt to its internal physiological environment. This is not easy and as a consequence, most parasites are highly host-specific. However, host-specificity places the parasite in a difficult situation because its existence then becomes dependent upon that of its host. Should the host become extinct, then its parasites will follow suit unless they are able to infect other organisms. Furthermore, for the individual parasite, finding hosts is seldom easy. Although many parasites produce huge numbers of offspring, the chances of any one of them managing to locate a suitable host and reproduce successfully are extremely small. The advantages and disadvantages of the parasite lifestyle are summarised in Table 1.1.

Table 1.1 Summary of advantages and disadvantages associated with the parasite lifestyle

Advantages	Disadvantages
Once host located, no need for further searching	Extreme host specificity can increase vulnerability to extinction
Food permanently available	
Limited requirement for complicated food capturing mechanisms	Must locate at optimal site on/in host to ensure food/survival
Reduced need for food processing	
Protection from environmental extremes	Must adapt to host's internal physiological environment (internal parasites only)
Protection from predators and diseases	Must overcome host's immune defences
Reduced need for dispersal because host (+ vector) carries the parasite.	Spread limited by host's geographic range
Can devote larger proportion of energy intake to reproductive output than a free-living organism	Transmission can be extremely risky and most offspring die before establishing in a new host

1.6 The economic cost of parasitic diseases

Parasitic diseases afflict large numbers of us humans: in 2004, the WHO estimated that on a global scale, infectious and parasitic diseases were responsible for 16.7% of male and 15.6% of female deaths. Malaria was the parasitic disease causing most deaths (1.5%) although it may come as a surprise that many more people die in traffic accidents (2.2%) and from diarrhoeal diseases (3.7%). In 2009, it was estimated that there were approximately 225 million clinical cases of malaria and these resulted in about 781,000 deaths. Most of these cases occurred in children younger than 5 years old living in Africa (http://www.rollbackmalaria.org). Some 50% of global deaths from malaria and 47% of all cases occur in just five countries: Nigeria, Democratic Republic of Congo, Uganda, Ethiopia, and Tanzania. Although these figures indicate the seriousness of malaria, they also demonstrate that malaria control programmes are working. In 2000, there were estimated to be 233 million clinical cases that resulted in approximately 985,000 deaths (WHO, 2011). Consequently, despite the rise in the human population in malaria-endemic regions, in the past decade there has been a reduction in the number of people developing clinical malaria and proportionately fewer people are dying from the disease.

1.6.1 Economic consequences of parasitic diseases of humans

Inevitably, parasitic diseases cause financial losses to both an individual, their family, and to the wider society as a consequence of both morbidity ('illness') and mortality ('death'). These costs can be divided into the direct costs associated with the diagnosis and treatment of the disease and indirect costs that result from a person's inability to work or reduced efficiency/productivity. Although the direct costs are relatively easy to measure, it is the indirect costs that usually have the most serious consequences for both an individual (personal mortality excepted) and society. This is because most parasites cause chronic disease that can persist for months or even years. For example, a study in Africa in 1987 found that the cost of a person suffering from a typical uncomplicated episode of malaria was US$9.87, of which the direct costs amounted to only 18.6% of the

total and the remaining 81.4% were owing to the indirect costs (Shepard *et al*., 1991). Although US$9.87 might sound like a small sum of money, at the time it was equivalent to 12 days of productive work which was being lost. Indeed, the enormous cost of parasitic diseases in developing countries is seldom appreciated. For example, in Southern India, lymphatic filariasis is estimated to cost in the region of US$811 million per year and cause productivity losses as high as 27% in the weaving sector (Ramaiah *et al*., 2000). Parasitic diseases that cause disfigurement, such as lymphatic filariasis, can result in social exclusion that further traps the sufferer in poverty. Perera *et al*. (2007) relate how patients suffering from lymphatic filariasis in Sri Lanka can become so isolated that they will not venture out to seek freely available treatment at government clinics, let alone to look for paid employment.

1.6.2 Economic consequences of parasitic diseases of domestic animals

Similarly, for domestic animals, there are the direct costs of diagnosis and treatment along with mortalities, but the losses that result from lost productivity (e.g. milk yield, live weight gain) or work capacity (e.g. draught oxen, camels, donkeys) are much greater. For example, in 1997, losses in the dairy and beef industry in Australia owing to abortion outbreaks induced by neosporosis (*Neospora caninum*) were in the region of Aus$25 million per year (Ellis, 1997).This figure, like most cost estimates of the effects of neosporosis, does not take into account sporadic abortions or the losses owing to reduced productivity (Reichel, 2000). There are no figures for the economic cost of *Neospora caninum* infection in dogs but many dog owners will spend large sums of money on the welfare of their pets and pedigree dogs can sell for hundreds or even thousands of pounds. Consequently, control of the disease in dogs is of concern to owners as well as a means of preventing its transmission to cattle. In developing countries, the economic costs of parasitic diseases of livestock can have consequences for the expansion of agriculture and also the ability of human populations to feed and clothe themselves. For example, in Africa, trypanosome parasites cause the wasting disease known as Nagana in cattle and other domestic livestock. The condition is debilitating and potentially fatal and it has been estimated to result in annual losses of over US$ 1.3 billion (Shaw, 2004). Similarly, East Coast fever in cattle (caused by *Theileria parva*) is currently mainly restricted to East, Central, and Southern Africa where it causes annual losses of hundreds of millions of pounds and prevents large areas of land from being used for farming. East Coast fever is mainly controlled through the use of acaricides to kill the tick vectors but because the tick populations are becoming increasingly resistant to these chemicals, there is a fear that the ticks will spread and consequently so will the disease.

1.6.3 Estimating the costs of morbidity due to disease

It is currently common practice to measure the consequences of disease and other causes of morbidity in terms of disability-adjusted life years (DALYs). These are calculated by summing an estimate of the disease's or condition's potential for reducing lifespan and an estimate of the amount of time a person suffering from the disease/cause is disabled (www.who.int/evidence/bod). One DALY is the equivalent of the person losing a year of healthy life:

$$\begin{aligned}\text{DALY} = {} & \text{number of years of life lost through premature mortality} \\ & + \text{years of life lived with disability}\end{aligned}$$

In some studies, the DALY model is refined to place greater value on the life of a young person than of an older person. This is done on the basis that a young person has (potentially) a longer productive life in front of them than an older person. DALYs enable the researcher to compare a wide variety of mortality factors. For example, a person committing suicide or dying in a traffic accident would suffer premature death but there would be little or no disability (assuming they died instantly) while a person with malaria may suffer prolonged ill health and ultimately die prematurely years later. The results of comparative studies can help prioritise funding and policy decisions and determine the effectiveness of health initiatives. For example, in 2006 it was estimated that mass drug administration to prevent the transmission of geo-helminths (e.g. *Ascaris* and *Trichuris*) would cost between US$2 and US$9 per DALY averted. By comparison, the frequent blood transfusions that are required to treat certain forms of thalassaemia can cost over US$10,000 per DALY averted and are therefore only available to the most affluent families or those where the treatment is provided by the government (Laxaminarayan *et al*., 2006).

Although DALYs can yield useful information, their application to parasitic diseases has been criticised (e.g. Hotez, 2007; Payne *et al*., 2009). In particular, the estimation of the years of life with disability includes a weighting factor that is meant to account for the severity of the disease. This is difficult to estimate for many parasitic diseases. For example, DALY calculations for the global effect of schistosomiasis vary from 3 million to 70 million and for hookworm infection from 1.5 million to 22.1 million. If the upper figures are used, then schistosomiasis and hookworm infections would rank alongside malaria (DALY = 46.5 million) as the most important of all parasitic diseases but if the lower figures are used, then their importance is considerably reduced (Hotez *et al*., 2010). The estimate is further complicated by the fact that people are often co-infected with a variety of parasite species and parasite–pathogen interactions (e.g. *Leishmania*–HIV) can have major implications for disease progression and outcome.

1.6.4 Economic consequences of parasitic diseases of wildlife

Whether it is appropriate to use economic costing when considering wildlife is a controversial topic: how much is a blackbird worth? Nevertheless, parasitic diseases can have profound effects on wildlife populations and these must be borne in mind in wildlife management and conservation. For example, 20–80% of white-tailed deer fawns in the Southern USA used to die each year as a consequence of infection (mostly in the umbilical region) with the larvae of the New World Screw-worm (*Cochliomyia hominivorax*) (Fuller, 1962). The fly also caused enormous economic losses to the cattle industry and therefore a control programme was instigated. This was so successful that *Cochliomyia hominivorax* was eradicated from the USA in 1964 and therefore (and unintentionally) a previously major cause of deer mortality was reduced to zero. Before the control programme commenced, *Cochliomyia hominivorax* was often spread to new locations through the transport of infected cattle. This movement of parasites can have devastating effects for local wildlife if they are susceptible. For example, on the Galapagos Islands, the populations of several of the species of Darwin's finches have been devastated following the arrival of *Philornis downsi* – a fly which has ectoparasitic larvae. The fly probably came to the islands in the 1960s among imported fruit and vegetables. The adult flies are free-living but their blood-feeding larvae are ectoparasitic on nestling birds and have caused high mortalities (Fessl *et al*., 2010). Wildlife tourism is big business and a major source of income in both developing and developed countries and therefore parasitic diseases that afflict wildlife can have serious economic consequences.

Box 1.5 How malaria has influenced the course of history

Malaria has influenced the course of history for thousands of years and remains relevant today. The symptoms of chronic and repeated infections of malaria are quite distinct and the disease can be identified with almost complete certainty from historical descriptions from Ancient civilisations of Egypt, Sumeria, China and India. For example, a Chinese medical text, the *Nei Cheng*, written in approximately 2700 BC refers to epidemics of the 'Mother of Fevers' that is undoubtedly malaria. The authors describe the cyclical fevers and enlarged spleen that are features of malaria. Similarly, the symptoms of malaria can be identified from the writings of Hippocrates in Ancient Greece (~500 BC). Some authors have suggested that the decline of the Ancient Greek and Roman civilisations was associated with effects of malaria epidemics (Poser and Bruyn, 1999). More recently, in 1943, an outbreak of malaria among Allied troops during the Second World War seriously compromised their attempts to invade Sicily. American troops suffered similar problems with malaria during the Viet Nam War in the 1960s. Although malaria caused only about 0.2% of fatalities among the American troops, the debilitating effects reduced the combat strength of some units by up to 50%. The Viet Cong were aware of the problems that malaria caused the American troops and intentionally sabotaged local mosquito and malaria control programmes. They were successful in undermining malaria control over large areas, but as a consequence the combat strength of their own troops was also severely compromised (Drisdelle, 2010).

Today malaria is often thought of as a tropical disease but it used to be a common problem in many of the temperate regions of the world. Malaria was a major cause of mortality in parts of Italy and Greece as late as the 1920s (Snowdon, 2006). Malaria existed in the UK until the early years of the twentieth century, particularly in the fenland regions, and was known as 'the ague' (Reiter, 2000). The potentially fatal consequences of the ague are alluded to in works by Geoffrey Chaucer (c.1343–1400) and William Shakespeare (1564–1616) so the disease was obviously common enough for their audiences to be familiar with it. Samuel Pepys (1633–1703) describes suffering from the ague in his diaries and Oliver Cromwell (1599–1658) died as a consequence of an attack of the 'tertian ague' in September 1658.

1.7 Why parasitic diseases remain a problem

Whenever a seemingly simple but intractable problem arises, a commonly heard refrain is 'If we can put a man on the moon, why can't we . . .?' As we have seen, parasitic diseases cause suffering to us and to our domestic animals and the economic costs are enormous. Furthermore, many parasitic diseases could be controlled by simple measures such as providing safe drinking water and appropriate waste disposal facilities. So, one might ask, why do parasitic diseases continue to afflict so many people and impact so heavily on agriculture?

As with so many apparently simple questions, the reason parasitic diseases are such a problem does not have a single simple answer and is also tied up with the most exasperating factor of all – human behaviour (Table 1.2). To begin with, human parasitic diseases are predominantly (although not entirely) a problem of poor people who live in insanitary conditions and do not have a healthy diet. The diseases are therefore most prevalent in developing countries and neither

Table 1.2 Summary of factors contributing to the problems of parasitic diseases

Poverty
Lack of sanitation
Complacency
Poor nutrition
Lack of health infrastructure
Lack of government interest
Corruption
Urbanisation
Social conflict/wars
Movement of non-immune people to regions where they become infected from the resident population.
Movement of infected people to regions where they infect non-immune resident population
Man-made environmental damage
Natural disasters
Lack of effective drugs/parasite resistance
Increasing resistance of vectors/intermediate hosts

the government nor individual people have money to spare. For example, according to the World Health Organisation (WHO, 2009), in 2006 the total healthcare expenditure in Zimbabwe as a percentage of the GDP (gross domestic product) was higher than it was the UK (9.3% vs 8.2%). However, in terms of total expenditure per capita, Zimbabwe was able to devote only US$38 per person per year to healthcare while the UK spent US$2815. It goes without saying that US$38 does not buy a lot of medicines.

Humans are extremely adaptable creatures and will survive under remarkably harsh environmental and political regimes. This adaptability can degenerate into acceptance and complacency on the parts of both individuals and governments. Because parasitic diseases are so prevalent in developing countries, there is a tendency not to prioritise them: periodic fevers and diarrhoea become an accepted part of everyday life. Furthermore, parasitic diseases tend to cause chronic disease and although the patient may ultimately die, the condition does not capture the attention of the local or world media. For example, the Ebola virus is well known in the developed world because of its appalling pathology and images of patients being treated by nurses and doctors dressed in spacesuit-like protective clothing. However, although the Ebola virus causes about 70% mortality, the numbers of people who have actually died of the infection are relatively few. The fact that Ebola virus has been touted as a possible biological warfare agent has also helped to engender interest in the disease and funds to study and control it. By comparison, Human African Trypanosomiasis (often referred to as ‘sleeping sickness’) causes 100% mortality if untreated and kills many more people than Ebola but is seldom mentioned in the media. The reason is simple, Human African Trypanosomiasis kills slowly by comparison and those who suffer are among the poorest in Africa and live in some of the most war-torn and disorganised regions of the continent.

In addition to being poor, the countries in which parasitic diseases are most problematic are often unstable and suffer high levels of corruption. Consequently, those in control often devote much of their revenue to the trappings of power and military spending: many developing

countries spend only 2–3% of their GDP on healthcare. This means that even less of not very much money is available for the treatment and control of parasitic diseases. The instability of the regimes and conflicts which can last for decades make it difficult to provide health services and co-ordinate control strategies. They also lead to the destruction of basic infrastructure and the decline in agricultural and commercial activity – and this contributes to poverty and malnutrition. At its worst, conflicts lead to large numbers of refugees who are frequently housed in squalid campsites which lack proper sanitation. These displaced people are often in poor health and malnourished, they take their parasites with them wherever they go and they are highly vulnerable to the local strains of parasites wherever they are relocated to. For example, the civil wars in the Central Asian states such as Tajikistan, which occurred after the break-up of the Soviet Union in the early 1990s, displaced people to neighbouring countries including Afghanistan. The most common type of malaria in Tajikistan at that time was caused by *Plasmodium vivax,* whereas in Afghanistan, the more virulent *Plasmodium falciparum* was found and drug-resistant strains were circulating. Some of the refugees who returned home in the late 1990s were infected with drug-resistant *Plasmodium falciparum* and since there was a suitable mosquito vector, this form of malaria was transmitted among people who had never left Tajikistan (Pitt *et al*., 1998). Natural disasters, such as cyclones and earthquakes, can lead to similar destruction of infrastructure and refugee problems. Widespread flooding can also provide suitable breeding conditions for mosquitoes and thereby increase the spread of mosquito-borne diseases such as malaria. For example, the widespread flooding in Mozambique which occurred in 2000, led to an increase in malaria and water-borne diseases.

Damage to the environment caused by humans can also encourage the spread of disease by making conditions more suitable for vectors and intermediate hosts and/or the survival of parasite eggs and cysts. For example, clearance of the rainforests in the Amazon produces open sunlit pools that are ideal breeding grounds for the mosquito vector of malaria *Anopheles darlingi* (Harris *et al*., 2006). Also, as people move into these clearings to live or work, they come into contact with infectious agents which are not adapted to living in humans but can still cause disease (i.e. zoonoses). Climate change is also thought to be affecting the transmission and range of a number of parasitic diseases (Patz, 2001, 2004; Patz *et al*., 2002; Haines and Patz, 2004).

The way we live and organise our societies also contributes to the spread of parasitic diseases. Throughout the world there is an increase in urbanisation which means that more people are living in close proximity together and where sanitation remains poor, this can facilitate the spread of contaminative diseases and those vector-borne diseases in which the vector can survive in an urban environment (e.g. certain mosquito species). The increased use of cars and motorised transport has resulted in large numbers of used tyres entering the ecosystem. Used tyres retain water after it has rained and make excellent breeding grounds for some mosquitoes. There is a huge international market in used tyres that are loaded onto ships and moved between countries. In the process, mosquitoes are also moved around the world and notorious vectors of disease such as *Aedes albopictus* are now established in countries (e.g. Spain) where they were formerly absent (Roiz *et al*., 2007). People are also much more mobile than they used to be and cheap air travel means that millions of people are now rapidly travelling between countries for leisure and business but also, and in large numbers, as economic migrants and political refugees. Consequently, they become exposed to diseases to which they have no previous experience, and hence immunity, and are therefore vulnerable to infection. Similarly, those who are already infected (but may not be aware of the fact) carry their diseases with them and could potentially transmit their infections to

a non-immune population on arrival (Myers, 1999, 2000a, 2000b, 2000c). For domestic animals it is possible to instigate legislation that governs their movement and a 'passport scheme' that ensures that they have received appropriate vaccinations and/or drugs and/or undergo a period of quarantine upon arrival in their country of destination. However, this cannot be done so easily for humans. Some countries insist that all persons entering their borders have documentation proving they have received certain vaccinations (e.g. yellow fever) but there are few anti-parasite vaccines and even where effective prophylactic medicines are available to treat parasites (e.g. antimalarials), it is notoriously difficult to persuade people to take them as prescribed.

Another of the major reasons why parasites remain a problem is the lack of suitable drugs and vaccines to treat them. The development of drugs for use in human medicine takes many years and is extremely expensive. Consequently, the drug companies need to be sure that they can obtain a good rate of return for their investments. Unfortunately, the people who suffer most severely from parasitic diseases are usually poor and cannot afford expensive drugs. Similarly, the development of anti-parasite vaccines has been hampered by a combination of cost and the difficulty of generating protective immunity against parasitic infections. These issues are dealt with in detail in Chapter 10.

The control of parasites by targeting their vectors/intermediate hosts is also becoming more problematic. For a number of years this approach proved highly effective and in the 1950s it was even believed it might be possible to eradicate malaria by killing the anopheline mosquito vectors. However, some vectors are exhibiting increasing resistance against a wide range of insecticides and new chemicals are not being developed to replace those in current use. Furthermore, there are mounting concerns for the environmental damage that can result from inappropriate use of insecticides and fears over the risks they pose to human health.

1.8 Taxonomy

Correct diagnosis is essential for treatment and control of any disease and that means there needs to be consensus on the names and terms used in the identification process. Therefore, before we begin to consider specific parasites, it is necessary to have an understanding of how the taxonomic system works and its relevance to parasitology. Those who study the identification of organisms are called taxonomists and they arrange organisms into a hierarchy of categories to demonstrate their relationship to one another (Table 1.3). Not all taxonomists agree on the appropriate division for a grouping (taxon). For example, some workers consider there to be two suborders of Diptera: the Nematocera and the Brachycera and that the term Cyclorrhapha should be considered a division of the Brachycera rather than a suborder.

There is some debate about how many kingdoms exist although most modern textbooks refer to six: Archaea, Bacteria, Protista, Fungi, Plantae, Animalia. Parasitic species are common in all the kingdoms but we will be concentrating on the kingdoms Protista and Animalia. Viruses are not usually considered to be living entities and therefore do not have a kingdom of their own. However, Didier Raoult and his co-workers argue that the giant viruses called 'nucleocytoplasmic large DNA viruses' (NCLDVs) should be considered as an additional distinct domain of living organisms (Boyer *et al*., 2010). NCLDVs are so large that they can be mistaken for bacteria and their genomes are typically twice the size of other viruses. The suggestion that a specific group of viruses might be 'living organisms' has generated a great deal of controversy (Zakaib, 2011). According to Williams *et al*. (2011), the large size of the NCLDVs is a consequence of capturing

Table 1.3 The taxonomic hierarchy with specific reference to the sheep nasal bot fly *Oestrus ovis*

Taxonomic division	Taxon name	Common name
Kingdom	Animalia	Animals
Subkingdom	Bilateria	
Branch	Protostomia	
Infrakingdom	Ecdysozoa	Moulting invertebrates
Phylum	Arthropoda	Arthropods
Subphylum	Hexapoda	Insects and related species
Class	Insecta	Insects
Infraclass	Pterygota	Winged insects
Division	Neoptera	
Subdivision	Endopterygota	
Superorder	Panorpita	
Order	Diptera	True flies
Suborder	Cyclorrhapha	Higher flies
Superfamily	Oestroidea	Bot flies
Family	Oestridae	
Subfamily	Oestrinae	
Genus	*Oestrus*	Sheep nasal bot fly
Subgenus		
Species	*ovis,* Linnaeus, 1758	
Subspecies		

Note that only the genus name and lower taxonomic descriptors are in italics.

host DNA through horizontal gene transfer and there is insufficient evidence to consider NCLDVs as a separate domain.

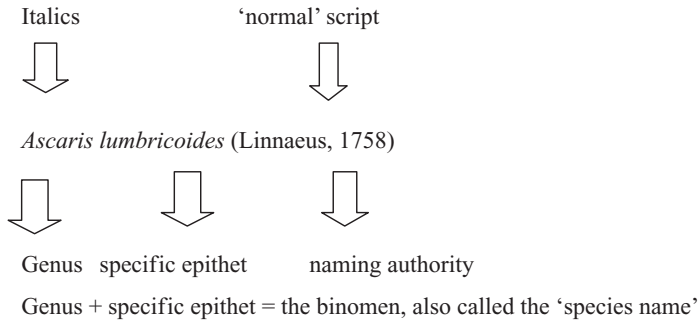
The kingdoms are subdivided into units or taxa (singular taxon) such as class, family, genus. There are no rules about how many species constitute a genus, how many orders constitute a class, or whether families are divided into subfamilies. However, it is essential that the taxon forms a natural grouping. Consequently, research, especially using molecular techniques, causes taxonomists to regularly re-arrange the hierarchy of individual species and groups of organisms. A class, family or any other category within one group of organisms is therefore not evolutionarily comparable with those in another group.

The International Commission on Zoological Nomenclature (ICZN) provides rules on legal aspects of nomenclature (e.g. precedence). However, it is not unusual for workers to continue using old names that have been superseded or to fail to agree on an accepted single name. For example, the blowflies known as *Lucilia cuprina* and *Lucilia sericata* within the UK and Europe are often called *Phaenicia cuprina* and *Phaenicia sericata* by workers in USA.

1.8.1 The binomen system

All organisms (apart from viruses) have a two-part name, or binomen – hence the term binomial nomenclature. The two parts consist of the generic (or genus) name and the trivial name (also called the specific epithet or specific name).

The trivial name may be followed by the naming authority, i.e. the name of the person who first described the organism, along with the date the description was published – this is placed in brackets.



Surprisingly, there is no universally accepted definition of what is meant by the term 'species'. Indeed, there are currently over 20 different definitions. Furthermore, over the course of thousands of years there is never a single point at which one species becomes two: it is like attempting to identify the day one ceases being a child and becomes an adult. To further complicate matters, some species have distinct forms that are called sub-species, and these are distinguished through the use of trinomens. For example, the human body louse *Pediculus humanus humanus* and the head louse *Pediculus humanus capitatis* are usually distinguished as separate sub-species. However, for many years there has been a debate about whether the reported differences in their morphology and behaviour are consistent enough to justify them being considered closely related sub-species or separate species in their own right. Current molecular evidence suggests that they are morphotypes of a single species (Light *et al*., 2008). Similarly, there are two physiological variants of the mosquito *Culex pipiens*: *Culex pipiens pipiens* which bites only birds and *Culex pipiens molestus* which bites only humans. The two variants of *Culex pipiens* cannot be differentiated morphologically. They can be crossed in the laboratory, but, in the UK, the populations remain genetically isolated in the wild. Distinguishing between the variants is important because this mosquito is capable of acting as a vector for the potentially fatal West Nile Virus and therefore its biting behaviour has a major impact on whether the disease spreads from birds to humans.

The difficulty of differentiating between species and sub-species can give rise to 'taxonomic inflation' in those groups that are particularly well studied. For example, ant taxonomists tend not to recognise sub-species so everything is separated at the species level. By contrast, butterfly taxonomists are enthusiastic users of trinomens. Not surprisingly, this often results in ecological surveys revealing a greater species diversity of ants than butterflies.

Some scientists have suggested that taxonomic inflation is being driven by the increasing use of the phylogenetic concept of species rather than the older biological species concept. The traditional biological species concept operates on the premise that two organisms should be considered different species if either they are incapable of mating or, if they do mate, then their progeny are infertile. Allowances have to be made for the likelihood of gene flow between populations. For example, the fact that tigers and lions can hybridise does not mean that they are the same species, as this would never happen in the wild. By contrast, the phylogenetic species concept is based on the fact that separate populations of organisms often have distinct inheritable differences (for example, a colour pattern or the size of a body part). What constitutes an inheritable difference

sufficient to qualify a population as a 'species' would depend upon the views of the taxonomist. The increasing use of DNA analysis by taxonomists has undoubtedly contributed to the popularity of the phylogenetic species concept, because it often identifies differences in gene sequences between populations. Some workers have suggested that the adoption of a phylogenetic species concept can result in up to 48% more species than the biological approach for the same group of organisms (Marris, 2007). It also causes complications by suggesting some unlikely taxonomic relationships. For example, a particular clinical presentation of an 'atypical' pneumonia is common among AIDS patients. It is associated with infection with an organism of *Pneumocystis* spp. which was originally classified as a protozoan parasite and called *Pneumocystis carinii*. DNA analysis yielded evidence that this organism is much more closely related to fungi than protozoa. Reclassification also brought about a change in species name to *Pneumocystis jirovecii* (Stringer *et al*., 2002).

Questions

1. With the aid of named examples, distinguish between the terms intra-specific and inter-specific animal associations.
2. Distinguish between the terms facultative and obligatory parasitism.
3. Should the red-billed oxpecker (*Buphagus erythrorhynchus*) be considered to be in a commensal relationship with cattle? Explain your answer.
4. What is the difference between morbidity and mortality?
5. Briefly explain why haematophagous organisms usually have a symbiotic relationship with microorganisms.
6. What advantage does *Hydra viridis* gain from its association with *Chlorella* when maintained in (a) constant darkness with food; and (b) constant light with food? Explain your answers.
7. What is a paratenic host and what purpose does it serve in parasite transmission?
8. Briefly explain why harm is a difficult term to define in relation to parasitism.
9. State two advantages and two disadvantages of the parasitic lifestyle.
10. Give four reasons why parasites remain a problem in developing countries.

2

Parasitic protozoa, fungi and plants

2.1 Introduction

In this chapter we provide a summary of the life cycles and biology of some of the most important parasitic protozoa in human and veterinary medicine. We also briefly mention fungi and plants but there is insufficient space to deal with these topics in any depth. Readers requiring more details on parasitic fungi and plants should therefore consult specialist texts such as Reiss *et al*. (2011) and Press and Graves (1995). Although they consist of just a single cell, parasitic protozoa exhibit a wide variety of morphological forms and some of them have exceptionally complex life cycles. These complexities are often part of the reason they are successful as parasites. They exhibit a vast array of immune-avoidance mechanisms and some species are able to reproduce in both invertebrate and vertebrate hosts. Parasitic protozoa can be found living in all the organs of the body and cause diseases that range from benign to rapidly and incurably fatal. They also exhibit every imaginable means of transmission from contamination to sexual and vector-assisted. As a consequence, this is unavoidably the longest chapter in the book and many species and topics will be returned to in later chapters.

2.2 Parasitic protozoa

2.2.1 Kingdom Protista

The kingdom Protista is a loose assemblage of organisms that have in common their shared characteristic of possessing a membrane-bound nucleus – which therefore makes them eukaryotes – and their lack of the organisational features that are found in the kingdoms Fungi, Plantae, and Animalia. Although they are for the most part single-celled, some are colonial and in the case of the brown seaweeds and kelp, these can form structures that extend to several metres in size. The lack of unifying morphological and molecular features leads to the conclusion that the kingdom is polyphyletic – that is, it is composed of individuals that arose from a variety of different ancestors – and there are frequent calls for it to be split into several separate kingdoms or clades to reflect these differences.

Parasitology: An Integrated Approach, First Edition. Alan Gunn and Sarah J. Pitt.

2.3 Phylum Rhizopoda

This phylum, which used to be known as the Sarcomastigophora, contains the amoebas. The name 'Rhizopoda' translates as 'root-like foot' and refers to the process by which the cytoplasm flows within the cell to cause projections of the body wall called pseudopodia (false feet) that are used for both movement and the ingestion of food. Food is ingested in a process called phagocytosis and results in the food item being enclosed within a membrane-bound vesicle called a food vacuole. In some species, movement is also aided by one or more flagellae. Although the amoebas are sometimes said to be primitive, ultrastructural and molecular studies indicate that this is not the case. It is a relatively small phylum consisting of about 200 described species, most of which are free-living or commensal but the phylum includes important parasitic species such as *Entamoeba histolytica*.

2.3.1 Genus *Entamoeba*

Entamoeba histolytica *Entamoeba histolytica* is the causative agent of amoebic dysentery. Dysentery is a general term that is used to describe a serious inflammatory disorder affecting the intestines that results in intense diarrhoea and is often accompanied by pain and fever. It can result from a variety of causes and amoebic and bacterial dysentery occur in both temperate and tropical regions. Dysentery has long been known as a 'handmaiden of war', often inflicting more casualties than bullets and bombs. Accounts of epidemics of dysentery accompany nearly every account of war from antiquity to the present day. Wherever large numbers of people (especially if they are malnourished) live in close proximity and squalid conditions, the situation is ripe for an outbreak of dysentery of epidemic proportions. Not surprisingly, the increased migrations and deteriorating economic conditions in many developing countries have led to enhanced levels of amoebic dysentery in recent years; tourists travelling on 'exotic holidays' have also found themselves victims of amoebic dysentery.

Box 2.1 Entamoebas and amoebic dysentery

It used to be estimated that about 10% of the world's population were infected with *Entamoeba histolytica* although the majority of these never expressed any disease symptoms. For many years, the reason so many apparently infected people remained asymptomatic was put down to the variable virulence of different strains of the parasite. It is now clear that there are three species of morphologically identical *Entamoeba* commonly found in the human intestine: *Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii*. These three species can only be distinguished by detecting parasite-specific antigens or DNA analysis (Jackson, 1998; Martinez-Palomo and Espinosa-Cantellano, 1998). Pathogenic symptoms are owing to infection with *Entamoeba histolytica*, whereas *Entamoeba dispar* is rarely, if ever, invasive in humans. However, intestinal pathology and liver abscesses have been described in hamsters infected with *Entamoeba dispar* (Shibayama *et al*., 2007). The pathogenic status of *Entamoeba moshkovskii* remains uncertain. About 9 in 10 infections with *Entamoeba histolytica* are now believed to be of

Entamoeba dispar and infections with *Entamoeba moshkovskii* are being increasingly reported (e.g. Fotedar *et al*., 2008; Karim *et al*., 2003). Consequently, it is difficult to make sense of literature in which the three species have not been distinguished. Nevertheless, strain differences do occur between populations of *Entamoeba histolytica* and these are reflected in the pathology they cause. Many people who are genuinely infected with *Entamoeba histolytica* fail to show disease symptoms and while this may in some circumstances be owing to host factors, it is also apparent that there are also avirulent strains of this parasite (Ali *et al*., 2007; Escueta-de Cadiz *et al*., 2010). A characteristic feature of virulent strains is that they over-express genes that code for lysine-rich factors and glutamic-rich and lysine-rich proteins. The function of these genes, that are referred to as KRiPs and KERPs respectively is not known but one of them, *kerp1*, is associated with the ability of *Entamoeba histolytica* to cause liver abscesses in hamsters (Santi-Rocca *et al*., 2008). A detailed review of amoebiasis is provided by Ximénez *et al*. (2009).

Despite the problems of identification, there is no doubt that *Entamoeba histolytica* is a major cause of disease in many parts of the world but particularly in developing countries. Every year, many millions develop amoebic dysentery or hepatic amoebiasis. The debilitating symptoms of these conditions can last for months or even years and up to 100 000 people die each year as a consequence of their infections (WHO, 1997).

There are two stages in the life cycle of *Entamoeba histolytica*: the actively growing and feeding stage referred to as the trophozoite form and the transmission stage called the cyst form (Figure 2.1). Like all other parasitic protozoa (but unlike the free-living forms), *Entamoeba histolytica* has no contractile vacuole. It also lacks mitochondria and this led some workers to suspect that it diverged from the path of evolution of other eukaryotes before these organelles were acquired. Molecular analysis has since demonstrated the presence of genes that code for

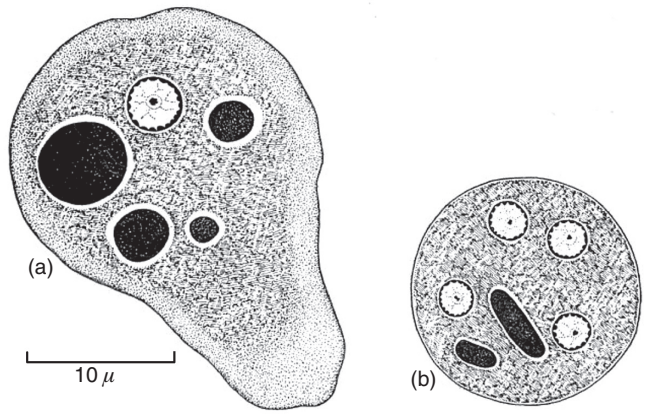


Figure 2.1 *Entamoeba histolytica* trophozoite (a) and cyst (b). It can be difficult or impossible to distinguish the chromatoidal bodies in the cysts using light microscopy and their nuclear structure may be lost after prolonged storage. Source: Chandler and Read, 1961

proteins of mitochondrial origin within the *Entamoeba histolytica* genome and the presence of an organelle called a 'mitosome'. Mitosomes are double-walled structures that lack DNA and have been described from a number of other organisms that were previously thought to lack mitochondria. Their function is uncertain but they are thought to represent the remnants of mitochondria.

The trophozoite is 12–60 μm in size and has a clear granular outer cytoplasm, a more densely granular inner cytoplasm, and there is an aggregated region of chromatin referred to as a karyosome centrally located within the nucleus. Reproduction takes place asexually by cell division and through cyst formation. The stimuli that cause the trophozoites to transform into cysts are uncertain but it is an essential part of the life cycle. This has led to the suggestion that if drugs could be developed that would prevent cyst formation, it might be possible to reduce parasite transmission. The cysts are 10–15 μm in diameter and (when mature) contain four nuclei and characteristic bar-shaped chromatoidal bodies that serve as a store of nucleoprotein. The cell wall contains chitin which provides protection and enables the cyst to survive in the outside environment for prolonged periods. The output of cysts can be enormous and an infected person may excrete over 10 million cysts per day in their faeces (Martinez-Palomo and Martinez-Baez, 1983). Transmission to a new host is a result of faecal-oral contamination (remember: 'flies, fingers, faeces, food') with infective cysts. Once ingested by a suitable host, the amoeba travels to the small intestine, where it emerges from the cyst and then undergoes a complicated series of divisions to produce eight trophozoites. The amoebae are subsequently swept down to the large intestine (colon) where they multiply in the lumen and may also invade the gut wall. The trophozoites are unable to survive outside the host and therefore usually play no part in the transmission process.

Avirulent strains of *Entamoeba histolytica* remain in the lumen of the colon and cause their host no harm. Those that are virulent attack and ingest the epithelial cells lining the gut wall and then proceed to spread through underlying layers. In the process of invasion, flask-shaped ulcers are formed which can become widespread with consequent bleeding over large areas of the intestine. It is therefore not unusual to find ingested red blood cells inside the food vacuoles of the trophozoites of virulent strains, and this can be useful in laboratory diagnosis. The loss of functional mucosal surface within the patient's gut and the loss of blood and fluid may cause death by dehydration and emaciation. A great deal of water is normally re-absorbed in the colon so, with the loss of functional surface, re-absorption is reduced. Patients suffering from amoebic dysentery therefore frequently complain of gastric pain and pass stools that are loose and contain mucus and blood mixed with faecal material. These symptoms are distinct from bacterial dysentery in which there is no cellular exudate.

The ulcers in the intestine often suffer secondary invasion by bacteria – this extends and deepens the ulcers and leads to increased blood and fluid loss. When the flask-shaped ulcers start to heal, they are replaced with fibrous scar tissue and this reduces gut elasticity and, if extensive, may impair peristalsis in the colon and even cause a potentially fatal gut blockage.

Secondary ulcers occur as a result of the amoebae damaging the lining of blood vessels and then being swept up in the bloodstream and setting up infections elsewhere in the body. The secondary ulcers can be potentially life-threatening. They are most common in the liver, although the lungs and brain may also be affected; a problem for the patient is that it is possible to mistake the symptoms for those of other diseases, such as cancer (e.g. Yapar *et al*., 2010). In hepatic amoebiasis there is usually just a single liver abscess that is located on the right lobe. Liver abscesses

can become extensive and produce copious amounts of purulent exudate (i.e. a thick fluid containing white blood cells, cell debris, and dead and dying cells) that is often described as resembling chocolate sauce. Depending on the site of the abscess, it can drain into the peritoneal cavity or the lungs – in which case it may be coughed up. Pulmonary amoebiasis often results from the extension of a pre-existing hepatic infection and therefore the majority of cases afflict the right lobes of the lungs. Cutaneous amoebiasis can result from the infection in the bowel spreading and colonising the perianal regions (Magana-Garcia and Arista-Viveros, 2008; Bumb and Mehta, 2006). Men can acquire cutaneous infections of the penis following anal intercourse probably from trophozoites colonising lesions on the penis (Parshad *et al*., 2002).

Entamoeba histolytica infections appear to be restricted to humans. The trophozoites voided with faeces soon die but the cysts are very resistant to environmental conditions and it is these that are the main source of infection. The cysts are usually transmitted via drinking water (or ice made from contaminated water), as contamination on vegetables grown on land fertilised with human faeces or via the bodies of insects that have moved between faeces and human food. Because of this, the safe and sensible disposal of human faeces is a crucial factor in reducing the transmission of *Entamoeba histolytica*.

Entamoeba dispar *Entamoeba dispar* is morphologically indistinguishable from *Entamoeba histolytica* and has the same life cycle but it is non-pathogenic. It is much more prevalent than *Entamoeba histolytica* (e.g. Samie *et al*., 2006) and therefore it is essential to be able to distinguish between the two species in order to avoid a false diagnosis of amoebic dysentery and thereby initiating inappropriate treatement. In mixed laboratory cultures *Entamoeba dispar* soon outgrows *Entamoeba histolytica* – which could cause problems where the amoebas are cultured to confirm an initial diagnosis by microscopy (Pysova *et al*., 2009). Whether or not this reflects better fitness or whether *Entamoeba dispar* influences the establishment of *Entamoeba histolytica* is not known.

Entamoeba moshkovskii For many years this species was considered to be a free-living amoeba. It was originally described from Moscow sewage and subsequently identified from a variety of ponds and sediments around the world. It has also been recorded in humans although the difficulty of distinguishing it from *Entamoeba histolytica* and *Entamoeba dispar* has undoubtedly led to it being under-reported. It was initially considered that, like *Entamoeba dispar*, it was a harmless commensal but there are reports of it causing diarrhoea (Fotedar *et al*., 2008; Tanyuksel *et al*., 2007). It is possible that once molecular-based diagnostic techniques are widely used that more cases of diarrhoea caused by *Entamoeba moshkovskii* will be reported.

Entamoeba gingivalis This amoeba is commonly found in swab samples taken from the gingival crevices of the human mouth. *Entamoeba gingivalis* does not form cysts and therefore transmission is probably through kissing or sharing food and eating implements. Although it is often considered to be involved in the development of periodontitis (an inflammatory disease that affects the gums and the bone surrounding the teeth), it is found in both healthy and diseased individuals. Part of the problem in determining its association with disease is that there is such a wide variation of rates of recovery of *Entamoeba gingivalis* from samples reported in the literature. This is probably owing to the collection techniques employed and it is recommended that 5–10 samples are taken from each person (Linke *et al*., 1989).

2.3.2 Other species of pathogenic amoebae

Naegleria fowleri Although over 30 species of *Naegleria* have been described, only one of these, *Naegleria fowleri*, is pathogenic. Like the other members of the genus, *Naegleria fowleri* is a free-living amoeboflagellate, i.e. it is an amoeba which, in one of its life cycle stages, possesses flagellae. It is a cosmopolitan species that is normally found in freshwater ponds and lakes but it has also been recovered from a wide range of wet or moist environments such as swimming pools, humidifier systems and damp soil (Marciano-Cabral and Cabral, 2007). There are three-life cycle stages: (1) the active amoeboid trophozoite; (2) the non-feeding flagellate stage that is produced when the food supply runs low and acts as a dispersal stage; and (3) a cyst stage that is formed in response to adverse environmental conditions.

Humans usually become infected when they swim in infected water. The trophozoite is the infective stage and it migrates from the nasal mucosa along the olfactory nerves, through the cribiform plate and thence into the brain where it causes a condition called primary amoebic meningoencephalitis. This term is used to distinguish it from encephalitis caused by *Entamoeba histolytica* in which invasion of the brain is a secondary consequence of infection in the gut. The trophozoite of *Naegleria fowleri* must move remarkably quickly, as the time between initial exposure and first symptoms may take as little as a 24 hours and death often follows after 4–10 days. The symptoms of infection are non-specific and often start with neck stiffness followed by headaches, photophobia, confusion, seizures, and the patient then enters into a coma from which he/she seldom recovers. Although other animals, such as mice and monkeys, can be infected under laboratory conditions it is not known whether *Naegleria fowleri* causes disease naturally to wild or domestic animals.

In the wild, *Naegleria fowleri* feeds on bacteria that it ingests using special 'feeding cups' on the outer cell membrane and it is often packed with vacuoles containing microbes. When it is a pathogen, it ingests host tissues and red blood cells and causes a serious inflammatory reaction that contributes to the pathology. Highly pathogenic strains are capable of killing cells on making contact – presumably by secreting toxic substances.

Balamuthia mandrillaris This is another cosmopolitan amoeba that is capable of causing fatal encephalitis (*Balamuthia* amoeba encephalitis) as an apparently opportunistic infection. Its name is derived from it being discovered as the cause of a fatal brain infection of a mandrill baboon that died at San Diego Wild Animal Park in California in 1986. Although it is usually described as free-living, particularly in association with soil, there are many more reports of it causing infections than of it being recovered from the environment (Matin *et al*., 2008). There are two life cycle stages – the trophozoite and the cyst stage but which of these is infective is not known. Many amoebae feed on bacteria but while *Balamuthia mandrillaris* ingests them, they do not appear to sustain growth. By contrast, at least in cultures, *Balamuthia mandrillaris* grows well when fed other species of amoebae or human tissue culture cells. Unlike *Naegleria fowleri*, human infections with *Balamuthia mandrillaris* tend to cause chronic disease that may last up to two years – although with a similar almost invariable (>98%) fatal outcome. The mode of entry is uncertain but in several cases it appears to have been via the puncture wounds in the skin and it then spreads via the bloodstream. *Balamuthia mandrillaris* has been recovered from a range of tissues other than the brain, including the kidneys, pancreas and the skin. It probably gains access to the brain via the choroid plexus (Jayasekara *et al*., 2004). Once established in the brain,

the amoebae cause a granulomatous reaction which results in the site of the infection being surrounded by macrophages. The pathology is therefore sometimes called granulomatous amoebic encephalitis.

Natural infections have been reported in a wide range of wild and domestic animals (Visvesvara *et al*., 2007) in which it has similar fatal effects to those in humans. As with other amoebas, *Balamuthia mandrillaris* can be a host for bacteria, including *Legionella pneumophila* (Shadrach *et al*., 2005) although its importance as a transport host for microbes is uncertain.

Acanthamoeba The genus *Acanthamoeba* contains over 20 species and they are among the most common free-living soil amoebae. They are also found in a variety of freshwater habitats and several have been recorded as opportunistic pathogens. There are two life cycle stages: the active feeding and dividing trophozoite stage and the cyst stage. Opportunistic infections arise through skin wounds and possibly via breathing in the cysts and it is also possible that infections result from swimming in infected water. From the initial entry site the amoebae then disseminate through the body in the bloodstream and they may gain entry to the brain in a similar manner to *Balamuthia mandrillaris*, although this is not yet certain. *Acanthamoeba* tends to cause chronic disease and granulomatous amoebic encephalitis that has a high fatality rate. In addition, and more commonly, *Acanthamoeba* can cause keratitis (inflammation of the cornea) in the eye (Dua *et al*., 2009). Infection results from the amoebae invading the surface of the cornea following trauma to the eye and/or using contaminated contact lenses. Soft contact lenses are said to be particularly likely to harbour the parasite and transfer it to the wearer's cornea. The infection can be extremely painful and, if not successfully treated, leads to the loss of the eye. *Acanthamoebae* are extremely common organisms so it is surprising that human infections are not more frequent than they are – presumably this is at least in part owing to host immune response factors.

2.4 Phylum Metamonada

This is a large group of anaerobic flagellate protozoa of uncertain composition and most probably derived from a variety of ancestors (i.e. it is polyphyletic). They lack mitochondria though this is almost certainly a derived characteristic rather than them never having possessed them during their evolution. They have groups of four flagellae and/or basal bodies that are often arranged in association with their nucleus to a form a structure called the karyomastigont.

2.4.1 Order Diplomonadida

Most protozoa belonging to this order are parasites, the best known of which is the genus *Giardia*. They are characterized by the presence of two haploid nuclei, each of which is associated with four basal bodies and flagellae (Figure 2.2). They lack golgi apparatus and mitochondria but organelles called mitosomes, that are thought to represent relict mitochondria, have been identified in some species. As in other organisms in which they occur, mitosomes do not undertake oxidative phosphorylation but appear to be involved in the formation of iron-sulphur proteins that undertake essential tasks in the cytosol (Goldberg *et al*., 2008; Tovar *et al*., 2003). In common with many other protozoa, the taxonomic position of the diplomonads is under constant revision (e.g. Siddall *et al*., 2007a; Kolisko *et al*., 2005).

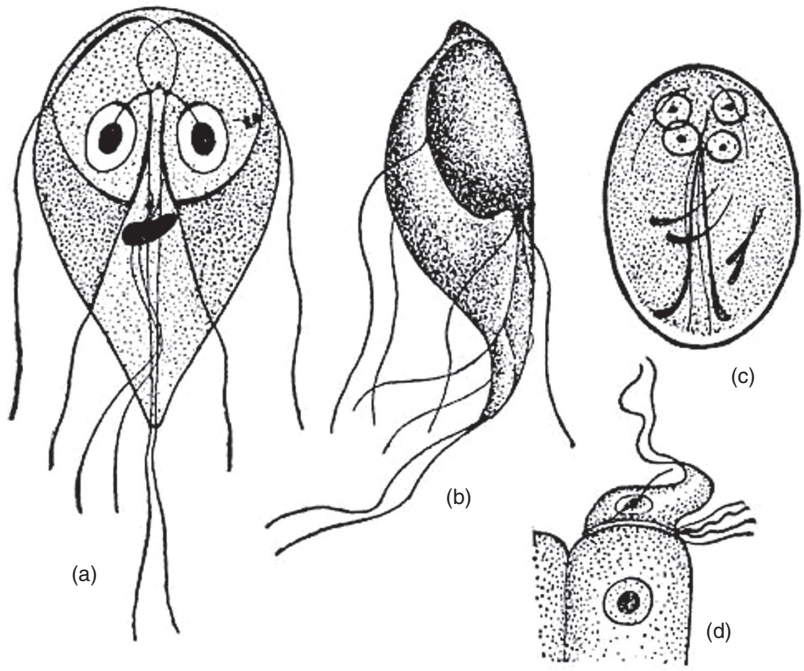


Figure 2.2 *Giardia duodenalis*. (a) = Face view. Note the two nuclei and the central dark 'median body'. The trophozoite stage is 12–15 μm in length. (b) = Profile view. (c) = Mature cyst stage with four nuclei. (d) = Profile view of a trophozoite resting on a gut epithelial cell. Note how the concave profile of the ventral surface enables a close association with the underlying host cell. Source: Chandler and Read, 1961

Genus Giardia The taxonomy of *Giardia* spp. is an interesting illustration of the insights that molecular phylogenetics can provide. At a fundamental level, recent work has shown that despite the morphological similarities between *Giardia* spp. and other flagellates (e.g. *Trichomonas* spp., *Trypanosoma* spp.), it is actually a much more primitive organism.

Within the genus *Giardia*, allocating isolates into clear species groups has proved very difficult. The first attempts at distinguishing species were based on apparent host specificity, since cysts are not distinguishable morphologically and growth *in vitro* to produce trophozoites was often unsuccessful. As culture methods and resolution of microscopy improved, it was possible to obtain and examine trophozoites. On this basis, there are six recognised species of G*iardia*, only one of which is known to infect humans. However, it is often difficult to gain consensus when naming organisms. For historical reasons, in Europe and Australia, the name *Giardia duodenalis* is used, although North Americans favour *Giardia lamblia*, while other authors use *Giardia intestinalis* to refer to the same organism. These species names are synonyms and there is no 'correct' one; the only important issue is that locally, clinicians and scientists should make sure that they use the same one.

Giardia spp. have now been isolated from a wide range of mammals. Organisation of the species into groups and strains within those groups is mainly determined by comparison of genetic sequences (Monis *et al*., 2009). However, it is important to note that many of the published studies to date have involved characterisation and genetic sequencing of small numbers of isolates – in

some cases only one parasite from one animal. This is a serious limitation and the area of *Giardia* taxonomy clearly requires further work. Nevertheless, the ability to type isolates and classify them more precisely has raised the possibility that giardiasis could be a zoonosis and has provided parasitologists with the tools to investigate this question.

Giardia duodenalis *Giardia duodenalis* was one of the first protozoan parasites to be discovered, having been described by van Leeuwenhoek in 1681 from a sample of his own diarrhoea. It is normally found in the human duodenum and upper small intestine although the stomach, ileum and colon can also become infected. The parasites attach to the surface epithelium and overlying mucus layer and although they may completely cover the surface of the gut, they do not invade the underlying tissues. Many people are non-symptomatic carriers of the parasite, but some develop an acute form of enteritis, referred to as giardiasis, that is manifested as profuse watery diarrhoea. The diarrhoea has a characteristic foul smell that is probably related to the fact that the infection interferes with the absorption of fats. If undiagnosed and untreated, the infection can become chronic. This is characterised by epsiodes of abdominal pain and passing of loose, clay-coloured stools which have a smell reminiscent of bad eggs. The consequence of a long-term infection can be malnourishment due to malabsorption and deficiency in certain vitamins such as Vitamin K. The reason some people become more ill than others following exposure to *Giardia* may be related to parasite strain differences and also their own immune status. It is also possible that the gut microflora may play a part in both protection and exacerbating the infection. This has led to several workers investigating the possibility of using probiotics to either prevent infections occurring or treat established infections (Roxström-Lindquist *et al*., 2006).

The trophozoite of *Giardia duodenalis* is pear-shaped 12–15 μm in length and equipped with four pairs of flagella. Its ventral surface has a concave profile on which there are two depressions that are often referred to as 'adhesive discs' or 'suckers' although they have a supportive function rather than being contractile. A pair of flagella, which are located within the 'ventral groove' work as a 'pump' that removes fluid from underneath the adhesive discs and may also facilitate the removal of nutrients from the underlying host mucosa. The oval-shaped cyst stage is 8–12 μm in size and initially contains two nuclei but once they are mature, four nuclei can be seen along with several prominent axonemes (microtubules that constitute the core of the flagella): the flagella and adhesive discs are broken down and stored as fragments during the cyst stage. The cysts are shed in large numbers in the faeces – possibly as high as 1×10^8 viable cysts per gram of faeces. Cyst shedding is intermittent and laboratory confirmation of the diagnosis often requires the patient to produce more than one faecal sample. Transmission is usually through the consumption of these cysts in food and water, or through touching contaminated surfaces.

Giardiasis is one of the most common parasitic diseases of humans in the world and it is characterised by diarrhoea, abdominal pain and loss of weight, although many of those infected are asymptomatic carriers of the parasite. It has been estimated that asymptomatic giardiasis affects around 200 million people in Africa, Asia, Central and South America, with at least 500,000 new cases each year. It is currently considered to be a re-emerging infection (Thompson *et al*., 2008). This is mainly due to the increased incidences reported in developed areas (Australasia, North America and Western Europe), to which a number of factors appear to be contributing. The most obvious is that global travel has become accessible to large proportions of the populations of these countries, which enhances their exposure to the parasite in known endemic areas. Also giardiasis is being attributed more frequently as the cause of outbreaks of diarrhoea among young children in day care facilities or following contamination of domestic water supplies. However, in the

United Kingdom, the number of laboratory-confirmed cases reported to the central surveillance unit, then called the Public Health Laboratory Service, peaked in the mid-1990s and has since declined. For up-to-date information, see the website for the organisation collecting this data – currently the Health Protection Agency: www.hpa.org.uk. Advances in taxonomy have also highlighted the potential for transmission of the disease between humans and other mammals (Thompson *et al*., 2009).

2.4.2 Order Trichomonadida

Most species within this group are parasites or endosymbionts within vertebrates and invertebrates. Several species are important parasites of domestic animals (e.g. *Histomonas meleagradis*, *Tritrichomonas foetus*) and humans (e.g. *Trichomonas vaginalis*). The trophozoites are often ovoid or pear-like in shape: the anterior is usually rounded and the posterior pointed, although amoeboid forms can be found in some species. The number of flagella varies between species but there are often between four and six emerging at their anterior apex. In addition, one flagellum usually curves backwards so that it runs along the cell wall to form an undulating membrane – this flagellum is therefore said to be 'recurrent'. Most species do not form cysts. They have a single nucleus and internally a prominent median tube-like organelle called the axostyle can be seen.

Histomonas meleagradis This parasite infects a wide range of birds but for some reason it is particularly pathogenic in young turkeys, in which untreated infections are usually fatal (McDougald, 2005). It is found within the lumen of the caecum and the liver parenchyma and gives rise to the disease 'histomoniasis'. Infected birds lose condition, become listless and suffer from anorexia, poor growth and sulphur-yellow diarrhoea. The neck and head often become black – and hence the infection is commonly known as 'blackhead disease'. The pathogenicity is often linked to concurrent infections with other parasitic protozoa, such as *Coccidia* spp., and pathogenic bacteria such as *Escherichia coli* and *Salmonella typhimurium*.

The morphology of the parasite is somewhat variable (pleomorphic) and depends upon the organ that is infected and the stage of the disease. For example, the form found free within the lumen of the caecum (and in culture) is amoeboid, 5–30 μm in diameter, with a clear outer ectoplasm, a more granular endoplasm and with one or two flagella emerging from close to the nucleus. The invasive form that is found within tissues is also amoeboid but it is smaller (8–15 μm) and the flagellum is absent.

Trichomonas vaginalis This is an extremely common parasite of humans and despite its name it is frequently found in men – though it tends to be less harmful in them. The parasite is 'tear-drop' shaped, with five flagella emerging at the anterior end: four of these flagella are free while the fifth curves back to form a short undulating membrane that extends just over half the length of the cell (Figure 2.3). Only one body shape is expressed, although the size can vary considerably: the length can be 7–32 μm while the width is 5–12 μm. In common with other Diplomonads, there are no mitochondria but they do possess hydrogenosomes that are arranged in a row alongside the axostyle. This arrangement distinguishes *Trichomonas vaginalis* from other Trichomonad species. Hydrogenosomes are believed to be derived from mitochondria and unlike the more degenerate mitosomes, they are capable of making ATP – as well as the hydrogen which gives them their name (Embley *et al*., 2003).

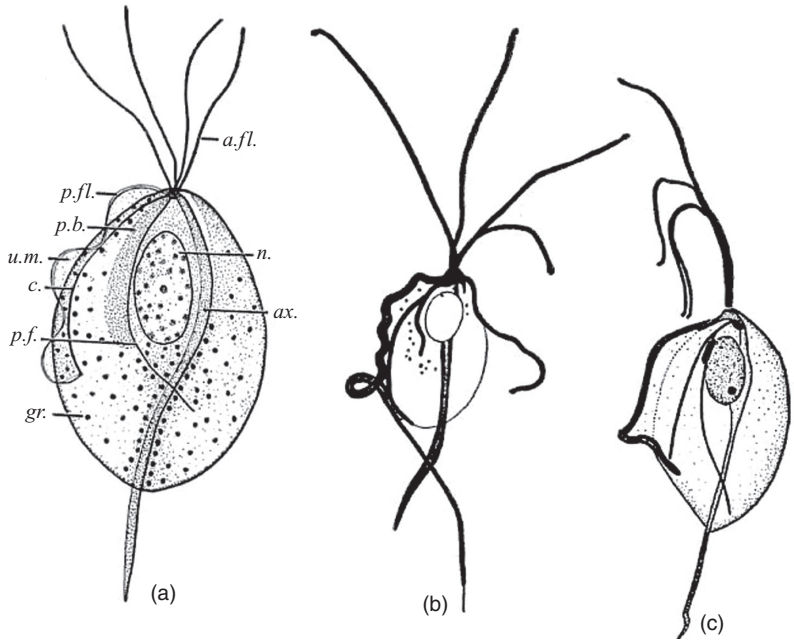


Figure 2.3 Trichomonad parasites of humans. (a) = *Trichomonas vaginalis*; (b) = *Trichomonas tenax*; (c) = *Pentatrichomonas hominis*. Note: Abbreviations: a.fl = anterior flagellum; ax = axostyle; c = costa; gr = metachromatic granules; n = nucleus; p.b. = parabasal body; p.f. = parabasal fibril; p.fl. = posterior flagellum; u.m. = undulating membrane. Source: Chandler and Read, 1961

Although millions of women are infected with *Trichomonas vaginalis* every year, in the majority of cases these are asymptomatic. The protozoa feed on bacteria and sloughed off epithelial cells present in the reproductive tract. However, in some cases the parasites cause a severe inflammatory response that is manifested as a copious frothy white or greenish vaginal discharge. There appears to be interaction between the protozoan and other sexually transmitted organisms. In particular, *Trichomonas vaginalis* is thought to enhance the transmission of HIV (Lewis, 2010). Infection during pregnancy is often associated with poor outcomes such as premature delivery and below-average birth weight for the baby, although whether the parasite actually induces these effects is uncertain.Trichomoniasis can be treated effectively with metronidazole.

Trichomonas tenax This is a common parasite of humans and is found throughout the world. It is rather similar to *Trichomonas vaginalis* in appearance although it is slightly smaller (5–16 μm long, 2–15 μm wide), has a somewhat shorter undulating membrane, and the hydrogenosomes are arranged differently (Figure 2.3). Molecular studies indicate a close similarity between the two species and *Trichomonas tenax* may be a variant of *Trichomonas vaginalis* (Kucknoor *et al*., 2009). *Trichomonas tenax* is usually restricted to the oral cavity where it feeds on bacteria and tissue debris. It does not form cysts and cannot survive passage through the digestive tract so it must be transmitted via kissing and the sharing of food and eating/drinking utensils. The reported prevalence varies from 4–53% (Hersh, 1985). Although it is often associated with dental disease, it is frequently found in people with good dental hygiene. There are occasional reports

of bronchopulmonary infections (e.g. Mallat *et al*., 2004) but these are usually associated with pre-existing pulmonary conditions, such as cancer.

Trichomonas gallinae This is a common parasite of poultry and many other birds, in which it is found predominantly in the upper digestive tract. In contrast, the related species *Tetratrichomonas gallinarum* tends to be found in the lower digestive tract, caeca, and sometimes the liver. The trophozoites of *Trichomonas gallinae* are usually ovoid in shape, 7–11 μm in length and have four free flagella with a fifth recurving to form an undulating membrane (Figure 2.4) (Melhorn *et al*., 2009). There are pathogenic and non-pathogenic strains and, among the many bird hosts, it is the pigeons that tend to be the worst affected (Stepkowski and Honigberg, 2007). However, trichomoniasis (trichomonosis) is also an emerging parasitic disease in finches in the UK, Europe and Canada (Forzán *et al*., 2010). In the UK, the British Trust for Ornithology (www.bto.org/) has estimated that about 500,000 finches died of the disease in 2007. The pathogenic strains can spread around the body and cause liver pathology similar to that of *Histomonas meleagradis*. Young birds are the worst affected and while adults are often infected, they do not show evidence of disease,

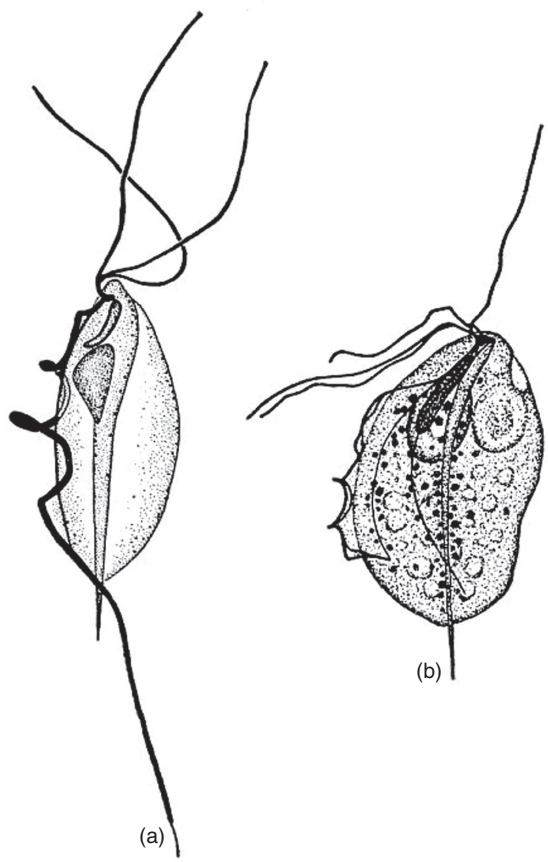


Figure 2.4 Trichomonad parasites of animals. (a) = *Tritrichomonas foetus*; (b) = *Trichomonas gallinae*. Source: Chandler and Read, 1961

though they act as carriers of infection. In badly affected birds, necrotic lesions to the intestine and mouth can extend to the bones. The parasite is thought to spread from pigeons to finches through infected pigeons contaminating garden bird baths. In the UK, there has been a marked rise in the population of wood pigeons and this has probably increased the levels of contamination. Raptors such as peregrine falcons (*Falco peregrines*) and sparrowhawks (*Accipiter nisus*) that feed on pigeons are also vulnerable to infection with *Trichomonas gallinae* and it can cause mortality in their chicks (Sansano-Maestre *et al*., 2009).

Tritrichomonas foetus This is a sexually transmitted parasite of cattle, and though it is also found as a sexually transmitted infection in other animals (e.g. horses), it is not usually pathogenic in them. There are increasing reports of *Tritrichomonas foetus* causing large bowel diarrhoea in domestic cats in the UK, the USA and parts of Europe (Frey *et al*., 2009). How cats could become infected with a sexually transmitted parasite of cattle is something of a mystery. It is unlikely to be acquired by consuming infected meat or living on or near farms on which infected cattle can be found. *Tritrichomonas foetus* does not form cysts so presumably the cats pass the infection between one another through contamination. Experimental studies have demonstrated that *Tritrichomonas foetus* isolated from cats are capable of infecting cows and *Tritrichomonas foetus* infecting cattle can infect cats but there are differences between the isolates and they may represent distinct strains/sub-species (Stockdale *et al*., 2008).

Tritrichomonas foetus is a pear-shaped organism 10–25 μm long and 3-15 μm wide with four anterior flagella, three of which are free and one flagellum curves backwards to form an undulating membrane that extends the length of the body and then projects freely from the posterior apex (Figure 2.4). In bulls, the parasites are usually found in the preputial cavity and cause little harm, though there are reports of inflammation that causes painful urination and unwillingness to copulate. In cows, the parasite causes much more serious pathology. The infection begins with vaginitis and then spreads to the uterus where they can cause early abortion and permanent sterility. The parasite remains in the lumen and does not penetrate the underlying tissues and pathology is probably induced by secretions such as DNAases (Greenwell *et al*., 2008).

Pentatrichomonas hominis This is also often referred to as *Trichomonas hominis* (Figure 2.3). In humans, it is normally considered to be a harmless commensal of the large intestine and caecum and although it is also found in cases of diarrhoea, it is uncertain whether it is the actual cause of the condition. It is a common organism and it is also often found in a range of other mammals including dogs, cats and pigs, although whether it is transmitted zoonotically is uncertain.

2.5 Phylum Apicomplexa

The Apicomplexa is one of the largest of the protozoa phyla and includes species that are important parasites of humans and domestic animals (Table 2.1).

As with many other areas of taxonomy, the arrangements within the Apicomplexa are still uncertain (Morrison, 2009). All members of the phylum are obligate intracellular parasites of invertebrates and vertebrates. A common feature shared by all apicomplexans is the presence within their invasive stage of a unique intracellular structure called the apical complex that is composed of a group of secretory organelles called the micronemes and rhoptries. The complex is situated at the anterior apex of the cell, where it is associated with a region called the oral structure.

Table 2.1 Representative examples of parasitic protozoa belonging to the phylum Apicomplexa to illustrate the wide variety of hosts and transmission strategies

Genus	Example	Host	Transmission	Disease
Plasmodium	*Plasmodium falciparum*	Humans	Vector: Anopheline mosquitoes	Malaria
Cryptosporidium	*Cryptosporidium hominis*	Humans	Contamination	Cryptosporidiosis
Toxoplasma	*Toxoplasma gondii*	All warm-blooded animals	Contamination, congenital, ingestion of infected flesh	Toxoplasmosis
Neospora	*Neospora caninum*	Dogs, cattle	Contamination, congenital	Neosporosis
Cyclospora	*Cyclospora cyetanensis*	Humans	Contamination	Cyclosporosis
Eimeria	*Eimeria tenella*	Poultry	Contamination	Coccidiosis
Theileria	*Theileria parva*	Cattle	Vector: *Rhipicephalus* ticks	East Coast fever
Babesia	*Babesia bigemina*	Cattle	Vector: *Rhipicephalus* ticks	Texas fever
Isospora	*Isospora belli*	Humans	Contamination	Isosporosis

The secretions of the micronemes and the rhoptries are believed to play an important role in the invasion of red blood cells by the malaria parasites (Singh *et al*., 2010).

Box 2.2 Plastids in parasites

Apicomplexans contain a unique organelle called the apicoplast that is thought to have evolved from a chloroplast (plastid), although it does not contain any pigments. The apicoplast has four membranes and contains DNA although most of the genes that code for proteins within it have been transferred to the nucleus. Moore *et al*. (2008) have described a free-living protist (*Chromera velia*) that is phylogenetically related to the Apicomplexa, and contains a functioning plastid that is morphologically similar to that found in the Apicomplexa. The apicoplast is of particular interest because it has no equivalent in the parasite's animal hosts and therefore it might be a target for specifically designed chemotherapeutics (Ralph *et al*., 2004). The precise function of the apicoplast is uncertain but the parasites are killed if they are exposed to drugs that interfere with the functioning of the apicoplast genome (Lim and McFadden, 2010).

Molecular phylogenetic studies have suggested that some of the Kinetoplastida may have contained plastids at an early stage in their evolution but these have since been lost. Chloroplasts are found in many of the euglenid protozoa (e.g. *Euglena gracilis*) and these are closely related to the Kinetoplastida but ultrastructural studies suggest that the euglenids acquired their plastids after the point where they diverged from the Trypomastigota (Leander, 2004).

2.5.1 Genus *Plasmodium*

The genus *Plasmodium* is believed to have evolved hundreds of millions of years ago and long before the arrival of the vertebrates (Escalante and Ayala, 1995). Over 200 species have been described, most of which are parasites of birds although there are also many that infect reptiles, rodents, and primates, and they are transmitted by blood-feeding invertebrates – especially mosquitoes. There are four principal species that infect humans: *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale,* and *Plasmodium malariae*. There is a fifth species, *Plasmodium knowlsei* which is normally considered to be a parasite of monkeys that can cause fatal infections in humans and certain other species which are usually parasites of other animals that cause occasional infections.

In most vertebrate hosts, *Plasmodium* parasites are not particularly pathogenic but in humans they cause the disease malaria – often referred to as 'the ague' or 'marsh fever' in historical literature. Malaria is one of the main causes of morbidity and mortality in the developing world. Despite this huge mortality, like all tropical diseases, malaria is above all a disease of poor people. Consequently, it is sometimes said that malaria does not 'make the headlines' and that the funding is insufficient. For example, global spending on malaria in 2000 was estimated at approximately $60 million a year; by comparison, at the same time spending on AIDS research in the USA alone was 64 times that. This situation is no longer the case and the combination of the Roll Back Malaria Partnership and the Bill and Melinda Gates Foundation has given malaria a much higher profile. For example, funding for malaria in 2007 was estimated at US$ 1.5 billion.

Plasmodium falciparum, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* exhibit marked differences in their biology, and molecular evidence suggests that they evolved from separate lineages. That is, they are more closely related to *Plasmodium* species that parasitise other animals than they are to one another (Waters *et al*., 1993). Interestingly, molecular evidence indicates that *Plasmodium falciparum* originated in gorillas and humans acquired the disease in a single cross-species transmission. Therefore, contrary to previous assumptions *Plasmodium falciparum* was not derived from chimpanzees nor did it originate in primitive human ancestors (Liu *et al*., 2010).

Plasmodium falciparum This causes the most dangerous form of malaria, as it can develop into the potentially fatal cerebral malaria – also sometimes referred to as malignant tertian malaria. Its distribution has become restricted in recent years although it is still very common in tropical and sub-tropical regions such as Sub-Saharan Africa, South-East Asia, and parts of South America (Snow *et al*., 2005). It is the main cause of the mortality associated with malaria. It does not form hypnozoites (see later) but sudden relapses (recrudescence) can occur months or even a year or more after the last fever episode as a result of the parasite persisting in the blood at low sub-clinical levels. The merozoites are able to invade and develop in both young red blood cells (reticulocytes) and mature red blood cells. Consequently, *Plasmodium falciparum* malaria is often characterised by high levels of parasitaemia.

Plasmodium vivax The name *vivax* is derived from the lively nature of the trophozoites in the red blood cells and they often have an amoeboid appearance in blood films. *Vivax* malaria was once the most widespread form of the disease and was even common as far north as Norway and in Siberia. However, as the temperate countries have developed, they have largely eradicated malaria

and *vivax* malaria is now mostly restricted to Asia and the countries bordering the Mediterranean. However, it is still the most common species in the majority of countries in which malaria remains endemic. It is not found in West Africa because the parasite can only penetrate red blood cells carrying the Duffy buffer blood group antigens Fy^a and Fy^b – and most West Africans do not express these. The merozoites are not able to penetrate mature red blood cells and therefore have to invade the developing reticulocytes. *Plasmodium vivax* is well known for causing latent infections in which the hypnozoite stage remains quiescent within the liver and then, after years of apparent good health, the patient suddenly develops the symptoms of malaria. The factors that determine the length of the latent period may be related to the genetic differences between the parasites causing the initial infection and/or sudden changes in the host's immune status.

Plasmodium ovale *Plasmodium ovale* is found in many parts of the world although it is not as common as *Plasmodium vivax* or *Plasmodium falciparum* and its natural distribution is Sub-Saharan Africa and the western Pacific (Collins and Jeffery, 2005). The merozoites are only able to develop in reticulocytes and the parasitaemia tends to be low. Long-lasting latent infections can develop as a consequence of hypnozoites being formed in the liver.

Plasmodium malariae This species has a world-wide distribution but it is not common anywhere and it is normally considered to be relatively benign (Collins and Jeffery, 2007). Natural infections are common in chimpanzees and, as in humans, latent infections can persist in them for many years (Hayakawa *et al*., 2009). Chimpanzees do not live in close proximity to human dwellings and they are not thought to be an important reservoir of infection. Unlike *Plasmodium vivax* and *Plasmodium ovale*, the sudden occurrence of malarial symptoms after a period of good health is not a result of activation of hypnozoites. Instead, the parasite persists within the blood for years, possibly even for life, at a very low parasitaemia and it is only when some change in the immune status of the host occurs that the numbers increase sufficient to cause fever. Unlike *Plasmodium vivax* and *Plasmodium ovale*, the merozoites of *Plasmodium malariae* preferentially invade older red blood cells.

Plasmodium knowlsei This is a zoonotic species which normally infects monkeys such as the long-tailed macaque (*Macaca fascicularis*) in many parts of South-East Asia while human infections are a particular problem in Sarawak and peninsular Malaysia. In humans it is often mistaken for *Plasmodium malariae* but unlike this species, the consequences of infection can be fatal (Cox-Singh *et al*., 2008). Like *Plasmodium vivax*, the merozoites of *Plasmodium knowlsei* can only invade red blood cells that carry the Duffy buffer blood group antigens Fy^a and Fy^b.

2.5.2 *Plasmodium* life cycle

The *Plasmodium* species that infect humans have a complex life cycle that involves both asexual and sexual reproductive stages (Figure 2.5). They are all transmitted by anopheline mosquitoes and multiplication takes place in both humans and the mosquito vector. Only female mosquitoes feed on blood, as they require the nutrients contained in it to produce eggs and therefore they are the ones that transmit the disease.

Humans become infected when they are bitten by a female mosquito harbouring the sporozoite stage of the parasite. Once they have gained access to the bloodstream, the sporozoites are

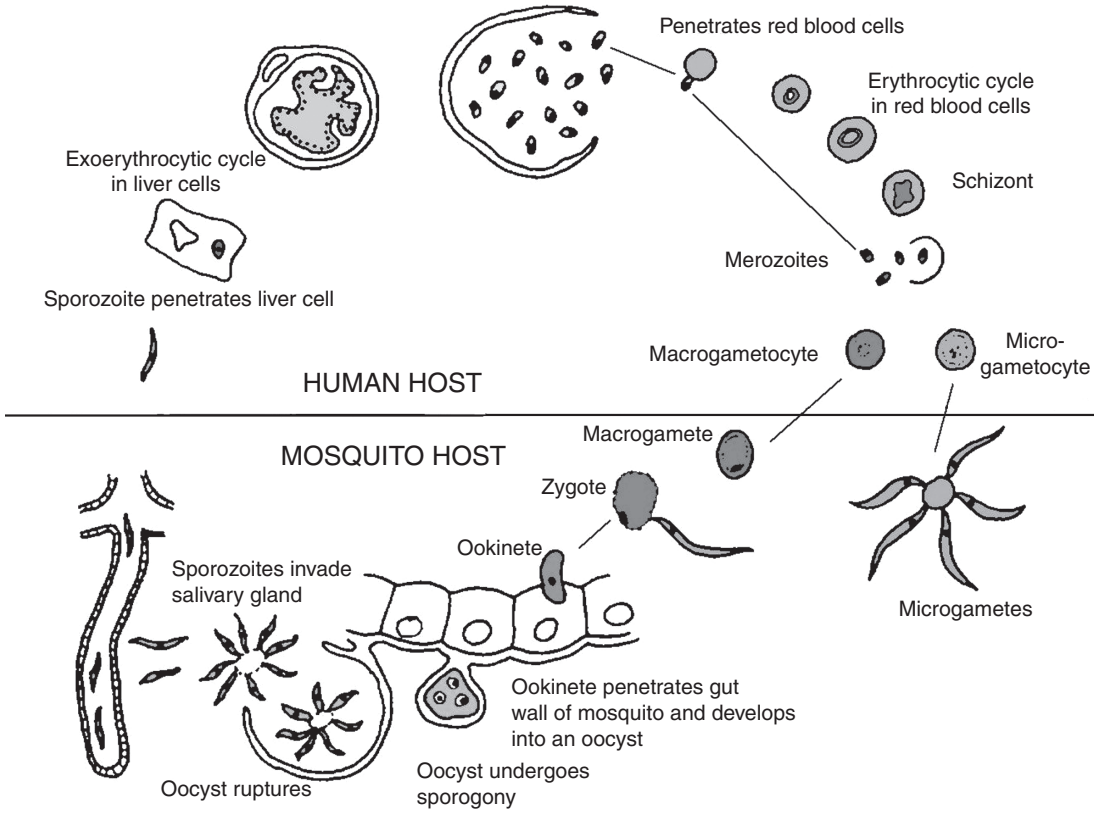


Figure 2.5 Generalized *Plasmodium* life cycle. Redrawn from Cox, 1993

transported to the liver where they penetrate the hepatocytes (liver cells). Within the hepatocytes the sporozoites undergo morphological change and multiply asexually in a process called exoerythrocytic schizogony to form thousands of merozoites (see Colour Plate 1). The term exoerythrocytic indicates that the reproduction takes place in cells other than the red blood cells. Schizogony is a form of asexual reproduction in which the nucleus divides several times and then the parent cell divides into as many individual merozoites as there are nuclei. Schizogony also occurs in many other apicomplexan parasites. In *Plasmodium vivax* and *Plasmodium ovale* not all of the parasites immediately undergo schizogony but instead some remain in a quiescent state referred to as the hypnozoite form. These hypnozoites can remain in the liver for weeks or even years before undergoing further development and are therefore responsible for the onset of illness/relapses long after the initial infection.

After the merozoites are produced, they leave their host cell and invade red blood cells in which they transform into another morphological form called the trophozoite stage and these also reproduce asexually by schizogony. Because these divisions take place in red blood cells, this is referred to as erythrocytic schizogony. The ingestion of host cytoplasm by a trophozoite results in the formation of a large food vacuole, giving the parasite the appearance of a ring of cytoplasm with the nucleus conspicuously displayed at one edge – this is sometimes referred to as the 'signet ring stage'. As the trophozoite grows, its food vacuole becomes less noticeable by light

microscopy, but pigment granules of haemozoin in the vacuoles become apparent. Haemozoin is an insoluble polymer of haem and represents an end product of the parasite's digestion of the host's haemoglobin. The growth of the parasites destroys the red blood cells, releasing the merozoites in the process. These merozoites then infect other red blood cells and the process of infection, replication, and destruction is repeated many times. At some point in this cycle, certain merozoites transform into sexual stages referred to as macrogametocytes (female) and microgametocytes (male). These gametocytes do not develop any further or fuse but remain within their host red blood cell until it is ingested by a suitable female anopheline mosquito.

Shortly after they are ingested by the mosquito, the male and female gametocytes swell, leave their host red blood cells, locate one another, and fuse together to form a diploid zygote. This is the only diploid stage in the whole *Plasmodium* life cycle. The zygote then differentiates into a morphological form called an ookinete. The ookinete is capable of movement and it bores through the mosquito's gut until it comes to rest at the outer wall of the midgut epithelium where it transforms into an oocyst. Inside the oocyst further rounds of asexual multiplication take place called sporogony that result in the formation of numerous sporozoites. Once it is mature, the oocyst bursts and the sporozoites migrate through the mosquito's haemolymph (blood) to the salivary glands. The sporozoites are then injected along with the saliva the next time the mosquito feeds. Depending upon the species of *Plasmodium* and mosquito, the oocyst stage can last between 8 and 35 days, thereby making it the longest stage in the life cycle. It also means that transmission is heavily dependent on the lifespan of the mosquito because it must survive long enough after its initial infected blood meal for the sporozoites to form and then reach its salivary glands. Infection stimulates mosquitoes to feed more frequently, thus increasing the chances of transmission. Once infected, a female mosquito remains infective for the rest of her life.

2.5.3 Genus *Theileria*

Several species in this genus are parasites of domestic animals but the best-known is *Theleria parva* which causes East Coast fever (theileriasis) in cattle in Sub-Saharan Africa. Other important species include *Theileria annulata* that is parasitic in cattle and *Theileria hirci* in sheep and goats that are both capable of causing fatal infections, and are found in parts of North Africa, the Middle East, Europe and Asia. *Theileria* parasitise red blood cells, lymphocytes and tissue macrophages (histiocytes) in mammals and are transmitted by Ixodid ticks (so-called 'hard ticks'). Human infections with *Theileria* either do not occur or are extremely rare. The genomes of both *Theileria parva* and *Theileria annulata* have been sequenced (Gardner *et al*., 2005; Pain *et al*., 2005) and indicate several differences from other apicomplexans sequenced to date. For example, although the *Theileria parva* genome is much smaller (36.5%) than that of *Plasmodium falciparum*, it contains 76.6% of the number of protein-encoding genes and this results in the genes being packed extremely closely together. In addition, some metabolic pathways are abbreviated/absent, which indicates considerable metabolic dependence upon the host.

Theileria parva *Theileria parva* is principally a disease of cattle and as it is transmitted by ticks belonging to the genus *Rhipicephalus* (mainly *Rhipicephalus appendiculatus* and *Rhipicephalus zambesiensis*). Its distribution is largely determined by the presence of its vectors.

Theileria parva exhibits considerable genotypic diversity and there is a lack of cross-protective immunity between different strains of the parasite (Katzer *et al*., 2010). The virulence of the

disease varies between regions and may be related to the ecology of the tick vector. Where the tick life cycle stages do not usually coincide (e.g. adults plus nymphs/nymphs plus larvae/adults plus larvae), the disease tends to be less virulent (Tindih *et al*., 2010). This is because a highly virulent parasite would kill its cattle host before there was the opportunity for the next generation of ticks to become infected. *Rhipicephalus appendiculatus* and *Rhipicephalus zambesiensis* are typically three host ticks and each stage (larva, nymph, adult) attaches to a different host, engorges, and then drops off to moult or in the case of the adult females, to lay their eggs. In subtropical and southern regions of Africa, they are seasonal with only one generation per year (i.e. they are unimodal), but in tropical regions where there is high rainfall, up to three generations may occur.

The *Theileria* life cycle begins when a tick injects infectious sporozoites into the bloodstream of a suitable mammal host. *Theileria parva* sporozoites are non-motile and, unlike many other apicomplexans, their invasion of the host cell is not an active process on the part of the parasite (Shaw, 1999). Instead they make random contact with T and B lymphocytes and attach to host cell receptors, after which they become internalised and are surrounded by a vacuole membrane of host origin. The parasites do not orientate themselves in relation to the host cell and it is only after entering into it that they discharge the contents of their rhoptries and micronemes. These chemicals cause the vacuole membrane to disperse so that they lie free within the cytoplasm of the lymphocytes. The sporozoites then transform into multinucleate schizonts and induce the host cell to proliferate: the parasites are closely associated with the lymphocyte mitotic apparatus and divide with their host cell so that daughter lymphocytes are also infected (Schneider *et al*., 2007; von Schubert *et al*., 2010). The first generation of schizonts are called 'macroschizonts' and these give rise to 'macromerozoites' that invade other lymph cells and give rise to either more macroschizonts and macromerozoites or to microschizonts that give rise to micromerozoites. The micromerozoites may either invade lymphocytes or red blood cells. Those invading lymphocytes continue to multiply by schizogony but those invading red blood cells differentiate into the 'piroplasm' form and these do not divide any further (Figure 2.6). It is these piroplasm forms that are found in circulating red blood cells that are infectious to the tick vector. The piroplasms are extremely small, typically 1.5–2 μm long and 0.5–1 μm wide and rod-shaped, although oval, comma, and ring-shaped forms may also be found. They are a characteristic diagnostic feature of East Coast fever. Once an infected red blood cell enters the tick gut lumen, the piroplasms are released and these differentiate into either microgametes or macrogametes. The microgametes and macrogametes fuse together to form a diploid zygote and this invades the tick gut epithelial cells where it transforms into a motile 'kinete' form. The kinetes make their way through the body to the tick salivary glands where they invade the type III acini cells and undergo sporogony to produce numerous infectious sporozoites.

2.5.4 Genus *Babesia*

There are numerous species of *Babesia*; some reports state that there are over 100, others estimate it at somewhat fewer, but they are all parasites of mammalian erythrocytes and they are transmitted by ticks. In some species, other blood cells, such as lymphocytes and histiocytes, may also be parasitised. Human infections can occur and may be fatal, but the genus *Babesia* is primarily of economic importance as parasites of cattle, sheep, and other domestic animals. The most important species are *Babesia bigemina*, *Babesia bovis*, and *Babesia divergens*. There are three sub-species of *Babesia canis*: *Babesia canis rossi*, *Babesia canis canis*, and *Babesia canis*

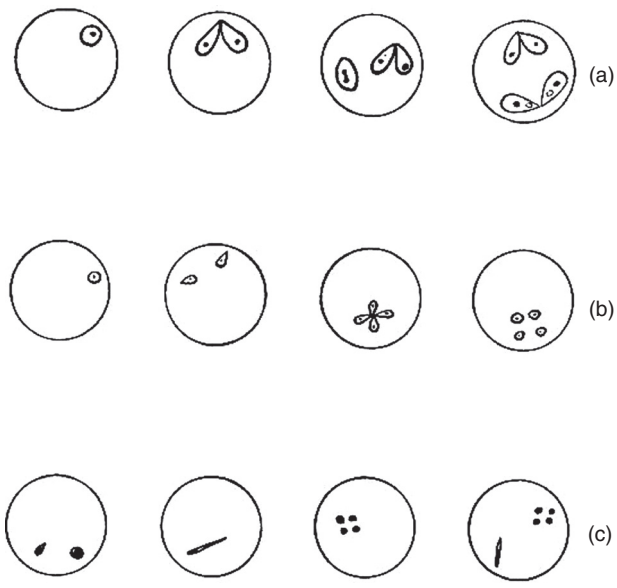


Figure 2.6 Diagrammatic representation of *Babesia* (a, b) and *Theileria* (c) parasites in red blood cells. 'a' illustrates the 'large type' of *Babesia* such as *Babesia bigemina* that is 4–5 μm in length, pear-shaped and usually found in pairs. 'b' illustrates the medium to smaller sort of *Babesia* such as *Babesia bovis* which is only 1.5 μm in length and *Babesia equi* which grows to 2 μm and often forms crosses. 'c' illustrates the piroplasms of *Theileria parva*. These are only 1.5–2 mm in length and do not divide but are infectious to the tick vector. Source: Cameron, 1934

vogeli, which are parasites of dogs and vary in their distribution and pathogenicity. *Babesia canis rossi* can cause a severe and potential fatal disease, while infection with *Babesia canis vogeli* is often asymptomatic. Infection with *Babesia canis canis* causes variable symptoms ranging from slight fever to fatal haemolytic anaemia. *Babesia microti* is sometimes referred to as *Theileria microti* in the literature and is the cause of many hundreds of human infections in the north-eastern USA. It has been isolated from ticks in Europe and a case of human infection has been reported in Switzerland (Meer-Scherrer *et al*., 2004). Table 2.2 shows the distribution, vectors and host ranges of *Babesia*.

Babesia bigemina The different species of *Babesia* vary in their pathogenicity and distribution but they share many similarities, so we shall discuss only one of them, *Babesia bigemina*, in detail. This species was once a major cause of disease in North America, where infected cattle were said to suffer from 'Texas fever'. Following successful eradication campaigns, *Babesia bigemina* is of much less economic importance in the USA today but it remains a serious problem in Central and South America, North and South Africa, Australia, and Asia. It is principally a disease of cattle, although water buffalo, zebu and deer may also be infected. It is mostly transmitted by ticks belonging to the genus *Rhipicephalus* and these have a widespread distribution in tropical and subtropical countries.

The life cycle begins when the infective sporozoites are injected into the mammal bloodstream and these then invade the red blood cells (Figure 2.6). Initially, the sporozoites are enclosed

Table 2.2 The distribution, vectors, and host ranges of representative *Babesia* species of medical and veterinary importance

Species of *Babesia*	Distribution	Mammal host	Tick vector
B. bigemina	Central and South America, North and South Africa, Australia, Asia (not in the UK)	Cattle, water buffalo, zebu, deer	Various species of *Rhipicephalus* and also *Ixodes ricinus*
B. bovis	Central and South America, North and South Africa, Australia, Asia, Southern Europe (not in the UK)	Cattle, deer	Various species of *Rhipicephalus* and also *Ixodes ricinus*
B. divergens	Northern Europe, including UK	Cattle	*Ixodes ricinus*
B. microti	North-eastern USA, Europe	Rodents, human infections increasingly reported	*Ixodes dammini, Ixodes scapularis*
B. ovis	Southern Europe, Middle East, Africa	Sheep, goats	Various species of *Rhipicephalus*
B. canis	Southern Europe, Middle East, Africa, Asia, Central, South and North America	Dogs and other caniids	Various species of *Ixodes, Dermacentor variabilis, Haemaphysalis leachi*

within a membrane-bound vacuole of host origin but they escape from this and are therefore (like *Theileria*) in intimate contact with the cytoplasm of the erythrocyte. The sporozoites transform into the merozoite stage that divides by binary fission and accumulate within the cell and eventually kill it. These merozoites are usually pear shaped (4–5 μm long × 2–3 μm wide) and can be seen singly or in pairs and, following their release, reinfect other red blood cells. The infected red blood cells can be mechanically transmitted to another suitable host, for example, via re-used needles and veterinary instruments and blood transfusion. In susceptible hosts, the parasitaemia builds up rapidly and 70–80% of the red blood cells can become infected. Some of the merozoites transform into oval-shaped gamonts and these develop no further unless they are ingested by a suitable tick vector.

When a tick ingests blood from an infected host, the *Babesia* merozoites are lysed along with the red blood cells they are parasitising. The gamonts, however, survive and invade the cells lining the tick's gut. Within these intestinal cells the gamonts differentiate into gametocytes that are referred to as 'ray bodies' (derived from the original German word *Strahlenkörper* that is still used by some authors), which resemble globules with one or two thorn-like projections. Once they are mature, the ray bodies leave the gut cells and two of them fuse to produce a zygote. The zygote then transforms into a motile kinete which invades a number of other cell types including muscles, Malpighian tubules and in female ticks the ovaries and oocytes. Within these cells they transform and multiply to form numerous secondary motile kinetes, and when these are then released, they parasitise other cells including the salivary glands. Within the salivary glands the kinetes transform into sporonts and these give rise to numerous infectious sporozoites. Those primary kinetes that invaded the tick oocytes result in the young tick being infected and in these the parasites ultimately invade their salivary glands. For one-host ticks, trans-ovarial transmission of the infection is essential if they are to function as effective vectors. This is because they usually spend their whole life on a single animal and therefore cannot transmit infections directly from one host to another. For two- and three-host ticks, trans-ovarial transmission may be of less importance

for the parasite since the ticks drop off and re-attach to new hosts between moults and therefore have greater opportunity to spread the infection. Many blood-feeding vectors are harmed by the parasites they transmit (e.g. sandflies and *Leishmania* spp.) but *Babesia microti* facilitates the feeding and improves the survival of its tick host, *Ixodes trianguliceps*, (Randolph, 1991). It is not known whether this also occurs in other *Babesia*–tick relationships.

Cattle normally acquire their infection from others that have recovered from the disease but continue to harbour a sub-clinical infection. The pathology associated with *Babesia bigemina* is unusual in that adult cattle tend to be more severely affected than the young. This is particularly the case if the cattle were not previously exposed to the parasite or the local strain, for example, because they were transported to a new region or country or infected ticks carrying the disease were introduced into the area. High performance milking cows that are imported into Africa from temperate regions are known to be at particular risk of succumbing to severe disease symptoms. In Mexico, white-tailed deer (*Odocoileus virginianus texanus*) have high seroprevalence for both *Babesia bigemina* and *Babesia bovis* but it is not known how important these (and other wildlife) might be as reservoirs of infection (Cantú-Martinez *et al*., 2008).

The consequences of infection are affected by a host of variables including the age and immune status of the host (e.g. previous exposure and vaccination status) and the strain of the parasite.The damage is primarily associated with the loss of function and destruction of the red blood cells. When the infected erythrocytes are lysed, they release haemoglobin into the bloodstream. The destruction of small numbers of red blood cells has little effect and many infections are sub-clinical but if numerous cells are destroyed in a short period of time, it can overload the body's ability to remove the waste material. Consequently, haemoglobin and its breakdown products accumulate, resulting in jaundice and their appearance in the urine – and hence the common name for the infection of 'red water fever' – or haemoglobinuria. The loss of functioning red blood cells also gives rise to severe haemolytic anaemia. The infected animal develops a fever and cerebral involvement is possible. The precise mechanism by which *Babesia* induces brain pathology is uncertain, although it has been suggested that it might provide a useful animal model for human cerebral malaria (Schetters and Eling, 1999).

Box 2.3 Human babesiosis

Human babesiosis is a rare condition that is caused predominantly by *Babesia divergens* in Europe and *Babesia microti* in North America (see review by Gorenflot *et al*., 1998). There are no *Babesia* species in which humans are the principal host. Humans usually become infected following a tick bite although a few cases have arisen following blood transfusion. As might be expected, working or walking in the countryside places one at risk of infection since there is a greater chance of coming into contact with ticks.

As with cattle, humans infected with *Babesia* often suffer from haemolytic anaemia, jaundice and fever. The fevers are non-periodic and therefore distinct from malaria although sometimes mistakes in clinical diagnosis are made. Humans are usually treated with atovaquone and azithromycin or quinine and clindamycin and, following treatment, most cases resolve within 1–2 weeks without complications. However, in patients who are immunocompromised, the disease can follow a persistent, relapsing course over several weeks (Krause *et al*., 2008).

2.6 Subclass Coccidiasina

Commonly known as the Coccidia, this is the largest group group of the Apicomplexa. All members are intracellular parasites of vertebrates and invertebrates and are principally associated with the intestinal cells though other cell types may also be infected. Some species have only a single host while others employ two – commonly a vertebrate and an invertebrate, although it may be two vertebrates, one of which feeds on the other. The life cycle usually begins with the invasion of a host cell by a sporozoite stage and is followed by a cycle of merogony, gametogony, and sporogony. The group used to contain just the *Eimeria,* the *Isospora* group and the haemogregarines (mainly parasites of red blood cells of amphibians and reptiles) but now includes *Cryptosporidium, Sarcocystis* and *Toxoplasma*.

2.6.1 Suborder Eimeriorina

This is a large group that contains thousands of species. Only a few species are parasites of humans but as a group they are of extreme importance in veterinary medicine as there are many species that infect domestic animals. The group includes species with either a single host (i.e. monoxenous) or two or more hosts (i.e. heteroxenous).

Family Eimeriidae, Genus Eimeria There are probably tens of thousands of species of *Eimeria* – more species are being described on a regular basis – and there are the usual problems with taxonomy and species identification (Long and Joyner, 2007). The host range encompasses fish, lizards and mammals and most species are host-specific or infect a few closely related host species. Not surprisingly, among the large number of species, several of them are of economic importance. For example, estimates for the annual worldwide losses owing to avian coccidiosis in commercially reared chickens and other birds are probably in the region of £500 million (Shirley *et al*., 2007). In addition, some *Eimeria* species play an important role in wildlife ecology (Hudson *et al*., 2002; Friend and Franson, 1999). By contrast, there are few reports of *Eimeria* infections in primates (Duszynski *et al*., 1999) and human infections either do not occur or are exceedingly rare. We will only consider one species, *Eimeria tenella* as a representative example.

Eimeria tenella This is the commonest and most pathogenic of the seven species of *Eimeria* that infect domestic poultry. Each *Eimeria* species develops in a different region of the bird's digestive tract and co-infections with two or more species are common. *E. tenella* is found throughout the world and is responsible for a great deal of economic loss. Although the parasite is capable of causing high flock mortality, the availability of vaccines and anticoccidial drugs, coupled with effective hygiene, has meant that most of the losses result from chronic and sub-clinical infections that cause reduced growth and egg production (e.g. Haug *et al*., 2008).

The life cycle is monoxenous and begins when a bird ingests an infective oocyst with its food or in its water. The oocyst contains the infective sporozoites and these are released when the oocyst reaches the small intestine. The sporozoites are transported down the intestinal tract with the digesta and once they reach the caecum, they invade the intestinal epithelial cells. In common with the other species of *Eimeria*, all the intracellular stages are surrounded by a membrane-bound

parasitophorous vacuole of host cell origin (Shi *et al*., 2009). Parasite proteins are incorporated into the vacuole membrane and this prevents the fusion of lysosomes or other vesicles (Beyer *et al*., 2002). The sporozoites travel through the epithelial cells and emerge into the lamina propria where they are promptly ingested by macrophages which act as a sort of transport host and move the parasites to the glands of Lieberkuhn where they escape and invade the glandular epithelial cells. Within the epithelial cells the parasites transform into meronts and undergo merogony to produce numerous merozoites. This kills the host cell and the merozoites are released to invade other caecal epithelial cells within which they produce another generation of merozoites. These second generation merozoites are then released and invade new epithelial cells but at this point, for some reason, the subsequent development can follow one of two paths. Some of the merozoites will give rise to a third generation of merozoites while others undergo gametogony to become either macrogametocytes (female) or microgametocytes (male). The microgametocytes leave their host cell and invade those containing a macrogametocyte and fuse with it to effect fertilisation. After fertilisation, the macrogamete transforms into a zygote and then into an oocyst. The oocyst is shed with the host's faeces and contains only a single cell that is referred to as the sporont. The oocyst is not infectious at this stage and must undergo a process of sporogony in which four sporocysts, each of which contains two sporozoites, are formed. This takes two or more days depending upon the environmental temperature and means that prompt removal of faeces and good farm hygiene can be effective at preventing the transmission of disease both within and between rearing sheds.

The disease primarily affects young birds and is most frequently seen in those between 3 and 8 weeks of age. Older birds can suffer badly if they have not previously been exposed to the parasite. Infected birds become listless, cease to feed and huddle together to keep warm. Damage to the caecum results in bleeding into the gut and the birds' faeces becomes stained with blood. The damage allows secondary invasion by bacteria present naturally in the gut and this can extend the lesions and cause further pathology. Acutely infected birds often die 5–6 days after becoming infected as a result of blood loss. In addition to the haemorrhages, the gut swells and becomes thickened and appears 'sausage-like'.Those birds that are still surviving 9 days after infection will usually recover: a caseous (cheese-like) plug may form in the lumen of the caecum which is ultimately voided with the faeces.

Birds that recover develop protective, species-specific, cell-mediated immunity to re-infection that is based on CD4+ and CD8+ T cells found in the lymphoid tissues associated with the gut (Shirley *et al*., 2007). Co-infections with two or more species of *Eimeria* do not necessarily compromise the development of immunity (Jenkins *et al*., 2009).

2.6.2 Isospora group

Genus Isospora There are currently about 250 described species belonging to this genus and most of them are parasites of birds though there are several that can infect mammals, including humans. They are all monoxenous and can therefore complete their life cycle in a single host, though some exploit paratenic hosts to effect transmission. Important species include *Isospora canis* which causes watery/bloody diarrhoea in puppies, *Isospora suis* that causes neonatal piglet diarrhoea, and *Isospora belli* which causes diarrhoea in humans and is frequently associated with persons suffering from AIDS or immunosuppressive illnesses. A review of the genus is provided by Lindsay *et al*. (1997).

Isospora belli This species is found throughout the world but is particularly common in tropical countries. The first human cases were recognised in people returning from the battlefields during the First World War, hence the species name (Latin *bellum* = war). It appears to only infect humans although other animals may act as paratenic hosts. This suggestion has been prompted by the observation that the sporozoites of some species of *Isospora* invade the lymph nodes, liver, muscles and other tissues where they become dormant and do not divide or develop further.

The life cycle of *Isospora belli* begins with the ingestion of oocysts containing infective sporozoites. The sporozoites emerge in the small intestine and invade the epithelial cells where they transform into merozoites which divide to produce more merozoites which in turn divide in a cycle that can continue many times. When the infected cell is destroyed, the parasites are released and they invade new cells. In serious infections this can result in the loss of large areas of the gut lining and allow secondary invasion by gut microbes. At some point, the merozoites transform into multinucleate meronts and these give rise to microgametes (male) or macrogametes (female). Unless the microgametes and macrogametes occur within the same cell (which is possible), the microgametes leave their host cell to locate a macrogamete and their fusion results in the formation of a zygote that then develops into an oocyst. The oocyst is then released into the gut lumen when the host cell dies and it is shed with the faeces. The oocyst then undergoes sporulation to produce two sporocysts, each of which contains four sporozoites. Transmission is therefore passive, by faecal-oral contamination. The time taken for sporulation of *Isospora belli* is not known but in other species it is influenced by environmental conditions. For example, in some species, sporulation is inhibited if the temperature falls below 20°C but could take less than 16 hours at 30°C. This is therefore distinct from *Cryptosporidium parvum* in which the oocysts are immediately infective after being shed with the host's faeces. It is clear, therefore, that transmission of Isospora belli is probably through infected food or water rather than direct contact (e.g. through not washing one's hands after defecation or by making contact with an article touched by such a person).

2.6.3 Genus *Cyclospora*

This genus currently contains 13 species which are parasitic in a range of vertebrates (e.g. snakes, rodents) but the most important is *Cyclospora cayetanensis* which is found only in humans.

Cyclospora cayetanensis This is an emerging parasitic disease which was not described until 1979 but has since been found to have a cosmopolitan distribution (Ortega and Sanchez, 2010). The life cycle begins with the ingestion of oocysts (8–10 μm diameter) containing infective sporozoites. The sporozoites are released in the duodenum and upper small intestine where they invade the gut epithelial cells. After entering the host gut epithelial cells, they transform into type I merozoites and these divide to give rise to type II merozoites. The type II merozoites transform into meronts and these give rise to microgametocytes and macrogametocytes. Fusion of a microgametocyte with a macrogametocyte gives rise to zygote and this develops into an oocyst which is shed with the faeces. The oocyst undergoes sporulation after it is shed – a process that takes 7–15 days depending upon the environmental conditions – to produce two sporocysts each containing two sporozoites. Because of the time taken for the oocyst to sporulate after being passed, it is thought most likely that transmission takes place through contaminated food and water rather than direct person-to-person or contaminated object contact.

The symptoms of infection are non-specific and resemble those of many other gastrointestinal diseases. Patients suffer from abdominal pain, watery diarrhoea, flatulence, low grade fever, anorexia, and weight loss. In endemic regions the symptoms tend to be worse in young children and in non-endemic regions most people who become infected express symptoms. Persons suffering from AIDS are more seriously affected (Stark *et al*., 2009).

2.6.4 Family Sarcocystidae

Genus Sarcocystis Members of this genus are obligate intracellular parasites with a life cycle that involves two hosts – a herbivore intermediate host in which only asexual multiplication occurs and a carnivore definitive host in which sexual reproduction takes place. Most *Sarcocystis* species are very host-specific and infect a limited number of closely related intermediate/final hosts. The taxonomy is confused and care has to be taken when using the older literature, since some host animals that were thought to harbour just a single species of *Sarcocystis* have since been found to contain several species. In addition, some species are morphologically identical and others have synonyms (e.g. *Sarcocystis cruzi* is also known as *Sarcocystis bovicanis* – a reflection that the intermediate and definitive hosts are cattle and dogs/other canines respectively). Table 2.3 summarises the most important species of *Sarcocystis* in human and veterinary medicine.

The life cycle of *Sarcocystis bovicanis* is typical of most *Sarcocystis* species. It begins when sporulated free sporocysts or oocysts, each containing four sporozoites, are shed in the faeces of the canine definitive host. These sporocysts or oocysts are then consumed by the cow intermediate host and when they reach the small intestine, the sporozoites are released. The sporozoites invade the gut epithelial cells and then make their way to the blood vessels and thereby become distributed around the body. The parasites invade the endothelial cells of the blood vessels that serve many of the body's organ systems. Within the endothelial cells the parasites undergo four cycles of merogony in which merozoites are produced. After each cycle, the merozoites are released and infect new endothelial cells downstream of the initial infection. After the last cycle, the merozoites invade skeletal and cardiac muscle cells. Occassionally, smooth muscle, the brain and spinal chord are also infected. Within these cells the merozoites transform into metrocytes or 'mother cells', each of which divides asexually to form a structure called a sarcocyst. With repeated asexual division a sarcocyst steadily becomes larger and larger and in some species may become so big that it is visible to the naked eye. After a period of time the globular metrocytes cease producing new metrocytes and form crescent-shaped bradyzoites. The time taken for this depends upon the species of *Sarcocystis* but can be around 2 months. During this development, the sarcocysts are non-infectious since only bradyzoites can transmit the infection.

Table 2.3 Summary of the most important species of *Sarcocystis* in human and veterinary medicine

Species of *Sarcocystis*	Synonym	Intermediate host	Definitive host
S. bovicanis	*S. cruzi*	Cattle	Dogs and other canines
S. bovihominis	*S. hominis*	Cattle	Humans
S. bovifelis	*S. hirsuta*	Cattle	Cats and other felines
S. suihominis	*Isospora hominis*	Pigs	Humans and some primates
S. ovifelis	*S. tenella*	Sheep	Cats and other felines
S. hovathi	*S. gallinarum*	Chicken	Dogs and other canines

The life cycle is completed when flesh containing the bradyzoites is consumed by a suitable canine host (or other carnivore in the case of other *Sarcocystis* species). Once the sarcocyst is digested within the dog's small intestine, the bradyzoites are released and become motile. The bradyzoites initially invade the gut epithelial cells and then make their way to the *lamina propria* region where they transform into either male or female gametes. After the gametes have fused, the parasite undergoes sporogony to form an oocyst that contains two sporocysts. The oocysts are shed into the lumen of the gut and passed with the faeces. The oocyst has a thin wall and often ruptures to release the sporocysts and it is therefore these that are usually seen in faecal samples. The sporocysts measure 16.3 μm × 10.8 μm and contain four sporozoites.

Box 2.4 Human *Sarcocystis* infections

Humans are definitive hosts for several species of *Sarcocystis* the infections are usually acquired from eating raw or poorly cooked meat. There have been relatively few surveys (Fayer *et al*., 2004) but the prevalence can be expected to relate to farm hygiene and culinary practices. The symptoms of infection are non-specific and typically include nausea and diarrhoea. The infections are usually self-limiting. *Sarcocystis* does not appear to cause serious pathology in other definitive hosts.

Humans can act as intermediate hosts but the *Sarcocystis* species responsible have not been identified. In this instance, humans are effectively 'dead end' hosts because they are seldom consumed by predatory animals. Presumably, humans become accidentally infected with the sporocysts or oocysts and the normal intermediate hosts are other species of primates.The symptoms of infection depend upon the site at which the sarcocysts are growing and their abundance but typically they induce inflammatory responses that result in pain, fever, and swelling at the infected site.

In intermediate hosts, the consequences of infection vary between species, the level of challenge, and the species of *Sarcocystis* infecting them. However, most pathology is usually associated with damage caused to the vascular epithelium during the second stage of merogony. Heavy infections of *Sarcocystis bovicanis* in cattle can result in widespread haemorrhages afflicting virtually every organ in the body.This results in anaemia, emaciation, and the animal can become anorexic; abortion can occur in breeding cattle. The immune reponse results in lymphadenopathy and submandibular oedema while the hair at the end of the tail is often lost. Most infections in domestic livestock, however, are sub-clinical and are not discovered until the sarcocysts are detected during meat hygiene inspections after the animal has been slaughtered.

2.6.5 Genus *Toxoplasma*

This genus contains just a single species, *Toxoplasma gondii* but what it lacks in member species it makes up for in numbers of hosts, since it is probably capable of infecting all mammals and birds.

Toxoplasma gondii This intracellular parasite was initially described from a desert rodent, the North African *gondi* (*Ctenodactylus gondi*) (sometimes spelled '*gundi*' – vowels are not used in

written Arabic so some words appear in a variety of spellings when translated into English) in Tunisia in 1908, but the life cycle was not established until 1969 or 1970. The name '*Toxoplasma*' has nothing to do with toxins but is derived from the curved shape of the tachyzoite stage of the life cycle. '*Toxon*' (*Τόξον*) is the Greek word for a 'bow or something that is crescent shaped' and '*plasma*' (πλάσμα) is Greek for 'creature'. Since these humble beginnings, it has become apparent that not only does *Toxoplasma gondii* have a cosmopolitan distribution but it has perhaps the widest host range of any parasite.

The life cycle of *Toxoplasma gondii* has two parts and three infectious stages. The two parts of the life cycle are the sexual cycle that occurs within cats and other felines which are the definitive hosts and the asexual cycle that occurs in the intermediate hosts – which are virtually any warm-blooded animal, including humans. The three infectious stages are the sporulated oocysts that contain the sporozoites, the tachyzoites, and the tissue cysts that contain bradyzoites: all three stages are infectious to both the feline definitive hosts and the intermediate hosts. Cats acquire their infection by consuming either sporulated oocysts passed in another cat's faeces (e.g. through contamination) or an intermediate host containing the tachyzoites and bradyzoites. Following ingestion, the parasites invade the epithelial cells lining the cat's small intestine and undergo a complex series of asexual divisions. The first step is multiplication by endodyogeny – this is an unusual form of division in which two daughter cells develop within a mother cell which is then consumed by her offspring before separation takes place. Endodyogeny is followed by endopolygeny in which the mother cell produces several daughter cells simultaneously. This is then followed by gametogenesis and the formation of microgametes and macrogametes. After fusion of the gametes, a zygote is formed and this develops into an oocyst that is shed with the faeces while it is still unsporulated. If the cyst is ingested by a new host at this stage, it is not infectious. Sporogony therefore happens outside the host and takes about 2–3 days under ideal conditions. The infectious sporulated oocysts are oval in shape (10–13 μm × 9–11 μm) and contain two sporocysts, each of which contains four sporozoites. The sporocysts can remain infectious in the environment for months and possibly several years. The shedding of oocysts starts 3–10 days after initial infection and continues for only 1–2 weeks although some estimates suggest that a cat may shed up to 100 million oocysts in its faeces during that time (Tenter *et al*., 2000). After an initial infection, a cat is usually resistant to reinfection. The prolonged survival of the oocysts in the environment compensates for the short period over which they are shed. In some cats, developmental stages may persist in a dormant state and give rise to another batch of oocysts at a later date following their reactivation. This could happen as a result of a change in the host's immune status following challenge by another infectious disease. Immunity can also wane naturally with time and a cat may be reinfected several years after the first challenge.

Domestic cats normally bury their faeces and although this might be considered to reduce the chances of contaminative transmission of the oocysts, it could help to contribute to their survival by reducing exposure to environmental conditions such as desiccation and UV light. Mice and voles are repelled by the smell of cat faeces and therefore if the oocysts are to be consumed by them, it is essential that they survive until the faeces breaks down and is dispersed in the soil. Dogs consume cat faeces (Lewin, 1999) and therefore might be expected to be at particular risk of serious infections. The seroprevalence of *Toxoplasma gondii* in dogs is often high, especially in strays (e.g. Ali *et al*., 2003) but this is probably due to their tendency to scavenge dead animals and waste food rather than consumption of cat faeces – although the latter habit is unlikely to help.

In recent years a number of outbreaks of toxoplasmosis in humans have been linked to contaminated water sources (e.g. Tenter *et al*., 2000) and it is therefore likely that many wild and domestic

animals also become infected this way. Presumably, the oocysts are washed from the soil into ponds, streams, reservoirs and it is known that they can survive the chlorination of drinking water and sewage treatment (Aramini *et al*., 1999). The oocysts are also able to survive for prolonged periods in brackish water and at least a year in sea water (Lindsey and Dubey, 2009).

Within the cat, some parasites invade other tissues, including the muscles and nervous tissues where they divide asexually to produce tachyzoites and tissue cysts containing bradyzoites – just as in the intermediate hosts. If the cat is pregnant, it is also possible for the developing kittens to be infected *in utero*. Most cats, however, become infected as a consequence of preying on mice and rats. As might be expected, the prevalence of infection is higher in stray cats and those which are good 'mousers'. Many bird species, from sparrows and pigeons to ducks and owls, are naturally infected with *Toxoplasma gondii* (Dubey, 2002) but they are probably not as important as rodents as sources of infection to cats.

When an intermediate host ingests an infectious oocyst, the sporozoites are released in the host's intestine and these then invade the gut epithelial cells. They then leave these cells and invade macrophages and many other cell types within which they transform into tachyzoites (Figure 2.7) which multiply by endodyogeny. In mammals, *Toxoplasma gondii* does not invade the red blood cells. However, bird red blood cells, which are nucleated, can be infected. The parasites continue multiplying until the host cell is entirely consumed and all that is left is the host cell membrane and at this point the host cell is sometimes referred to as a pseudocyst. When the pseudocyst membrane breaks down, the tachyzoites are released and they invade new host cells and repeat the process. As in other apicomplexan parasites, the tachyzoites actively invade their

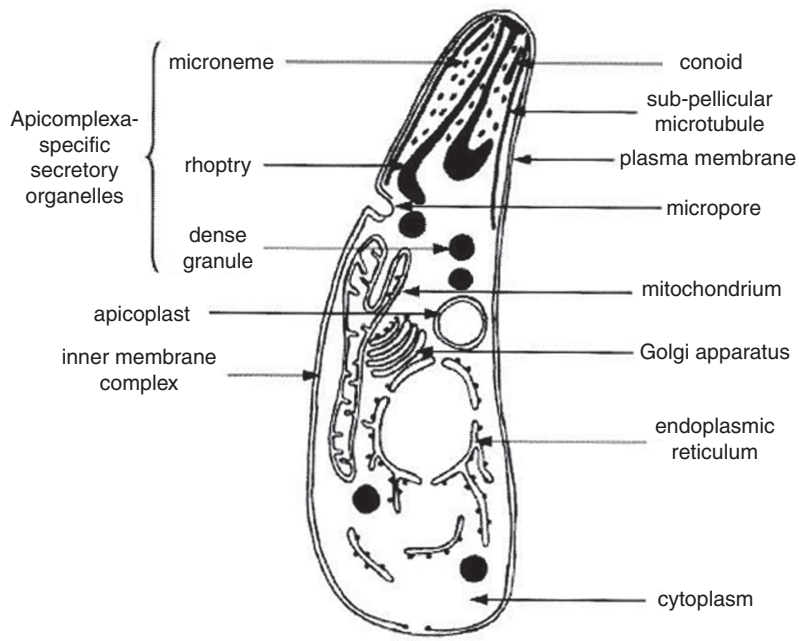


Figure 2.7 Diagrammatic representation of a *Toxoplasma gondii* tachyzoite. Source: Mercier *et al*., 2010. (Reprinted from Parasitology Reserach Trends, Mercia, C. *et al*., The dense granule proteins of Toxoplasma gondii, De Bruyn, O. & Peeters, S., 2010, pp 1–31, Reproduced by permission of Nova Sciences Publishers Inc)

host cells. Having made initial contact with a suitable cell, the tachyzoites re-orientate themselves and then discharge the contents of their micronemes, rhoptries, and dense granules. This enables the parasite to attach to the host cell surface after which it forces its way inside and becomes enclosed within a parasitophorous vacuole. Because *Toxoplasma gondii* infects such a wide variety of cell types and species of animal, it suggests that it must identify host cell receptors that have a widespread distribution in warm-blooded vertebrates. Blumenschein *et al*. (2007) have found that one of the micronemal proteins, TgMIC1, has a cell-binding motif called 'microneme adhesive repeat' (MAR) that binds to a group of carbohydrates called sialylated oligosaccharides. Sialic acid oligosaccharides are common components of cell surface membranes and play important roles in a variety of carbohydrate-mediated cell surface interactions (e.g. Varki, 1997).

After a series of parasite division cycles, the host's immune system starts to exert an effect and as a consequence *Toxoplasma gondii* forms tissue cysts (zoitocysts) containing bradyzoites. These tissue cysts are found predominantly within nervous (e.g. central nervous system and eyes) and skeletal and cardiac muscle tissue but can also be found elsewhere. The tissue cysts are thought to persist for life in some intermediate hosts although their presence may be sustained by periodical re-activation, transformation into tachyzoites, followed by the formation of new tissue cysts.

Intermediate hosts can also become infected by consuming meat containing the tachyzoite and/or bradyzoites. In the case of humans, this occurs through the consumption of raw or undercooked meat. Infections have also resulted from blood transfusions and organ transplants. For an in-depth review of how humans become infected with *Toxoplasma gondii*, see Tenter *et al*. (2000). Once the parasites reach the small intestine, they invade the surface epithelium and the life cycle takes place as described above. In pregnant mammals, vertical transmission can occur when tachyzoites cross the placenta and infect the developing embryo. Most of the work on transplacental transmission has been done on humans and the extent to which it occurs in other animals and its importance in the epidemiology of the parasite are uncertain. Acquistion of primary toxoplasmosis during pregnancy is, however, a significant cause of congenital infection in humans. In some countries, such as France, screening for the infection is a routine part of antenatal care (Pappas *et al*., 2009). Although there are sporadic calls for *Toxoplasma* testing for pregnant women in the UK, the incidence is too low to make it cost-effective. It is considered a better use of resources to identify and treat individuals. *Toxoplasma gondii* is susceptible to treatment with antibiotics such as spiramycin which are relatively safe to administer during pregnancy and help to reduce the chances of congenital transmission, if the mother is exposed to infection during the early stages of pregnancy. However, the outcome is often not a happy one; if that foetus is infected it can lead to spontaneous (or therapeutic) abortion or a severely handicapped baby.

Box 2.5 Population structure of *Toxoplasma gondii*

The population structure of *Toxoplasma gondii* is highly clonal. In Europe and North America most strains of *Toxoplasma gondii* studied to date belong to one of three distinct genotypes: Types I, II, and III (Dubey *et al*., 2002). However, other genetically distinct strains have been described in Brazil and French Guiana (Khan *et al*., 2006). There is limited variability within genotypes I, II, and III and this suggests that they may have arisen relatively recently (Boothroyd and Grigg, 2002; Lehman *et al*., 2006). Furthermore, this polymorphism appears to be limited to just two alleles: these have been called A and E – short for Adam and Eve – on the basis that all

extant strains are derived from mixtures of two ancestral founder strains. The continued existence of these separate strains is probably a result of the ability of asexually reproducing populations of the parasite to be maintained, possibly indefinitely, by horizontal and vertical transmission between intermediate hosts and the limited opportunity for genetic mixing during sexual reproduction within the cat definitive host (Boothroyd and Grigg, 2002). Because *Toxoplasma gondii* is haploid, genetic mixing can only occur if the cat is simultaneously infected with two different strains of the parasite. Furthermore, the development of the two strains would have to be synchronous because the production of gametes occurs over a relatively short period of time. This scenario might occur if the cat had consumed a mouse harbouring two or more strains of the parasite or had consumed two mice harbouring different strains in a short period of time. There is limited evidence of intermediate hosts harbouring mixed strain *Toxoplasma gondii* infections but more work is needed in this area.

2.6.6 Genus *Neospora*

This is a genus of 'emerging parasites', and their first existence became apparent in 1984 with the description of a cyst-forming protozoan parasite that was assumed to be responsible for encephalomyelitis and myositis in a litter of puppies in Norway. Since then, the parasite responsible has been given the name *Neospora caninum* although Heydorn and Melhorn (2002) consider this to be a '*nomen nudum*', that is, it fails to conform to the International Code of Zoological Nomenclature. Despite the taxonomic uncertainty, most workers continue to refer to the parasite as *Neospora caninum* and we shall do the same. Until recently the parasite must have been overlooked because it has rapidly been recognised that not only does *Neospora caninum* have a cosmopolitan distribution but it is also a major cause of abortion in cattle. Another species of *Neospora*, *Neospora hughesi,* has also been described, which causes myeloencephalitis in horses (Marsh *et al*., 1998) but very little is known about its biology.

Neospora caninum *Neospora caninum* is essentially a disease of dogs and cattle (neosporosis) though other mammals may also be infected. On a world-wide basis it causes losses of hundreds of millions of pounds per year (Dubey *et al*., 2007) and is a particular problem in cattle-breeding countries.

The life cycle of *Neospora caninum* resembles that of *Toxoplasma gondii* and apart from having different definitive hosts, they occupy a very similar ecological niche. It is therefore not surprising that there is some evidence that competition between the two species could occur in the intermediate hosts (Sundermann and Estridge, 1999). Sexual reproduction in *Neospora caninum* occurs within dogs and other canines (rather than cats in the case of *Toxoplasma gondii*) and they are therefore the definitive host. A separate asexual cycle occurs within cattle, particularly dairy cattle, but can also occur within a number of other mammals, including dogs, in which transmission takes place vertically via the placenta. There have been no cases of human infection to date.

Dogs normally acquire an infection by consuming meat that contains the tissue cysts. When these cysts reach the small intestine, they release bradyzoites which invade the intestinal cells and reproduce by schizogony to form schizonts. The subsequent stages that lead to the formation of the oocysts are not clearly identified yet (Dubey *et al*., 2007) but presumably the schizonts give

rise to microgametes and macrogametes and fusion of the gametes results in the formation of oocysts that are shed in the dog's faeces. The oocysts are 10–11 μm in size and sporulate in the environment to produce two sporocysts, each containing four sporozoites.

A cow (or other suitable host) can become infected when it ingests a sporulated oocyst, for example, while grazing or through contaminated water. The oocyst releases the sporozoites when it reaches the cow's small intestine and these invade the intestinal epithelium and ultimately find their way to a wide variety of cell types around the body. The majority of parasites locate themselves in the reticulo-endothelial system where they transform into tachyzoites which multiply asexually by endodyogeny until they kill their host cell, after which they invade other cells. Healthy cows usually do not exhibit any signs of infection. The parasites induce a strong gamma-interferon-based cell-mediated immune response and this causes *Neospora caninum* to form tissue cysts containing bradyzoites. These tissue cysts are approximately 100 μm in size and are found predominantly in nervous tissue but they may be located elsewhere. If the cow was pregnant at the time of her initial infection, the tachyzoites can cross the placenta and infect the developing embryo. Alternatively, if she became pregnant some time after being infected, the resting tissue cysts are activated as a consequence of the normal reduction in cell-mediated immunity that takes place during the first trimester of pregnancy. Within the tissue cysts, the bradyzoites transform into tachyzoites which find their way to the developing calf. The consequences depend upon the number of invading tachyzoites and the stage of pregnancy. The earlier in the pregnancy that the calf is infected, the worse the prognosis. Infection during the first and second trimester is most likely to result in abortion while infection during the third trimester may have apparently no harmful effects – although 80–95% of calves born to infected mothers are infected at birth. Occasionally a calf that is infected during the third trimester may abort or be born with neurological symptoms, such as paralysis. Within infected but otherwise healthy calves, the parasite forms dormant tissue cysts. When infected female calves reach adulthood and become pregnant, these cysts will be activated and the parasite will be transferred to the next generation. High levels of seroprevalence have been detected in sheep and goats in Jordan and some other parts of the world (e.g. Abo-Shehada and Abu-Halaweh, 2010). Although *Neospora caninum* can cause abortion and birth defects in sheep and goats, there are fewer published reports on this than in cattle.

Tachyzoites have been detected in the colostrum but it is not known how important this is as a source of infection – given that over 80% of calves born to infected mothers are already infected at birth, it is doubtful whether it is a major factor. Although the parasite has been detected in the semen of infected bulls it is not known whether it is possible for the parasite to be transmitted sexually – and if it is whether it happens sufficiently often to be a significant epidemiological factor.

Because so many calves are born infected, this is thought to be the principal means by which the infection is maintained within herds. This would occur through the normal farm practice of retaining a proportion of the female calves as replacement heifers (female cows). However, modelling studies suggest that this is not sufficient to maintain the infection over prolonged periods and there has to be periodic horizontal transmission from infected dogs (Reichel, 2000).

Within dogs, tissue tachyzoite stages may also be formed and neurological symptoms can occur if neurological tissues are infected. In addition, in pregnant bitches the tachyzoites can invade across the placenta and infect the developing pups. The consequences of congenital infection in dogs can result in the pups being born with serious damage to nervous and skeletal muscle tissue that results in paralysis.

2.6.7 Family Cryptosporidiidae

Genus Cryptosporidium Cryptosporidiosis is a diarrhoeal disease of humans and many other mammals as well as amphibia, reptiles and birds, caused by protozoa of the genus *Crytosporidium* (Fayer, 2010). Cryptosporidiosis is an emerging infection (Thompson *et al*., 2008) and the taxonomy of the genus *Cryptosporidium* is also a fast-moving area in which the most important species in humans was not described until 2002. The first species to be recognised in the genus was *Cryptosporidium muris* which was isolated from mice and described by Tyzzer in 1907. Five years later he described *Cryptosporidium parvum*, also from mice. A number of other species were subsequently added to the genus later in the century until work in the 1980s concluded that there may actually be only a single species of *Crytptosporidium*. However, it is now thought that the samples used were misidentified and these conclusions were therefore invalid. Although traditionally considered to be coccidian parasites, albeit somewhat aberrant ones, molecular and ultrastructural studies indicate that the cryptosporidians have much closer affinities to the gregarines (Carreno *et al*., 1999; Thompson and Smith, 2011), a group of intracellular parasites that infect a wide range of invertebrates but not vertebrates. For more details on gregarines, see Leander (2008).

On the basis of appearance and dimensions of the oocyst and the usual animal (or bird) host, ten species of *Cryptosporidium* are distinguishable. Molecular studies have confirmed the existence of the ten species and added several more (Egyed *et al*., 2003). *Cryptosporidium parvum* emerges as three distinct genotypes: 'human', 'bovine', and 'pig', with the human and bovine genotypes closely related (Figure 2.8). The human *Cryptosporidium parvum* genotype (also called *Cryptosporidium parvum* genotype I) has been described as a separate species, *Cryptosporidium hominis* (Morgan-Ryan *et al* 2002), and this is now accepted as its correct name. See Fayer (2010) for a recent review of the genus. Table 2.4 presents selected species of *Cryptosporidium*, indicating their usual host and zoonotic potential.

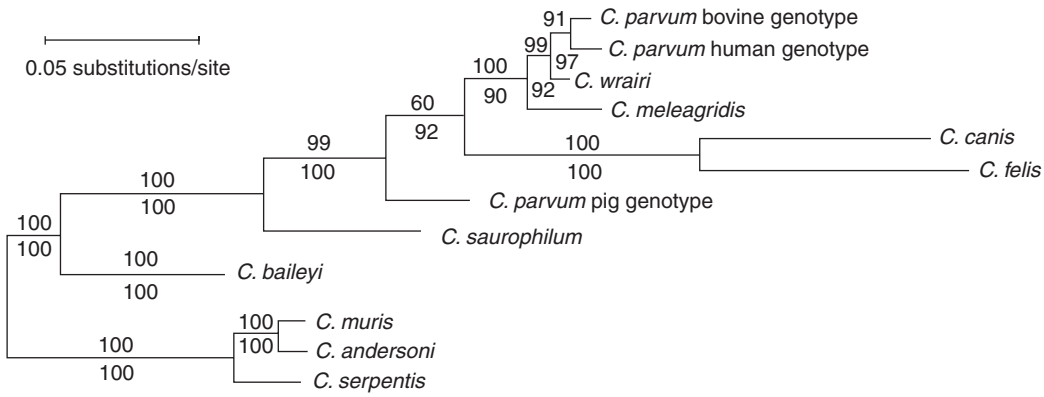


Figure 2.8 Phylogenetic relationships between the most important species of *Cryptosporidium* inferred from Maximum Likelihood Analysis of Hsp 70 (heat shock protein) gene sequences. Numbers above the nodes represent neighbour-joining values and those below the nodes represent parsimony bootstrap values. Only bootstrap values over 50% are shown. Source: Egyed *et al*., 2003. (Reprinted from Veterinary Parasitology, 111, Egyed, Z. *et al*., 103–114, 2002, with permission from Elsevier)

Table 2.4 Selected species of *Cryptosporidium* indicating their usual host and zoonotic potential

Species name	Usual host	Zoonotic potential
C. muris	Mouse	Yes
C. parvum	Mouse	Yes
C. meleagridis	Turkey	Yes
C. wrairi	Guinea pig	No
C. felis	Cats	Yes
C. serpentis	Snakes	No
C. baileyi	Chicken	No
C. saurophilum	Skink	No
C. andersoni	Cattle	Yes
C. canis	Dog	Yes
C. hominis	Humans	Yes

The life cycle of *Cryptosporidium parvum* is typical for the genus. When an oocyst reaches the small intestine or the respiratory system, it releases four sporozoites and these adhere to the surface mucus lying above the host epithelial cell's apical membrane. They then release enzymes that break down the mucus and this enables the sporozoites to make contact with and bind to the host cell membrane through receptor–ligand interactions. The sporozoites then discharge the contents of their apical organelles and these include chemicals that induce the host cell membrane to form protrusions which surround the parasites so that they become encapsulated within a parasitophorous vacuole. Although the host cell initially encapsulates *Cryptosporidium*, the membrane that ultimately surrounds the parasitophorous vacuole contains both parasite and host cell components. This parasitophorous vacuole sits just inside the host cell apical membrane and is separate from the host cytoplasm. This location is unusual as it means that the vacuole is effectively extracytosolic. There is a spot, known as the annular ring, where the host apical membrane and the parasitophorous vacuole membrane fuse. In addition, the parasite develops a unique organelle called a 'feeder organelle' through which it derives nutrients from the host cell. More details of the invasion process can be found in Borowski *et al*. (2008). Within the parasitophorous vacuole, the sporozoite transforms into a trophozoite and then into a meront that divides asexually by merogony to produce type 1 meronts that contain eight merozoites and then type 2 meronts that contain four merozoites. Further cycles of merogony might take place or the type 2 meronts may undergo gametogony to produce microgamonts (male) and macrogamonts (female). The microgamonts produce microgametocytes and these are released so that they can find macrogamonts with which they fuse. Once a macrogamont has been fertilised, it develops into a zygote. About 20% of zygotes form thin-walled oocysts that are able to reinfect the host. The other 80% of zygotes develop into thick-walled oocysts that are shed into the environment where they are immediately infectious although they can survive for prolonged periods. Unlike *Cyclospora*, *Isospora*, and *Sarcocystis*, parasites of the genus *Cryptosporidium* do not form sporocysts.

Most *Cryptosporidium* infections are acquired by ingesting oocysts which are transmitted by the faecal-oral route although they may also be inhaled, and auto-infection with oocysts is also possible. In immuno-compromised humans, the oocysts may be shed via the sputum. Outbreaks of infection in humans are often associated with contamination of drinking water and many other animals probably also acquire their infections this way. The oocysts can survive in sea water and

are resistant to chlorination. Consequently, infection can also result from ingesting oocysts while swimming in recreational pools, lakes, or the sea. Flies and other invertebrates may also pick up and transmit the oocysts to food (e.g. Graczyk *et al*., 2000). Clams, oysters and other shellfish are able to concentrate *Cryptosporidium* oocysts from the surrounding water but it is not known whether they pose a significant source of infection.

While cryptosporidiosis is unpleasant (watery diarrhoea, abdominal cramps, slight fever) in immuno-competent adults, it is usually self-limiting. In the United Kingdom, as well as continual low-level transmission between humans, there are sporadic outbreaks associated with contaminated drinking water, which are likely to be zoonoses. Detection of *Cryptosporidium* spp. in patients' samples or water sources can be difficult, because it relies on the identification of oocysts which are only ~5 μm long and often present in low numbers.

2.7 Phylum Kinetoplastida

The Kinetoplastida are a large diverse group of protozoa that includes both plant and animal parasites (Table 2.5). Some authors consider the Kinetoplastida to be a phylum, while others refer to it as a class or an order. They are commonly known as the trypanosomes after the genus *Trypanosoma* that includes the causative agents of Human African Trypanosomiasis (sleeping sickness) and several other parasites of medical and veterinary importance. A comprehensive review of all aspects of trypanosome biology and their transmission is provided by Maudlin *et al*. (2004).

Table 2.5 Examples of kinetoplastid parasites of medical, veterinary, and agricultural importance and the diseases they cause

Genus	Example	Host	Vector/transmission	Disease
Leishmania	*L. donovani*	Humans, dogs, rats	*Phlebotomus* sandflies	Kala azar (visceral leishmaniasis)
	L. major	Humans, monkeys, dogs, rodents	*Phlebotomus* sandflies	Cutaneous leishmaniasis
	L. tropica	Humans, monkeys, dogs, rodents	*Phlebotomus* sandflies	Cutaneous leishmaniasis
	L. braziliensis	Humans, sloths, monkeys, opossums and many others	*Lutzomyia* sandflies	Cutaneous/ mucocutaneous leishmaniasis
Trypanosoma	*T. brucei gambesiense*	Humans	Tsetse flies *(Glossina* spp.)	African trypanosomiasis (sleeping sickness)
	T. congolense	Cattle	Tsetse flies *(Glossina* spp.)	African trypanosomiasis (nagana)
	T. equiperdum	Horses	sexual	Dourine
	T. cruzi	Humans, dogs, cats, rats, and many others	Triatomid bugs	Chagas' disease
Phytomonas	*P. staheli*	Coconut palms	*Lincus lobuliger* (Pentatomid bug)	Hartroot

The Kinetoplastida are characterised by the fact that they have flagellum and a unique intracellular structure called a kinetoplast. The kinetoplast is a disk of interlocking DNA circles (kDNA) located within a large mitochondrion. The structure of kinetoplast DNA is unlike that found in any other organism and its complex replication involves special proteins (Liu *et al*., 2005). It may therefore be possible to design drugs to interfere with the replication of kinetoplast DNA. The mitochondrion is situated so that the kinetoplast is located just underneath a structure called the kinetosome that is itself situated underneath the base of the flagellum. The kinetosome (sometimes called the basal body), is a structure found in many organisms and is homologous with the centriole; it is involved in the formation of the flagellum (Chapman *et al*., 2000). The Kinetoplastida always have a flagellum which may be long and free, incorporated into the cell surface to form an undulating membrane, or small and enclosed within a pocket. The inner core of the flagellum is called the axoneme and alongside this, and connecting to it, is a lattice-like crystalline array of structural proteins called the paraxial rod. In the promastigote, epimastigote, and trypomastigote stages the flagellum emerges at the anterior end of the cell and therefore acts as a propeller that pulls the cell along rather than pushing it from behind.

The Kinetoplastida have a unique organelle called the glycosome which may be related to the peroxisomes (which they do not have) found in other organisms. The glycosomes are about 0.25 μm in diameter and contain glycolytic enzymes that are normally present in the cytoplasm of other organisms (Hannaert and Michels, 1993). The bloodstream forms of trypanosomes are extremely metabolically active and will soon die if they run out of glucose to metabolise. The glycosomes are the site of glucose metabolism and therefore, like the kinetoplast DNA, they are an obvious target for antiparasite drug design.

Many species within the Kinetoplastida are only parasitic in insects. For example, members of the genus *Leptomonas* are found in the gut of a variety of insects including blood-feeding reduviid bugs (Yurchenko *et al*., 2006). Because they have only a single host, these species are said to be monoxenous although rare cases of human infections have been reported in patients who are HIV positive (e.g. Boisseau-Garsaud *et al*., 2000; Jimenez *et al*., 1996). A large number of the Kinetoplastida alternate between an invertebrate such as a blood-feeding insect or leech, and a vertebrate, with development occurring in both of them. Species that have more than one type of host are said to be heteroxenous. Heteroxenous species often exist in two or more morphological forms with one form present in the invertebrate and the other in the vertebrate (Table 2.6). There is a suspicion that some members of the Kinetoplastida can exhibit sexual reproduction, or something similar, but it is not known whether it is a widespread phenomenon in the group (e.g. Kreutzer *et al*., 1994).

2.7.1 Genus *Leishmania*

Members of the genus *Leishmania* exhibit two morphological forms: the amastigote form which is found in the vertebrate host and the promastigote form which is found in the invertebrate vector. The vertebrate hosts are mostly mammals while the invertebrates are various species of sandflies. Those species that are parasitic in reptiles are currently separated into a subgenus – the *Sauroleishmania* – and they are not thought to have any epidemiological relevance to human infections (Belova, 1971). There is some debate over whether the *Leishmania* evolved from the *Sauroleishmania* or *vice versa* (Tuon *et al*., 2008). Perhaps counter-intuitively, current molecular evidence suggests that the *Leishmania* initially evolved in the Neotropical regions as parasites of

Table 2.6 Morphological forms of Kinetoplastida parasitic in humans and domestic animals

Morphological form	Description	Example
Amastigote	Kinetoplast and kinetosome above the nucleus, flagellum short and confined in pocket. Cell is globular	*Leishmania donovani* inside vertebrate macrophage *Trypanosoma cruzi* in human spleen, liver, muscle and other cell types
Promastigote	Kinetoplast and kinetosome at anterior end of cell, flagellum free and long. Cell is elongate	*Leishmania donovani* in sandfly gut
Epimastigote	Kinetoplast and kinetosome close and anterior to the nucleus. There is a short undulating membrane before the flagellum emerges at the anterior of the cell. Cell is elongate	*Trypanosoma cruzi* in triatomid gut
Trypomastigote	Kinetoplast and kinetosome at posterior end of cell. Flagellum forms an undulating membrane that runs the length of the cell and may continue free when it reaches the anterior end. Cell is elongate	*Trypansoma cruzi* in human bloodstream

Note: Other morphological forms (e.g. choanomastigote, opisthomastigote, paramastigote) are exhibited by those parasitic in invertebrates.

mammals and that the parasites of reptiles, the *Sauroleishmania*, subsequently evolved from them (Noyes *et al*., 2000).

Box 2.6 How *Leishmania donovani* got its name

Although an epidemic disease known as kala-azar in the Indian subcontinent had been known for hundreds of years, the causative agent was not discovered until the beginning of the twentieth century. Another name for the disease was Dum Dum fever after the town 'Dum Dum' on the outskirts of Calcutta where the disease was a serious problem. Dum Dum has not gained a good reputation in history as it also gave its name to the infamous rounds of ammunition known as dum-dum bullets. In 1900, a British soldier suffering from kala-azar was shipped home from Dum Dum to England, where he died in the military hospital at Netley. His body was autopsied by the Scottish doctor Dr William Boog Leishman and he described circular bodies within the macrophages present in the spleen. Dr Charles Donovan, who was working in Madras, was the first to confirm Leishman's findings and he was also able to demonstrate that these circular bodies could only be found in the spleen of patients suffering from kala-azar. Subsequently, the 'circular bodies' received the name Leishman-Donovan bodies. Initially, Leishman believed that these bodies were degenerating trypanosomes that had been ingested by the macrophages. However, they were subsequently recognised as being parasitic intracellular protozoa and they were given the name *Leishmania donovani* by Laveran and Mesnil in 1903.

Table 2.7 Taxonomic divisions within the genus *Leishmania*

Genus	Subgenus	Disease	Example
Leishmania	*Leishmania*	Visceral	*L. donovani* phenetic complex
			L. infantum phenetic complex
		Old World cutaneous	*L. major* phenetic complex
			L. tropica phenetic complex
		New World cutaneous	*L. mexicana* phenetic complex
	Viannia	New World	*L. braziliensis* phenetic complex
	Sauroleishmania	Lizard leishmanisis	*L. tarentolae*

The taxonomy of the genus *Leishmania* is complex and it is extremely difficult or even impossible to distinguish many of them on the basis of their morphology. The genus was comprehensively reviewed by Lainson and Shaw in 1987 and since then numerous molecular and phylogenetic studies have largely supported their proposals for how it should be divided (Table 2.7). However, agreement concerning the status of several species is far from complete. Lainson and Shaw (1987) identified two subgenera: *Leishmania* and *Viannia*. In the subgenus *Leishmania*, the parasites begin their development in the insect vector's midgut and then move forwards to the pharynx, from which they are injected into the vertebrate host when the sandfly feeds. By contrast, in the subgenus *Viannia*, the parasites begin development in the insect vector's hind gut and then move forward to the pharynx.

Those species within the subgenus *Viannia* (e.g. *Leishmania braziliensis*, *Leishmania peruviana*, *Leishmania guyanensis*, *Leishmania panamensis*) are restricted to South America and are primarily responsible for cutaneous disease. Species belonging to the subgenus *Leishmania* (e.g. *Leishmania donovani*, *Leishmania major*, *Leishmania infantum*, *Leishmania tropica*, *Leishmania mexicana*) have representatives in both the New World and the Old World and include agents of both visceral and cutaneous disease. Because of the difficulties associated with identifying the parasites and the diversity of the pathologies they cause, there is a tendency to refer to *Leishmania* 'phenetic complexes'. That is, a species exhibits a range of phenotypes that may or may not be related to underlying genotypic differences. More than 30 species of *Leishmania* have been described to date and 10 of these are of medical or veterinary importance and several of these are zoonotic.

Present-day members of the genus *Leishmania* are responsible for a great deal of morbidity and mortality in humans and some cause serious disease in domestic and wild animals. It has been suggested that it was not a meteor that killed off the dinosaurs but ancestral versions of *Leishmania* (with help from some other parasites) (Poinar and Poinar, 2008). In humans, leishmaniasis is a complex of diseases caused by various species of *Leishmania*. The diseases, in their various forms, are found in over 80 countries; approximately 350 million people are at risk of infection and there are about 2 million new cases diagnosed every year (WHO, 1990). While it is convenient to group those species of *Leishmania* which invade organs as 'visceral' and those which affect the outer body surface as 'cutaneous' the distinctions are far from clear. For example, *Leishmania donovani* is normally considered to cause visceral leishmaniasis but it can become cutaneous – as in the development of post-kala-azar dermal leishmaniasis (see later). Similarly, *Leishmania tropica* usually causes cutaneous leishmaniasis but can infect the viscera. Some 12 million people are infected with cutaneous leishmaniasis, which leaves long-lasting sores, and a further 500,000 with

the potentially fatal visceral leishmaniasis of whom up to 80,000 die every year. The incidence of HIV/*Leishmania* co-infections is also increasing and is a serious cause for concern.

2.7.2 *Leishmania* life cycle

In the vertebrate host, *Leishmania* exists in the amastigote form (2.5–5 μm) within mononuclear phagocytes and in particular in the macrophages (see Colour Plate 2). These are a sub-group of the leucocytes (white blood cells) that have an essential role in the immune response and they are normally responsible for the phagocytosis and destruction of foreign organisms. The parasites multiply by binary fission within a phagocyte until the host cell is destroyed, after which they are released to be ingested by, and subsequently invade, new phagocytes. The infection is normally transmitted between individuals by sandflies. It is the blood-feeding female sandflies that transmit the disease – the males are harmless nectar feeders. Parenteral transmission, for example, via contaminated needles or blood transfusion, has been described in some *Leishmania* species.

The sequence of development within the sandfly varies slightly between species but in all cases involves transformation, replication, and subsequent movement to the anterior region of the gut (Bates, 2008, 2007). A sandfly becomes infected when it ingests infected mononuclear phagocytes during feeding. Like mosquitoes, sandflies are 'batch processors' that take in a large blood meal that is then enclosed within a peritrophic membrane when it enters the midgut. The blood meal is held within the midgut where it is digested over the whole of its surface after which the products are absorbed. There is therefore usually a gap of several days between blood meals. When the blood meal first reaches the insect's midgut, the amastigotes transform into the procyclic promastigote stage in response to the decrease in temperature and increase in pH. The procyclic stage has a relatively short flagellum, is not very motile and multiplies by binary fission within the blood meal. The promastigotes undergo a series of morphological transformations and multiplications as they move up from the midgut to the region of the stomadael valve which marks the boundary between the foregut and the midgut. Along the way, some of the parasites attach to the lining of the gut and stomadeal valve using their flagellae and this attachment is thought to be an important part of the life cycle. In those species belonging to the sub-genus *Viannia*, the majority of parasites make their way to the pylorus region (hind triangle) at the posterior of the midgut before moving forwards. Unlike *Plasmodium* (in which the infective stages invade the salivary glands of the vector mosquito), the metacyclic stages of *Leishmania* do not, as a rule, infect the salivary glands of the sandflies. However, sandfly saliva does play an important role in the transmission process.

Box 2.7 How harming the vector can facilitate transmission

On reaching the stomadeal valve, the *Leishmania* promastigotes secrete a gel-like substance called promastigote secretory gel, the main component of which is filamentous proteophosphoglycan and some of them transform into the infective metacyclic promastigote stage (this has a long flagellum and is very active). The gel physically blocks the gut and this, together with the sheer numbers of parasites, severely compromises the fly's ability to feed. Further compounding this, the parasites also produce chitinase enzymes that physically damage the peritrophic

membrane and stomadeal valve. Because the insect's ability to ingest food is impaired, it becomes hungry thereby increasing its probing and number of visits to hosts, all of which increases the chance of transmission. Physical probing probably does not transfer many parasites but in order to ingest food, the infected fly has to first expel some of the promastigote secretory gel – and this is packed with the infective metacyclic stage as well as other non-infective stages. The secretory gel also appears to facilitate the establishment of the infection in the vertebrate host so it has a dual role in both the invertebrate and vertebrate host (Rogers *et al*., 2004).

The transmission mechanism of the *Sauroleishmania* is poorly understood. Within the sandfly vector, these species tend to remain in the posterior regions of the gut and therefore may not be transmitted when the sandfly feeds. It is possible that transmission might resemble that of *Trypanosoma cruzi* by triatomid bugs but as sandflies do not normally defecate while feeding, this is unlikely. A third possibility is that the transmission occurs through the lizards consuming infected sandflies.

Much of the work on the establishment and development of *Leishmania* within its mammalian hosts has been done on those few species where it is possible to establish laboratory cultures and using mice as model organisms. It should be remembered that although mice and humans are both mammals, it cannot be assumed that their immune systems will react in an identical fashion to the same infectious agent. Furthermore, leishmaniasis manifests itself in a wide variety of forms and therefore it is to be expected that the different species and strains will show variations in the manner in which they establish themselves and interact with the host immune system. Nevertheless, all species follow a basic pattern of development following their entry into the bloodstream which involves morphological and physiological transformations and establishment in the mononuclear phagocytes and in particular the macrophages.

Box 2.8 How *Leishmania* establishes within mammalian phagocytes

Once injected into the mammalian host, the promastigotes are detected and ingested by mononuclear phagocytes. Initial attachment to the phagocytes begins at the tip of the parasite's flagella (Miller *et al*., 2007) and is mediated via a ligand-receptor process. The principal ligands are phosphoglycans and the zinc metalloprotease enzyme gp63 on the surface of the promastigote, while on the phagocytes a variety of complement receptors have been implicated in the attachment process. The composition of the cell surface phosphoglycans changes following transformation to the amsatigote stage while the enzyme gp3 ceases to be expressed there. Consequently, the invasion of phagocytes by amastigotes involves a different set of ligands and receptors (Handman and Bullen, 2002; Kaye and Scott, 2011). After attaching to the phagocyte the parasite is ingested by phagocytosis and held within a membrane-bound vesicle called a phagosome (Lodge and Desconteaux, 2008). Lysomes then fuse with the phagosome and discharge a range of hydrolytic enzymes and microbicidal peptides into it. They also cause the contents to become acidified and the structure then becomes known as a parasitophorous vacuole or phagolysosome. Different species of *Leishmania* cause the formation of different types of phagolysosome: those produced

by *Leishmania mexicana* tend to be large and contain many parasites while those formed by *Leishmania donovani* tend to be much smaller (Handman and Bullen, 2002).

The combination of a rise in temperature associated with moving to a mammalian host and the drop in pH caused by being enclosed in a phagolysosome induce the parasite to change into the amastigote form. This transformation takes about 1–4 hours following ingestion (Miller *et al*., 2007) and is essential if the parasite is to survive the acidic pH and hydrolytic enzymes within the phagocytes. In particular, there is a change in the nature of the parasite's cell surface membrane and the expression of new cell surface components. For example, some of these, the GIPLs (glycoinositol phospholipids) have been shown to directly inhibit the production of nitric oxide (NO) which has microbiocidal properties (Späth *et al*., 2003). Despite these changes, phagocytes are able to destroy amastigotes and the reason why some people develop serious or even fatal infections while in others the infection is resolved, possibly without any symptoms being displayed, is not known.

Visceral Leishmaniasis The classical visceral form of leishmaniasis produces the disease commonly known as kala-azar that has febrile symptoms similar to those of malaria. Malaria and kala-azar occur in the same geographical areas so it is important for doctors to distinguish between them. The name kala-azar (black head) is derived from the symptomatic darkening of the forehead and mouth of patients suffering from visceral leishmaniasis. In India great plagues of visceral leishmaniasis occurred in Assam in the late nineteenth and early twentieth centuries that resulted in whole villages being depopulated. Serious outbreaks still occur today and visceral leishmaniasis remains an important cause of morbidity and mortality in over 70 countries around the world (McGregor, 1998). Most cases of visceral leishmaniasis occur in South Asia ($\sim$ 67%) but it is also a big problem in parts of East Africa where its incidence is increasing in some areas (e.g. Sudan, in the region bordering with Ethiopia) (Hotez *et al*., 2004). There is also a focus of infection in South America, in particular, in Brazil.

The clinical picture of visceral leishmaniasis differs geographically. There is, nonetheless, a basic pattern to the course of the disease. The first stage begins when a papule develops at the site of the sandfly bite but this eventually regresses. Low grade recurrent fevers then develop anything from 10 days to two years or more afterwards and these persist throughout the course of the disease. Within the spleen, red blood cells are destroyed owing to an immune-related response and this causes anaemia. In addition, the liver becomes enlarged (hepatomegaly) as does the spleen (splenomegaly). Enlargement of the spleen results from a combination of hyperplasia induced by the need to produce new mononuclear phagocytes and also from infected mononuclear phagocytes filling with parasites. The patient often suffers from diarrhoea and this, together with the fever, leads to anorexia, malnutrition, and dehydration. If the disease is not treated, 90% of people suffering visceral leishmaniasis will die. However, recovery can be rapid and complete with or without treatment. However, in many cases, the parasite persists and may appear on the skin in raised macules, causing the disfiguring condition post-kala-azar dermal leishmaniasis.

Post-kala-azar dermal Leishmaniasis Post-kala-azar dermal leishmaniasis (PKDL) is a condition that is usually associated with *L. donovani*. It is characterised by the development of nodules and/or macules that can be extensive and cover any area of the body and may be mistaken for leprosy. The nodules take the form of irregular raised masses on the skin surface while macules

(Latin *macula* = blemish or small spot) are flat, discoloured areas on the skin surface. These regions are heavily parasitised with amastigotes and where the nodules or macules are on exposed parts of the body, they are a ready source of infection for sandflies. The identification and treatment of patients suffering from PKDL are therefore an important part of any control programme (Salotra *et al*., 2003; Thakur, 1992). There are marked differences in the occurrence and development of PKDL between countries which suggests that host and parasite factors may be important in whether or not it develops. For example, the majority of cases of PKDL (~50%) are found in the Sudan and the condition tends to develop more rapidly there than in India.

PKDL is often said to develop as a sequel to visceral leishmaniasis (hence 'post-kala- azar') and may manifest itself anything from immediately afterwards to several years following the condition. Some workers consider that the development of PKDL is associated with the incomplete or inefficient treatment of visceral leishmaniasis and in particular where the drugs sodium stibogluconate and pentamidine have been employed. However, cases have also been recorded where miltefosine has been used (Das *et al*., 2009). In India about 20% of PKDL cases occur in people for whom there is no record of either visceral leishmaniasis or the prescription of the drugs used to treat it. However, these people are infected with *Leishmania donovani* and therefore must be carrying an asymptomatic infection (Das, *et al*., 2009).

Cutaneous leishmaniasis Cutaneous leishmaniasis can manifest itself in a variety of different forms depending upon the species of *Leishmania* (reviewed by Reithinger *et al*., 2007). Medically, cutaneous leishmaniasis is divided into three basic types depending upon how the disease presents. Localised Cutaneous Leishmaniasis (LCL) generally takes the form of a dry ulcer that develops at the bite site of the sandfly vector and which usually heals by itself, though this may take some time and may leave permanent scarring. This form of disease is common in India, Central Asia, the Middle East and parts of southern Europe and is often caused by *Leishmania major* and *Leishmania tropica*. LCL is also common in South America where species such as *Leishmania venezuelensis* and *Leishmania mexicana* are responsible. Diffuse (disseminated) Cutaneous Leishmaniasis (DCL) is a rarer and much more serious condition than localised cutaneous leishmaniasis. It is typified by the formation of numerous raised (but not ulcerating) papules and nodules that spread to cover large areas of the body. The condition is often associated with immune suppression and there are several reports of HIV co-infection (e.g. Chaudhary *et al*., 2008; Niamba *et al*., 2007). Unlike localised cutaneous leishmaniasis, patients with diffuse cutaneous leishmaniasis seldom recover without treatment. In the 'Old World', *Leishmania aethiopica* is the most common cause of DCL while in South America, *Leishmania mexicana* and *Leishmania amazonensis* are implicated. As mentioned earlier, it is not unusual for any one species of *Leishmania* to cause different types of leishmaniasis.

Mucocutaneous leishmaniasis occurs where the infection induces the formation of an ulcerative lesion that afflicts the mouth, palate and nose. As a rule, the condition develops and spreads slowly over a period of years and can lead to the total destruction of the affected area. It is most common in South America, particularly Brazil and the Amazon regions of Peru, Ecuador, Colombia and Argentina where it is caused primarily by *Leishmania braziliensis* and the condition is known as 'espundia'.The discovery of pre-Inca pottery illustrating disfigured faces has been used as evidence that the disease pre-dates the European invasion of South America, but this is still disputed. Some workers claim that the disease was introduced into South America by the Conquistadors and early Spanish settlers. Provided that medical care is available, espundia has a low mortality (5%) but would be undoubtedly higher without it. Death from mucocutaneous

leishmanisis is most commonly as a result of complications such as aspiration pneumonia although some sufferers have suffocated owing to laryngeal closure. Mucocutaneous leishmaniasis may also be caused by *Leishmania major* and other species of *Leishmania* but these cases are rare (e.g. Kharfi *et al*., 2003).

2.7.3 Genus *Trypanosoma*

All members of this genus are parasites of vertebrate animals and almost all of them are transmitted by invertebrate vectors. Asexual reproduction usually takes place in both the vertebrate host and the invertebrate vector (i.e. the parasites are heteroxenous). However, if the parasite is mechanically transmitted, such as *Trypanosoma evansi* which is transmitted via the contaminated mouthparts of biting flies, there is no development outside the vertebrate host. There is some evidence of sexual reproduction (or something like it) occurring in the invertebrate host in some trypanosome species. *Trypanosoma equiperdum* is something of an exception to this general lifestyle because it is a sexually transmitted parasite of horses and other equids. Nevertheless, the majority of species are heteroxenous and two distinct groups can be identified on the basis of where they develop within their invertebrate host. Those that develop within the anterior regions of the gut and are transmitted by the vector's bite are said to exhibit 'anterior station' development and belong to the 'Salivaria' group. Species that develop in the vector's hind gut and are transmitted via its faeces are said to exhibit 'posterior station development' and belong to the 'Stercoraria' group. Both groups include species of medical and veterinary importance (Table 2.8). It should come as no surprise that the taxonomy of trypanosomes is in a state of flux and it is highly likely that some well-known 'species' are actually 'sub-species' or 'synonyms' but for the sake of comparison with past literature we have retained the most common species' names.

The trypanosome kinetoplast has become modified or lost in several species and this has had a dramatic effect on their subsequent spread and evolution. In the case of *Trypnaosoma brucei*, during the vertebrate stage of development the mitochondria is down-regulated but it is fully functional during the procyclic stage that develops in the tsetse fly vector. The partial loss of kinetoplast DNA (dyskinetoplastidy) or its complete loss (akinetoplastidy) means that the mitochondria cannot fully function and therefore the parasite can only develop within its vertebrate host. Consequently, trypanosomes which lack a kinetoplast or the kinetoplast are only partially functional and must rely on mechanical vector transmission or sexual transmission. However, this should not be considered to be a 'retrograde step' because the evolution of these modes of transmission broke the link with the African tsetse fly belt and allowed the parasite to spread to other countries. Dyskinetoplasty can arise naturally in wild populations by mutation, in response to treatment with certain anti-trypanosome drugs (e.g. triacetylbenzene hydrochloride [TBG]), and through long-term *in vitro* culture.

Trypanosoma brucei *Trypanosoma brucei* is responsible for the diseases commonly referred to as Human African Trypanosomiasis, or more colloquially 'sleeping sickness' and the wasting disease in cattle known as 'nagana'. Human African Trypanosomiasis is predominantly a disease that affects poor people living in rural environments in some of the most politically unstable parts of Africa and consequently there is widespread under-reporting of the disease. In the absence of treatment, Human African Trypanosomiasis is almost always fatal. In 2006, the WHO estimated that there were around 20,000 cases of Human African Trypanosomiasis every year (WHO, 2006a)

Table 2.8 Examples of *Trypanosoma* species of medical and veterinary importance

Parasite	Transmission group	Vector/means of transmission	Host
T. brucei brucei	Salivaria	Tsetse flies (*Glossina* spp.)	Ruminants (cause of 'nagana')
T. brucei gambiense	Salivaria	Tsetse flies (*Glossina* spp.)	Humans (cause of 'sleeping sickness). No important animal reservoir of infection
T. brucei rhodesiense	Salivaria	Tsetse flies (*Glossina* spp.)	Humans (cause of 'sleeping sickness). Reservoir of infection in cattle, wild game, lions, hyenas, etc.
T. congolense	Salivaria	Tsetse flies (*Glossina* spp.)	Cattle, pigs, wild game. Cause of 'nagana'
T. evansi (*T. brucei evansi*)	Salivaria	Tabanid and other biting flies (mechanical transmission)	Horse, cattle, pigs, dogs, rodents. Cause of 'surra'
T. equinum (synonym of *T. evansi*)	Salivaria	Tabanid and other biting flies (mechanical transmission)	Horses, donkeys, cattle, dogs. Cause of 'mal de caderas'
T. equiperdum (*T. brucei equiperdum*)	Salivaria	Sexual transmission	Horse, asses. Cause of 'dourine'
T. cruzi	Stercoraria	Reduviid bugs (e.g. *Triatoma infestans*)	Humans (cause of Chagas' disease). Reservoir of infection in many domestic and wild animals
T. theileri	Stercoraria	Tabanid flies	Cattle

while more recently Brun *et al*. (2010) suggest that there are between 50,000 and 70,000 cases. Because of the difficulty in treating Human African Trypanosomiasis, its debilitating effects and potentially fatal outcome, this makes the disease of considerable medical concern. Until recently, the most commonly used drugs for treating the disease were developed over 60 years ago and are so toxic that they would never have been approved if they were required to pass today's stringent safety standards. For example, suramin can cause anaphylactic shock and kidney failure while melarsoprol (Mel B) causes seizures and kills 1 in 20 of the patients who receive it. Although this might sound unacceptable, it should be remembered that in the absence of treatment, there is a 100% certainty that the patient will die of the disease. There is, however, reason to hope that things may improve since a drug discovery scheme has identified a novel group of chemicals called the benzoxyboroles that are highly effective at treating stage 2 Human African Trypanosomiasis (Jacobs *et al*., 2011). Not only can these chemicals be taken orally (suramin and melarsoprol have to be given as a series of intravenous injections), but they also do not produce the harmful side-effects of current drugs. The benzoyboroles also include chemicals which show promise for the treatment of malaria, filarial nematodes, and bacteria (Zhang *et al*., 2011). Consequently it may become possible to treat co-infections with a single safe drug.

There are three morphologically identical sub-species of *Trypanosoma brucei*: *Trypanosoma brucei brucei*, *Trypanosoma brucei gambiense*, and *Trypanosoma brucei rhodesiense*. The different sub-species vary in their geographical distribution, ability to infect mammalian hosts

and their pathology, but they are all transmitted by a variety of tsetse fly species (e.g. *Glossina palpalis*) with different species being of particular importance as vectors in different areas. Tsetse flies are only found in Sub-Saharan Africa within the latitudes 14° North and 29° South – an area that encompasses about 10 million km^2. Because of their absolute dependence upon tsetse flies to effect transmission, the parasites are also limited to this region. Tsetse flies have specific environmental requirements to complete their life cycles and they are therefore not found in urban environments. Consequently, Human African Trypanosomiasis is acquired by people working in the fields or visiting the countryside and game reserves.

Trypanosoma brucei brucei is essentially a parasite of wild and domestic animals and it does not infect humans. Infections in wild game (e.g. kudu [*Tragelaphus strepsiceros*], warthog [*Phacohoerus aethiopicus*]) and some native cattle breeds (e.g. N'Dama, Muturu, Maasai Zebu) do not always lead to serious disease symptoms and they are therefore said to be trypanotolerant. By contrast, many introduced varieties of domestic animals are severely affected by *Trypanosoma brucei brucei* and succumb to a condition called 'nagana' – a word derived from the Zulu language and meaning 'to be in low or depressed spirits'. Horses, sheep, goats, and dogs are particularly severely affected and often suffer an acute disease which culminates in the death of animal within 20 days to a few months of becoming infected. In cattle, *Trypanosoma brucei brucei* tends to cause chronic disease that lasts several months and the infected animal may ultimately recover.

Trypanosoma brucei rhodesiense is genetically very closely related to *Trypanosoma brucei brucei* and is found mainly East Africa, principally Tanzania and Uganda (Simarro *et al*., 2008). In addition to infecting humans, it can also infect a range of wild game animals. Consequently, *Trypanosoma brucei rhodesiense* is a potentially zoonotic disease with numerous reservoirs of infection. In humans, *Rhodesiense* Human African Trypanosomiasis develops rapidly and can prove fatal within a matter of weeks or months of infection although in some geographical regions a less severe disease occurs (Ormerod, 1967).

Trypanosoma brucei gambiense is the principal cause (~90% of cases) of Human African Trypanosomiasis. *Gambiense* Human African Trypanosomiasis usually follows a chronic course over a period of years and severe nervous system impairment does not occur until the late stages of the disease. It is found mainly in West and Central Africa with the majority of cases in the Democratic Republic of Congo, Angola, and Sudan and significant numbers of new cases per year (more than 100 but less than 1000) in Central African Republic, Congo, Chad, and Uganda (Simarro *et al*., 2008). Although *Trypanosoma brucei gambiense* can infect a variety of wild animals, the importance of zoonotic transmission in the epidemiology of *Gambiense* Human African Trypanosomiasis is not certain (Njiokou *et al*., 2006).

Within the mammal host, the trypomastigotes of *Trypanosoma brucei* exhibit a variety of morphological forms although they all have a prominent undulating membrane and multiply by longitudinal fission (Figure 2.9). The slender forms are 25–35 μm in length, have a pointed posterior end and a long free flagellum. The mitochondrion of the slender form is poorly developed, has few cristae and lacks a functional cytochrome chain and tricarboxylic acid cycle (i.e. TCA or Krebs' cycle). Stumpy forms are about 15 μm long, have a broad, blunt posterior end and lack a free flagellum. The mitochondrion of the stumpy form is more developed than in the slender form and has many cristae but the cytochrome chain is still absent and the TCA cycle is incomplete. Intermediate forms averaging 23 μm in length can also be found which have a blunt posterior end and a free flagellum. All three forms can be found at the same time although usually one of them predominates at any single time interval.

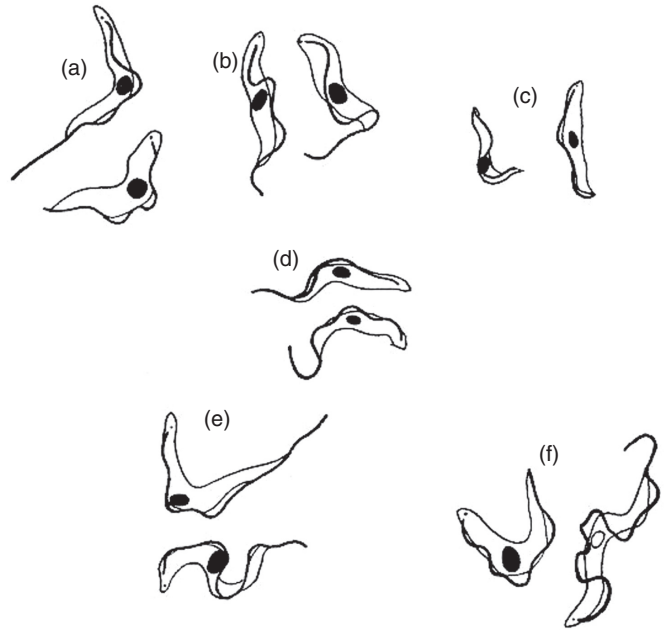


Figure 2.9 Diagrammatic representation of some trypanosome species. All are drawn to the same magnification. (a) = *Trypanosoma brucei*; (b) = *Trypanosoma vivax*; (c) = *Trypanosoma congolense*; (d) = *Trypanosoma equinum*; (e) = *Trypanosoma equiperdum*; (f) = *Trypanosoma evansi*. Note the small size and lack of a free flagellum in *Trypanosoma congolense*. Source: Cameron, 1934

Box 2.9 Genomic regulation in trypanosomes

The genome of *Trypanosoma brucei* has been sequenced and key features are discussed by Berriman *et al*. (2005) and Jackson *et al*. (2010). It is becoming increasingly apparent that in most eukaryotic organisms the regulatory processes that take place after the conversion of DNA into RNA are of greater complexity and importance than transcription itself. In trypanosomes this has been taken to its limits and genome regulation is undertaken almost entirely at the post-transcriptional level (Walrad *et al*., 2009). The formation of proteins in trypanosomes is not regulated by the rate at which mRNA is synthesised but occurs through factors that control the stability of the mRNA molecules (i.e. alter their half-life and hence concentration) and the rate at which mRNA is translated into protein. Much of this post-transcriptional regulation is performed by RNA binding proteins and adaptions to environmental change such as the movement between vector and mammalian host are accomplished through rapid changes in the half-life of mRNA molecules and translational control (D'Orso *et al*., 2003).

Once they have become established within their vertebrate host, the trypanosomes become rapidly disseminated throughout the body via the blood and lymphatic system. Unlike *Trypanosoma cruzi* and *Leishmania*, *Trypanosoma brucei* remains an extracellular parasite and never invades cells in

its vertebrate host. It does, however, invade most of the organs of the body by colonising the intercellular spaces (cf. *Trypanosoma congolense* which tends to remain within the circulatory system). In humans, *Trypanosoma brucei gambiense* crosses the blood–brain barrier and colonises the intercellular spaces in the brain. In so doing it causes the classic symptoms of Human African Trypanosomiasis (sleeping sickness). By contrast, *Trypanosoma brucei rhodesiense* does not usually colonise the nervous system in humans though this may be at least partly because the patient dies before this can happen.

In addition to the species of trypanosome, the development of Human African Trypanosomiasis also depends upon the genetic strain of the parasite as well as host health and genetic factors (MacLean *et al*., 2007). In the case of *Gambiense* Human African Trypanosomiasis, a red sore develops at the site of the tsetse fly bite and over the subsequent weeks or months the patient develops a fever, their lymph glands swell, and they suffer from aches, pains, and headaches. This is often referred to as stage 1 Human African Trypanosomiasis. These symptoms are non-specific and the disease often remains undiagnosed. In the absence of effective treatment the disease develops remorselessly to stage 2 Human African Trypanosomiasis in which the symptoms become severe with prolonged fevers, weight loss, anaemia and damage to the central nervous system.

Following ingestion by a tsetse fly, the parasites differentiate into procyclic trypomastigotes within the midgut region. This change is mainly stimulated by the drop in temperature ($\sim$10°C) that the parasites experience when they move from the warm mammalian bloodstream to the much cooler insect gut. There are also significant changes in the parasite's metabolism as they have moved from a hot environment in which glucose is plentiful to a cooler one in which glucose is in much lower concentration. The predominant blood sugar in insects is usually the disaccharide trehalose rather than the monosaccharide glucose, and tsetse flies are relatively unusual among insects in using the amino acid proline as the principal flight fuel. Consequently, in mammals the trypanosome mitochondrion is poorly developed since the parasite derives all its ATP from glycolysis, using the abundantly available glucose obtained from its host and most of the trypanosome's glycolytic enzymes are located within their glycosomes. The process of glycolysis is remarkably inefficient (the metabolism of one mole of glucose yields only two moles of ATP), and the pathway ceases at pyruvate which is excreted from the cell. By contrast, in tsetse flies the trypanosome mitochondrion is a much bigger organelle and has well-developed cristae as oxidative catabolism becomes more important as a source of ATP (Sharma *et al*., 2009).

Having successfully transformed into procyclic trypomastigotes, the parasites reproduce by longitudinal fission. Interestingly, the procyclic forms can exhibit apoptosis – a phenomenon that is normally associated with metazoan animals – but its function is not fully understood (Figarella *et al*., 2008). The promastigotes penetrate the fly's peritrophic membrane after which they migrate forwards to the proventriculus (Vickerman *et al*., 1988). At this point they cease dividing and transform into mesocyclic trypomastigotes and these penetrate back through the peritrophic membrane and make their way further forwards to the salivary glands. Once this point is reached, the parasites transform into epimastigotes. The epimastigotes attach themselves by their flagellum to the epithelial cells lining the tsetse fly salivary gland, multiply by longitudinal fission and then transform into non-dividing metacyclic trypomastigotes. Some form of mating involving meiosis often takes place during the epimastigote stage but it is not obligatory and the extent that it occurs varies between populations (Tait *et al*., 2007). This has considerable relevance for the transfer of genetic traits such as drug resistance between trypanosome lineages. It is the metacyclic trypomastigote stage which is infective to susceptible mammalian hosts and these trypanosomes express a specific subset of genes coding for variant surface glycoproteins (VSGs) which will help protect

them from the mammalian immune system after they are injected by the tsetse fly when it takes its next blood meal.The life cycle within the tsetse flies therefore involves a complex sequence of migrations and transformations and typically takes about 3–5 weeks. Consequently, effective transmission is heavily affected by the lifespan of the tsetse fly. Male tsetse flies usually only live for about 2–3 weeks in the wild and, while female flies can survive for up to 4 months, most die within 20–40 days (Krinsky, 2002).

Box 2.10 How trypanosomes alter tsetse fly physiology to facilitate transmission

Despite all the factors that can prevent the establishment of trypanosomes in the tsetse fly, provided the fly is susceptible to infection, the ingestion of even a single trypanosome in the blood meal is sufficient to infect it and thereby ensure it becomes a vector (Maudlin and Welburn, 1989). Once infected, a tsetse fly remains infected for the rest of its life. Unlike sandflies infected with *Leishmania*, tsetse flies do not appear to be unduly harmed by the trypanosome parasites (Moloo and Kutuza, 1985). The trypanosomes do, however, alter the protein composition and antihaemostatic properties of the tsetse fly saliva. This reduces the tsetse fly saliva's capacity to prevent coagulation of the blood and vasoconstriction and the consequence of this is that the flies spend more time probing for a suitable blood vessel. This may either provide more time for parasite transmission to take place or encourage the tsetse fly to move more frequently between hosts. The mechanisms by which the parasites alter the composition of the tsetse fly saliva are not known but it is possible that they trigger a stress response that decreases the expression of certain genes (Van Den Abbeele *et al*., 2010).

Trypanosoma congolense Measuring only 9–18 μm in length, *Trypanosoma congolense* is the smallest of the African trypanosomes (Figure 2.9). It is monomorphic, though short and long strains have been described. One of its characteristic features is the absence of a free flagellum though the cell tapers finely at the anterior end so it is possible to mistakenly believe one is present. The undulating membrane is not pronounced but this, together with the absence of a free flagellum, does not restrict its ability to move, although authors differ in whether its activity should be described as active or sluggish. The posterior end is blunt and the kinetoplast marginal.

Within the blood and lymphatic system of the mammal host, the trypomastigotes of *Trypanosoma congolense* multiply by dividing by longitudinal fission. Transmission is brought about by a range of tsetse fly species (e.g. *Glossina morsitans*) with different species being of particular importance as vectors in different areas. Following ingestion by a tsetse fly, the parasites differentiate into procyclic trypomastigotes and undergo a similar migration pattern a sequence of transformations to *Trypanosoma brucei*. However, the tsetse fly salivary glands are not invaded and the epimastigotes attach themselves to the walls of the proboscis and once they have transformed into the metacyclic stage the parasites migrate to the hypopharynx region (Vickerman *et al*., 1988). As in *Trypanosoma brucei*, a form of mating occurs in some strains of *Trypanosoma congolense* (Morrison *et al*., 2009).

Trypanosoma congolense is found throughout Southern, East and West Africa and infects a range of domestic mammals (e.g. cattle, sheep, horses, pigs, dogs) and wild game (e.g. antelopes,

warthogs). Humans are usually not thought to be at risk from this parasite although Truc *et al*. (1998) report a case of a mixed infection of *Trypanosoma brucei* and *Trypanosoma congolense* in a woman with advanced Human African Trypanosomiasis. This, of course, does not mean that *Trypanosoma congolense* was responsible for the woman's disease but it does indicate that human infections can occur. In addition, although *Trypanosoma congolense* is normally killed by the trypanolytic activity of normal human serum, some strains are resistant to it (Van Xong *et al*., 2002).

Trypanosoma congolense is of primary concern for its effect on domestic cattle and it is a major cause of economic loss to cattle farmers throughout the affected regions (Kristjanson *et al*., 1999; Shaw, 2004). Other domestic animals and wild game tend to be less harmed. In susceptible cattle, *Trypanosoma congolense* causes similar symptoms to *Trypanosoma brucei* and infected animals are also said to suffer from 'nagana'. The disease may manifest itself in acute, chronic forms, and mild forms. In the acute form, the disease is marked by anaemia, emaciation and a high parasitaemia in the peripheral circulation. The liver, lymph nodes and spleen become enlarged, haemorrhages occur in the heart muscle and kidneys, and the infected animal may die within 10 weeks of becoming infected. In the chronic form the symptoms are less severe and it may be difficult to find the parasites in the blood. There is enlargement of the lymph nodes and liver and signs of degeneration in the kidney but the infected animal can recover after about a year. In mild infections it may not be obvious that the animal is actually infected with the parasite. Pathology that results from *Trypanosoma congolense* differs from that caused by *Trypanosoma brucei* in that the parasites remain within the circulatory system and the central nervous system is not affected. Anaemia is the most characteristic feature of *Trypanosoma congolense* infection and results from the destruction of red blood cells in the liver and spleen although other mechanisms (e.g. inflammatory processes) may also be involved (Noyes *et al*., 2009). Indiginous breeds of cattle, such as N'Dama, are not actually resistant to infection with *Trypanosoma congolense* but have a genetic ability to limit the development of anaemia (Naessens, 2006).

Trypanosoma evansi *Trypanosoma evansi* is monomorphic, 14–33 μm in length and 1.5–2.2 μm in width and is morphologically indistinguishable from *Trypanosoma equinum*, *Trypanosoma equiperdum* and the slender forms of *Trypanosoma brucei* (Figure 2.9). Molecular evidence suggests that it may be a variant of *Trypanosoma brucei* and should be considered a sub-species with the name *Trypanosoma brucei evansi* (Lai *et al*., 2008). In addition, it is now thought that *Trypanosoma evansi* and *Trypanosoma equinum* are actually synonymous and that *Trypanosoma equinum* should no longer be considered as a separate species or sub-species. *Trypanosoma equinum* lacks a kinetoplast (i.e. it is 'akinetoplastic') and this was one of the reasons for separating it from *Trypanosoma evansi* in which both kinetoplastic and dyskinetoplastic forms are found.

Trypanosoma evansi has an extremely wide distribution being found in Africa, Asia, and Central and South America. It is spread mechanically by biting flies such as tabanids and stable flies and reproduction takes place by longitudinal binary fission within the vertebrate host. It is particularly pathogenic in horses but it also causes considerable morbidity and mortality in camels, cattle, pigs, dogs, and cats and has also been found in a range of wild animals such as deer, tapir and capybara.

The disease caused by *Trypanosoma evansi* infection is commonly known as '*surra*' – which is the Hindi word for 'rotten' or 'emaciated' although other terms are also used such as

el debab in many Arabic-speaking countries. It is the cause of death of many thousands of animals every year and a great deal of morbidity (Brun *et al*., 1998). Horses are particularly susceptible to infections and there are claims that the inoculation of even a single parasite can prove fatal (http://www.fao.org/docrep/006/x0413e/X0413E06.htm). The disease is often acute in horses and the animal dies within a few weeks to 2 months. Chronic infections lasting over a year may also occur but also often end with the death of the horse. The disease is characterised by the development of anaemia, emaciation, and oedema that may vary from urticarial plaques on the neck and flanks to widespread swelling of the legs and lower body. The plaques may subsequently become necrotic and bleed while encephalitis and demyelination can occur in the brain and spinal chord that result in staggering and paralysis. Affected animals may also express abnormal behaviour such as hyperexcitability, head tilting, and circling (Rodrigues *et al*., 2009).

Surra is an important cause of morbidity and mortality in camels in which it tends to be a chronic wasting disease that is accompanied by fever, anorexia and the development of oedema. Cattle can be severely affected when exposed to the parasite for the first time but in endemic areas the disease tends to be sub-clinical and reduce productivity rather than cause major morbidity and and mortality. Dogs are susceptible to an acute and rapidly fatal form of the disease that causes nervous signs which may be misdiagnosed as rabies.

Trypanosoma equinum *Trypanosoma equinum* (Figure 2.9) is now considered to be synonymous with *Trypanosoma evansi* but it is dealt with separately here because the literature tends to give different accounts of the host range and pathogenic effects. *Trypanosoma equinum* was only ever described from various South American countries where it causes a disease commonly known as '*mal de caderas*' in Spanish and '*mal de cadeiras*' in Portuguese – which translates as 'illness of the hips'. *Trypanosoma equinum* is principally a parasite of horses. Other equids and a variety of domestic and wild animals (e.g. capybara) can also be infected, but they do not tend to suffer severe disease. The parasite is spread mechanically by biting flies such as tabanids and is a particular problem in swampy areas where these flies are most numerous.

Unlike *surra*, *mal de caderas* is normally described as a chronic disease in horses which develops over a period of months but usually has a fatal outcome. Symptoms begin with a fever and loss of weight and then the hindquarters become progressively weaker (hence the name '*mal de caderas*') resulting in staggering and then an inability to walk. Horses can also exhibit conjunctivitis, the eyelids become oedematous (filled with fluid) and transient plaques form on the neck and flanks. In addition, the kidneys, brain and spinal chord show signs of inflammation and necrosis.

Trypanosoma equiperdum *Trypanosoma equiperdum* is monomorphic and morphologically indistinguishable from *Trypanosoma equinum* (Figure 2.9). It exhibits dyskinetoplastidy – that is it retains only fragments of kinetoplastid DNA. It is found in a range of equids although the domestic horse (*Equus cabalus*) is more severely affected than others such as asses, donkeys, etc. Dogs can be infected by some strains and it is possible to establish laboratory cultures in rats and mice. It is sexually transmitted and causes a condition called 'dourine' – a word that is derived from the Arabic for 'unclean'. The disease has been spread around the world with the use of horses in agriculture and warfare but nowadays has a more restricted distribution in parts of Africa, Asia, southern Europe and South America following breeding programmes aimed at eliminating infected horses.

Box 2.11 Is *Trypanosoma equiperdum* a distinct species?

Claes *et al*. (2005) propose that the various strains of *Trypanosoma equiperdum* are in fact varieties of *Trypanosoma brucei* and *Trypanosoma evansi*. The disease known as dourine would therefore need to be considered to be a host-immune reaction to trypanosome infection rather than a response to a specific species of parasite. Their suggestion was initially criticised (Li *et al*., 2006) but Lai *et al*. (2008) have subsequently presented molecular evidence that both *Trypanosoma equiperdum* and *Trypanosoma evansi* should be considered strains of *Trypanosoma brucei*. They therefore suggested that two new sub-species be recognised: *Trypanosoma brucei evansi* and *Trypanosoma brucei equiperdum*.

Trypanosoma equiperdum is primarily a tissue parasite and not usually present in the circulating blood but it can often be found in smears taken from the genitalia or plaques. Diagnosis has traditionally been based on serological complement fixation tests and in some countries where dourine occurs, they are mandatory. However, such tests will not reliably distinguish *Trypanosoma equiperdum* from other trypanosomes such as *Trypanosoma evansi* and *Trypanosoma brucei*, which is hardly surprising if they are all closely related sub-species. Consequently, there is a move to using PCR-based tests that are both rapid and more reliable (Zablotskij *et al*., 2003) and commercial versions of these are now available.

Dourine typically manifests itself in three stages. The first stage begins with swelling of the genitalia, patchy depigmentation of the penis and vulva and in mares there is a vaginal discharge. The horse may also exhibit a slight fever and loss of appetite. After about a month the second stage of the disease begins with the development of circular fluid-filled (oedematous) plaques 2.5–10 cm in diameter underneath the skin. They usually form on the flanks although any part of the body can be affected. The plaques may last a few hours or days after which they disappear and then reappear again later. This stage is sometimes called the ‘urticarial stage’ because of the rash-like swellings. The plaques are not always formed but when they are, they are considered to be a reliable indicator of the nature of the disease. The third stage of the disease is marked by the onset of paralysis. Often this begins in the nose and face and then spreads to affect the rest of the body and can result in complete paralysis affecting all the limbs. The course of the disease may take as long as two years and mortality can be as high as 70%.

Trypanosoma cruzi *Trypanosoma cruzi* is the causative agent of Chagas’ disease – a potentially fatal infection that afflicts around 11 million people in the New World from Argentina to the southern United States of America. In addition, owing to migration, the disease can now be found in many other parts of the world with important foci in Canada, North America, Europe, and Australia (Coura and Viñas, 2010).The name of the disease is derived from that of Carlos Chagas who first described the parasites in 1910. Chagas initially believed that the parasites underwent schizogony within their mammalian host and hence named them *Schizotrypanum cruzi* – ‘*cruzi*’ being derived from the famous Oswaldo Cruz Institute in Brazil. It has since become apparent that the parasite’s life cycle does not include schizogony and most workers now refer to it as *Trypanosoma cruzi*.

In addition to humans, *Trypanosoma cruzi* infects a wide range of domestic and wild mammals including dogs, cats, bats, rats and armadillos. This makes control of the disease difficult because there are so many potential reservoirs of infection. The parasite is transmitted by blood-feeding reduviid bugs (Order Hemiptera, Family Reduviidae, Sub-Family Triatominae) that from their appearance are sometimes called cone-nosed bugs. Over 130 species of reduviid bug are capable of acting as vectors although it is only those that live in close proximity to humans that are medically important. Although Chagas' disease now occurs in many countries around the globe, its potential for transmission outside Central and South America is limited by the availability of suitable vectors. There is therefore concern that vector species could be transported by air or sea (the bugs can survive for weeks without feeding) to other countries with luggage or farm produce. A number of other blood-feeding invertebrates can also be infected including the bed bug (*Cimex lectularius*; Order Hemiptera, Family Cimicidae), the argasid tick *Ornithodorus moubata*, and the medicinal leech *Hirudo medicinalis* but these are not thought to be important in the transmission of the disease to humans.

The life cycle of *Trypanosoma cruzi* differs from the other trypanosome species discussed so far in that it involves stercorarial transmission from the invertebrate vector to the vertebrate host. Reduviid bugs often defecate during or immediately after feeding and the infective metacyclic stage trypanosomes are voided with the bug's faeces. The bugs feed at night and are sometimes referred to as 'kissing bugs' because they often bite their victims around the face. On waking the natural reaction is to rub or scratch the site where one has been bitten and this can result in the parasite-infected faeces being rubbed into the wound site. However, it is thought that most infections result from the faeces or parasites contaminating the fingers and from there being transferred to the eyes and mucous membranes where they are able to penetrate the skin more easily. Human infections have also arisen from organ transplants and contaminated blood being used during transfusions (Coura and Viñas, 2010). In endemic areas, where the likelihood of acquiring *Trypanosoma cruzi* infection from blood transfusion is high, units of blood from donors are sometimes treated with gentian violet. This does kill the parasites, but as the blood infuses through the tissues of the recipient, it also has the unfortunate effect of turning the patient purple temporarily. Reservoir animals can become infected by consuming bugs containing the parasites. Congenital infection via the placenta is possible and can result in spontaneous abortion or serious disease of the infant at birth.

Box 2.12 Invasion of vertebrate host cells by *Trypanosoma cruzi*

Once *Trypanosoma cruzi* enters a suitable mammalian host, the trypomastigotes invade both phagocytic and non-phagocytic cells. Indeed, they are apparently capable of invading all nucleated cells although most of the pathology arises from the invasion of smooth, cardiac, and skeletal muscle cells, nerve cells, and cells comprising organs such as the liver, spleen, and lymphatics. Within the host cell, the parasites become enclosed within a parasitophorous vacuole. The precise mechanism by which the trypomastigotes enter the host cell varies between cell types and may involve active invasion by the parasite and passive internalisation through endocytosis/phagocytosis. Within the parasitophorous vacuole the parasites transform into the amastigote stage (1.5–4 μm in size) and multiply by binary fission. When large numbers

of amastigotes accumulate within a cell, the structure is sometimes referred to as a pseudocyst. Eventually, the parasites kill their host cell and they transform back to trypomastigotes which then invade new cells and repeat the process. This distinguishes *Trypanosoma cruzi* from *Leishmania* in which the parasites are principally found within phagocytic cells and they are always in the amastigote stage within the vertebrate host. The trypomastigotes of *Trypanosoma cruzi* are not themselves able to reproduce, although large numbers of them may be seen in the bloodstream, especially during the early stages of infection. Broad and slender morphotypes of the trypomastigotes can be found, although in both cases these are relatively small compared to those of some other trypanosomes, being around 16.3–21.8 μm in length (including the flagellum) and have a characteristic curved 'C' shape. By contrast, the kinetoplast is exceptionally large and the cell membrane sometimes bulges around it.

The reduviid bug vector becomes infected when it feeds on blood containing the trypomastigote stage. These bugs can take in large blood meals several times their own body weight and this increases their chances of ingesting a trypomastigote. In some populations, over half the bugs are infected with *Trypanosoma cruzi*. In addition to feeding on blood, reduviid bugs will also attack one another and can become infected by ingesting parasites from one of their brethren. However, this is probably not a common means of infection. When the parasites reach the posterior region of the insect midgut, they transform into short epimastigotes and these divide by longitudinal fission to form long epimastigotes. Ultimately, the epimastigotes move to the rectum where they transform into the infective short metacyclic trypomastigote stage. The gut of infected bugs can contain huge numbers of parasites but unlike some of the other trypanosome species, *Trypanosoma cruzi* does not appear to harm its invertebrate host.

Box 2.13 Population structure of *Trypanosoma cruzi*

Trypanosoma cruzi reproduces asexually in both its vertebrate and invertebrate host and has often been considered to have an essentially clonal population structure. However, it is now recognised that genetic hybridisation between parasites takes place (Sturm and Campbell, 2010) and this has implications for studies on the epidemiology of the disease (Macedo *et al*., 2004). Up until recently, molecular and isoenzyme research indicated the existence of two sub-specific groups: *Trypanosoma cruzi* I and *Trypanosoma cruzi* II. The *Trypanosoma cruzi* I strains were usually associated with sylvatic transmission (i.e. in the forests and countryside) and normally infected marsupials and other wild animals. Human infections with *Trypanosoma cruzi* I strains were relatively rare. By contrast, *Trypanosoma cruzi* II, was divided into subgroups a, b, c, d, and e, and was more commonly associated with transmission in human habitations and was the main cause of human infections. However, there were insufficient differences between the sub-specific groups to consider them as separate species. It has now been recommended that *Trypanosoma cruzi* is divided into six separate groups that are designated TcI, TcII, TcIII, TcIV, TcV, and TcVI and further details of this can be found in Zingales *et al*. (2009).

The development of Chagas' disease varies considerably and there are marked differences between individuals and geographic localities. This suggests that genetic differences on the parts of both the parasite and the host are important in the way the disease manifests itself. Two phases of the disease are recognised: an acute phase and a chronic phase. The initial invasion of parasites may give rise to an acute infection or symptoms so general that it may not be obvious that infection has occurred. The acute phase is characterised by high levels of parasitaemia and in a small percentage of cases can prove fatal. There is often an initial localised inflammatory response with swelling of the nearest lymph node. Where infection occurs via the insect bite wound, a raised red nodule develops that is called a chagoma. If the infection occurs via the eye, the eyelid and preauricular lymph node swell so that eye becomes closed – this is known as Romaña's sign. As the acute phase of the disease progresses, the parasites can be found in all organs of the body but it is their tendency to localise within and destroy heart muscle and cardiac ganglion cells that results in the most severe consequences. The pathological mechanisms are not fully understood, but it is possible that the damage to cardiac muscle during chronic Chagas disease may have an autoimmune basis. If the brain tissues are invaded, meningoencephalitis can develop with similar potentially fatal or long-term damage as a result. The patient often develops a fever and their liver and spleen become enlarged; they may also start to suffer from diarrhoea and exhibit evidence of respiratory infection. The acute phase is most commonly seen in children younger than 5 years old but unless their heart or nervous tissues are severely infected, most of them recover even if they do not receive adequate medical treatment (Prata, 2001).

2.8 Phylum Chlorophyta

Commonly known as the green algae, the Chlorophyta is a paraphyletic phylum, that is, it contains some but not all of the species that have descended from a common ancestor. Because most species within the phylum contain chloroplasts, they are often referred to as plants. Indeed, the chloroplasts found in the Chlorophyta are not only similar in appearance to those found in multicellular plants such as wheat and roses, they also have a very similar physiology and contain both chlorophyll a and chlorophyll b. However, the Chlorophyta are single-celled organisms (although some are colonial) and are classed within the kingdom Protista rather than Plantae, although some workers consider them to belong among the kingdom Plantae. A number of species of green algae are in close symbiotic relationships with invertebrates. For example, the alga *Chlorella* spp. lives in association with the cnidarian *Hydra viridis* and some sea slugs extract the chloroplasts from their algal food and utilise them as photosynthetic organelles within their own cells (Rumpho *et al*., 2000). Although a variety of algae grow on the body surface of sloths and certain lizards there are few reports of them becoming intracellular symbionts of vertebrates. An instance where it does occur is between the alga *Oophila amblyostomatis* and amphibian egg masses (Pinder and Friet, 1994). This alga has also been observed living within the embryos of the spotted salamander *Amblyostoma maculatum* and may be maternally transmitted (Petherick, 2010).

Some species of algae have lost their chloroplasts during the course of evolution and among these are members of the genus *Prototheca* which includes species that are parasites of humans and other mammals (Lass-Flörl and Mayr, 2007) and the genus *Helicosporidium* that are parasitic in insects (Pombert and Keeling, 2010).

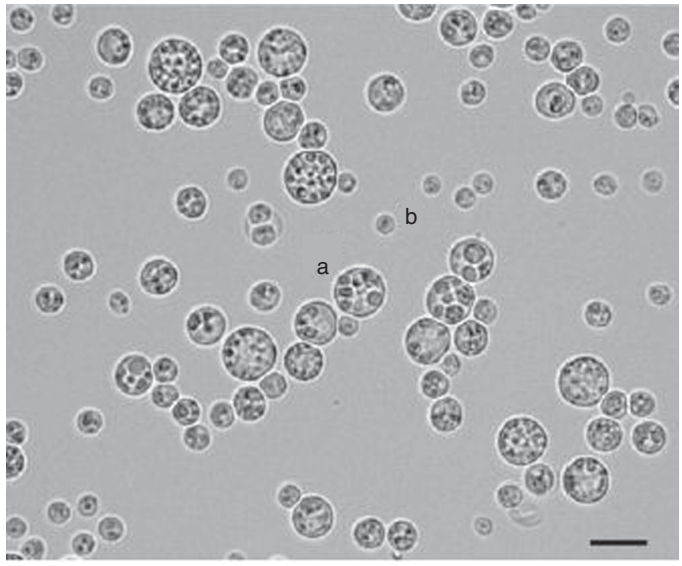


Figure 2.10 *Prototheca cutis* cultured from the infection illustrated in Plate 3. Abbreviations are 'a' = sporangia and 'b' = sporangiospores. Source: Satoh *et al*., 2010. (Satoh, K. *et al*. (2010) Prototheca cutis sp. Nov., a newly discovered pathogen of protothecosis isolated from inflamed human skin. International Journal of Systematic and Evolutionary Microbiology 60, 1236–1240. Reproduced by permission of Society for General Microbiology)

2.8.1 Genus *Prototheca*

Members of this genus are closely related to the well-known alga *Chlorella*, but they have lost their chloroplasts and most species survive as saprophytes feeding on dead organic matter in a similar manner to free-living fungi. They are found throughout the world and can be isolated from the soil, slime, sludge, faeces, marine and freshwater, swimming pools, and virtually anywhere which has high organic matter content (Pore *et al*., 1983). Some species are facultative parasites and can infect a range of animals, with consequences that range from mild disease to fatalities. Human infections are often due to *Prototheca wickerhamii* and *Prototheca zopfii* and are usually associated with patients who are immuno-compromised through disease (e.g. HIV infection) or medical treatment (e.g. chemotherapy for cancer). A new species, *Prototheca cutis* (Figure 2.10 and see Colour Plate 3), has been described from a patient in Japan (Satoh *et al*., 2010) and further species will probably be described in the future now that the genus is receiving more attention. The algae gain entry to the body via the skin – usually through an existing wound – and cause a localised cutaneous infection. This is often manifested as dermatitis with the formation of pustules, ulcers, and erythematous plaques. Occasionally, the infection becomes disseminated throughout the body and can cause a potentially fatal meningitis (Kaminski *et al*., 1992).

Prototheca wickerhamii, *Prototheca zopfi*, and *Prototheca blaschkeae* are responsible for sporadic cases of mastitis in cows in many parts of the world. Protothecosis is not one of the common causes of mastitis and therefore it is often not diagnosed because vets do not think to test for it. This can cause problems because the algae do not respond to normal treatments for mastitis and

therefore the course of the disease can be prolonged and in some dairy herds severe economic losses have occurred (Thompson *et al*., 2009).

2.9 Kingdom fungi

Some estimates suggest that there may be over a million species of fungi although less than 10% of these have so far been described. Unlike plants, fungi are heterotrophs, that is, they cannot make their own food and have to gain their nutrients by breaking down existing organic matter. The majority of fungi gain these nutrients by acting as saprophytes, that is, they break down dead organic matter. In addition, many species are in symbiotic relationships with plants and invertebrates while some are parasites of other fungi, plants, and invertebrate and vertebrate animals. Some of these parasitic species are important in human and veterinary medicine as well as wildlife ecology. For example, *Pneumocystis* (which was once thought to be a protozoan) is a major cause of morbidity and mortality in AIDS patients (Morris, 2008), the skin disease 'ringworm' in cattle is caused not by a helminth but fungi such as *Trichophyton verrucosum* (Silver *et al*., 2008), and chytridiomycete fungi are responsible for widespread and catastrophic levels of mortality among amphibians in many parts of the world (Briggs *et al*., 2010; Vredenburg *et al*., 2010). However, only the Microsporidia will be covered here.

2.9.1 Microsporidia

The Microsporidia are a cosmopolitan group of obligate intracellular parasites that infect a wide variety of invertebrate and vertebrate animals. Over 1200 species have been described but the majority of these are parasites of invertebrates and fish. Several species are of medical, veterinary, and commercial importance. For example, *Nosema apis* and *Nosema ceranae* are major causes of disease in honeybees while several species, such as *Nosema locustae* (in locusts) and *Nosema algerae* (in mosquitoes) have been investigated for their potential as biological control agents. Fourteen species have so far been found to infect humans although *Enterocytozoon bieneusi* is responsible for the majority of clinical cases. *Enterocytozoon hellem* and *Encephalitozoon intestinalis* cause occasional infections and the other microsporidia species are rarely found in humans.

Although they were once designated as protozoa, the molecular evidence now indicates that microsporidia are actually fungi (Keeling *et al*., 2000; Keeling and Fast, 2002). Precisely where they fit within the taxonomy of fungi is uncertain although they show some resemblance to the zygomycetes (Corradi and Keeling, 2009; Dyer, 2008). The zygomycetes also have relevance to parasitologists since they include genera such as *Pilobolus* that helps to spread the infective nematode larvae of the lungworm *Dictyocaulus viviparus* (Doncaster, 1981) and *Entomophthora* that parasitise insects and have been investigated for their potential to control vector insects. As with *Entamoeba histolytica*, the microsporidia were once thought to have split off from other organisms at an early stage in their evolution because they did not appear to contain mitochondria. However, they too have been found to contain genes that have mitochondrial functions and putative relict mitochondria called 'mitosomes' have been identified in *Encephalitozoon cuniculi* and *Trachipleistophora hominis* (Goldberg *et al*., 2008; Williams *et al*., 2002). They also have one

of the smallest genome sizes of all the eukaryotic organisms: in *Encephalitozoon intestinalis*, the genome is only 2.3 megabases (Mb) in size although in *Glugea atherinae*, a fish parasite, it is almost ten times larger at ~20Mb (Williams *et al*., 2008a).

The spore is the only microsporidian life cycle stage capable of surviving in the external environment. Immediately above the spore's plasma membrane are two protective layers, the first of these is the 'endospore' which contains chitin and is electron luscent when viewed with a transmission electron microscope and above this is the 'exospore' that contains glycoprotein and is electron dense so it appears dark in transmission electron micrographs. The spore walls must provide excellent protection, as in some species they can remain infective for over a year.

Microsporidia are normally transmitted horizontally as a result of the host coming into contact with the infective spores; humans usually become infected by ingesting or breathing them in. Vertical transmission has been described in those species infecting other animals as a result of crossing the placenta in mammals or infecting the eggs while they are still in the ovary in invertebrates. Transovarial transmission is common among endosymbiotic bacteria such as *Wolbachia* but very rare among protozoan parasites (Dunn *et al*., 2001). Like *Wolbachia*, some of the microsporidia species that are transmitted transovarially affect the sex ratios of their hosts (Terry *et al*., 2004).

The spores, like the other life cycle stages, usually contain either a single nucleus (monokaryon) or two adjacent nuclei (diplokaryon) that function as a single unit. The spores also contain a posterior vacuole and a structure called the 'polaroplast' that is thought to be derived from modified Golgi apparatus. In addition, there is a coiled polar tubule (polar filament) that is attached to the anterior end of the spore by an 'anchoring disc complex'. The polar tube is hollow and unique to the Microsporidia but in terms of appearance and function, it bears more than a passing resemblance to the nematocysts found in jellyfish (Cnidaria). When the spore receives the correct stimulation (presumably a combination of pH and chemical factors), the posterior vacuole and polaroplast absorb water and start to swell. Because the tough spore wall prevents it from expanding, the pressure within the spore starts to rise. Ultimately the spore wall is ruptured at the anterior end where the spore wall is thinnest and the polar tubule is shot out through the break as if from a harpoon gun. The polar tubule may be discharged with sufficient force to pierce the adjacent host cell or alternatively it may be subsequently ingested by receptor-mediated endocytosis (Bigliardi and Sacchi, 2001). At the same time, the pressure within the spore forces the nucleus and cytoplasm, now referred to as the 'sporoplasm' down the everting polar tubule and thence into the host cell. The spore is able to ensure its proximity to a suitable host cell by binding onto host-cell sulphated glycosaminoglycans (GAGs). In addition, the exospore and endospore both contain an adherence protein called 'endospore protein 1' (EnP1) (Southern *et al*., 2007). It is also possible that the whole spore may be ingested by the host cell but infection still results from the discharge of the sporoplasm into the host cell cytoplasm (Couzinet *et al*., 2000).

Once within the host cell cytoplasm the sporoplasm differentiates into a stage called a 'meront' and undergoes a series of cycles of asexual reproduction called merogony in which numerous meronts are produced. In the case of *Entercytozoon* and *Nosema*, the meronts are in direct contact with the host cell cytoplasm while in *Encephalitozoon* they are localized within a membrane-bound parasitophorous vacuole of host cell origin. The meronts of both *Nosema* and *Encephalitozoon* divide by simple binary fission but those of *Enterocytozoon* have a more complex development which produces multinucleate cells. After a period of merogony, the parasites start to produce spores in a process called sporogony. The meront transforms into a sporont which produce sporoblasts that then mature into spores. The spores steadily accumulate in the host cell and

may eventually fill it. When the host cell membrane eventually ruptures, the spores are released and may infect an adjacent cell or be released into the environment.

In humans, microsporidia usually cause persistent or self-limiting infections of the gastrointestinal tract. However, they are an important cause of gastrointestinal pathology in AIDS patients (Cheesbrough, 2005). They can cause extensive damage to the mucosal surface of the intestine and symptoms therefore typically include diarrhoea, abdominal pain, malabsorption and wasting. There are also records of them invading the eye, muscles and causing systemic infections.

2.10 Kingdom plantae

Although plants are normally considered to be primary producers that provide for their needs by photosynthesis, there are a number of species that supplement this either partially or entirely by parasitising other plants (Press and Graves, 1995). In extreme cases, some of these species no longer produce chlorophyll. For example, the 'Indian pipe' or 'corpse plant' (*Monotropa uniflora*) obtains its energy from mycorrhizal fungi that are themselves in a symbiotic relationship with tree roots (Young *et al*., 2002). Plants do not, however, parasitise animals although some, such as the Venus fly trap (*Dionaea muscipula*) are carnivorous and obtain some of their nutrients by capturing invertebrates and digesting them.

Questions

1. Why is it necessary to distinguish between *Entamoeba histolytica* and *Entamoeba dispar*?
2. What is amoebic encephalitis? Name two species of parasite responsible for the condition.
3. Match the parasite with the vector. Each parasite and vector can only be used once.
   Parasite: *Theileria parva*, *Plasmodium vivax*, *Trypanosoma cruzi*, *Leishmania major*
   Vector: *Phlebotomus papatasi, Anopheles gambiae, Rhipicephalus appendiculatus, Triatoma infestans*
4. In which host species does *Neospora caninum* cause epidemic abortion?
5. Briefly describe the life cycle of *Plasmodium falciparum*.
6. Explain the importance of cats in the life cycle of *Toxoplasma gondii*.
7. Briefly explain what is meant by the term 'post-kala azar dermal leishmaniasis' and why identifying a person suffering from this condition is important for disease control.
8. Explain why *Trypanosma brucei gambiense* is limited to certain regions of Africa but *Trypanosoma evansi* is found in many parts of the world.
9. What is meant by the term stercorarial transmission?
10. Make a sketch to illustrate the difference between the amastigote, promastigote and trypomastigote forms of trypanosomes.

3

Helminth parasites

3.1 Introduction: invertebrate taxonomy

Invertebrates are multicellular (i.e. metazoan) animals that are united simply in their lack of a backbone rather than the presence of any commonly shared morphological features. Over the years various invertebrate phyla have been recognised and grouped together in many different arrangements. Molecular phylogeny can confirm some of these arrangements while questioning others, consequently, readers should not be surprised by the lack of consistency between texts. For the purposes of this book, we shall be following the arrangement suggested by Brusca and Brusca (2002) that is based on morphology and embryology. Under this scheme, animals are initially placed into one of two groups: the Parazoa and the Eumetazoa. The Parazoa are organisms that lack true tissues and this group contains only the phylum Porifera, better known as the sponges. All sponges are aquatic and they gain their nutrition through filter feeding and, in some cases, symbiosis with algae or bacteria. Sponges which belong to the family Clionidae bore into the shells of molluscs and penetrate the calcareous skeleton of corals and they have been held responsible for causing mass mortalities in commercial oyster beds. Other sponges will, in their turn, exploit the burrows and also grow over them. Sometimes this is referred to as an example of parasitism although this is debatable (Annandale, 1915; Le Cam and Viard, 2011). The Eumetazoa are those animals in which muscles, nerves, and other tissues are recognisable and this group contains the vast majority of animal phyla, i.e. all vertebrates and most invertebrates. The eumetazoans can be divided into two sub-groups – the diploblasts and the triploblasts – on the basis of their embryonic development. Diploblastic animals are those in which only two germ layers are formed during embryonic development. These layers are the outer *ectoderm* and the inner *endoderm*. There are only two phyla that exhibit this form of development, the Cnidaria (jellyfish, sea anemones, and hydra) and the Ctenophora (sea gooseberries). Virtually all members of these two phyla are free-living and the true taxonomic position of the best-known parasitic species (*Polypodium hydriforme*) is a source of ongoing debate (e.g. Evans *et al*., 2008). Triploblastic animals are those in which a third germ layer develops during embryonic development: this is the *mesoderm* and it forms between the outer ectoderm and the inner endoderm layers. The majority of invertebrate species and all vertebrate species are triploblastic organisms.

Triploblastic animals can be split into three broad categories on the basis of their internal morphology: acoelomates, pseudocoelomates, and coelomates. The acoelomates are those that lack a body space other than the gut (e.g. phylum Platyhelminthes: tapeworms, flukes). The pseudocoelomates are also known as the blastocoelomates and they are characterised by the presence of a blastocoel (pseudocoelom) between the gut and the body wall (e.g. phylum Nematoda:

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nematodes). A blastocoel is a body cavity that develops in most metazoan animals during embryonation but in the blastocoelomates it persists into adulthood. The coelomates are sometimes referred to as the 'eucoelomates' and are those in which a true coelom (body space surrounded by mesoderm) develops between the gut and the body wall (e.g. phylum Annelida [earthworms], phylum Arthropoda [scorpions, crabs, insects], phylum Vertebrata [fish, amphibians, reptiles, birds, mammals]). There are numerous examples of triploblastic invertebrate species that are parasites of other organisms as well many that act as the intermediate hosts or vectors of parasites. In this chapter we shall concentrate upon those that, although they are taxonomically distinct, are collectively known as the 'helminths' or 'parasitic worms'.

3.2 Phylum Platyhelminthes

Members of the phylum Platyhelminthes are commonly known as the 'flatworms' on the not unreasonable basis that they are dorso-ventrally-flattened and worm-like in appearance. They are acoelomate soft-bodied animals that are bilaterally symmetrical (i.e. their left side is the same as their right side) with an obvious head-end. Most platylhelminths have a mouth at the anterior end although in some free-living species the mouth is situated at or close to the centre of the body while in tapeworms the mouth is absent. Tapeworms also lack a gut although this is present in most other platyhelminth species and usually consists of a blind-ending sack or series of branching tubules. The lack of an anus means that waste material is egested through the mouth. Platyhelminths do, however, have a ramifying series of tubules that constitute their 'excretory system'. Flame cells (protonephridia) maintain the movement of fluid within the excretory system and the waste is removed through excretory pores. Because the excretory system is used to remove excess water and ions, it is sometimes referred to as the osmoregulatory system. Most platyhelminth species are hermaphrodites, containing both male and female reproductive organs although in a few species there are separate male and female sexes.

Until recently the phylum Platyhelminthes was divided into four classes: Turbellaria, Monogenea, Trematoda (Digenea), and Cestoda. Apart from the usual exceptions, this roughly translated into those that are free-living (Turbellaria), those that live as ectoparasites on a single host (Monogenea), those living as endoparasites whose life cycle includes two or more hosts but which always includes a mollusc as the first intermediate host (Trematoda), and the tapeworms (Cestoda). This arrangement has now been superseded (e.g. Philippe *et al*., 2007) but there is not yet a firm agreement as to the form the taxonomic hierarchy should take. For a more detailed discussion, readers should consult Baguñà and Riutort (2004) and Littlewood and Bray (2001). We shall therefore consider just two Classes: Class Trematoda and Class Cestoda (Cestoidea). These two classes have the advantage of containing the bulk of the platyhelminths of medical and veterinary importance and for which there is more or less agreement among taxonomists of their validity as taxonomic groupings.

3.3 Class Trematoda

The parasites belonging to the Class Trematoda are commonly known as the 'flukes': those living in the blood are therefore called 'blood flukes', those living in the gut are 'intestinal flukes', while those associated with the liver and gall bladder are called 'liver flukes'. The term 'fluke' has

been used in common language to describe the parasite we now know as *Fasciola hepatica* for hundreds of years. The word is probably derived from the Old English '*flōc*' that was used as a term for flatfish such as the 'flounder' (*Pleuronectes flesus*), to which the liver fluke *Fasciola hepatica* has a superficial resemblance. Before the advent of scientific taxonomy, it was a common belief that animals that shared a similar appearance or mode of locomotion were related.

The Class Trematoda is divided into two Subclasses: the Aspidobothria and the Digenea. The Aspidobothria are also known as the Aspidogastrea and the Aspidocotylea and are of biological interest because several species are apparently making the transition between a free-living and a parasitic lifestyle. Most species are parasitic in fish and turtles and they include species with fascinating life cycles but we are not able to discuss them further here. Further details can be found in Kearn (1998).

The majority of the trematodes (~24,000 species) are members of the Subclass Digenea and as adults these are all obligate parasites of vertebrates (Poulin and Morand, 2004). They have complex life cycles that include one or two intermediate hosts, the first of which is invariably a mollusc, in which asexual reproduction takes place (Table 3.1). Intriguingly, Hechinger *et al*. (2011) have demonstrated that in some trematode species the asexual stages developing in the snail intermediate host exhibit evidence of social organisation. After the initial invasion of the snail, the parasite reproduces asexually and some of the progeny develop as 'reproductives' while others form non-reproducing 'soldiers'. The soldiers are smaller than the reproductives but they are more active and have larger mouthparts. The soldiers congregate in regions where miracidia (the stage that invades the snail host) are likely to penetrate and they attack the asexual stages of other trematodes of both the same species as themselves and those of other species. This

Table 3.1 Life cycle stages of digenetic trematodes

Life cycle stage	Description	Reproduction
Adult	Lives in the definitive host. Usually hermaphrodites but sexes separate in some species. Has mouth and gut though may also absorb nutrients across the body surface. Motile. Produces eggs	Yes. Usually sexual reproduction but may be parthenogenic
Egg	Contains the miracidium. May hatch in the environment or within gut of the first intermediate host	No
Miracidium	Infective stage. Covered in cilia, motile, invades the first intermediate host	No
Sporocyst	Lacks a mouth and gut; absorbs nutrients across body wall. Reproduces asexually within first intermediate host	Yes. Asexual reproduction to form daughter sporocysts or rediae
Redia	Has a mouth and gut. Motile. Reproduces asexually within first intermediate host. Evidence of caste system in some species	Yes. Asexual reproduction to form daughter rediae or cercariae
Cercaria	Infective stage. Usually motile with a propulsive 'tail'. Often leaves first intermediate host and invades second intermediate host or definitive host	No
Metacercaria	Infective stage. Not motile once encysted and covered with protective wall. Develops in the environment or within the second intermediate host	No

Note: Not all species produce rediae and metacercariae.

indicates that the asexual stages are able to distinguish 'self' (i.e. colonies with the same genetic constitution as themselves) from those of non-self (e.g. the same species but developing from a different miracidium). The development of distinct castes is probably widespread among trematodes and they offer excellent models for the study of social organisation (Hechinger *et al*., 2011).

Olson *et al*. (2003) divide the Digenea into two clades: the Diplostomatida and the Plagiorchiida. The Diplostomatida contains the blood flukes, of which the Schistosomatidae (schistosomes) are of importance as the cause of schistosomiasis in humans. The Plagiorchiida contains the majority of digenean species of which the Families Fasciolidae (e.g. *Fasciola hepatica*), Dicrocoeliidae (e.g. *Dicrocoelium dendriticum*), and Paragonomidae (e.g. *Paragonimus westermani*) are of particular importance in human and veterinary medicine (Keiser and Utzinger, 2009).

3.3.1 Family Fasciolidae

This family includes some of the largest flukes: *Fasciola gigantica* can reach 75 mm in length and 12 mm in breadth while *Fasciola magna* is even bigger and can be up to 100 mm in length and 26 mm in breadth (Figure 3.1). Adult fasciolids are usually found in the liver and bile ducts

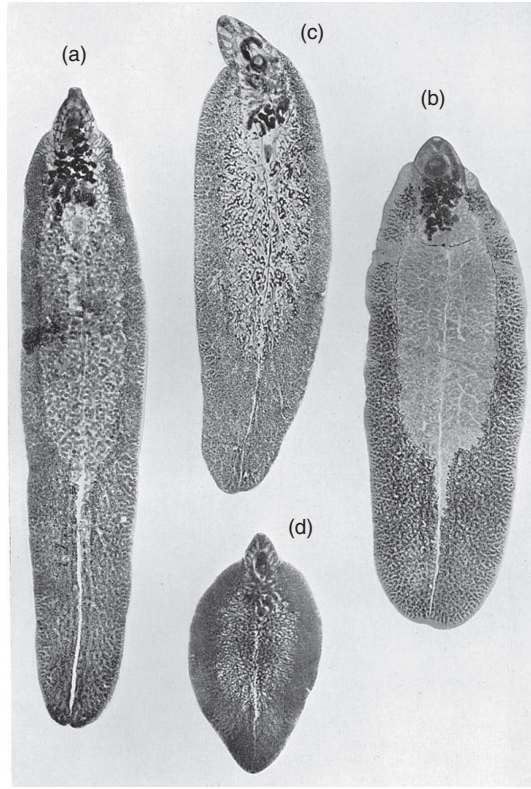

Figure 3.1 Adult *Fasciola hepatica* and *Fasciola gigantica*. (a) = *Fasciola gigantica* from an African ox; (b) = *Fasciola gigantica* from a Burmese ox; (c) = *Fasciola hepatica* from a British ox; (d) = *Fasciola hepatica* from a British sheep. All parasites are to the same scale. Note how there can be a considerable difference in size and shape between specimens. Source: Cameron, 1934

of ungulates and other herbivorous mammals – although omnivores such as pigs and humans (and occasionally carnivores) are sometimes used as definitive hosts. *Fasciolopsis buski* is an exception where the adults are found in the intestines of pigs and humans. The adults are usually leaf-shaped with the body broadening out immediately behind the anterior end and then tapering gradually towards the posterior. Their tegument is covered with large scale-like serrated spines and surface folds. The tegument is metabolically active and plays an important role in the absorption and exchange of nutrients and ions. The spines are thought to facilitate movement by enhancing the parasite's grip on the host's tissues. There is an oral sucker surrounding the mouth and a short distance behind there is a well-developed ventral sucker (Figure 3.2). The adult worms lack teeth or cutting mouthparts but do have a large muscular pharynx. Shortly beyond the pharynx, the gut divides up into a series of ever smaller branches throughout the body. These branches ultimately end blindly – there is no anus. These organisms lack a circulatory system and nutrients have to reach all the cells through diffusion. Consequently, because the worms are so large if the gut was divided into just two caecae (as is the case with some smaller digenean parasites), the distance between the gut and the most distant body regions would be too great. The Fasciolidae are hermaphrodites, containing both testes and ovaries. Their vitellaria are well developed and ramify throughout the body in the region beneath the ventral sucker. The eggs have thin shells and have a 'lid' (operculum) through which the miracidium emerges. Fasciolids have only one intermediate host which is an aquatic or semi-aquatic snail and their transmission to the definitive host is therefore heavily influenced by the availability of a suitable snail intermediate host species and its population dynamics.

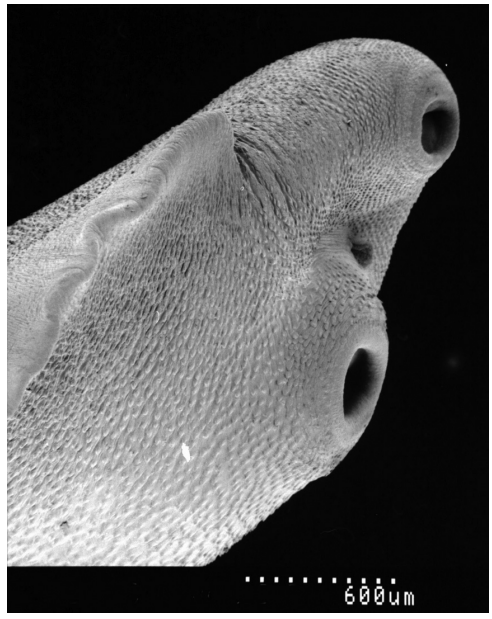

Figure 3.2 Scanning electron micrograph of an adult *Fasciola hepatica* from sheep. Note the oral sucker and the anterior of the body and the large ventral sucker. The genital pore is immediately above the ventral sucker. Note also the dense array of spines coating the surface of the worm

Fasciola hepatica/Fasciola gigantica *Fasciola hepatica* and *Fasciola gigantica* cause the disease 'fascioliasis' or 'liver rot' in sheep, cattle and other ruminants. *Fasciola hepatica* has a widespread distribution and is found in Europe, Africa, Asia, Australia, and North and South America. By contrast, *Fasciola gigantica* is most prevalent in the tropics, such as parts of Africa, the Indian subcontinent, and certain islands in the Pacific. In some parts of the world the two parasites co-exist but it is not certain to what extent competition between them occurs. The prevalence rates for fascioliasis can be as high as 30–90% and it is often considered to be the most important cattle helminth infection. In 1985, it was stated that these two species were responsible for world economic losses of approximately $2000 million every year and there is little reason to believe that they are now any less important (Boray, 1985).

The adult flukes live in the bile ducts and *Fasciola hepatica* feed on blood that is released from ulcers that develop on the lining of the bile duct: the worms are thought to feed repetitively in the same region until ulcers develop. *Fasciola hepatica* does not appear to ingest bile although it can absorb nutrients from the bile across its tegument. Less is known about the feeding behaviour of *Fasciola gigantica* but it is probably very similar to that of *Fasciola hepatica*. The adult worms release eggs that pass with the bile into the duodenum and are then voided with the host's faeces. If the eggs fall into freshwater they continue their development and under ideal circumstances they hatch after about 9–10 days to release the free-living but non-feeding miracidium stage (Figure 3.3). The miracidium is covered in cilia and actively searches for a suitable lymnaeid aquatic or semi-aquatic snail that will act as the intermediate host. In the UK, the snail *Lymnaea truncatula* is the most important intermediate host of *Fasciola hepatica* while in North America *Fossaria modicella* and *Stagnicola bulimoides* are utilised. In many parts of the world, *Lymnaea auricularia* is the main intermediate host of *Fasciola gigantica*. The miracidium chemically and physically bores its way into the snail and then sheds its cilia and transforms into the sack-like mother sporocyst stage. The mother sporocyst lacks a mouth and gut and absorbs nutrients across its tegument. Within the mother sporocyst the next life cycle stage is the first generation redia (plural 'rediae') that develops through asexual reproduction. The rediae are motile and after they are released from the mother sporocyst, they move through the snail host's tissues. The rediae also have a mouth and gut and are able to ingest snail tissues although they can continue to absorb nutrients across their tegument. The rediae then undergo a complex pattern of reproduction in which they form second and sometimes third generation rediae (Rondelaud and Barthe, 1982). Ultimately, each redia gives rise to several cercariae and these physically and chemically burrow out of the snail and swim away. The cercaria consists of two body regions: a globular body and a long, stoutly built, 'tail'. The 'body' has a mouth surrounded by an oral sucker and a ventral sucker. There is a pharynx and the gut divides into two caecae, but the cercaria does not feed. The powerful 'tail' contains striated muscle (unusual in platyhelminths) that enables the cercariae to swim actively. The cercariae of *Fasciola hepatica* usually emerge from their snail host during the night and after swimming for a while they settle on blades of grass, or other plants, just below the water surface. Here they lose their tails and transform into metacercariae. Sometimes they encyst on the surface of the water and, subsequently, sink to the bottom.

The definitive host becomes passively infected when it eats plants contaminated with the metacercariae. For example, human fascioliasis is relatively rare and usually associated with eating semi-aquatic plants such as water cress (e.g. *Nasturtium officinale*). Cattle can also become infected when they wade into ponds to drink and, in the process, stir up cysts lying at the bottom and swallow them. After ingestion, the metacercariae hatch in the duodenum in response to specific physical (e.g. temperature) and chemical stimuli (e.g. bile composition). The young flukes

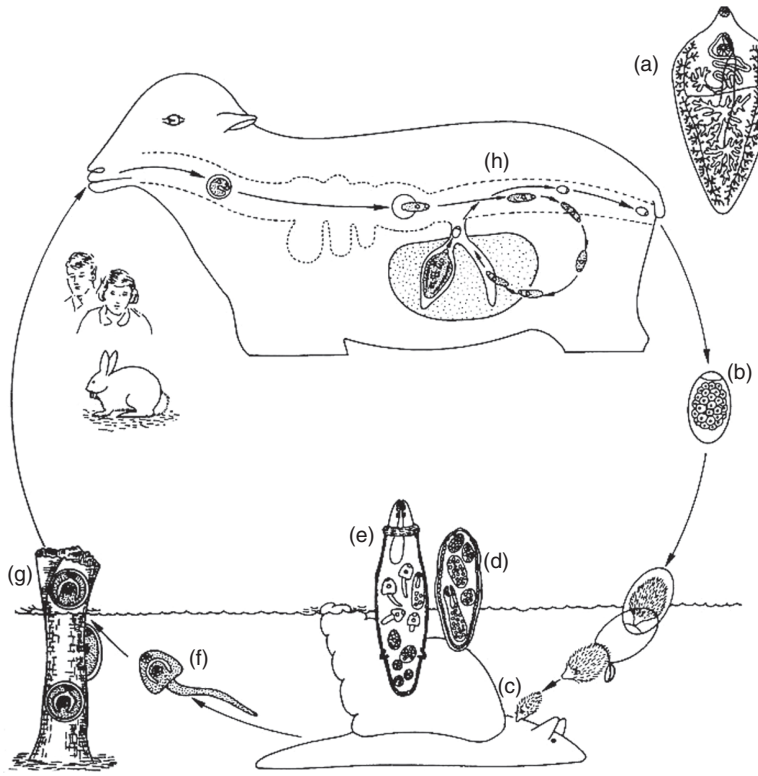

Figure 3.3 Life cycle of *Fasciola hepatica*. For full details see text. (a) = adult; (b) = egg (note the operculum/'lid'); (c) = miracidium; (d) = sporocyst; (e) = redia; (f) = cercaria; (g) = metacercaria; (h) = young fluke migrating to the liver. Source: Kearn, 1998. (Reproduced with kind permission from Springer Science+Business Media B.V.)

then physically and chemically bore their way through the gut and enter the abdominal body cavity. They probably reach the liver indirectly via the body wall, and they then penetrate the liver capsule and begin migrating through the liver parenchyma. The young flukes take about 5–6 weeks to reach the gall bladder and the simultaneous migration of large numbers of flukes is responsible for the condition 'acute fascioliasis'. Once the flukes reach the gall bladder, they become sexually mature and commence laying eggs. The adult worms can live for several years and during this time they give rise to the condition 'chronic fascioliasis'. There is no synchrony in the rates at which the young flukes move through the liver and then become mature. Consequently, following an initial infection young flukes will arrive at the bile ducts and then become mature over a period of two or more months.

Fasciolopsis buski *Fasciolopsis buski* is a large fluke that can reach up to 75 mm in length and 25 mm in breadth. The definitive hosts are pigs and humans, it is predominantly found in India, Bangladesh, China, and other South-east Asian countries. The life cycle of *Fasciolopsis buski* is similar to that of *Fasciola hepatica* except that the adult worms are typically found in the duodenum and upper regions of the small intestine. However, in heavy infections the parasites may also

be found in the stomach and as far down the intestinal tract as the colon. *Fasciolopsis buski* also differs from other adult fasciolids in having unbranched digestive caecae. This may be because it lives in the digestive tract and is therefore able to absorb more nutrients across its tegument than flukes that live in the bile ducts. The intermediate hosts are semi-aquatic snails belonging to the genera *Planorbis* and *Segmentina* within which sporocysts, rediae and finally cercariae are formed; the cercariae then bore their way out and encyst as metacercariae on surrounding vegetation. The snails feed on water caltrop (*Trapa natans*) and water chestnut (*Eliocharis tuberosa*) that are widely cultivated throughout South-East Asia for food and are consumed raw. Furthermore, these plants are often fertilised with human and pig faeces and therefore there is a perfect combination of all the elements needed to ensure transmission. Not surprisingly, infection rates in humans and pigs can be as high as 60%. In addition, conflict and economic problems in the region have led to widespread migrations and this has led to the parasite being spread to regions in which it did not previously occur (Wiwanitkit *et al*., 2002). The worms feed on the lining of the gut and the irritation causes excessive mucus secretion. In severe cases there is erosion and ulceration of the gut wall and it may even become perforated. The pathology, coupled with the large size of the flukes, can lead to gut obstruction and the patient may suffer from diarrhoea, vomiting, and abdominal pain. In severe cases infection with *Fasciolopsis buski* can prove fatal although most people are infected with small numbers of flukes and express few or no signs of infection. In addition, some people develop an allergic reaction to the parasite antigens that results in facial oedema and generalised ascites and this too can be fatal.

3.3.2 Family Cathaemasiidae: Genus *Ribeiroia*

This family of trematode parasites does not include any species of notable medical or veterinary significance. However, one particular species, *Ribeiroia ondatrae*, is of interest to parasitologists because of its role in causing morphological deformations in frogs (Figure 3.4). The populations of many amphibian species are declining around the world and the reasons for this are all too obviously a consequence of habitat destruction, pollution, and the intentional or unintentional introduction of predators, competitors, and diseases. However, after initial reports in 1995 from schoolchildren who were shocked to find affected frogs while doing pond surveys in Minnesota in the USA, large numbers of similarly deformed frogs were subsequently found in many other parts of the USA and Canada. Some of the affected frogs had malformed limbs, some had extra limbs and some had no limbs at all. Initially it was thought that the deformations were a consequence of an environmental pollutant and there were concerns about whether humans might also be at risk. However, it is now almost certain that the majority if not all of the deformities are a consequence of infection with the metacercariae of the trematode belonging to the genus *Ribeiroia*, probably *Ribeiroia ondatrae*.

Under experimental conditions the adults of *Ribeiroia ondatrae* can parasitise mammals and birds although the normal definitive hosts are uncertain. The eggs are passed in the faeces and these give rise to miracidia that infect aquatic snails such as *Planorbella*. Within the snail, the parasite develops first into a sporocyst and this gives rise to rediae that in turn give rise to cercariae. The cercariae then leave the snail and infect frog tadpoles within which they transform into metacercariae. The life cycle is completed when the definitive host consumes an infected frog. What is so interesting about the life cycle is that the metacercariae tend to cluster around

Figure 3.4 The metacercariae of *Ribeiroia ondatrae* cause deformations such as these in frogs. Source: Johnson and Sutherland, 2003. (Reproduced with kind permission from Springer Science+Business Media B.V.)

the pelvic girdle and hind limbs where they appear to act as a physical 'roadblock' that disrupts the growth of the developing limb buds – similar deformities can be induced experimentally by implanting resin beads (Sessions and Ruth, 1990). It is possible that the metacercariae may also release chemicals that affect the growth of surrounding host cells. Under experimental conditions, Johnson *et al*. (1999, 2002) showed that exposure of frog tadpoles to *Ribeiroia* spp. induced limb malformations in the majority of frogs that survived to adulthood and the level of malformation was related to parasite density. By contrast, malformations were not induced by metacercariae of the trematode *Alaria* spp. and none occurred in the controls. Obviously, by inducing limb deformities the parasite is increasing the likelihood that the intermediate host would be consumed by the definitive host – and this has clear evolutionary advantages. However, it does beg the question why so many frogs should suddenly be found with deformities. Scientists and schoolchildren have been observing pond-life for generations and yet so few people had seen deformed frogs in the past that their apparently sudden appearance was considered worrying and newsworthy. As with any disease the obvious reasons put forward included a sudden increase in parasite abundance, an increase in parasite pathogenicity, and an increase in host susceptibility. However, proving which, if any, of these factors is responsible is not easy. One suggestion is that the increasing use of agrochemicals has resulted in wildlife being exposed to low levels of numerous pollutants. These may not be sufficient to cause obvious toxicity but they compromise immunity and thereby increase susceptibility to pathogens. Rohr *et al*. (2008) found good evidence for this through a series of laboratory (mesocosm) and field experiments in which they demonstrated that tadpoles exposed to the herbicide atrazine were more vulnerable to infection with *Ribeiroia* spp. In addition, atrazine was shown to increase the growth of algae (periphyton) and these are an important source of food for the snail intermediate host. Previous workers have shown that amphibians exposed to low concentrations of atrazine are more vulnerable to viral diseases and it is therefore possible that there may also be interactions with co-infections.

3.3.3 Family Dicrocoeliidae

The adult flukes belonging to the Dicrocoeliidae are rather delicate small to medium-sized organisms that parasitise amphibians, reptiles, birds, and mammals. The adult worms are usually found in the ducts leading from the pancreas and gall bladder although some species live in the intestine itself. There is a large oral sucker around the mouth and the ventral sucker is situated a short distance behind it. They do not usually have the 'broad shoulders' of the fasciolids and the body tapers to a point at both the anterior and posterior ends. Beyond the pharynx, the gut divides into two simple caecae that end blindly before they reach the end of the body. Their tegument often lacks spines. The first intermediate hosts are usually terrestrial snails and there is also a second invertebrate intermediate host. Most species are parasites of wild animals and of no medical or veterinary importance. However, *Dicrocoelium dendriticum* is an important pathogen of sheep and there are isolated cases of humans infections.

Dicrocoelium dendriticum This has a widespread distribution including Europe, Asia, and North America but appears to be absent from Australia and Central and South Africa. Adults grow to about 10 mm in length and 2.5 mm in width and the adults can be found in a wide range of mammals including sheep, cattle, deer, donkeys and rabbits. It is predominantly a problem in sheep but the wide range of wild reservoir hosts makes control difficult. The adults live within the bile ducts and their eggs are passed with the host's faeces. Unlike fasciolid eggs, those of *Dicrocoelium dendriticum* do not hatch in the environment. Instead the eggs must be consumed by an appropriate terrestrial snail intermediate host (Figure 3.5). Therefore, infections with *Dicrocoelium dendriticum* are common among sheep and animals that graze on well-drained pasture. In Europe, *Zebrina detrita* is the principal snail intermediate host while *Cionella lubrica* is the main intermediate host in North America. However, a wide range of other snail species can also act as intermediate hosts. The eggs hatch within the snail's gut to release a miracidium that bores through the gut wall and transforms into the mother sporocyst stage. The mother sporocyst reproduces asexually to produce daughter sporocysts (there is no redia stage) and these in turn produce cercariae that move to the snail's lung. The cercariae are of a type called xiphidiocercariae – that is they have a piercing stylet at the anterior margin of their oral sucker. The cercariae accumulate in groups within the snail's lung where they become coated in mucus that is formed from their own glands and that of the snail host. They are then forcibly ejected from the snail's pneumostome (breathing pore) as 'slime balls' in an event that can be likened to the snail sneezing. The slime balls stick to the vegetation and are attractive to ants belonging to the genus *Formica*. The ants eat the slime ball and the cercariae then penetrate the ant's crop and enter the haemocoel where they transform into metacercariae. Several of them move to the ant's head and one of these penetrates the ant's brain and encysts within the sub-oesophageal ganglion. The remaining metacercariae travel back to the haemocoel of the abdomen and encyst within thick cyst walls. As night falls, the ants normally return to their nests but those infected within metacercariae remain outside and as the temperature drops and the humidity increases they climb up vegetation. They then clamp their mandibles onto the plant and remain firmly attached until the morning arrives and the temperature rises once more: at which point they release their grip and return to their normal tasks. This presumably increases their chances of being consumed by herbivores grazing during dusk or early morning.

After a sheep (or other definitive host) consumes an infected ant, the metacercariae excyst within the duodenum and the young flukes travel up the bile duct until they reach the finer branches where they become adults. Therefore, unlike *Fasciola hepatica*, there is no migratory

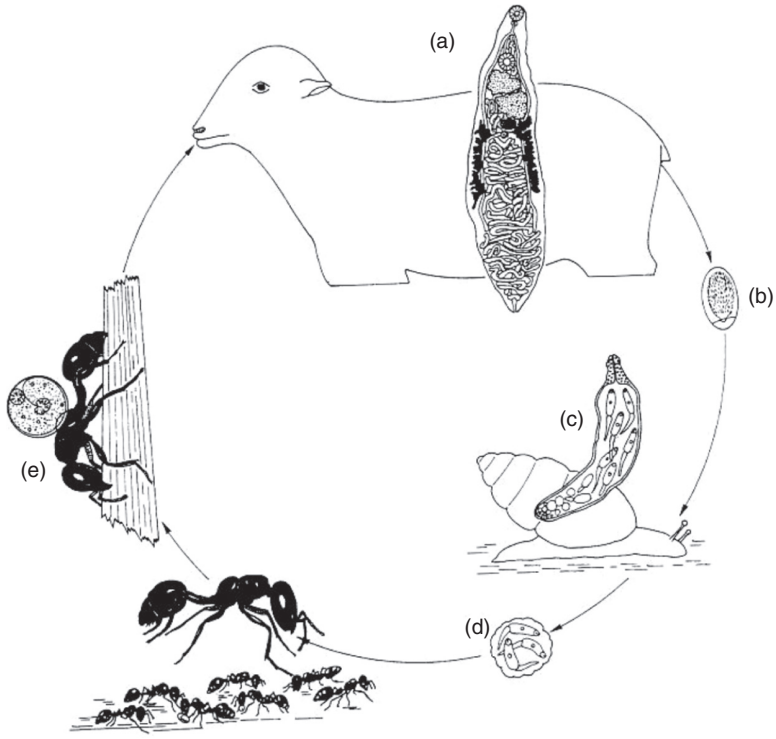

Figure 3.5 Life cycle of *Dicrocoelium dendriticum*. For full details see text. (a) = adult; (b) = egg (note the operculum/'lid') – this is consumed by the snail intermediate host; (c) = sporocyst; (d) = slime ball containing cercariae; (e) = metacercariae in ant. Source: Kearn, 1998. (Reprinted from Trends in Parasitology, 19, Johnson, P.T.J. & Sutherland, D.R.332–335, 2003, with permission from Elsevier)

phase through the liver parenchyma. Several thousand worms can develop within a single sheep and heavy infections cause inflammation, fibrosis, and biliary dysfunction. Long-standing infections can cause cirrhosis and the surface of liver becomes fibrotic. Humans can be infected by *Dicrocoelium dendriticum* but genuine cases are rare, since it is usually obvious that an ant is on one's food and we seldom consume raw *Formica* spp. ants intentionally.

3.3.4 Family Opisthorchiformes

This family contains 33 genera and includes parasites of fish, reptiles, and a range of mammals (King and Scholz, 2001). However, the best-known species are *Clonorchis sinensis*, *Opisthorchis viverrini*, and *Opisthorchis felineus* that infect humans. The body of adult worms belonging to the Opisthorchiformes is usually very thin with weak musculature so they are often translucent. The oral and ventral suckers are poorly developed and the testes are situated in the posterior half of the worm, usually close to the hind end. The gut divides shortly after the pharynx into two long, blindly ending caecae that terminate near to the posterior of the worm. The parasites have a complex life cycle that contains two intermediate hosts (the first of which is a snail) and a definitive vertebrate host. The adults usually live in the bile ducts or intestine and their eggs are already embryonated when passed within the host faeces. Although the first intermediate host is usually an

aquatic snail belonging to the family Bithyniidae, Hydrobiidae, or Thiaridae, the eggs do not hatch until they are consumed by the snail. However, as the snails are coprophagic (i.e. feed on faeces), it is not usually long before this occurs. After hatching, the miracidium transforms to a sporocyst: this stage lacks a mouth and gut and therefore absorbs nutrients across its body wall. After a few days the sporocyst gives rise to several rediae through asexual reproduction and these in turn can give rise to daughter rediae. It is difficult to distinguish between mother and daughter rediae and therefore it is often not known how many generations of rediae are formed. Each redia gives rise to several cercariae and when these are mature, they burrow out of the snail. The cercariae are of a type termed 'pleurolophorcercous': basically, this means that they have a globular body and a long thick muscular tail which has dorsal and ventral fin folds that presumably aid swimming. The body is equipped with an oral sucker and a ventral sucker and, unusually for cercariae, has two pigmented eyespots. After leaving their snail host, the cercariae hang upside down in the water and allow themselves to sink slowly for a while before swimming back upwards. They continue this sink and swim behavior, but contact with a solid object or experiencing the water currents produced by an animal swimming close by stimulates more activity. This presumably increases the chances of the cercariae making contact with the second intermediate host. Most second intermediate hosts are various species of freshwater fish (usually in the family Cyprinidae) although some Opisthorchiformes species will also use freshwater crabs or crayfish and even mammals may be infected. In those species that use fish as the second intermediate host, the cercariae latch onto the skin of the fish using their suckers and by a combination of releasing the contents of their penetration glands and physical action, they force their way into the fish's body. The cercariae then lose their tails and encyst as metacercariae within the musculature. The definitive host then becomes infected when it consumes the infected fish. Not surprisingly, fish-eating animals such as certain birds and mammals are common definitive hosts for these parasites. The consumption of raw fish is widespread among various populations in the Far East and is also becoming increasingly popular among many in Western societies. As a consequence, humans become vulnerable to contracting these parasites. *Clonorchis sinensis*, *Opisthorchis viverrini*, and *Opisthorchis felineus* together are thought to infect about 30 million people with a further 290 million people are at risk of infection.

Clonorchis sinensis This is an extremely common fluke throughout South-East Asia in countries such as China, Japan, Thailand, Cambodia, Laos, and Viet Nam. In addition to humans it is also a parasite of dogs, cats, pigs, and a variety of other mammals. Consequently, there are a large number of reservoir hosts and this can make control problematic. Prevention of infection is also made difficult because the metacercariae are resistant to harsh environmental conditions and can survive in salted and pickled fish. *Clonorchis sinensis* is a medium-sized worm that grows up to 25 mm long and 5 mm in breadth and has a pinkish, almost transparent coloration while still alive. The metacercariae excyst within the duodenum and then, like *Dicrocoelium dendriticum*, the young flukes make their way up the bile duct to the larger proximal bile ducts and their main branches where they become adults. Heavy infections cause inflammation and fibrosis that disrupts the flow of bile and this can lead to jaundice. In addition, there is pain and the patient can suffer fever, diarrhoea and consequent malnutrition. The liver can become swollen and cirrhotic – this in turn can result in disruption to the portal circulation and the formation of ascites. Sometimes the worms will also parasitise the pancreatic ducts and cause pancreatitis (inflammation of the pancreas). Although much of the damage is caused by the physical actions of the parasites, some of the pathology results from the host's immune response to parasite antigens. In common with *Opisthorchis viverrini* and *Opisthorchis felineus*, chronic infection with *Clonorchis sinensis*

has been linked to the development of cancer of the bile duct (cholangocarcinoma). At present, there is no effective chemotherapy for cholangiocarcinoma and therefore it is important to reduce the likelihood of its occurrence by preventing infection with parasites that are linked to its occurrence. The mechanism by which they induce cancer is not known but may be linked to increasing the susceptibility of DNA to damage by carcinogens (Young *et al*., 2010).

Opisthorchis viverrini *Opisthorchis viverrini* is very similar to *Clonorchis sinensis* in terms of its biology and pathology. Human infections are particularly common in parts of Thailand and in the Khon Kaen region it is thought to be responsible for exceptionally high levels of cholangiocarcinoma (Sriamporn *et al*., 2004). *Opisthorchis viverrini* tends to be smaller than *Clonorchis sinensis* and grows to only 12 mm in length and 2.5 mm in breadth. Living specimens have a reddish coloration and the testes are lobed rather than branched as is the case in *Clonorchis sinensis*.

Opisthorchis felineus *Opisthorchis felineus* is also very similar to *Clonorchis sinensis* in terms of its biology and pathology. It typically grows up to 12 mm in length and 2.5 mm in breadth and has a reddish coloration and lobed testes. *Opisthorchis felineus* has a more widespread distribution than *Opisthorchis viverrini* and *Clonorchis sinensis* and can be found in Siberia, Kazakhstan, Europe and North America as well as South-East Asia. Its distribution is spreading through Europe owing to the increased popularity of the consumption of raw fish and the movement of infected migrant workers from endemic regions (e.g. Armignacco *et al*., 2008). It has also been linked with the development of cancer (Mordvinov *et al*., 2010).

3.3.5 Family Paragonomidae

The Family Paragonomidae was initially erected by Dr Robert Ph. Dolfus in 1939 but many workers continue to place the genus *Paragonimus* in the Family Troglometridae (Blair, 2008). The genus *Paragonimus* contains several species that infect a variety of mammals and birds and some of these species are zoonotic. Several million people are thought to be infected with paragonomiasis and almost 300 million are at risk of infection (Procop, 2009). In Africa, *Paragonimus africanus* is important, while in Mexico and Central America *Paragonimus mexicanus* causes occasional human infections. Similarly, in the Midwest states of the USA, a number of human infections with *Paragonimus kellicotti* have been reported (Procop, 2009). However, *Paragonimus westermani* is the best known and the most important species as a human pathogen. We shall therefore consider this species in detail – the other species have a similar appearance and biology.

Paragonimus westermani This fluke is an important human parasite in those Asian countries in which freshwater crabs form a significant part of the diet. These countries include Korea, Japan, China, Taiwan, and the Philippines. In common with other members of this genus, the adults of *Paragonimus westermani* are oval-shaped and have an unusually stocky body for a trematode that can be up to 5 mm thick. They typically grow up to 12 mm in length and 6 mm in breadth, have a dense covering of spines and are reddish in colour. In humans and fish-eating mammalian carnivores they are normally found in the lungs, although they may also occur in other organs such as the liver, spleen, gut wall, muscles, kidney, and brain. The adults create a space around them that is surrounded by dead and dying cells and these (plus the worms) become walled off by a granulomatous immune reaction that is sometimes referred to as a ‘cyst’. Because the

parasites become enclosed by a host reaction rather than by material produced by the parasites, some workers consider the term 'cyst' in this instance to be inappropriate.

Usually one, two, or three worms are localised within a granuloma: the number varies with the host and other biological factors. *Paragonimus westermani* can be diploid, triploid, or even tetraploid and this influences not only its ability to undergo sexual reproduction but also other biological characteristics. For example, triploid individuals are unable to produce spermatozoa and therefore they can only reproduce parthenogenetically. Triploids also tend to develop to maturity faster than diploids and are more pathogenic in humans (Dreyfuss and Rondelaud, 2008). Triploidy also occurs in several other trematode species including *Fasciola* (Terasaki *et al*., 2000) but there is little research on the topic. Egg production usually commences before the adults are completely surrounded by fibrous host tissue but even so some eggs are often retained within the lungs. These eggs also induce a chronic immune response and become enveloped within granulomas. Most eggs reach the bronchial tubes and they are then transported upwards with sputum and are then spat out or swallowed and passed out with the faeces. If they are in freshwater, the eggs hatch after anything from 16 days to several weeks and the miracidia invade aquatic snails such as *Melania* spp., *Semisulcospira* spp., and *Brotia* spp. Within the snail, the miracidium first develops into a sporocyst and then there are two generations of rediae, the last of which gives rise to cercariae. The cercariae are of a type called 'microcercous' because the 'tail' is so reduced that it has become a non-functional appendage. Microcercous cercariae are therefore unable to swim but they have a large globular body and can crawl across solid surfaces using their well-developed oral and ventral suckers. There is some uncertainty in the literature as to what happens next, with some texts indicating the cercariae bore their way out of the snail and then creep off in search of a suitable crustacean host (e.g. Roberts and Janovy, 2006). Other authors suggest that the cercariae remain within the snail and infect the crustacean second intermediate host when it is consumes the infected snail (Kearn, 1998). A wide range of freshwater crab and crayfish species can be used as second intermediate hosts. Once they have penetrated the body of the crustacean, the cercariae transform into thick-walled metacercariae within the muscles and the viscera. When the infected second intermediate host is consumed by a suitable definitive host, the metacercariae excyst in the duodenum and transform into juvenile worms. The juvenile worms then penetrate the gut wall and enter the coelomic cavity; here they often pair up before making their way through the diaphragm and entering the lungs. If they are unable to pair up, they will often remain in the coelom awaiting the arrival of a suitable mate (Blair *et al*., 1999). The worms become mature about 8–12 weeks after reaching the lungs. However, sometimes the juveniles 'get lost' and ultimately end up in one of the other body organs, which are referred to as 'ectopic sites'.

The consequences of infection with *Paragonimus westermani* infection depend upon the number of worms and their location. In humans, cats, and dogs, small numbers of worms in the lungs may induce few symptoms (Procop, 2009; Taylor *et al*., 2007), whereas a large collection of parasites in the lungs can induce breathing difficulties, coughing, and the sputum may contain blood. However, even a single worm locating within the eye, brain or spine can have serious consequences including blindness, paralysis, and even death.

3.3.6 Family Schistosomatidae

These unusual parasites are commonly known as the blood flukes because the adult worms live in the blood vessels of birds and mammals. The phylogeny of the Family is reviewed by Zarowiecki

et al. (2007) and a comprehensive discussion of their biology is provided by Basch (1991) and Loker (1983). There are about 100 distinct schistosome species although natural hybrids can occur when a host is invaded by cercariae of two different species. Most of the schistosomes that infect wild animals are not very pathogenic but those that infect humans cause a disease called schistosomiasis that is responsible for considerable morbidity and mortality. Schistosomiasis is also known as Bilharzia or bilharziosis after the German physician Theodor Bilharz who in 1851 identified a schistosome as the causative agent of a disease which we now know as urinary schistosomiasis. Bilharz named the parasite *Distomum haematobium* but this was then changed to *Bilharzia haematobium* in honour of its discoverer, only for it then to be changed to *Schistosoma haematobium*.

Schistosomiasis is one of the most important human parasitic diseases: in 2010, it was estimated to afflict more than 207 million people and in the region of 700 million people were at risk of infection (WHO, 2010). Although schistosomes that infect domestic and wild animals can be found throughout the world, human schistosomiasis is almost entirely a disease of people living in developing countries with hot or tropical climates. Most cases of human schistosomiasis are caused by just three species of schistosome: *Schistosoma mansoni*, *Schistosoma japonicum*, and *Schistosoma haematobobium* (Table 3.2). In addition, *Schistosoma intercalatum*, *Schistosoma mekongi*, *Schistosoma malayensis* and *Schistosoma guineensis* can be locally important (Murinello *et al*., 2006). *Schistosoma mattheei* will infect humans but is normally a parasite of cattle, sheep, and goats. Kruger and Evans (1990) have suggested that the parasites described as *Schistosoma mattheei* recovered from humans may actually be hybrids of *Schistosoma mattheei* and *Schistosoma haematobium*.

Schistosoma mansoni is capable of infecting baboons, monkeys and some rodents but how important these are as reservoir hosts from which zoonotic transmission takes place is uncertain. Similarly, although *Schistosoma haematobium* can infect other primates, such as baboons and monkeys, these are unlikely to play an important role in human transmission cycles. By contrast, *Schistosoma japonicum* has been reported from over 30 species of domestic and wild mammals including dogs, pigs, cattle, and rats and therefore its control can prove difficult as a consequence of the large number of reservoir hosts.

Adult *Schistosoma mansoni*, *Schistosoma intercalatum* and *Schistosoma guineenensis* tend to be found in the inferior mesenteric veins draining the large intestine while adult *Schistosoma japonicum*, *Schistosoma mekongi* and *Schistosoma malayensis* are found in the superior and inferior mesenteric veins associated with the small intestine. Adult *Schistosoma haematobium* are commonly found in the veins of the vesical plexus around the urinary bladder and along the

Table 3.2 Distribution and intermediate hosts of schistosome species parasitising humans

Schistosome species	Snail host	Distribution
Schistosoma mansoni	*Biomphalaria, Tropicorbis*	Africa, Middle East, Caribbean islands, South America
Schistosoma japonicum	*Oncomelania*	South-East Asia
Schistosoma haematobium	*Bulinus, Physopsis*	Africa
Schistosoma intercalatum	*Bulinus*	Africa
Schistosoma mekongi	*Neotricula*	Laos, Cambodia
Schistosoma malayensis	*Robertsiella*	Malaysia
Schistosoma guineensis	*Bulinus*	Central West Africa, São Tomé
Schistosoma mattheei	*Bulinus*	Africa

ureters, but can also occur in the veins around the rectum. Adult schistosomes are unusual among the platyhelminths in having separate sexes (i.e. they are dioecious). The males are shorter but more robustly built than the females (see Colour Plate 4): male *Schistosoma mansoni* and *Schistosoma japonicum* are 10–15 mm long and 0.8 mm wide while the females are about 20 mm long and 0.25 mm wide; male *Schistosoma japonicum* are 12–20 mm long and 0.5–0.55 mm wide and the females are about 26 mm long and 0.3 mm wide. The males have a ventral longitudinal groove called the gynaecophoric canal (gynaecophoral channel) within which the female worm is normally found. The worms have two suckers, an oral sucker that surrounds the mouth and a ventral sucker situated close to the anterior end and used to grip the lining of the blood vessel they are living in. The suckers of the female are less well developed than those of the male. The gut divides into two caeca shortly after the mouth and these then rejoin about half way down the length of the worm and continue as a single tube to the posterior end where it ends blindly – there is no anus.

In the species that infect humans the large oval-shaped parasite eggs must traverse the tissues between the blood vessel walls and the gut lumen (*Schistosoma mansoni* and *Schistosoma japonicum*) or the bladder (*Schistosoma haematobium*). Adults of *Schistosoma nasalis* (*Schistosoma nasale*) live in the veins of the nasal mucosa of sheep, cattle and horses and the eggs are sneezed out with nasal secretions. Several schistosome species produce eggs that are equipped with a prominent spine and this was once thought to facilitate the egress of the eggs. However, the eggs of some species, such as *Schistosoma japonicum*, lack a spine (or it is rudimentary) and these have no difficulty exiting the host's body. It is now known that the eggs move through the tissues by stimulating a dramatic immune reaction; this is also responsible for much of the pathology associated with schistosomiasis. The function of the egg spine is therefore uncertain but would probably facilitate the egg sticking to the side of blood vessels. By the time they reach the outside world, the eggs are fully embryonated and they hatch when exposed to freshwater. The eggs release the free-living miracidium stage that is covered with cilia and swims actively in search of a suitable snail intermediate host. Schistosomes are very specific in their choice of intermediate host and some workers think that they may have originally been parasites of molluscs that subsequently added a vertebrate to their life cycle. Either way, the availability of a suitable mollusc intermediate host (Table 3.2) is a major factor determining the transmission and distribution of the different schistosome species. The extensive movement of people, and in particular slaves, from Africa to South America during the sixteenth and seventeenth centuries must have resulted in the importation of both *Schistosoma mansoni* and *Schistosoma haematobium*. However, only *Schistosoma mansoni* encountered a suitable local species of snail that could act as intermediate host (*Biomphalaria glabrata*) and therefore it was the only one to establish in the New World. Interestingly, the New World branch of *Schistosoma mansoni* is already diverging from its Old World roots and has evolved an enhanced affinity for the black rat (*Rattus rattus*) (Imbert-Establet and Combes, 1986).

The miracidium physically and chemically bores its way into the body of the intermediate snail host, loses its cilia and develops into the sack-like mother sporocyst stage. The mother sporocyst has no mouth or gut and absorbs nutrients across its epithelium. It grows to about 1 mm in length and reproduces asexually to give rise to daughter sporocysts and these reproduce asexually to produce cercariae. In those species that infect humans it normally takes about 4–6 weeks from initial infection for the cercariae to be produced. The mother sporocyst continues to produce daughter sporocysts and the snail remains infected for life. This means that a single miracidium can, ultimately, give rise to thousands of cercariae. Once they are mature, the cercariae bore their way out of the snail and swim off in search of the definitive host. Their emergence tends to exhibit daily rhythms related to the time of day the definitive host is most likely to be present. For example, the

cercariae of *Schistosoma mansoni* and *Schistosoma haematobium* tend to emerge mid-morning to shortly after midday when people are often outside collecting water, washing, fishing, paddling or swimming, etc. By contrast, the cercariae of *Schistosoma rodhaini* emerge at night because this is when its rodent definitive hosts are active. The cercariae of different schistosome species share a similar body plan that is called 'furcocercous' in which there is an anterior rather globular 'body' to which is attached a 'tail' that terminates in two short branches (furca). Unlike the 'body' of most trematode cercariae, the schistosome cercaria lacks an oral sucker and the ventral sucker is reduced in size and coated with tiny spines. The cercariae do not feed and die within 1–3 days if they do not find a suitable definitive host.

In those schistosome species that infect humans the cercariae swim to the surface of the water and then become motionless with their forked tail extended. They then passively sink for a short distance before actively swimming back up to the surface. By alternately rising and sinking the cercariae can be carried in water currents a considerable distance from their snail host. The cercariae are equipped with four types of glands: the 'escape glands' are so called because they are lost after emergence from the snail although their function is not known; preacetabular glands which contain enzymes and chemicals used in the process of invasion; postacetabular glands which secrete mucus that may aid purchase while invading the definitive host; and the head gland which produces secretions involved in the post-invasion transformation of the parasite. The host-finding strategies of the different schistosome species vary considerably. Somewhat surprisingly, the attachment of the cercariae of *Schistosoma mansoni* is stimulated by L-arginine despite the fact that this amino acid is not specific for human skin and is present at very low concentrations (Haas *et al*., 2002).

The cercariae physically and chemically penetrate through the skin of their definitive host. This normally takes place while the skin is fully submerged or from a film of drying water following bathing. The surface tension provided by a drying film of water facilitates invasion. The cercariae can also penetrate the lining of the throat and oesophagus if they are consumed in drinking water. The cercariae penetrate beneath the skin surface within 30 seconds and they then lose their tails and start to transform into schistosomules. This is associated with major changes in the surface covering that enable the parasite to survive the host's immune response and adapt to the new environmental conditions. The schistosomules migrate through the dermis until they locate a venule which they then move into and enter the peripheral circulation. Some may also enter the lymphatic system. Ultimately, the schistosomules reach the right side of the heart and from here they penetrate into the left side of the heart and thereby gain entry to the systemic (arterial) circulation. They are then swept to the lung capillaries where many of them die. This is because if they accidentally break through into the alveoli, they have difficulty regaining access to the circulation. The schistosomulae that successfully negotiate the lungs continue their migration until they reach the liver. They are metabolically quiescent while they are migrating through the systemic circulation but once they reach the liver, they start to feed on blood cells for the first time and their cell division and development recommences. They spend approximately 3 weeks living in the liver sinusoids after which they pair up and move to their species-specific adult breeding site.

Adult schistosomes that parasitise large mammals can live for many years and it is assumed that they remain together as monogamous pairs for much if not all of this time. However, divorce remains a possibility (Beltran *et al*., 2009; Tchuem-Tchuenté *et al*., 1996). Monogamy is unusual in the Animal Kingdom and schistosomes are the lowest taxonomic level at which it has been reported (Beltran and Boissier, 2008). The females are less muscular than the males and it is thought that they lack the physical strength to move against the flow of blood to reach the sites where they lay their eggs. They therefore have to 'pair up' with a strong male before they leave the

liver. Furthermore, if a female is separated from her mate, her reproductive system regresses. The reason for this is not known but it is independent of sperm transfer and it is not species-specific – if the female is paired with a male of another species, she can regain her reproductive health. It is therefore possible that by being enveloped in the male's gynaecophoric canal the female is exposed to the male's muscular pumping action and this has a growth stimulating effect.

3.4 Class Cestoda

Members of the Class Cestoda (also known as Class Cestoidea) are commonly known as the 'tapeworms'. All species are parasitic during both the adult and larval developmental stages. In most species the adult worm lives in the gut of a vertebrate while the intermediate host is either another vertebrate or an invertebrate. Some species go through a succession of larval stages in different intermediate hosts while *Hymenolepis nana* is an exception which does not have an absolute requirement for an intermediate host. A few species exhibit precocious development of the reproductive organs during their larval stage and are able to produce viable eggs before reaching the definitive host. One of the most extreme examples of this is *Archigetes cryptobothrium* that has dispensed with the need for a vertebrate definitive host entirely and sustains a life cycle within aquatic tubicifid worms (Olson *et al*., 2008; Kearn, 1998). All life cycle stages lack a mouth and gut and their body surface is composed of a metabolically active tegument across which nutrients are absorbed and waste products disposed. In most species, the adult worm consists of a scolex at the anterior end that is equipped with attachment organs and to which is joined a short 'neck' followed by a series of proglottids (segments) that become increasingly mature the further they are from the scolex. The neck plus proglottids are sometimes referred to as the 'strobila'. The adult worms are usually hermaphrodites and the individual proglottids are equipped with both male and female reproductive organs although these may mature at different times. Usually the male reproductive organs mature first. Sexual reproduction can occur through mating between two adjacent worms. A worm may also be able to mate with itself when the strobila is looped back so that proglottids with mature male and female reproductive organs are in close proximity. In some species asexual reproduction may also occur during the larval stage.

The classification of the tapeworms is in a state of flux and it is too complicated to go into the taxonomic disputes here. Fortunately, all species of major medical and veterinary importance belong to just two orders – the Pseudophyllidea and the Cyclophylidea – that are both within group known as the Eucestoda. Unfortunately, the validity of the order Pseudophyllidea has been questioned and it has been suggested that it should be split into two new orders: the Bothriocephalidea and the Diphyllobothridea (Kuchta *et al*., 2008). For our purposes we shall discuss just a few species that are of medical and veterinary importance and exemplify different aspects of tapeworm biology.

3.4.1 Order Pseudophyllidea/Diphyllobothriidea

Most members of this order are parasites of fish although a few species have mammals, birds, or reptiles as their definitive host. There are usually two intermediate hosts, the first of which is a crustacean and the second is a fish. In many species the adult worms are relatively short but some of them reach remarkable lengths: a specimen of *Polygonoporus giganticus* recovered from the gut of a sperm whale (*Physeter macrocephalus*) was 30 metres in length and had a strobila 5 cm in width at its widest point (Skrjabin, 1967): this species is also referred to as *Hexagonoporus*

physeteris in the limited literature available on it. The scolex of the adult pseudophyllidean tapeworm typically has two shallow longitudinal grooves called bothria and in the genus *Diphyllobothrium* each bothrium is used to clasp one or two intestinal villi.

Genus Diphyllobothrium There are several species of *Diphyllobothrium* and they are difficult to distinguish on the basis of their morphology. It is therefore highly likely that the literature contains many instances of misidentification. The best-known species is *Diphyllobothrium latum* in which humans, bears, dogs, and several other mammals that consume fish are the definitive hosts. The adult worms can grow up to 10 metres although some texts suggest that they may reach 20 metres. The proglottids nearest to the scolex are broader than they are long but they become square as they approach the posterior end. They are therefore commonly known as 'broad fish tapeworms'. The eggs are released into the host's gut via the genital pores on the gravid proglottids and are then passed with the host's faeces. The eggs have a superficial resemblance to those of *Fasciola hepatica* since they are ovoid and have an operculum (lid). However, the eggs of *Diphyllobothrium latum* are only 67–71 μm × 44–45 μm while those of *Fasciola hepatica* are about twice this size (130–150 μm × 63–90 μm). The eggs take several weeks before they are ready to hatch and they must be submerged in water. The eggs release a free-living stage called a coracidium that consists of the oncosphere that is covered by a ciliated embryophore. The coracidium does not feed and soon dies unless it is eaten by a suitable copepod crustacean that acts as the first intermediate host. The parasite then penetrates the copepod's gut and develops to the procercoid stage within its haemocoel. The next stage of the life cycle begins when the infected copepod is consumed by a suitable fish that will act as the second intermediate host, often members of the salmon and pike families (Family Salmonidae and Family Esocidae). The parasite penetrates the gut of the fish and makes its way to the muscles, gonads, and viscera where it develops to the plerocercoid stage. The mature plerocercoid is 1–2 cm in length and has rudimentary bothria. The life cycle is complete when the infected fish is consumed by the definitive host. Human infections can be common among societies in which there is a tradition of consuming raw or lightly cooked fish. For example, in the Baltic countries many people consume raw slightly salted roe and surveys have found that up to 91% of the fish gonads the roe is derived from can be infected with plerocercoids (Kearn, 1998). The plerocercoid has white coloration and is usually easy to spot when the fish is fresh. However, even light cooking changes the coloration of the fish tissues and identifying the plerocercoids becomes more difficult. Although the adult worm can grow to an enormous size, most people infected with *Diphyllobothrium latum* suffer few symptoms. However, some people develop pernicious anaemia and this is thought to be due to parasite metabolism reducing the availability of vitamin B_{12} in the gut (Scholtz *et al*., 2009). Several other species of *Diphyllobothrium* can parasitise humans and *Diphyllobothrium dendriticum* is actually more widespread and prevalent in humans than *Diphyllobothrium latum*. Some estimates suggest that as many as 20 million people may be infected with tapeworms belonging to the genus *Diphyllobothrium* and that the number of cases in developed countries is actually increasing owing to the popularity of meals containing raw or barely cooked fish (Scholz *et al*., 2009).

3.4.2 Order Cyclophyllidea

Most of the tapeworm parasites of birds and mammals belong to the Cyclophyllidea although the order also includes species that are parasites of amphibians and reptiles. The order contains several Families and most cyclophyllidean tapeworms are parasites of wild animals but a few of

Table 3.3 Metacestodes formed by cyclophyllidean tapeworms

Metacestode	Description	Example
Cysticercoid	Solid bodied with a single invaginated scolex. Asexual reproduction reported in some species but this is the exception rather than the rule	*Moniezia expansa, Hymenolepis nana*
Strobilocercoid	Cysticercoid in which some segmentation occurs behind the scolex	*Schistotaenia*
Cysticercus	A single scolex develops on a germinal membrane and is both introverted and invaginated. The germinal membrane encloses a fluid-filled space (bladder)	*Taenia solium, Taenia saginata, Taenia hydatigena*
Strobilocercus	Like a cysticercus but some segmentation occurs behind the scolex	*Taenia taeniaeformis*
Coenurus	An enlarged version of a cysticercus in which tens or hundreds of protoscolices develop from the germinal membrane through asexual reproduction	*Taenia multiceps*
Unilocular hydatid	Single defined cyst within which brood capsules develop. Thousands or even millions of protoscolices form on germinal membranes of brood capsules	*Echinococcus granulosus*
Multilocular hydatid	Cyst lacks a defined shape and ramifies through the host tissue	*Echinococcus multilocularis*

them are of relevance in human and veterinary medicine. The adult tapeworm usually has four circular or oval-shaped suckers (acetabula) that are used to grip the lining of the small intestine. In some species, the suckers are supplemented by a rostellum that is armed with hooks. The rostellum is a rounded protuberance at the top of the scolex and is equipped with muscles that enable it to be retracted and extended, thereby facilitating the use of the hooks. However, some species have a rostellum but no hooks and others lack a rostellum. A more reliable morphological diagnostic feature for cyclophyllidean tapeworms is the presence of a vitelline gland beneath the ovaries. The vitelline glands produce components used in the manufacture of the yolk and shell of the developing embryo; in other tapeworm species these glands are scattered throughout the proglottids.

The typical cyclophyllidean life cycle consists of the adult worm living in the gut of the definitive host producing eggs that are passed with the host's faeces. The eggs are then consumed by the intermediate host which may be an invertebrate or a vertebrate, depending upon the tapeworm species. Within the intermediate host the larval metacestode can take many forms and some of them are capable of asexual reproduction (Table 3.3). The life cycle is completed when the intermediate host is consumed by the definitive host.

3.4.3 Family Taeniidae

The taeniid life cycle typically involves a mammalian carnivore that acts as the definitive host and herbivorous or omnivorous mammalian intermediate hosts. The adult worms live within the small intestine and vary in size from species such as *Taenia saginata* that can grow to 20 metres in length

to *Echinococcus multilocularis* that is typically only 1.2–3.7 mm long. The adult tapeworm reproduces sexually and its eggs are passed with the host's faeces. The eggs are subsequently consumed by the intermediate host through faecal-oral contamination. The egg then hatches within the small intestine of the intermediate host to release an oncosphere that penetrates the gut and is swept in the circulation to a point in the body where it develops into the metacestode stage. In some species, the metacestode exhibits asexual reproduction. Interestingly, the extent to which asexual reproduction takes place is related to the size of the adult worm. Those species that produce large adult tapeworms, such as *Taenia saginata* and *Taenia solium*, do not exhibit asexual reproduction during the metacestode stage. By contrast, medium-sized species, such as *Taenia multiceps* (0.4–1 metre) may exhibit some asexual reproductive capacity during the metacestode stage while species that have very small adults, such as *Echinococcus granulosus*, undergo extensive asexual reproduction (Moore and Brooks, 1987). The life cycle is completed when the metacestode stage is consumed by the definitive host.

Taeniids are extremely important in both human and veterinary medicine and several species are zoonotic. Humans are the definitive hosts for three taeniid species: *Taenia solium*, *Taenia saginata*, and *Taenia asiatica*. Molecular evidence suggests that the switch from carnivores to humans as definitive hosts occurred twice during the evolution; *Taenia saginata* and *Taenia asiatica* are extremely closely related and may originate from a single initial colonisation event while *Taenia solium* probably colonised humans independently (de Queiroz and Alkire, 1998). The adult worms can co-occur in the same individual and inter-specific competition probably influences their transmission dynamics (Conlan *et al*., 2009). In addition, humans can act as intermediate hosts for several taeniid species including *Taenia solium*, *Echinococcus granulosus*, and *Echinococcus multilocularis*.

Taenia saginata *Taenia saginata* has a worldwide distribution and is one of the commonest tapeworm parasites of humans. The adult worm is capable of growing to over 20 metres in length although 3–5 metres is more common. Despite its large size, the scolex is only 1–2 mm and unlike *Taenia solium,* the grip provided by the four suckers is not supplemented by the presence of hooks and there is no rostellum. A fully-grown adult worm may consist of over 2000 proglottids and the final gravid segments are 16–20 mm long and 4–7 mm wide (Figure 3.6). After the gravid segments become detached, they are passed with the host's faeces or may even make their own way out of the anus. The detached proglottids are remarkably active and an infected person is often made first aware of their condition when they find a proglottid busily crawling around in their underwear, across the bedcovers or within the toilet bowl. A single proglottid can contain up to 200,000 eggs and these are dispersed into the surroundings as the proglottid crawls around: the eggs are forced through surface cracks that form as the proglottid starts to dry out. If the eggs are subsequently consumed by cattle, they hatch within their small intestine. The oncospheres then penetrate the gut, enter the circulation and are swept around the body. Ultimately, the parasites penetrate muscle fibres in which they develop into cysticerci. The heart, tongue, masseter muscles and intercostal muscles are common sites of infection although non-muscular tissues such as the liver and kidneys may also be parasitised. A cysticercus takes about 2 months to reach the infective stage by which time it is approximately 2 mm in diameter and contains a single invaginated protoscolex. The cysticerci have a pearly white coloration and heavily infected meat is sometimes said to be afflicted with 'beef measles'.

The adult worms are seldom seriously pathogenic unless the patient is already malnourished or undergoes an allergic reaction to the tapeworm antigens. Similarly, cattle with cysticercosis rarely

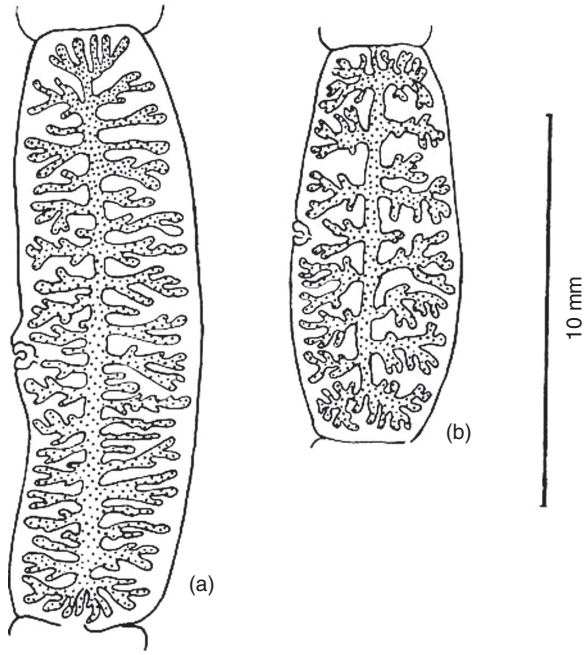

Figure 3.6 Gravid proglottids of *Taenia saginata* (a) and *Taenia solium* (b) Source: Cameron, 1934

exhibit clinical signs unless they are subjected to extremely high parasite burdens. Occasionally, cysticercosis 'storms' are reported in which a high proportion of the cattle on a farm become infected with large numbers of parasites. This is often a consequence of untreated human sewage being used as a fertiliser or the presence of an infected farmer or farmer-worker who is not careful about where he defecates.

Taenia solium *Taenia solium* is the most important tapeworm parasite of humans and it is the cause of considerable morbidity and mortality. Meat hygiene regulations have resulted in it becoming rare in developed countries but it remains common in South and Central America, India, and parts of India, Asia and Melanesia. It is a highly unusual tapeworm in that it is able to exploit humans as both a definitive host and an intermediate host. The normal life cycle involves humans acting as definitive host and pigs as the intermediate host – and hence it is commonly known as the pork tapeworm. The adult worm lives in our small intestine and can grow up to 10 metres in length although most only reach 2–3 metres. The scolex of adult *Taenia solium* is smaller (∼1 mm) and rounder than that of *Taenia saginata*, and its rostellum is armed with 22–32 curved hooks set out in two rows. The gravid proglottids are shorter than those of *Taenia saginata* and are typically 10–12 mm in length and 5–6 mm wide (see Figure 3.6). Each gravid proglottid contains about 40,000 eggs but it is not motile and may break up before it is shed with the faeces. Pigs become infected through faecal contamination and the cysticerci usually develop within the skeletal and cardiac muscles although the liver, lungs, and brain may also become infected (see Colour Plates 25 and 26). The oval-shaped cysticerci are much bigger than those of *Taenia saginata* and typically grow to 20 mm by 10 mm in size. The life cycle is completed when

humans consume raw or undercooked pork that is infested with mature cysticerci. Dogs and cats can also act as intermediate hosts and where these are also part of the human diet (e.g. parts of Asia), they can also be a source of human infection (Ito *et al*., 2002). Humans can also act as intermediate hosts and this usually occurs through the ingestion of the eggs following faecal contamination. Because the proglottids are not motile and the eggs are not dispersed, a pig or human can be simultaneously infected with large numbers of eggs when contact is made with infected faeces. Auto-infection can also occur when a gravid proglottid is transported back to the stomach through reverse peristalsis. This results in the eggs receiving the physical and physiological stimuli to hatch to release the oncosphere without leaving the host's body. Again, this can result in sudden massive infection. In humans, the cysticerci of *Taenia solium* can be found in virtually all body tissues and organs including the muscles, subcutaneous tissues, liver, spleen, eyes, and brain. The cysts take about 3 months to develop to maturity and the consequences depend upon their number and location. Those developing in the brain and spinal cord can cause a condition known as neurocysticercosis that results in seizures, epilepsy and can prove fatal (Wandra *et al*., 2000). Cysts that form in the brain can grow unusually large and exceed 50 mm in size. This increases their pathogenicity since it results in pressure atrophy of the surrounding tissues and restricts the local blood circulation.

Genus Echinococcus The adults of this genus are among the smallest tapeworms: they often have only two to four proglottids and are no more than a few millimetres in length (see Colour Plate 5). By contrast, their larval stages (hydatid cysts) are among the largest known and can grow to 50cm or more in diameter. The taxonomy of the genus has been complicated by disputes about whether worms recovered from different hosts represent different strains, sub-species, or should be considered as separate species. Currently, seven species are recognised, of which the best known are *Echinococcus granulosus* and *Echinococcus multilocularis* (Thompson, 2008). There are several distinct strains/genotypes of *Echinococcus granulosus* with their own transmission characteristics but there is less evidence of genetic distinctiveness among populations of *Echinococcus multilocularis*.

As a species, *Echinococcus granulosus* is found throughout the world and especially in sheep-farming regions although the various strains tend to have more localised distributions. The adults of the various strains of *Echinococcus granulosus* are usually found in domestic dogs (*Canis familiaris*) although some strains also occur in other caniids such as wolves (*Canis lupus*), foxes (e.g. *Vulpes vulpes*), and hyenas (e.g. *Crocuta crocuta*). Cats and other felids are usually poor definitive hosts although in parts of Africa there is a strain for which lions (*Panthera leo*) are the principal hosts. The adult tapeworms attach to the mucosa of the host's small intestine using their suckers and the double row of hooks on their rostellum. They are typically 2–7 mm in length and have 3–4 proglottids (see Colour Plate 5). The penultimate proglottid is mature while the final proglottid is gravid (full of eggs) and usually constitutes about half the length of the worm. After it has detached, the final (gravid) proglottid disintegrates within the intestine. This means that eggs become thoroughly mixed in with the host's faeces. Dogs can be infected with hundreds or even thousands of adult worms but they seldom cause serious pathology although heavy infestations can result in diarrhoea, weight loss, and poor condition.

The intermediate host becomes infected with *Echinococcus granulosus* when it ingests the eggs through faecal-oral contamination. The different strains tend to be associated with different intermediate hosts but they are all large herbivorous mammals such as sheep, cattle, camels, and pigs. Humans can also be infected by some of the strains. The eggs hatch within the intestine of

the intermediate host to release the oncosphere stage and this then penetrates the gut and enters the blood circulation. The oncosphere is then swept around the body to the point at which it will develop into the metacestode (larval) stage, which is commonly called a 'hydatid cyst' (see Colour Plate 6). Most cysts develop in the liver although other organs may be affected, including the lungs, spleen, and brain. The cyst is usually unilocular – that is, it is more or less spherical and has a thick laminated outer membrane within which is an inner germinal membrane that surrounds a fluid-filled cavity. The cysts grow slowly and usually are 5–10 cm in diameter although there is a record of a 50-cm cyst containing 16 litres of fluid being removed from an African patient. Brood capsules within which protoscolices develop by asexual reproduction are formed from the germinal membrane (see Colour Plate 7). In this way a single egg gives rise to a single hydatid cyst which, in turn gives rise to hundreds of protoscolices (singular: 'protoscolex') – each of which has the potential of developing into an adult worm.

The consequences of a unilocular hydatid cyst for the health of the intermediate host are dependent upon the number and size of the cysts and the organ in which they develop (see Colour Plate 7). Small cysts within the liver may have little impact, while the same sized cysts within the brain could prove fatal. The cysts cannot survive for long after the death of their host and therefore the life cycle depends upon the final host either killing and eating the intermediate host or consuming it shortly after its death (e.g. after it died of natural causes or being killed by humans). The transmission of most strains of *Echinococcus granulosus* is therefore strongly affected by human activities such as farming practices and our relationships with dogs. However, in Australia, the recent introduction of *Echinococcus granulosus* to the continent has led to the establishment of a sylvatic cycle between kangaroos and dingoes (*Canis lupus dingo*) that is based on predator–prey dynamics (Barnes *et al*., 2007). Similarly, in Africa there is a strain that has a sylvatic transmission cycle between lions and big game animals such as zebra. Hydatid disease in humans continues to be a serious problem in certain parts of the world and in particular in those countries where meat hygiene legislation is not enforced and dead farm animals are left where dogs can eat them.

Echinococcus multilocularis tends to be associated with cold climates and has high prevalence in Alaska, Siberia and Northern China. The adult worms have a similar morphology to those of *Echinococcus granulosus* but the larval stage forms a multilocular (alveolar) cyst that consists of a mass of separate vesicles (Figure 3.7) and unlike *Echinococcus granulosus*, the cyst contents are gelatinous rather than fluid. The adult worms are usually found in the small intestine of foxes such as the red fox (*Vulpes vulpes*) and the arctic fox (*Alopex lagopus*) although coyotes (*Canis latrens*), domestic dogs and wolves can also be infected. In Germany and other parts of Central Europe, the raccoon dog *Nyctereutes procyonoides* is becoming an important definitive host in the transmission cycle (Schwarz *et al*., 2011). (The raccoon dog was intentionally introduced into Central Europe from its native East Asia on several occasions during the years 1928–1953. Raccoon dogs are hunted for their luxurious fur. Since their initial introductions they have become abundant and widespread.) The intermediate hosts of *Echinococcus multilocularis* are usually wild rodents such as voles (Tribe Microtini) and lemmings (Tribe Lemmini) and parasite transmission is heavily affected by predator–prey (e.g. fox–rodent) relationships.

The multilocular cysts formed by *Echinococcus multilocularis* have been likened to neoplasms from the way in which they grow and ramify through the affected tissues. Most cysts occur in the liver although other organs may be affected. The cysts lack a limiting membrane and consist of a constantly growing sponge-like mass of parasite tissue. Furthermore, like certain cancers, fragments of the germinal membrane can break away and be carried in the bloodstream to other regions

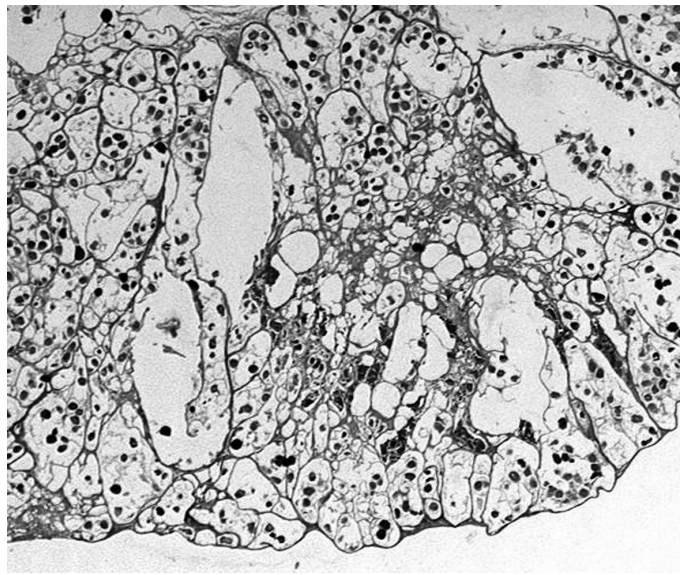

Figure 3.7 Multilocular cyst formed by *Echinococcus multilocularis* in the liver of a rodent

of the body where they initiate secondary cysts. Humans who ingest the eggs of *Echinococcus multilocularis* can develop multilocular cysts and the consequences may be fatal. Although the cysts are slow-growing in humans, they can completely destroy the liver or any other organ they infect. In humans and other atypical intermediate hosts, the germinal membrane of multilocular cysts usually does not produce protoscolices.

3.4.4 Family Anoplocephalidae

Adult anoplocephalid tapeworms have a scolex that is equipped with suckers but lacks a rostellum or hooks. The proglottids are usually wider than they are long and each proglottid has one or two sets of genital organs. The presence of two sets of genitalia probably increases reproductive output. The eggs have three protective layers: an external 'shell', a middle 'sub-shell layer' containing lipid droplets, and innermost sub-shell membrane. These layers are probably important in protecting the oncosphere when it is consumed by the intermediate host.

The adults of most anoplocephalid species are parasites of herbivorous mammals although *Bertiella studeri* infects a variety of primates including monkeys, baboons, and gibbons and there are occasional reports of humans becoming infected (e.g. Achir *et al*., 2008). The larvae develop as cysticercoids within mites (Acari) belonging to the Family Oribatidae. These mites are extremely common on the surface and upper layers of soil and can be found throughout the world. They are detritivores that feed on decaying organic matter and are important in nutrient recycling. Herbivores ingest the mites accidentally while grazing and primates presumably ingest them when foraging for roots, tubers, and food lying on the ground. Important anoplocephalid tapeworms include *Anoplocephala perfoliata* that has been implicated in the development of colic in horses,

Cittotaenia pectinata that can cause fatal infections in rabbits and *Moniezia expansa* and *Moniezia benedeni* that are common parasites of sheep and cattle.

Moniezia expansa and Moniezia benedeni Both *Moniezia expansa* and *Moniezia benedeni* are found throughout the world and are common parasites of sheep, goats, and occasionally cattle. The adult worms live in the small intestine and their eggs, along with strings of gravid proglottids, are shed with the faeces. Wild birds such as starlings have been implicated in the dissemination of *Moniezia* by consuming the gravid segments voided in the faeces. The eggs are not affected by passage through the bird's gut and so may be spread over a wide area (Kozlov, 1974). The eggs are consumed by oribatid mites (Acari) such as *Galumna*, *Oribatula*, and *Zygoribatula*. Exactly how the mites become infected is uncertain because the anterior section of their gut is probably too narrow to permit them to swallow the eggs whole. It is possible that the mites pierce the eggshell and suck out the embryophore without damaging it and the oncosphere then penetrates the mite's gut (Caley, 1975). Within the mites the parasites develop into cysticercoids over a period of 1–4 months depending on the temperature and can remain viable for at least 15 months (Al'kov, 1975). The simultaneous development of several cysticercoids can prove fatal for the mite (Xiao and Herd, 1992). The life cycle is completed when the infected mites are consumed by the definitive host. The adult worms grow rapidly and reach maturity between 30–57 days after infection. They typically grow up to about 2 metres in length although some texts suggest that they can reach up to 6 metres. The scolex is globular in shape and 0.36–0.8 mm at its widest point. It is equipped with four well-developed suckers but there is no rostellum and there are no hooks. The mature proglottids of *Moniezia expansa* are about 1.6 cm wide while those of *Moniezia benedeni* are about 2.6 cm. A unique feature of the genus *Moniezia* is the presence of interproglottidal glands at the posterior margin of each proglottid that become fully developed in the mature proglottids (Figure 3.8). In *Moniezia expansa* the glands run the full length of each segment while in *Moniezia benedeni* they are concentrated more to the centre. The function of the glands is not known but

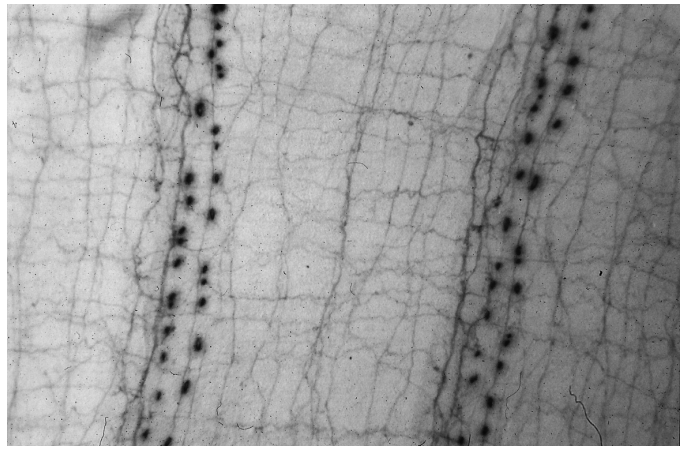

Figure 3.8 Mature proglottids of *Moniezia expansa*. The nerve net and interproglottidal glands stain strongly for acteylcholinesterase activity

they secrete a variety of vacuole-bound substances and a number of enzymes including alkaline phosphatase and acetylcholinesterase (Gunn and Probert, 1983).

There is some uncertainty about how long the adult worms can live although Kuznetsov (1968) suggested that this was up to 256 days. It is probable that the worms induce a reasonably good protective immune response since heavy infections are usually only found in young sheep. In the past, *Moniezia* infection rates in the UK were high and surveys often revealed over 80% of sheep were infected. However, with the advent of modern broad spectrum anthelmintics, they are becoming less common.

There are contradictory reports in the literature regarding the pathogenicity of *Moniezia expansa* and *Moniezia benedeni* (Elliott, 1986). In the UK, Australia, New Zealand and the USA, *Moniezia* is considered to be of minor importance and even where sheep were subjected to high worm burdens under experimental conditions, no serious effects were reported (e.g. Kates and Goldberg, 1951). Conversely, on the European continent, especially in Russia and the countries comprising the former USSR, *Moniezia* has often been described as seriously pathogenic. For example, Averkin *et al*. (1974) describe pathological changes in the intestine, pancreas, liver, spleen, kidney, and heart muscle of experimentally infected calves that would suggest immune-mediated pathology in response to parasite antigens. It is possible that the discrepancy may be a consequence of differences in pathogenicity between strains of the parasite and/or susceptibility of the sheep and cattle definitive hosts. It has also been alleged that because *Moniezia* are large and obvious that they get the blame for disease that is caused by other pathogens such as viruses, bacteria, protozoa, or even nematodes. Whether or not there are any relationships between *Moniezia* and other pathogens is not known.

3.5 Phylum Acanthocephala

The Acanthocephala are commonly known as the ‘thorny-headed worms’ or ‘spiny-headed worms’ because they have a proboscis at their anterior end that is armed with an array of hooks. The proboscis is eversible and can be retracted into a proboscis sac. Despite its sometimes fearsome appearance, the proboscis is used solely for attachment and acanthocephalans lack a mouth or alimentary tract. They are usually relatively small sausage-shaped creatures and grow to only a few millimetres or centimetres in length. There are, of course, exceptions and in pigs the males of *Macroacanthorhynchus hirudinaceus* can grow to 10 cm while the females may reach 60 cm in length. However, even these are dwarfed by *Oligoacanthorhyncus longissimus* which live in aardvarks (*Orycteropus afer*) and can grow to about 1 metre in length.

All acanathocephalan species are obligatory endoparasites and their life cycle usually involves a larval stage that develops in an invertebrate intermediate host while the adult stage lives within the gut of fish, amphibians, reptiles, birds, and mammals. About 1000 species of acanathocephalan have been described to date but the biology of most species is poorly understood. Adult acanthocephalans feed in the same manner as cestodes by absorbing nutrients across their body surface. However, unlike cestodes, acanthocephalans are psedocoelomate animals and have separate male and female sexes. Taxonomically, there is good morphological and molecular evidence for a close relationship between the Acanathocephala and the Rotifera although how the different groups are related is still uncertain (Fontaneto and Jondelius, 2011). This is at first sight a somewhat unexpected association because most rotifers are free-living organisms and have a fully-developed

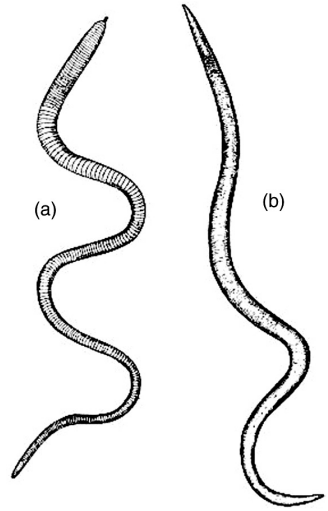

Figure 3.9 The acanthocephalan *Macroacanathorhynchus hirudinaceus* (a) bears a superficial resemblance to the nematode *Ascaris suum* (b) Source: Cameron, 1934

mouth and gastrointestinal tract. Rotifers are generally less than 1 mm in length and only a few species achieve 2 mm. They are usually found in aquatic ecosystems and there are a few ectoparasitic species. However, like acanthocephalans, rotifers are pseudocoelomates.

Several species of acanthocephalan are of relevance as parasites of domestic animals such as *Macroacanthorhynchus hirudinaceus* in pigs, *Oncicola canis* in dogs, and *Filicollis anatis* in ducks, geese and a range of wild aquatic birds (Taylor *et al*., 2007). Human infections are rare and probably the result of accidentally ingesting the invertebrate intermediate host. However, Haustein *et al*. (2010) report an unusual case in which a larval acanthocephalan was discovered living in the eye of a 24-year-old man.

Macroacanathorhynchus hirudinaceus is found in the small intestine of pigs and its size and pinkish coloration give it a superficial resemblance to the nematode *Ascaris suum* (Figure 3.9). It is not particularly host-specific and infections have also been recorded from dogs, other carnivores and even humans. After mating, the female worms release eggs that are passed in the host's faeces. The eggs are highly resistant to environmental conditions and can survive for several years. The eggs hatch to release the acanthor stage after being consumed by beetle (Coleoptera) larvae such as those of dung beetles and cockchafers. The acanthor bores through the gut of the insect and transforms into the acanthella stage. Subsequently, the acanthella develops into a juvenile and then becomes a cystacanth. At this point further development ceases until the insect is consumed by the definitive host – the cystacanth is then released and attaches to the host's gut wall where it becomes an adult.

In pigs, *Macroacanathorhynchus hirudinaceus* is not usually pathogenic although heavy infestations cause reduced weight gain. If the worms penetrate deep into the intestinal mucosa, they may ultimately perforate the gut, giving rise to potentially fatal peritonitis. Tamam (2009) considered that *Macroacanathorhynchus hirudinaceus* was responsible for the development of neoplasia in Egyptian long-eared hedgehogs (*Hemiechinus auritus*).

Box 3.1 Homosexual interactions in the acanthocephalan *Moniliformis dubius*

Homosexual interactions are common in many vertebrate and invertebrate groups, although their biological significance is a source of constant controversy. Among invertebrates most same-sex interactions occur between males and are often dismissed as a consequence of poor sex recognition and followed by the jaundiced observation that whatever their level of evolution, males will attempt to copulate with anything that is about the right size and shape and doesn't move out of the way fast enough! This, however, neglects the fact that the production of sperm, especially in some invertebrates, can represent a considerable metabolic investment and a male mating with another male is potentially wasting both time and energy.

Sexual interactions between adult male *Moniliformis dubius* are common but in this species they have a clear, albeit sinister, purpose. The adult worms are found in the intestine of rats and other rodents while the larval stages develop in a variety of insects, including the cockroach *Periplaneta americana*: it therefore makes a good laboratory model and has been studied extensively. During adult development the males and females are spatially segregated with the females developing in the anterior region of the small intestine where there is a higher carbohydrate concentration. When they are mature, the males migrate up the intestine to mate with the females and after mating a male seals the female's vagina with a cement cap using secretions from his cement glands. This prevents the female from mating with another male and thereby reduces the opportunity for sperm competition. All the female worms from a single infection tend to mature at about the same time and therefore the competition between males for access to the females can be considerable. Abele and Gilchrist (1977) observed that male worms would forcibly mate with one another in an attempt to seal off a competitor's genital opening. Although they found that only 2.5% of males were 'capped', they estimated that the true figure could be as high as 50%. They used the term 'homosexual rape' which is a dangerously emotive term but does indicate that the interaction is not accidental and neither is it without purpose.

3.6 Phylum Nematoda (Nemata)

Somewhat paradoxically, the nematodes are simultaneously one of the most abundant, important, and yet neglected groups of metazoan animals. Some estimates suggest that there may be over 100 million species but fewer than 30,000 have actually been described (Meldal *et al*., 2007). Nematodes occupy all terrestrial and aquatic ecosystems while one species, *Halicephalobus mephisto*, lives over a kilometre below ground in a gold mine in South Africa. In marine sediments, up to 95% of the organisms present can be nematodes and they are responsible for much of the benthic invertebrate biomass. Many nematodes feed on bacteria, fungi or are detritivores but there are also examples of predators and many are parasites of plants, invertebrates, and vertebrates.

Most nematodes, especially the free-living species, are microscopic in size but some of those parasitic in mammals can be several centimetres in length while some giants are over a metre long. For example, the females of *Placentonema gigantissima*, which lives in the placenta of sperm whales, can reach at least 8.4 metres in length and 2.5 cm in width (Gubanov, 1951). Nematodes are very uniform in their appearance and their thin, elongate and cylindrical shape

explains why they are commonly known as 'roundworms'. They lack a well-defined head and have a worm-like (vermiform) body that tapers at the anterior and posterior ends. The body is covered by a complex layered cuticle that is secreted by the underlying epidermis and is periodically shed during the juvenile stages to enable growth. The cuticle is proteinaceous but, unlike that of insects, it is not chitinous. Nematodes do, however, contain chitin and it is found in their eggshells, their pharynx, and in the sheath of microfilarial nematodes. In common with other higher invertebrates, nematodes are triploblastic (i.e. they have three primary germ layers during embryonic development) and they are bilaterally symmetrical (i.e. the left-hand side of the body is arranged the same as the right-hand side). Although some species have annulations on the surface of their cuticle, there are no body segments and unlike the trematodes and cestodes, they do not possess suckers. Their body cavity takes the form of a pseudocoelom (blastocoelom) that is filled with fluid (haemolymph) at exceptionally high pressure and serves to maintain the hydrostatic skeleton. For example, the pressure within *Ascaris suum* is 6.6–37.6 kN m^{-2}, which is considerably higher than that of other invertebrates with hydrostatic skeletons: in earthworms the pressure is only 0.28–2.8 kN m^{-2} (Moore, 2006). The high internal pressure necessitates the cuticle to be extremely strong and also affects other aspects of nematode biology (Lee, 2001).

The mouth of nematodes is usually surrounded by lips/mouthparts that are arranged symmetrically and there is a muscular pharynx that pumps food into the body. The gut is tube-like and terminates in an anus close to the posterior of the worm. The high internal pressure means that nematodes often project their waste with some force: *Ascaris suum* can eject a stream of liquid waste across several centimetres and it is not a good idea to peer too close to the hind end of a live worm (A. Gunn, pers. obs.). All nematodes have unique chemoreceptors at their anterior end called 'amphids' and some also have them at their posterior (caudal) region called 'phasmids'. The amphids are usually well developed in free-living marine nematodes but reduced in size in terrestrial and parasitic species. An amphid consists of an external opening that leads via a duct to an amphidial pouch within which there are sensory neurons. An amphidial gland is also associated with the amphidial pouch. In *Caenorhabditis elegans*, the amphids work as chemoreceptors involved in taxis (movement towards a stimulus) while the phasmids detect repellents and influence movement away from a stimulus (Hilliard *et al*., 2002). Nematodes have longitudinal muscles but lack circular muscles: they can therefore only bend their body from side to side and are unable to exhibit the creeping style of movement seen in earthworms (Annelida).

Although a few species of nematode are parthenogenic, most of them reproduce sexually and there are separate male and female sexes (i.e. they are dioecious). The males are usually smaller than the females and there is often marked sexual dimorphism. Female nematodes typically have a vagina, vulva and a pair of tube-like uteri, oviducts, and ovaries. In parasitic nematodes the female reproductive system is often extremely productive and a single worm may release hundreds or even thousands of eggs every day. Usually female nematodes release ovoid eggs, but in filarial nematodes the eggs hatch while still inside the female worm and she releases microfilariae. The males have a single testis and the ejaculatory duct empties into the rectum. Nematode spermatozoa are usually large and amoeboid in shape – even if they appear to have a tail, it is not used as a flagellum to provide propulsion. The hind end of male nematodes is usually curved and has a number of sensory papillae. In some species these papillae are little more than raised bumps, while in others they are much larger or reduced to pit-like depressions. In males belonging to the order Strongylida, the posterior of the worm is modified to form a 'copulatory bursa' that consists of two lateral lobes and a smaller dorsal lobe (Figure 3.10). The lobes are supported by fleshy rays and the whole structure envelopes the female's genital opening during mating. Male nematodes

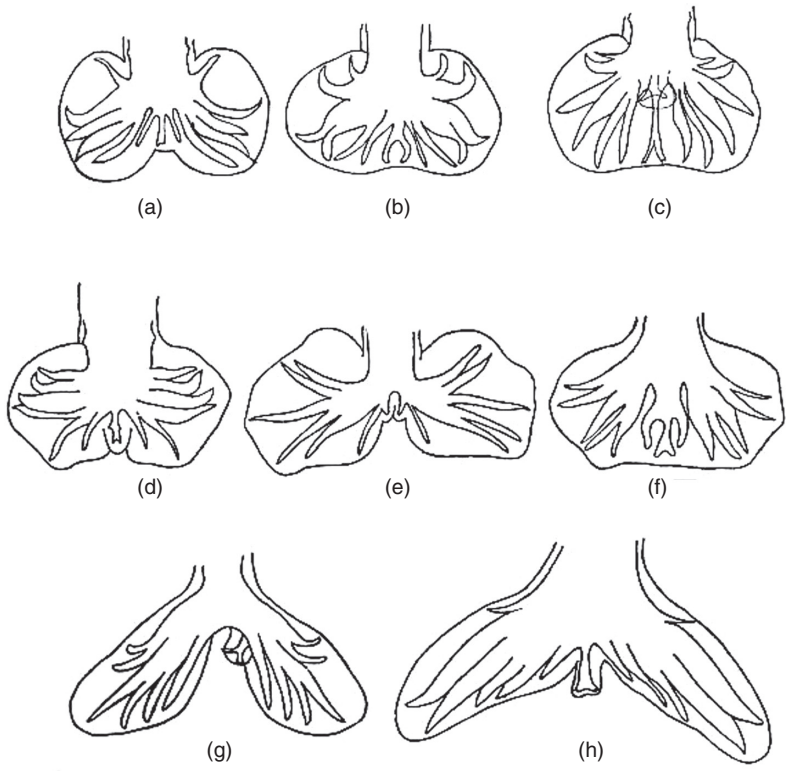

Figure 3.10 The structure of the copulatory bursa of strongylid nematodes can prove a useful taxonomic indicator. This is a diagrammatic representation of several trichostrongyle parasites of veterinary importance. (a) = *Trichostrongylus*; (b) = *Cooperia*; (c) = *Ostertagia*; (d) = *Hyostrongylus*; (e) = *Nematodirus*; (f) = *Ollulanus*; (g) = *Haemonchus*; (h) = *Mecistocirrus*. Source: Cameron, 1934

are equipped with one or two spicules (Figure 3.11) that are used to dilate the female's vaginal opening during copulation: this is necessary so as to overcome the high internal body pressure and allows the spermatozoa to be then rapidly and forcibly injected into the female. The spicules are enclosed in a fibrous sheath that opens into the rectum. The spicules vary enormously in size and shape between species, and are therefore very useful in species identification.

The taxonomy of nematodes is difficult owing to their small size and the limited number of defining morphological characteristics. However, their morphological similarity belies considerable underlying genetic diversity. Traditionally, the nematodes are divided into two classes: the Secernentea (Phasmida), which have cephalic amphids as well as caudal amphids, and the Adenophorea (Aphasmida), which, as the name implies, lack the latter. However, molecular analysis indicates that the Adenophorea is paraphyletic. Blaxter *et al*. (1998) recognised five distinct clades of nematodes, all of which include examples of parasitic species. They suggested that this indicates that animal parasitism probably evolved independently on at least four separate occasions. Subsequently, Holterman *et al*. (2006) proposed that the number of clades should be increased to 12 while further revisions to the taxonomy of nematodes have been suggested by Meldal *et al*. (2007). Some taxonomists divide the nematodes into two new classes: the Enoplea and the

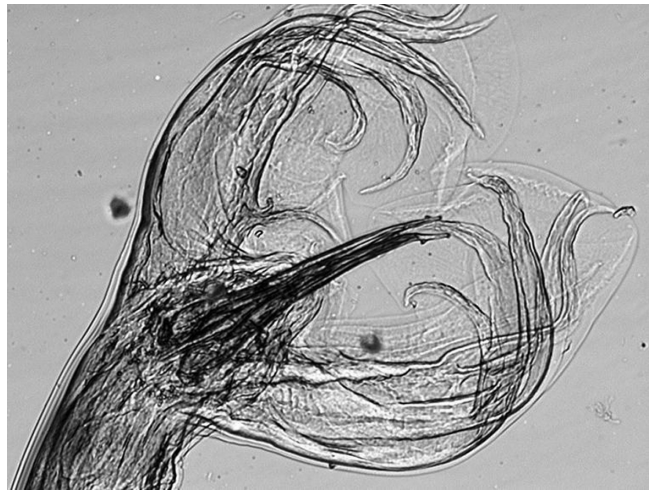

Figure 3.11 Copulatory bursa and spicules of *Haemonchus contortus*

Rhabditea (Coomans, 2002; Roberts and Janovy, 2006). The Enoplea supersedes the Adenophorea and contains those nematodes that, apart from certain parasitic species, have well-developed amphids but lack phasmids. The majority of nematodes in this class are free-living, but it includes the order Trichurida that contains important parasitic genera such as *Trichuris*, *Trichinella*, and *Capillaria*. The Rhabditea supersedes the Secernentea and contains both free-living and many parasitic species. Among the many important and diverse genera within the Rhabditea are *Strongyloides*, *Ancylostoma*, *Necator*, *Ascaris*, *Anisakis*, *Dictylocaulus*, *Dracunculus*, and *Wuchereria*. In view of the large numbers of parasitic species and the uncertainty concerning the higher taxonomy of the nematodes, we will only consider certain genera to illustrate the diversity in the biology of animal parasitic nematodes. Further details on the biology and classification of nematodes are available in Lee (2001).

3.6.1 Class Enoplea

Genus Trichuris Members of this genus are commonly known as the 'whipworms' because the anterior two thirds of their body is thin and whip-like, in comparison to the thicker posterior region (Figure 3.12). The adult worms lack lips and there are no cutting plates or other forms of mouthparts. The posterior of the males is tightly coiled and they have a single spicule that is contained within a protrusible membranous sheath covered in a dense layer of short chitinous spines. The females have their genital opening at the junction of thin anterior region and the thicker posterior region. *Trichuris* eggs have a distinctive barrel shape with clear opercular plugs at either end.

There are a number of species in the genus and they tend to parasitise groups of closely related hosts and have similar life cycles. For example, *Trichuris trichiura* infects humans and other primates, *Trichuris suis* infects pigs, *Trichuris ovis* infects sheep and other ruminants, while *Trichuris muris* infects rodents and is a good laboratory model for studying gastrointestinal nematode

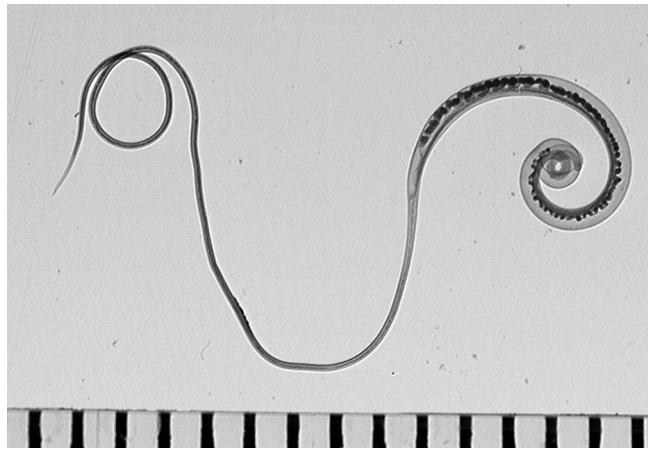

Figure 3.12 Adult male *Trichuris suis*. Note the long slender anterior end and much thicker posterior region. Scale in mm.

infections. The adult worms live in the caecum and their unsegmented eggs are passed with the host's faeces. If they are kept in the shade, in moist soil, the eggs embryonate to the infective first juvenile stage but do not hatch. The infective eggs are very resistant to environmental conditions and can remain viable for several years. Transmission is through contamination and the eggs hatch after they are consumed by a suitable definitive host. After hatching, the larvae invade the crypts of Lieberkühn and after penetrating the cells at the base of the crypt, they then tunnel their way back to the surface of the gut lumen. Although juvenile worms may be found in both the small and large intestine, only those in the caecum are able to complete their development to adulthood. Some texts continue to suggest that juveniles developing in the small intestine subsequently migrate to the caecum but Bundy and Cooper (1989) state that there is no evidence of this. As the worms become adults, the thick posterior region of the worm breaks through the surface mucosa into the lumen while the anterior portion remains buried within the host's tissues.

Some estimates suggest that over 1,000 million people are infected with *Trichuris trichiura* (Khuroo *et al*., 2010). The adult worms have a pinkish-grey coloration; the females are 30–50 mm in length while the males are slightly smaller (30–45 mm). Infections are common in people living in developing countries with poor sanitation and the incidence is often high among children. Light worm burdens are not particularly pathogenic but high worm burdens can induce diarrhoea, cause stunting and clubbing of fingers, rectal bleeding, anaemia, and even induce rectal prolapse (Khuroo *et al*., 2010). Co-infections of *Trichuris trichiura* and *Ascaris lumbricoides* are common since their eggs require similar conditions in which to embryonate and they are both transmitted through contamination.

Similar pathologies to *Trichuris trichiura* are caused by *Trichuris suis* in pigs, *Trichuris vulpes* in dogs, and *Trichuris ovis* in sheep. There is some interest in the potential of *Trichuris suis* for use in helminth therapy (see Chapter 8). This is controversial for a variety of reasons, which include concerns about whether or not people infected with *Trichuris suis* might transmit their infection to others. This is partly fuelled by an ongoing debate about whether or not *Trichuris suis* and *Trichuris trichiura* are actually the same species. However, there is now molecular evidence to conclusively prove that they are not (Cutillas *et al*., 2009).

Genus Trichinella For many years *Trichinella spiralis* was the only recognised species in this genus but it is now divided into two clades that contain a total of eight species and four distinct genotypes (Pozio *et al*., 2009). The division into two clades is based on whether or not the host encapsulates the larvae while they are developing within muscle cells. The larvae of the majority of species and genotypes of *Trichinella* are encapsulated within nurse cells that are surrounded by a fibrous collagen wall of host origin (see Colour Plate 8), but in three species (*Trichinella pseudospiralis*, *Trichinella papuae*, and *Trichinella zimbabwensis*), this does not occur. The various species vary in their distribution and normal hosts but they all share a similar life cycle and have a potentially wide host range – even if it is not always used under natural conditions.

Trichinella spiralis has an exceptionally wide host range and infects humans, pigs, dogs, cats, badgers, rats, and numerous other mammals. It is unusual in that an infected individual serves as both definitive host and intermediate host and it also has the distinction of being the largest intracellular parasite known. The life cycle begins with the host consuming raw or undercooked meat containing infective first-stage larvae that are encapsulated within the flesh. The larvae are released within the host's duodenum and then proceed to invade the glandular crypts of the upper region of the small intestine. The developing worms thread through adjoining villi by penetrating the cytoplasm of cells (rather than pushing the cells to either side of their body). The juveniles grow rapidly and within four days they become sexually mature adults. The worms are slender and there is not the obvious division between the anterior and posterior regions seen in *Trichuris*. The females are 3–4 mm in length and 60 μm in diameter while the males are about 1.6 mm long and 40 μm in diameter. The male lacks spicules but he has a pair of lateral flaps at the posterior end that can be used to grasp the female. The female has her genital opening in the middle of the oesophageal region. The male does not live for as long as the female, but he has sufficient time to mate with several females. The female can live for 4–16 weeks and during this time the eggs within her uterus mature and hatch *in situ* so she releases hundreds or even thousands of first stage larvae (80–160 μm in length). The larvae penetrate the intestinal mucosa and then enter the blood supply that distributes them around the whole body. Larvae that reach skeletal muscles proceed to penetrate individual muscle fibre cells. Although all skeletal muscle cells can be infected the diaphragm, jaws, tongue, extraocular muscles and larynx are often the most heavily parasitised.

It takes about 4–8 weeks for the larvae to reach the infective stage, at which point they enter a period of arrested development. The larvae can remain alive and infectious for many years. After 6–9 months the nurse cells often start to become calcified and this can be detected as a 'gritty' feeling when a sample of muscle is squashed between two glass slides. In heavy infections, the calcified nurse cells may be visible as a scattering of grey-white spots within the muscle. The life cycle is completed when the infected muscle is consumed by a suitable definitive host. Therefore, unlike many nematode parasites, none of the developmental stages are exposed to the environment and the whole life cycle takes place within the body of another animal.

There are essentially four life cycle types: domestic, sylvatic-temperate, sylvatic-torrid, and sylvatic-frigid and all of them can include humans – although usually as 'dead-end hosts'. The 'domestic' life cycle principally involves *Trichinella spiralis* and the parasite is transmitted from pigs to humans through the consumption of raw or poorly cooked pork. Humans are essentially dead-end hosts because pigs rarely get the opportunity to eat humans. Pigs become reinfected through feeding on scraps of infected meat and cannibalism may also be important: during fights they will consume one another's tails (and worse). Rats and other rodents can also figure in this cycle through eating discarded infected meat and then falling victim to a hungry pig at a later date. The sylvatic-temperate transmission cycle occurs between wild animals living in

temperate climates such as Europe and parts of Asia and North America. This cycle typically involves *Trichinella spiralis*, *Trichinella britovi*, and *Trichinella murrelli* and the parasites cycle between foxes, bears, wild boar, rodents and other carnivores/scavengers. Infection may take place through the consumption of prey or of animals that died from natural causes. Humans usually become infected through the consumption of poorly cooked game animals. The parasites may subsequently enter a domestic cycle if flesh from game animals is fed to pigs. The sylvatic-torrid transmission cycle occurs in tropical Africa and involves *Trichinella nelsoni*. Hyenas, lions, warthogs, and bush pigs are thought to be important in the transmission of this parasite and it is very pathogenic in humans. As one would expect, the sylvatic-frigid transmission cycle occurs in the Arctic and often involves *Trichinella nativa*. Normally the parasite is transmitted between predators and scavengers such as bears, wild cats, foxes, mustelids, and walruses. We shall only consider *Trichinella spiralis* since it has an almost worldwide distribution and is the most important species from both a medical and veterinary perspective.

Box 3.2 Invasion of muscle cells by *Trichinella spiralis*

Muscle cells are highly specialised and differentiated cells but when they are infected by *Trichinella spiralis* larvae, they are induced to de-differentiate into 'nurse cells'. The larvae accomplish this by altering the gene expression of parasitised cells and in particular the activity of those genes controlling cell cycle-related factors. In addition, there is down-regulation of genes coding for muscle cell-specific proteins and the muscle cell de-differentiates. That is, it ceases to be a muscle cell and re-enters the cell cycle. De-differentiation is to be expected since this is a normal part of the muscle cell repair process following damage – and the larvae damage the cells in the process of invasion. However, normally, de-differentiation is followed by regeneration but this does not take place in parasitised cells. Instead the larvae manipulate their environment to facilitate their own growth. The contents of the cell undergo apoptosis and the sarcomeres and myofilaments are broken and this presumably provides some nutrients required by the worms. The cell does not totally degenerate because there is a balance between the up-regulation of apoptosis-genes and anti-apoptosis genes (Boonmars *et al*., 2004). In addition, the parasite induces the nurse cell to secrete vascular-endothelial growth factor that stimulates the growth of small blood vessels around the nurse cell and this presumably increases the supply of oxygen and metabolites. Eventually, the nurse cells become up to 0.4–0.6 mm in length and 0.25 mm in width and contain infective first stage larvae that are about 1 mm in length. Further details are available in Wu *et al*. (2008) and Despommier (1998).

Adult *Trichinella spiralis* seldom cause clinical disease and are soon cleared by the host's immune response. However, heavy infections can cause enteritis with the production of excessive intestinal mucus and diarrhoea. Similarly, small numbers of larvae encysting within the muscles may cause no problems but the sudden invasion of large numbers of larvae can result in serious pathology and even prove fatal. This typically occurs in humans following the ingestion of poorly cooked heavily infested pork. The wandering larvae can induce pneumonia and their movement through tissues such as the heart, eye or brain can have serious consequences. The invasion of skeletal muscles causes myositis (inflammation of the muscles), fever, intense muscular pain and impairs the ability of the affected muscles to contract. Consequently, invasion of the diaphragm and

intercostal muscles compromises breathing while invasion of muscles in the tongue and oesophagus results in difficulty in swallowing. There is an ongoing debate in the scientific literature concerning the popular belief that the consumption of whisky will protect against contracting *Trichinella*. Campbell and Blair (1974) refer to unpublished studies that indicate that pigs are protected from *Trichinella* if given a suitable oral dose of Irish whisky shortly after exposure. Similarly, the low morbidity and mortality associated with a mass outbreak of trichinellosis in Laos was ascribed to the excessive inebriation of those exposed to infection (Barennes *et al*., 2008).

3.6.2 Class Rhabdita

Genus Strongyloides Members of this genus have an unusual life cycle that alternates between generations that are in a free-living cycle and those that are parasitic. Both free-living and parasitic generations involve four larval stages and the production of egg-laying adult worms. However, in the free-living cycle, males and females are formed and sexual reproduction takes place while in the parasitic cycle only females are formed and they reproduce by parthenogenesis. Over 50 species of *Strongyloides* have been described, of which *Strongyloides stercoralis* is the best known (Grove, 1996). *Strongyloides stercoralis* is primarily a parasite of humans although other primates as well as dogs and cats can also be infected. In addition, there are several species that parasitise other vertebrates that can cause zoonotic infections in humans. For example, *Strongyloides fuelleborni fuelleborni* is a usually a parasite of non-human primates but also infects humans in parts of Africa and South-East Asia. Intriguingly, a closely related species *Strongyloides fuelleborni kellyi* is a parasite of humans in certain restricted regions of Papua New Guinea. *Strongyloides papillosus* parasitises rabbits, sheep and other ungulates, but the larvae will invade humans although they may not give rise to adult worms.

Strongyloides stercoralis has a cosmopolitan distribution and is a common infection wherever there is poor sanitation and people walk barefoot. It is estimated that anywhere from 30–100 million people are infected with the parasite (Olsen *et al*., 2009). The free-living larvae stages have a rhabditform pharynx and live in the soil where they feed on bacteria. A rhabditiform pharynx is one in which the muscular pharynx has a prominent posterior bulb that is separated from a smaller anterior swelling called the metacorpus (in appearance this is a bit like two clubs balancing on top of one another). After mating, the female produces eggs that hatch to release the first-stage larvae. The free-living cycle is sometimes referred to as ‘indirect’ or ‘heterogonic’ development. Some species of *Strongyloides* can undergo repetitive free-living generations in which the larvae develop into free-living adults, provided the conditions are suitable, but if the conditions are unsuitable, then the larvae develop into infective filariform third stage larvae (Figure 3.13). However, according to Streit (2008) *Strongyloides stercoralis* has only a single free-living generation and the larvae invariably develop into infectious third stage larvae that are 490–630 μm in length and have a long cylindrical pharynx. If a suitable host passes by, the larvae burrow into the skin and therefore infections are commonly acquired through walking in bare feet in areas contaminated with faeces. Infections can also take place if the place if the larvae are consumed in drinking water or with food. Those larvae that invade through the skin make their way to the veins and enter the venous circulation and are swept to the heart and thence to the lungs. The larvae then penetrate the alveoli and make their way up the bronchioles to the bronchi and thence to the trachea where they are swallowed. When they reach the duodenum the worms burrow into the intestinal villi and may penetrate the crypts of Lieberkühn. Here the females mature and start to produce dozens of

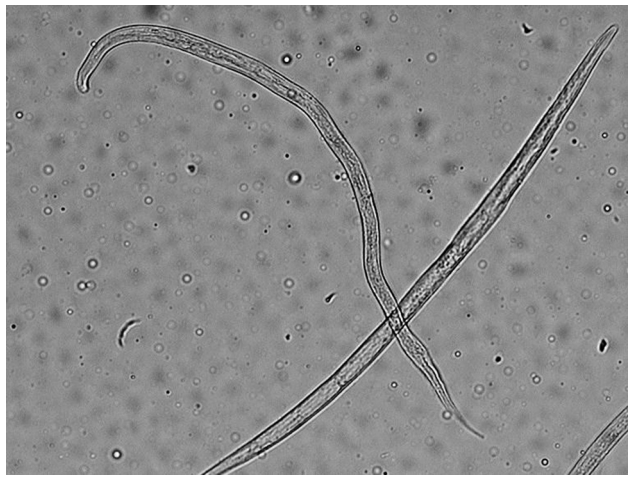

Figure 3.13 Larvae of *Strongyloides stercoralis*

eggs every day by parthenogenesis. The adult female worm is about 2.0–2.5 mm in length and 0.034 mm in width and has a long thin cylindrical oesophagus that is about a third the length of the body and lacks a posterior bulb. There is general consensus that none of the various species of *Strongyloides* produce parasitic males, although more work is required to confirm this. The process of invasion of the host and the subsequent production of parasitic adult worms is sometimes referred to as the 'direct' or 'homogonic' development. The eggs released by the female worms are already embryonated and usually hatch within the host's intestine to release first stage larvae. Some of these larvae pass out with the faeces where they either develop into free-living adults or into infective filariform third stage larvae. This begs the question of how parthenogenic reproduction can give rise to both male and female offspring, especially as *Strongyloides stercoralis* has a sex chromosome: females have six chromosomes (XX) while males have only five (XO). Certainly, environmental factors such as temperature, food availability, and population density can affect the development of the larvae either through previous impact on the mother or directly upon on the developing eggs or larvae. However, other factors such as the age and genetic constitution of the female worm and also the host immune response may also be important. More detail on this topic is provided by Streit (2008). Other first-stage larvae develop rapidly to the infective third stage and auto-infect the host by penetrating the intestinal mucosa or the peri-anal skin. These larvae then undergo visceral migration to the heart, lungs, and other organs, and become adults in the duodenum. Consequently, a host can remain infected for many years through repetitive auto-infection with successive generations of worms. Furthermore, an initial infectious dose of one or a few larvae can ultimately give rise to a large worm burden without the host being exposed to another external source of infection.

Strongyloides stercoralis can cause pathology at three points during its parasitic life cycle: (1) at the time of the initial penetration of the skin; (2) during migration through the lungs and other body tissues; and (3) as adults in the small intestine. Larval penetration through the skin can cause haemorrhage and an acute inflammatory response with resultant pain and swelling, while migration through the lungs can give rise to symptoms similar to asthma – although pulmonary symptoms are uncommon. Low worm burdens of adult *Strongyloides stercoralis* in the gut may cause few symptoms but as the numbers of worms increase, they can cause pain, nausea, diarrhoea,

intestinal bleeding, and obstruct the passage of digesta. Patients who are immuno-compromised can suffer from ongoing cycles of excessive auto-infection (hyperinfection) and the larvae may become disseminated around the body. This can result in the larvae invading other tissues such as the liver, kidneys, and central nervous system with debilitating and even fatal consequences. Hyperinfection can also occur in people who are not immuno-compronised and may be exacerbated by bacterial infections that cause septicaemia and bacterial meningitis (Smallman *et al*., 1986; Vadlamundi *et al*., 2006). The bacteria may invade through the damaged gut epithelium but it is more likely that the bacteria are transported into the body either by adhering to the cuticle of the invading larvae or by passing unharmed through their gut and being voided with their faeces.

Genus Ancylostoma This is one of the genera of nematodes that are commonly known as the 'hookworms'. There are several species belonging to the genus *Ancylostoma* although the best known of these is *Ancylostoma duodenale* that infects humans. Other important species include *Ancylostoma caninum* that is primarily a parasite of domestic and wild canines, *Ancylostoma tubaeforme* that usually infects cats, and *Ancylostoma braziliense* and *Ancylostoma ceylanicum* that both infect wild and domestic carnivores. The larvae of all these species can infect humans although they do not usually develop to adulthood.

The worms belonging to this genus have a well-developed buccal capsule that on its ventral margin is armed with 1–4 pairs of large chitinous 'teeth' which terminate in sharp backwardly curved points (Figure 3.14). Most authors state that it is these mouthparts that explain the name '*Ancylostoma*' which is derived from the Greek words '*ankylos*' (hook) and '*stoma*' (mouth). However, some authors state that the 'hook' actually refers to the fact that the worms have stout stiff bodies that are bent at the anterior end so that they resemble a crotchet hook. In common with other hookworms, male *Ancylostoma* have a well-developed copulatory bursa that has two broad lateral lobes and a small dorsal lobe and they also have two long needle-like spicules. Another explanation for the derivation of '*Ancylostoma*' is that the term was originally used in relation to a different genus of hookworm in which the rays of the male's copulatory bursa appear hook-like.

Ancylostoma duodenale has a widespread distribution and is found in Europe, Africa, Australia, Asia, China, and Japan and both North and South America. The larvae require a combination of moisture and a temperature of over 20°C in order to develop so it is less common in regions with cold or temperate climates. However, hookworm infection is a known risk for Northern European mineworkers because deep mines are hot and humid and the workers are often not provided with

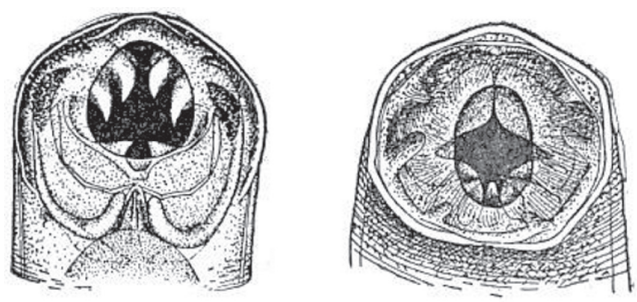

Figure 3.14 Diagrammatic representation of the mouth and buccal cavity of adult *Ancylostoma duodenale* (a) and *Necator americanus* (b) Source: Chandler and Read, 1961

toilet facilities underground (it can be over 1 km from the workface to the surface and mineworkers cannot be expected to make the trek every time that nature calls even if the management are willing to allow it – which they are not). The female worms are 10–15 mm in length and lay up to 30,000 eggs per day. The males are slightly smaller at 8–11 mm in length. The adults of both sexes have two large ventral teeth and one small ventral tooth on either side of the mouth.

Ancylostoma brazilense is found throughout the tropical regions of the world and is sometimes confused with *Ancylostoma ceylanicum*. The females are 7–10 mm in length and the males are 6–7.75 mm long. The adults can be distinguished by having one large and one small ventral tooth on either side of the mouth.

Ancylostoma caninum has a cosmopolitan distribution. The females are 14–16 mm in length while the males are 10–12 mm. The adults have a pair of triangular dorsal teeth and a pair of ventro-lateral teeth.

The adult worms live in the small intestine where they use their mouthparts to grasp onto the mucosa and lacerate the underlying tissue. The worms then feed on the blood that seeps from the wound. The worms do not remain in a fixed position but move around the gut and therefore leave wounds that continue to bleed. In addition, the worms consume more blood than they require and excrete much of it before it is completely digested. This inefficient process uses up a lot of the host's blood thereby increasing the risk of anaemia. There are separate male and female sexes and, after mating, the female releases eggs that are passed with the host's faeces. Most hookworm eggs are very similar in appearance and it is usually not possible to identify them to species. Provided they are maintained in a warm moist environment, the eggs continue to develop and then hatch after about 24–48 hours to release free-living first stage larvae. These larvae have a rhabditiform oesophagus and feed on bacteria and other material in the faeces and soil. In countries with warm climates fresh faeces instantly attracts large numbers of coprophagic insects that churn it up and in the process aerate it and incorporate the faecal material into the soil. This improves the conditions for the growth of the hookworm larvae. After 2–3 days the first stage larvae moult to the second stage and these then continue to feed before moulting after about 5 days to the infectious third stage. The third stage larvae are referred to as 'filariform' because their oesophagus lacks a terminal bulb or isthmus. They do not feed but there are sufficient food particles stored in their intestines to enable them to survive for several weeks. The third-stage larvae usually retain the cuticle shed by the second stage as a protective sheath (Figure 3.15) although this is lost when the worm invades a potential host. The infectious larvae position themselves in the top layer of the soil although they move up or down the profile depending upon how moist the soil is. When

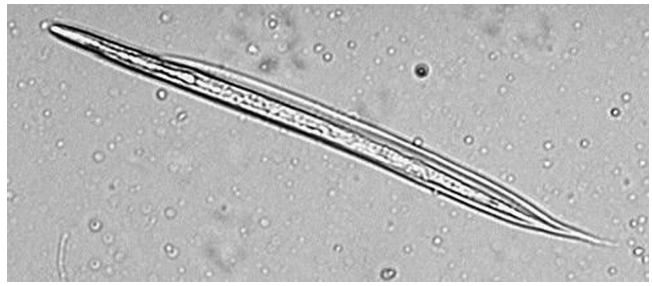

Figure 3.15 Infective larvae of *Necator americanus*. Note the sheath

a suitable host is detected, the larvae penetrate through the skin by a combination of physical boring and the release of chemicals. They are very thin and are easily able to make their way through cracks in the skin or penetrate the hair follicles. Once they have penetrated through the epidermis, they make their way to the blood vessels or lymphatic system and are transported in the circulatory system to the heart and thence to the lungs. The larvae then penetrate the alveoli and enter the air spaces after which they climb up the bronchioles to the bronchi and thence to the trachea, after which they are swallowed down the oesophagus. Once the filariform third-stage larvae reach the small intestine, they moult to the fourth stage that has an enlarged buccal cavity and attach to the intestinal mucosa. The fourth stage then moults to the adult stage and when they are mature, the worms mate and commence egg-laying. It typically takes about five or so weeks from initial invasion through the skin to egg-laying although in some species it takes much longer than this because the larvae enter a period of delayed development. Some texts indicate that adult *Ancylostoma* can live for several years but it is more probable that their lifespan is of the order of 6–12 months. Although infections can persist for many years, this could be explained by the periodic re-activation of larvae in arrested development (Loukas and Prociv, 2001).

Box 3.3 Arrested development (hypobiosis) in hookworm larvae

In some *Ancylostoma* species, a variable proportion of the migrating third stage larvae do not proceed to the lungs but disperse from the circulation into the skeletal muscles and enter a period of arrested development (hypobiosis). For example, the larvae of *Ancylostoma duodenale* may arrest their development for up to 38 weeks so that they do not become adults and commence egg-laying until the external environmental conditions are warm and moist enough for the survival of the thin-shelled eggs and free-living larvae. Similarly, some of the larvae of *Ancylostoma caninum* undergo developmental arrest while migrating through female dogs and are then re-activated during the host's pregnancy. These larvae then make their way to the mammary glands and are able to infect the pups through trans-mammary transmission over a period of about 3 weeks after birth. There is circumstantial evidence of trans-mammary transmission of *Ancylostoma duodenale* in humans. At the time of writing there is not yet good evidence for trans-placental transmission. Interestingly, the *Ancylostoma caninum* larvae are not directly reactivated by changes in hormonal titres in the pregnant bitch. Instead, they respond to the up-regulation of transforming growth factor-β in the uterus and mammaries brought about by oestrogen and prolactin (Arasu, 2001). The return to development is mediated through cyclic guanosine monophosphate (cGMP) in a similar manner to the mechanisms by which the free-living nematode *Caenorhabditis elegans* returns to normal development from the resting dauer larval stage (Hawdon and Datu, 2003). Arrested larval development is a feature of parasitic nematodes belonging to several genera and is an important consideration in determining their treatment and control. If these nematodes share a common basic mechanism, it is possible that *Caenorhabditis elegans* would be a suitable laboratory model for studying the factors that govern the onset into/emergence from arrested development. Prolactin stimulates the reactivation of arrested *Toxocara canis* larvae and thereby facilitates their transmission in the mother's milk but it is not yet certain whether this is a direct effect or indirectly through up-regulating the production of transforming growth factor-β as occurs in *Ancylostoma caninum* (Jin *et al*., 2008).

Hookworms cause pathology at three main points in their life cycle: during the initial invasion through the skin, during their migration through the body, and as adults consuming blood in the small intestine. Larvae that penetrate their normal definitive host do not usually cause a serious host reaction although they may introduce bacteria that set up a localised acute inflammatory response. In humans, penetration usually takes place through the feet although other parts of the body may be affected, for example, if the person was sitting, kneeling or lying on the ground. Humans who are infected by the larvae of *Ancylostoma caninum*, *Ancylostoma braziliense* and some of the other species that are normally parasites of other animals can suffer from a condition known as cutaneous larvae migrans. These larvae are unable to move beyond the basal layer and wander around just underneath the skin surface for a period of weeks or even months until they are eventually killed by the immune system. While they are wandering, they produce long serpentine tracks that are intensely itchy and painful as a consequence of the physical damage coupled with the acute immune response. The damaged tissue is often secondarily infected by bacteria that are either introduced by the worm or as a consequence of scratching and this exacerbates the pathology. The pathology caused during migration through the lungs, like that of the adults in the intestine, is very dependent upon the number of worms involved. Small numbers may cause so little damage that there are no clinical signs while large numbers can be fatal. If large numbers of larvae make their way through the lungs at the same time, they can cause respiratory distress and pneumonitis. The consequences of infection with adult worms depend upon the numbers involved and the nutrition of the host. An adult *Ancylostoma duodenale* consumes about 0.26 ml blood day^{-1} so the presence of a few worms has little impact on health and low worm burdens are even considered by some people to be beneficial to the maintenance of a healthy immune system. However, as the numbers of worms rise, then the amount of blood lost can quickly become significant – especially if the host does not have a nutritious diet. The loss of blood results in iron-deficiency anaemia and the faeces can turn red or black depending upon the amount of blood loss. Hookworm infection is also responsible for a range of other symptoms (e.g. fatigue, weakness, pallor), most of which can be linked to the consequences of iron-deficiency anaemia. One of the more unusual symptoms is 'reverse iris-dilation reflex' in which the pupil of the eye dilates in response to strong light rather than contracting. Hookworm infection has also been suggested to stimulate the consumption of soil, known as 'geophagia' or 'pica'. Chronic infections in children can result in stunted physical growth and mental retardation.

Genus Necator This genus of hookworms can be easily distinguished from those of the genus *Ancylostoma* from the structure of the mouthparts. The adults of *Necator americanus* are equipped with a pair of ventral cutting plates rather than teeth (see Figure 3.15) and at the base of the buccal capsule there is a pair of ventral teeth and a pair of subdorsal teeth. In addition, the copulatory bursa in the males is longer and narrower and the rays have a different arrangement; in the females the vulva is situated more towards the anterior of the worm. *Necator americanus* is somewhat smaller and more slender than *Ancylostoma duodenale*: the female worms average 10–11 mm in length while the males are typically 7–8 mm.

In Latin, '*necator*' is a noun that is translated as a 'murderer' or 'slayer'. *Necator americanus* can therefore be taken to mean 'Slayer of Americans'. However, the worm is anything but prejudiced in its relationships with humans. Indeed, it is the commonest hookworm parasite that afflicts humans with a widespread distribution in parts of North and South America, Africa, Asia, India, Australia, and south-west Pacific Islands. However, it requires even warmer (25–28°C) conditions than *Ancylostoma caninum* to complete the free-living stages of development and

therefore it is limited to regions with hot/tropical climates. Co-infections of *Necator americanus* and *Ancylostoma duodenale* can occur, but usually one of the two species is more abundant than the other. One of the theories of how humans came to colonise the Americas is that they migrated across the Behring land bridge. This would mean that any parasites which could not survive in the cold environmental conditions should, theoretically, be left behind. *Necator americanus* requires very warm conditions for transmission and therefore it has been suggested that *Necator americanus* was actually introduced into the Americas during the period of colonisation by Europeans and particularly as a consequence of the slave trade. However, native Paraguayan Indians living in comparative isolation have been found to suffer from *Necator americanus* and this is being used as evidence that human colonisation of South America may also have been accomplished by trans-Pacific migration of Asiatic populations during pre-Columbian times (Araújo *et al*., 1988).

The life cycle of *Necator americanus* closely resembles that of *Ancylostoma* but the larvae do not undergo hypobiosis and the adult worms can live for 5 or more years. It is primarily a parasite of humans and although it consumes less blood than *Ancylostoma duodenale* – typically 0.05 ml $worm^{-1}$ day^{-1} – large worm burdens can develop in certain people and it can then cause serious disease. Nevertheless, in regions in which the parasite is endemic, many people harbour relatively small worm burdens that cause little harm and Pritchard and Brown (2001) have even argued that it may be close to evolving a mutualistic relationship with us. In those people who harbour large worm burdens, the pathology of *Necator americanus* closely resembles that of *Ancylostoma*.

Genus Ascaris Members of this genus are large robust worms that are several centimetres long. For example, female *Ascaris suum* can grow to 41 cm in length and 5 mm in width while the males grow to 25 cm in length and 3 mm in width (some texts state that they can grow even larger). The cuticle is thick and covered in numerous fine striations. The body is rather rigid and has a cream or pale pinkish coloration. The adult worms have three curved lips and on the inner edge of each of them is a single row of tiny teeth (denticles). The oesophagus is a simple cylindrical muscular tube and does not have a bulb. The posterior of the male is curved and lacks a bursa although there are numerous papillae in the vicinity of the cloaca that probably serve as sensory structures during copulation. The male has two simple spicules that are about 2 mm in length. The vulva of the female opens in the anterior third of the body and leads to two uteri that occupy the bulk of the internal space of the worm.

This genus contains two of the best-known parasites: *Ascaris lumbricoides* that infects humans and *Ascaris suum* that infects pigs. For many years there was a debate about whether these were two distinct species and, if so, the extent to which they were host-specific. It is now generally accepted that they are two distinct species and although they are relatively host-specific, they can infect other mammals but may not reach sexual maturity in them. For example, there are occasional reports of *Ascaris suum* infecting sheep (Gunn, 1980). Older literature needs to be treated with care because the distinction between two species was not clear and consequently there are often references to *Ascaris suum* in humans and *Ascaris lumbricoides* in pigs.

Ascaris lumbricoides has a cosmopolitan distribution and is common wherever there is poor sanitation. Some estimates suggest that as many as 1.5 billion people are infected (Chan, 1997). Infection usually peaks during childhood and early adolescence. As with many parasites, within a population a small number of individuals are often much more heavily infected than everyone else. This is to a large part a consequence of genetic susceptibility (Williams-Blangero *et al*., 1999). The adult worms live in the small intestine where they consume the digesta; they may consume blood if it is leaking from an existing wound but there is limited evidence to indicate

that they 'intentionally' damage the gut mucosa to feed on the host tissues or blood. After mating, the female worms release their eggs and these are passed with the host's faeces. The reproductive potential of *Ascaris lumbricoides* is phenomenal: a single female worm may contain up to 27 million eggs at any one time and lay over 200,000 eggs day^{-1} (Brown and Cort, 1927). The eggs are roundish to oval in shape (45–75 μmm long × 35–50 μm wide) with a thick clear inner shell and a lumpy outer layer that is variously called the 'mammilated', 'uterine', 'albuminous', or 'proteinaceous' layer. This outer layer is often stained yellow or brown due to incorporating bile from within the digesta. When it is passed with the faeces, the egg contains a well-defined roundish cell. Unfertilised eggs are often seen in faecal samples and these usually have a more elongated profile, their outer mammilated layer is either pronounced or absent and the contents of the egg are amorphous. These unfertilised eggs are incapable of further development. The fertilised eggs are extremely resistant to environmental conditions and can survive for at least 5 years under suitable conditions although they are susceptible to desiccation and exposure to UV light. Studies on *Ascaris suum* have shown that embryonation and the early stages of larval development take place outside the host. Within the egg the first stage larva moults to the second stage and then to the third stage – and it is this which is the infectious stage (Kirchgäβner *et al*., 2008). However, some earlier workers state that the infectious stage is the second instar and the moult to the third instar takes place in the liver (e.g. Douvres *et al*., 1969). It is highly likely that *Ascaris lumbricoides* shows a similar sequence of development stages as *Ascaris suum*. Development to the infectious stage takes about 9–13 days under ideal conditions and consequently fresh faeces is not an immediate source of infection. Transmission is through passive faecal-oral contamination. The eggs hatch in the host's intestine and the larvae then burrow through the mucosa and enter the venous or lymphatic circulation. The larvae usually reach the liver via the hepato-portal blood supply about 24 hours after the eggs hatch and after travelling through the hepatic venules, they are transported to the right heart. Once they reach the heart, the larvae enter the pulmonary circulation. When the larvae reach the lungs, they are about 1.8 mm in length and burrow through the walls of the capillaries and enter into the air spaces. The larvae then make their way up the bronchial tree to the trachea and are then swallowed down the oesophagus. Once the larvae reach the small intestine, they moult to the adult stage, mate and commence egg-laying. The time between initial infection and the adult worms commencing egg production is in the region of 8–12 weeks. Most of the work on larval migration has been done on *Ascaris suum* and there is some variation in the literature about the sequence of developmental stages. Douvres *et al*. (1969) state that the migrating larvae reach the small intestine still in the third stage and subsequently moult to the fourth stage and then moult again to become adults. By contrast, Roberts (1934) states that the larvae moult to the fourth stage while still in the lungs and those that remain at the third stage are killed by the acidic pH of the stomach. The reason why some gastrointestinal nematodes undertake apparently pointless migrations around their host's body only to arrive back at the point where they began remains a mystery. Some workers consider it to be a 'phylogenetic reminiscence' but Read and Skorping (1995) have proposed that gastrointestinal nematodes whose larvae undergo a period of migration through the host's tissues are able to grow faster than those parasite species that remain solely in the gut lumen.

Ascaris lumbricoides and *Ascaris suum* mainly cause pathology at three distinct points during the life cycle: (1) during migration through the liver; (2) during migration through the lungs; and (3) as adults in the small intestine. Large numbers of larvae moving through the intralobular veins can cause haemorrhage and tissue damage. In pigs this gives rise to a condition known as 'white spot' and results in the liver being condemned as unfit for human consumption. More serious

pathology results in the lungs when the larvae penetrate into the air spaces. This can cause a condition known as Loeffler's pneumonia (pneumonitis) and in severe cases this can be fatal. The consequences of infection are dependent upon the number of worms involved and whether they go wandering. Large numbers of worms can cause potentially fatal intestinal blockage (see Colour Plate 27) but more normally the host suffers from malnutrition and digestive upsets. In young children the consequences of malnutrition can lead to stunted growth and cognitive impairment. Wandering worms can cause lead to appendicitis, blockage of the bile duct, or cause enormous psychological trauma when they are coughed up or the worm attempts to exit via the nose.

3.6.3 Family Onchocercidae

The parasites belonging to this group are commonly known as 'filarial nematodes' as a consequence of their characteristic larvae. There are about 80 genera within the family and the adult worms are tissue parasites that live in mammals, birds, reptiles and amphibians although not fish. Most, but not all, species are in a symbiotic relationship with *Wolbachia* bacteria that is essential for reproduction to take place and is also responsible for some of the pathology (Hise *et al*., 2004). The adult worms are usually long and slender and have a long cylindrical pharynx. The eggs hatch in the uteri of the females to release 'microfilariae' – these are not as developed as the first larval stage of other nematode species and some workers describe them as 'pre-larval' or 'advanced embryos'. In some species the microfilariae are enclosed in a 'sheath' that is derived from the egg membrane and is therefore distinct from the 'sheath' of third-stage nematodes such as *Ancylostoma duodenale*, in which it is formed by retaining the cast cuticle of the second juvenile stage. One of the ways of distinguishing between microfilariae is by the presence or absence of a sheath. For example, the microfilariae of *Mansonella* and *Onchocerca* do not have sheaths while those of *Wuchereria* and *Brugia* are sheathed (see Figure 9.3 on page 328). The larvae are transmitted by blood-feeding insects that also act as intermediate hosts within which development to the infectious stage takes place.

Although the majority of species within the family Onchocercidae are parasites of wild animals, it includes the important genera *Brugia*, *Dirofilaria*, *Loa*, *Mansonella*, *Onchocerca*, and *Wuchereria*. Details on the molecular taxonomy of filarial nematodes are covered by Morales-Hojas (2009).

Genus Wuchereria *Wuchereria bancrofti* is found in tropical and sub-tropical regions of Africa, India, China, Indonesia, South America, and certain Eastern Pacific Islands. Humans are the sole definitive host but over 70 species and sub-species of mosquito are known to be able to act as intermediate host and vector. The adult worms live tightly coiled together so that they form nodule-like masses within the afferent lymphatic ducts – especially in the lower parts of the body. The female worms grow up to 10 cm long but are only 0.3 mm wide while the male worms are 4 cm long and 0.1 mm wide (Figure 3.16). The female worm releases thousands of microfilariae into the lymph every day and these are then transported to the thoracic duct and enter the blood circulation. The microfilariae are 244–296 μm in length and move actively but they lack a functional gut and do not feed. The microfilariae exhibit marked periodicity in their appearance in the peripheral circulation that is related to the biting habits of the local mosquito vector(s). The appearance of microfilariae in the circulation is sometimes called 'microfilaraemia'. After being ingested by the mosquito, the microfilariae penetrate the intestine and make their way to

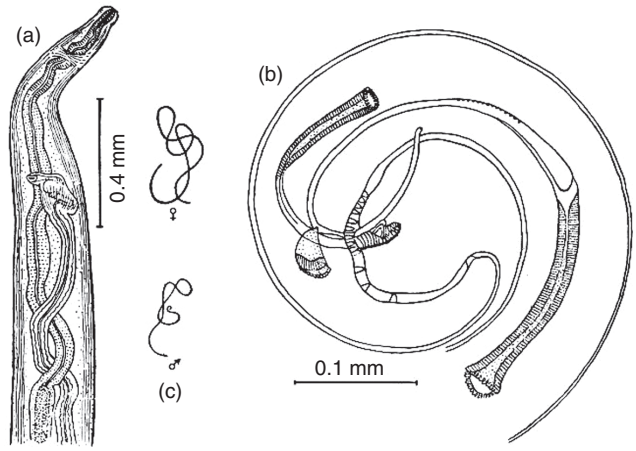

Figure 3.16 *Wuchereria bancrofti*: (a) = anterior of adult female worm; (b) = posterior end of adult male worm; (c) = adult male and female worms, natural size. Source: Chandler and Read, 1961

the thoracic flight muscles. Here they develop into the infectious third stage over a period of 7–21 days and during this time they damage the flight muscles. Over part of its range, *Wuchereria bancrofti* co-occurs with *Plasmodium falciparum* and they share the same vector mosquitoes (e.g. *Anopheles gambiae* and *Anopheles funestus*). In a survey in Kenya, Muturi *et al*. (2006) found that a small percentage (~1.1–1.6%) of mosquitoes were naturally infected with both parasites. Although mosquitoes infected with both *Plasmodium* and *Wuchereria* had higher sporozoite rates, they also had a higher mortality and therefore co-infections would appear to be deleterious to the chances of transmission for both parasites.

Once they are mature, the infectious larvae migrate to the mosquito's proboscis and from there they enter the definitive host when the mosquito feeds. The infectious larvae are 1.4–2.0 mm in length at this stage and after entering the host's circulation, they make their way to the peripheral lymphatic system and ultimately locate themselves in lymph vessels distal to the lymph nodes. They then continue their development to the adult stage, mate, and commence the production of microfilariae. The microfilariae can be detected after 8–12 months after the initial infection although it is not unusual for disease symptoms to develop in the absence of microfilariae in the circulation.

The pathology associated with *Wuchereria bancrofti* is to a large part associated with the immune reaction against the symbiotic *Wolbachia* bacteria (Hise *et al*., 2004). Blockage of the lymph drainage can lead to elephantiasis. Strictly speaking, 'elephantiasis' means to be 'afflicted by elephants' but is really a reference to the enormous swelling that occurs in the affected limbs (see Chapter 7).

Genus Brugia There are several species within this genus although *Brugia malayi* is the most important. As the species name intimates, this parasite is found in Malaya but it also occurs in India, Sri Lanka, Thailand, the Philippines, China, Korea, and Japan. Between them, *Wuchereria bancrofti* and the various species of *Brugia* are estimated to infect over 120 million people and over 40 million of these suffer from serious debilitating diseases (Anon, 2007b). The adult worms of *Brugia malayi* are very difficult to distinguish from those of *Wuchereria bancrofti* and

they also parasitise the lymphatics. However, although they also produce sheathed microfilariae, these can be distinguished from those of *Wuchereria* by being smaller (177–230 μm) and from the positioning of the nuclei in the tail region: in *Brugia malayi* the nuclei extend to the tip of the tail while in *Wuchereria bancrofti* they do not (see Figure 9.3 on page 328). The life cycle is similar to that of *Wuchereria bancrofti* but a key difference is that the adults are able to parasitise a range of animals including monkeys, pangolins (*Manis* spp.), and some wild felids. It is therefore a zoonotic parasite with a range of reservoir hosts and this can make control more difficult. It develops faster than *Wuchereria bancrofti* both within the mosquito vector and the definitive host: microfilariae can appear in the peripheral circulation in as little as 3 months after the initial infection. The pathology is similar to that of *Wuchereria bancrofti*.

Genus Onchocerca There are several species in this genus but it is *Onchocerca volvulus* that is the best known. *Onchocerca volvulus* is of medical importance as the cause of onchocerciasis that is more commonly known as 'river blindness'. *Onchocerca volvulus* is solely a parasite of humans and it is estimated to afflict about 18 million people (Anon, 2007b). It is particularly common in parts of Africa (e.g. Ghana, Uganda, Nigeria, Congo, Chad) but it is also found in certain regions of the Middle East (e.g. Yemen), and Central America (e.g. Mexico, Ecuador, Colombia, and Venezuela). The parasite was almost certainly introduced into South America as a consequence of the slave trade but has since evolved distinctive local characteristics. Adult female *Onchocerca volvulus* are up to 50 cm in length and 0.4 mm in width while the male worms are much smaller – 3–5 cm long and 0.2 mm in width. They live in pairs or groups tightly coiled together in the subcutaneous tissues. This can give rise to raised nodules on the scalp, torso, and limbs that can be anywhere from 0.5–10 cm in size. In Africa most of the nodules develop below the waist, while in Central America they tend to form above the waist. This variation is explained by the biting behaviour of the local blackfly vectors. The female worms release microfilariae but unlike many of the strains of *Wuchereria bancrofti* and *Brugia malayi*, these do not exhibit periodicity and they are not sheathed. The microfilariae are 220 – 360 μm in length and tend to remain within the subcutaneous tissues (Figure 3.17) in which they can live for up to a year. In common with other members of the genus, *Onchocerca volvulus* is transmitted by various species

Figure 3.17 Smear taken from a subcutaneous nodule formed by *Onchocerca volvulus*. The aspirate contains numerous microfilariae. Source: Kean *et al*., 1991

of blackfly (*Simulium* spp.). The larvae of these flies live within fast-flowing streams and rivers and therefore the parasites are also predominantly found in their locality. However, the adults of some species can migrate over 500 km from their site of emergence through being borne aloft on seasonal winds. Like mosquitoes, the females of many *Simulium* species need to take a blood meal in order to mature their eggs although there are a few species which do not, and these, obviously, are not important in disease transmission. Male blackflies do not feed on blood and are therefore not important in disease transmission (apart from fertilising the female flies). Female blackflies have short mouthparts and are pool-feeders: they acquire the microfilariae – which are in the tissues – while lacerating skin and imbibing the blood and serum that oozes to the surface. The microfilariae then penetrate their gut and make their way to the thoracic flight muscles where they develop to the infectious third stage. This typically takes about 6–12 days. The infectious larvae then make their way to the fly's mouthparts and are transferred to the definitive host the next time the blackfly feeds. Female blackflies typically feed only every 3–5 days and therefore they do not usually transmit an infection until at least their third blood meal. After invading a human host, the infectious larvae of *Onchocerca volvulus* moult to the fourth stage after about 7 days and then moult again to become adults after which they mature over a period of 12–18 months. The adult worms are very long-lived and can survive for up to 15 years.

The pathology associated with *Onchocerca volvulus* is mostly associated with the immune reaction against the microfilariae and includes blindness, 'leopard skin', and 'hanging groin' (see Chapter 7). Onchocerciasis has also been associated with the development of epilepsy in parts of West, Central and East Africa (Pion *et al*., 2009) although at the time of writing a causative relationship had not yet been established. Onchocerciasis has also been suggested as a factor involved in the development of the childhood disease 'nodding syndrome' that in 2011 was spreading rapidly throughout parts of southern Sudan and northern Uganda. Nodding syndrome causes seizures, stunted growth, and cognitive impairment. It gets its name from the way children experiencing seizures temporarily lose muscle tone in their neck so that their head rocks backwards and forwards every 5–8 seconds. The cause of the disease is not known and while there is not a clear link with infection by *Onchocerca volvulus*, there is increasing evidence that it could be involved (Kaiser *et al*., 2009; Winkler *et al*., 2008). The microfilariae do not appear to enter the brain but it is possible that they could be triggering an autoimmune reaction that results in nerve damage.

Although *Onchocerca volvulus* is essentially a 'tropical disease', there are a variety of other species of *Onchocerca* that afflict animals other than humans that occur in temperate countries. For example, *Onchocerca gutturosa* is a relatively common parasite of cattle in Europe and elsewhere in the world in which it causes raised nodules on the back ears and neck. It is not, however, considered to be pathogenic.

3.6.4 Family Dracunculidae

This family includes a number of species that are parasitic on wild animals but the best known is *Dracunculus medinensis*, commonly known as the Guinea worm. This parasite has a long history but a bleak future. Some people believe that *Dracunculus medinensis* was the 'fiery serpent' that afflicted the Jewish people while Moses was leading them through the Sinai wilderness to the Promised Land (Numbers XXI. v. 4–8). However, this is disputed by some workers not least because according to the Biblical account 'many of the people of Israel died' and while *Dracunculus*

does cause serious morbidity, it has a low mortality. Nevertheless, there are many more convincing accounts from antiquity that *Dracunculus medinensis* has been a serious problem for thousands of years (Adamson, 1988). Until recently it was a common problem in many parts of Africa, the Middle East, India and Pakistan but a highly effective control programme has reduced it to just a few villages in six Sub-Saharan countries (Solomon *et al*., 2009). Although the aim to eradicate the disease entirely by 2009 was not met, there were fewer than 1,800 cases in 2010 and it will probably not be long before *Dracunculus medinensis* becomes extinct.

Dracunculus medinensis causes the condition known as 'dracunculiasis' – that can be translated as 'afflicted by little dragons' – which is an apt description of what it feels like to be unfortunate enough to be parasitised by the worm. The adult worms live in the subcutaneous tissues and in particularly in those regions that are most likely to come into contact with the water such as the lower legs, ankles, and feet although other parts of the body can also be afflicted (Figure 3.18). The females grow to an enormous length and worms of 80 cm have been extracted although they are very thin – typically about 1.7 mm in width. The males are much smaller and are thought to be typically 1– 4 cm long although very few have actually been described. It is not known at what point in the life cycle that the male and female worms copulate but the scarcity of observable males may be a consequence of them dying soon after mating. The eggs hatch within the uterus of the female worm to release first-stage larvae that are 500 – 750 μm in length. In the older literature some authors refer to these larvae as microfilariae: the genus *Dracunculus* was once incorporated with the filarial nematodes but has since been moved to a totally separate sub-order – the Camallanina. The vulva is non-functional in gravid females and therefore the larvae are unable to exit this way. Instead, the high internal body pressure within the worm causes its cuticle and uterus to rupture near to the anterior end and thereby releases the larvae. The larvae induce a pronounced immune response but there are as yet no reports indicating that the worms have a symbiotic relationship

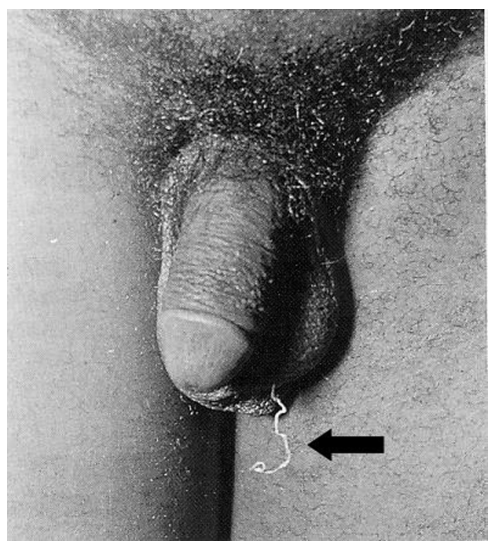

Figure 3.18 Adult *Dracunculus medinensis* emerging from the scrotum. This is an unusual site for the adult worm – usually they emerge in the lower leg. Source: Kean *et al*., 1991

with *Wolbachia* or any other bacteria. The inflammatory reaction must therefore be a consequence of excretory/secretory substances released by the parasites. The inflammatory response results in the formation of an ulcer (also sometimes referred to as a 'blister' or a 'bulla') that ultimately bursts and the larvae escape through the wound. If the wound is immersed in water, it stimulates the worm to contract and this pushes more larvae out of the uterus. While the ulcer is underwater, part of the uterus or worm's body often extends out of the ulcer but this either contracts back in when the affected body part is withdrawn or the exposed region shrivels and prevents further release of larvae. As the uteri release larvae, the empty regions atrophy and the posterior sections are pushed forward until the worm has released its full complement of larvae. At this point the worm dies and the ulcer heals. A traditional means of treating the condition was to grasp the part of the worm extending from the ulcer and wrap it around a thorn or (in the modern era) matchstick. The worm would then be slowly wound out a few centimetres a week over a period of time. This is a potentially high risk treatment since if the worm should break, it would die *in situ* and this could cause a serious immune reaction as its body decomposed and the wound often became infected with bacteria.

The larvae have to be released underwater if they are to survive. They are unable to feed and die within three days unless they are consumed by an appropriate species of *Cyclops*. *Cyclops* spp. are common freshwater Crustaceans belonging to the Copepoda. The first stage larvae penetrate the gut of the *Cyclops* and develop within the body cavity to the third stage: this usually takes about 3 weeks. The life cycle is completed when an infected *Cyclops* is accidentally consumed with drinking water. The larvae are released from the Cyclops when it is digested in the host's gut and they penetrate the gut wall and make their way to the subcutaneous tissues and then migrate to the axillary and inguinal regions. During this time they develop into adults and mating takes place. After about 8–10 months the fertilised female worms migrate to their final position in the subcutaneous tissues. It takes about 12 months between initial infection and the release of first-stage larvae.

Perhaps counter-intuitively for a parasite with an aquatic intermediate host, *Dracunculus medinensis* is (or rather was) a particular problem in regions with low rainfall. This is partly a consequence of the *Cyclops* growing best in still stagnant water. In addition, it is common practice for villagers without piped water to walk into a pond or step well (Figure 3.19) in order to draw water. This means that the adult worms are able to release their larvae from ulcers on a person's feet or ankles. Furthermore, *Dracunculus medinensis* is a zoonotic parasite and can also infect dogs, horses, cattle and a number of other animals and these can act as a reservoir of infection if they are also able to similarly walk into the local water supply. The parasite has been controlled by a concerted education campaign in which villagers are encouraged to filter their drinking water to remove potentially infected *Cyclops*. In addition, funds have been made available to modify wells and other sources of drinking water so as to prevent humans and animals from walking into them and thereby being infected by the *Cyclops* (Reynolds, 2007).

The pathology associated with *Dracunculus medinensis* infection occurs as a consequence of three processes: (1) migration of the adult worms to their 'emergence site'; (2) failure to migrate to the 'emergence site'; and (3) through the formation of an ulcer at the 'emergence site'. The migration of the adult female worms induces an allergic reaction that results in pain, nausea, and diarrhoea. Worms that fail to migrate ultimately die and often become calcified. The consequences of this depend upon the location of the worm. Sometimes there are no harmful effects but the decomposition of a worm close to joints or important nervous tissue can result in arthritis and

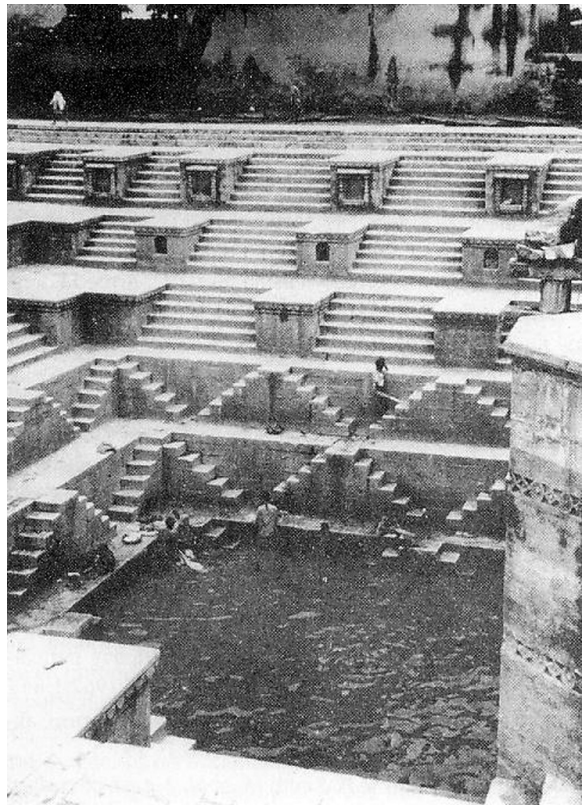

Figure 3.19 Traditional step well in Rajasthan, India. Most villagers walk barefoot and in the process of climbing down the well it is almost inevitable that their feet and lower limbs will be immersed in the well-water. Source: Kean *et al*., 1991

even paralysis. The formation of ulcers is extremely painful and debilitating and the ulcers can become secondarily infected by bacteria.

Questions

1. Briefly describe how one could distinguish between a trematode and a nematode on the basis of their morphology.
2. State three ways in which schistosomes differ from other trematode parasites.
3. *Opisthorchis felineus* infections are being increasingly reported in parts of Europe. Why is this, and should it be a cause for concern?
4. What aspect of the life cycle of *Hymenolepis nana* makes it different from all other tapeworms?
5. Why are large tapeworms less likely to produce metacestodes that undergo asexual reproduction?

6. What are the morphological differences between a typical adult *Taenia* tapeworm and an adult Acanthocephalan?
7. Compare and contrast the life cycles of *Trichuris trichiura* and *Trichinella spiralis*.
8. *Onchocerca volvulus* causes a condition known as 'river blindness'. Briefly explain the association between *Onchocerca* and rivers.
9. Why is an infection with 100 *Ancylostoma duodenale* potentially more serious than an infection with 100 *Necator americanus*?
10. Briefly explain how an understanding of the life cycle of *Dracunculus medinensis* has enabled it to be effectively controlled.

4

Arthropod parasites

4.1 Introduction

In terms of abundance, numbers of species, total biomass, and exploitation of ecosystems, the arthropods represent the most successful metazoan animals. Over a million species of arthropod have been taxonomically described and there are probably several times as many awaiting the attentions of biologists willing to study them. Some estimates suggest that there are currently about 1×10^{18} individual arthropods living at any one time and there is a high possibility that some of them will be living on us. As a group, modern-day arthropods are generally small creatures with only a few marine species such as the giant spider crab (*Macrocheira kaempferi*) reaching any appreciable size. However, 460–248 million years ago they included the Eurypterid 'water scorpions' which were among the largest creatures alive at the time and grew up to 2.5 metres in length.

Arthropods share many common morphological and developmental features and these are listed in Table 4.1. All arthropods share some of these traits – such as a coelom and a hardened exoskeleton – but as might be expected from such an abundant and diverse group of creatures, there are always some species that do not possess all the traits. For example, spiders (Aranea) have well-developed eyes but they do not have compound eyes. Similarly, some species of parasitic arthropod have become so modified that it can difficult to determine their taxonomic position from their external morphology.

The taxonomy of the arthropods is currently in a state of flux. To begin with, there is some uncertainty whether the Arthropoda is a monophyletic or polyphyletic clade. That is, whether the current members are derived from a single ancestor or from two or more different ancestors. The monophyly hypothesis is based on the observation that the arthropods share numerous morphological features and there is also some DNA evidence that indicates a common molecular heritage. By contrast, the polyphyly hypothesis is based on the fact that the current arthropods have many morphological and developmental differences. Furthermore, it is argued that many of the 'similarities' might have arisen because of convergent evolution. For example, if an organism has a hard exoskeleton, the only way it could move would be with specialised jointed appendages.

To further complicate taxonomic considerations, there is mounting molecular evidence for a new clade called the Ecdysozoa that incorporates all invertebrates that grow by moulting (ecdysis) (Aguinaldo *et al*., 1997). The Ecdysozoa would therefore comprise all of the arthropods, tardigrades, onychophorans, nematodes, nematomorphs, kinorhynchs and priapulids. This would suggest that there is a closer taxonomic relationship between a nematode and a butterfly than there is between a nematode and an earthworm. This might sound counter-intuitive but new techniques such as 'express sequence tag analysis' are rendering increasing support (Philippe *et al*., 2007).

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Table 4.1 Morphological and developmental features exhibited by the majority of arthropods

Features common to the majority of arthropods
Coelom (haemocoel is the primary body cavity)
Hardened chitinous exoskeleton (cuticle)
Moulting (ecdysis) of exoskeleton to allow growth
Foregut and hindgut lined with cuticle
Jointed appendages
Specialised body segments and appendages that interact with bundles of muscles
Compound eyes
Metamorphosis

Within the Arthropoda there is considerable uncertainty as to how the various groups relate to one another. For example, a common means of dividing the living arthropods is to recognise three phyla. These are the Chelicerata that includes the scorpions, spiders, and ticks; the Crustacea that includes the barnacles, lobsters, and crabs; and the Uniramia that includes the centipedes, millipedes (Myriapoda), and insects (Hexapoda). Unfortunately, this nice simple arrangement no longer appears to be tenable. Molecular evidence indicates that the Chelicerata could remain as a distinct entity. However, the relationship between the various crustaceans, myriapods, and insects is less certain. It would appear that the phylum Crustacea is not a monophyletic group (i.e. its current members are not derived from a common ancestor) and the insects are actually much more closely related to two obscure groups of Crustacea called the Remipedia and the Cephalocarida than they are to the millipedes and centipedes. Furthermore, the millipedes, centipedes, and other myriapods are closely related to one another and would probably be best considered as a distinct group within the Arthropoda (Regier *et al*., 2010). These issues will not be resolved for a long time and for the purposes of this book we will consider three taxonomic divisions: Chelicerata, Crustacea, and Hexapoda (Insects).

4.2 Phylum Chelicerata

The phylum Chelicerata consists of three somewhat disparate classes. The class Merostoma contains the horseshoe ‘crabs’; class Arachnida contains the scorpions, spiders, ticks, and mites; and class Pycnogonida contains the sea ‘spiders’. Members of the Chelicerata exhibit the basic arthropod characteristics but share several morphological characteristics that distinguish them from other arthropods (Table 4.2).

From a parasitological perspective, the most important members of the Chelicerata are the Acari. This includes the organisms that are commonly known as the mites, ticks, and chiggers. The Acari are also sometimes referred to as the ‘Acarina’ and the study of the Acari is known as ‘Acarology’. The Acari contains several thousand species that exhibit every possible lifestyle and occupy most ecosystems. There are ongoing disputes about the taxonomy of the Acari although one of the more widespread arrangements is to recognise two divisions (Table 4.3). Although the Acari is sometimes referred to as a single order, it is probably polyphyletic and its various members evolved from various other groups of arachnids. Most Acari are tiny creatures difficult to see with the naked eye and some are even smaller than large protozoa. The largest of the Acari

Table 4.2 Distinguishing morphological characteristics of the phylum Chelicerata

Morphological characteristics of chelicerates
Body divided into two regions: the cephalothorax (prosoma) and abdomen (opisthosoma)
Cephalothorax divided into eight segments.
No appendages on the first cephalothorax segment.
Chelicerae attached to second cephalothorax segment. Chelicerae are specialised appendages that are used for various functions in different species.
Pedipalps attached to third cephalothorax segment. Pedipalps are specialised appendages that perform different functions in different species.
Four pairs of walking legs (in adult) attached to cephalothorax segments 4–7. Some species, especially parasitic species, have only two or three pairs of legs as adults.
Lack antennae.

only reach about 2 cm in length and often owe their size to having ingested large quantities of their host's blood. Regardless of their lifestyle, most of the Acari feed by ingesting fluids and their mouthparts are adapted for this purpose. Their chelicerae are usually pincer-like or needle-like while their pedipalps take the form of leg-like or pincer-like appendages although in some they have regressed to vestigial structures that probably serve little if any function. It is often difficult to make out the two body divisions because the prosoma and opisthosoma have fused and/or the dorsal (upper) surface is covered by a hard chintinous plate.

The Acari tend to have simple life cycles and separate sexes. After mating, the female lays eggs that hatch to release 6-legged larvae. Usually, the eggs are laid in an appropriate location but in some species the eggs are retained in the female's body until they hatch. The larvae moult through a variable number of further developmental stages; these are known sequentially as protonymph, deutonymph, and tritonymph. The nymphal stages and the adults usually have four pairs of legs. The durations of the different stages of the life cycle are hugely variable and often affected by environmental conditions and, in the case of parasitic species, the behaviour and ecology of their host.

There are numerous species of Acari that are of medical and veterinary importance, especially as vectors of other pathogens and we can only cover a few of these. Those readers requiring more detail are advised to consult Mullen and Durden (2002).

4.2.1 Family Demodicidae

This is an unusual family of mites that have become specialised skin parasites of humans and other mammals and they tend to be host-specific. They have been described from a wide range of mammals (e.g. *Demodex cati* (cats), *Demodex equi* (horses), *Demodex phylloides* (pigs), *Demodex bovis* (cattle), *Demodex musculi* (mice), *Demodex caviae* (guinea pigs)) and it is probable that

Table 4.3 Taxonomic arrangement of the Acari

Super-order	Parasitic examples
Parasitiformes	Poultry mites (*Dermanyssus gallinae*), soft ticks, hard Ticks
Acariformes	*Demodex* spp., scabies mite, chiggers,

Figure 4.1 Adult *Demodex folliculorum* Source: Chandler and Read, 1961

most mammals have 'their own' species. There is even a marine species, *Demodex zalophi*, which parasitises Atlantic harbour seals (*Phoca vitulina*).

There are two species that live on humanss: *Demodex folliculorum* and *Demodex brevis* (Figure 4.1). *Demodex folliculorum* has a long (0.3–0.4 mm in length) carrot-shaped body and lives in the hair follicles of simple hairs. By contrast, *Demodex brevis* has a shorter more stumpy body (0.2–0.3 mm in length) and lives in the sebaceous glands of vellus hair. The life cycle of *Demodex folliculorum* takes about 14–18 days and consists of an egg, larval, protonymph, deutonymph, and adult stage. *Demodex* spp. mites feed on the host cells and they are linked with various dermal pathologies (see Chapter 7). They have very limited powers of movement and the eggs are laid within their host's skin, so it is probable that hosts often probably acquire infections from their mother (Mullen and Durden, 2002). If so, then it would make genetic mixing difficult for the mites and it would be interesting to compare the molecular profile of *Demodex* within and between communities.

4.2.2 Family Sarcoptidae

This family contains the well-known scabies mite *Sarcoptes scabei*, *Knemidocoptes mutans* that causes scaly leg in hens and a variety of other birds and *Notoedres cati* that afflicts domestic and wild cats as well as dogs and a number of other mammals.

Genus Sarcoptes Unlike *Demodex*, in which there are numerous host-specific species, *Sarcoptes scabei* exists as a variety of host-specific sub-species. For example, humans are infected by

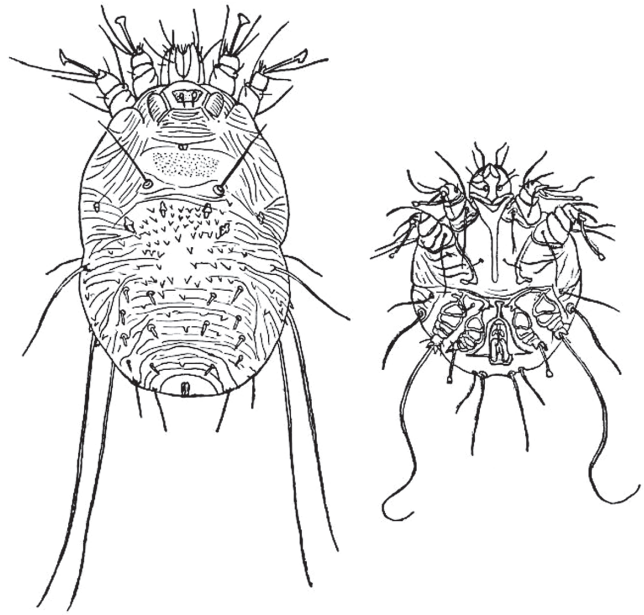


Figure 4.2 Adult *Sarcoptes scabei*. (a) = female, dorsal view; (b) = male, ventral view Source: Chandler and Read, 1961

Sarcoptes scabei var *hominis* while dogs are infected with *Sarcoptes scabei* var *canis*. Although humans are sometimes infected by other varieties of *Sarcoptes scabei* such as those normally found on dogs and horses, these tend to cause humans temporary infestations, although these may persist for a few weeks or months. *Sarcoptes scabei* var *hominis* is found throughout the world and is an obligate parasite of humans (Figure 4.2). As with other mite infestations, it is sometimes thought to be an indicator of poverty and/or neglect. However, it is now recognised that *Sarcoptes scabei* can and does infest people of all classes and levels of affluence. The mites are protected within their burrows and are not removed by frequent washing in hot soapy water. Scabies is, however, a recognised problem for people living in close association in cramped unhygienic circumstances such as urban squatter settlements and refugee camps.

Sarcoptes scabei is a small almost circular-shaped mite with miniscule legs. The front two pairs of legs terminate in structures called pedicels that look a bit like sink plungers. However, the mite's shape belies a remarkable turn of speed and the females have been timed at moving at 2.5 cm per minute. This might not sound very fast but the female mites are only 350–440 μm in length and the males are even smaller (180–240 μm). Assuming a body length of 350 μm (0.35 mm) this translates as 71.4 body lengths per minute – or the equivalent of 129 metres per minute (7.74 km per hour) for a 1.8 metre-tall human. Scabies infestations are generally considered to be transmitted through close physical contact. Although 15–20 minutes of close contact is usually quoted as being required, this presumably depends a lot on the numbers of mites involved and the individual circumstances. For example, if the donor is heavily infested, there is a higher chance that sufficient mites will 'make the jump' to establish an infestation sooner rather than later. Although the mites can be transmitted via bedding or shared clothing, this is not thought to be a major route

of infestation. Scabies can be a particular problem for poor people, especially children, living in tropical countries because they frequently wear few clothes. This means that there is an increased chance of skin: skin contact – especially at night when they often share a bed, bunk, or hammock.

In adult humans, the mites usually dig where the skin is thin and wrinkled. The majority of infestations (63%) are found on the webs between the fingers and on the wrists, after which the elbows are the second most common site (11%). Infestations are also found on ankles, scrotum, and buttocks while in women the mites may be found in the skin folds on the breasts and nipples. Young children have softer skin than adults and it is not unusual to find the burrows on the palms of their hands and soles of their feet as well as on the neck and head.

After mating, the female mite goes in search of a suitable place to dig a permanent burrow in which she can raise her family. She digs as far the boundary of the *stratum corneum* and *stratum granulosum* and then starts burrowing horizontally. She also starts laying eggs at a rate of two or three eggs every day until she dies after about 30 or so days. The eggs hatch about three or four days after they are laid and most of the larvae crawl up to the skin surface and move a short distance away before digging a shallow 'moulting pocket'. Each larva digs its own 'pocket' and within this it moults to a protonymph and then a tritonymph before becoming an adult. For *Sarcoptes scabei* var *hominis* it takes the female mites about 14 days to develop from egg to adulthood while the males can take as little as 10 days. The times vary slightly between the different sub-species. After becoming mature, the male mites leave their pocket and wander off in search of female mites. The females by contrast remain within their pocket until the males have found them and mating has taken place. After mating, the females leave their pocket and search for a place to make a permanent burrow.

The mites feed on the fluid exudates of damaged dermal cells. Although the physical damage is relatively minor, scabies infections cause intense itching as a result of the immune reaction to the cuticular components of the mites as well as their secretions and excretions. In humans, this gives rise to the formation of rashes that may develop at sites distant from the actual site of infestation and persist even after all the mites have died. Although the mites produce highly immunogenic substances, chemicals in their saliva modulate the immune response directed against them and this probably facilitates their initial establishment (Morgan and Arlian, 2010). Although there are differences between animals in their response to *Sarcoptes scabei*, on the whole, those that recover from an infestation are resistant to reinfection, probably as a consequence of immunoglobulin-E-mediated responses. There is therefore interest in developing vaccines to treat sarcoptic mange in domestic animals and scabies in humans (Rodríguez-Cadenas *et al*., 2010).

In humans, hyperinfections with huge numbers of mites gives rise to crusted scabies which is potentially fatal (see Colour Plate 18). In addition, the presence of such large numbers of mites means that people suffering from the condition are highly infectious to others. Although the mites are not considered to be disease vectors, the lesions caused by a combination of the mite and scratching and rubbing often result in secondary bacterial infections. In cattle, *Sarcoptes scabei* var *bovis* causes 'sarcoptic mange'; this is a notifiable disease in the UK and the import of cattle that are suffering from infestation is banned regardless of whether or not they are clinically affected by it. Serious disease is also caused by *Sarcoptes scabei* var *ovis* in sheep, *Sarcoptes scabei* var *cameli* in camels, and *Sarcoptes scabei* var *aucheniae* in the alpaca (*Vicugna pacos*). Serious disease is usually associated with animals in poor condition at the end of the winter period. In the UK and several other countries in northern Europe, the fox (*Vulpes vulpes*) population has been drastically affected by epidemics of sarcoptic mange which has had a consequent impact on the population of other wild animals (Carlsson *et al*., 2010).

Genus Knemidocoptes The name of this genus is also sometimes spelled *Cnemidocoptes*. There are several species that are important pests of domestic and wild birds. *Knemidocoptes gallinae* causes 'depluming itch' of domestic fowl. The mites burrow into the shafts of feathers thereby inducing an inflammatory response and itching. The feathers break easily and are pulled out by the affected bird resulting in bare patches on the back and wings although the head and neck may also be affected. *Knemidocoptes mutans* causes a condition known as 'scaly leg' in domestic hens, turkeys and a number of other birds. The mite probably initially infects a bird by crawling onto it from a surface since the lesions usually start in the toes and then progress up the leg. The mites penetrate under the scales and create tunnels in the underlying skin. As a consequence of the combination of the mites' feeding and the induction of inflammatory reactions, the scales are raised upwards and become loose. The *stratum corneum* becomes hyperkeratotic (i.e. the *stratum corneum* becomes thickened owing to the deposition of excess keratin) and it is not unusual for the lesions to become secondarily invaded by opportunistic bacteria and fungi. As a result, the affected limb becomes deformed, digits can become necrotic and fall off and the bird is rendered lame. Ultimately, the bird may cease feeding and die. The comb and neck may also be affected.

4.2.3 Family Psoroptidae

The family Psoroptidae includes several genera of importance in veterinary medicine. For example, the genus *Psoroptes* that includes *Psoroptes ovis* that causes sheep scab, genus *Chorioptes* that cause chorioptic mange and the genus *Otodectes* that includes *Otodectes cyanotis* that afflicts the ears of cats, dogs, and several other carnivores.

Genus Psoroptes The genus *Psoroptes* contains a number of important ectoparasites of domestic animals but they are not parasites of humans. There are several distinct host-specific species although these can be difficult to distinguish on the basis of their morphology. Indeed, it appears that some of the mites that were previously considered to be separate species such as *Psoproptes ovis* in sheep and *Psoroptes cuniculi* in rabbits are actually strains of the same species.

Psoroptes ovis is economically the most important member of the genus as it causes the condition known as sheep scab (Figure 4.3, and see Colour Plate 9). *Psoroptes ovis* lives entirely on the skin surface where it feeds on bacteria and skin secretions. The adult mites are small (~0.75 mm) and oval-shaped with relatively long legs and the mouth parts project to form a noticeable cone-shaped point at the front of the body. The female mite has pedicels on the first, second and fourth pair of her legs and long whip-like setae (hairs) on her third pair of legs. By contrast, the somewhat smaller male has pedicels on his first, second, and third pairs of legs and long setae on his fourth pair of legs. The male also has a pair of 'copulatory suckers' towards the rear of his ventral surface – he can spend a long time attached to a female so he needs to maintain a good grip.

The female mite lays her eggs on the surface of the skin. The eggs are approximately 0.25 mm in size – which makes them about a third the size of the adult female. The eggs hatch after about 1–3 days but may take longer if they are in a cooler microclimate. The eggs hatch to release a 6-legged larva which then moults first to a protonymph and then a tritonymph which then moults to become an adult – the whole process from egg to adulthood taking about 10 days. The adult males will attach themselves to a female while she is still a tritonymph or even a protonymph and

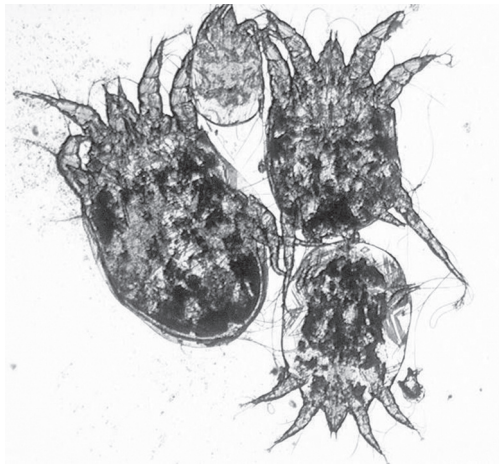


Figure 4.3 *Psoroptes ovis*: Adults and nymphs

when she finally moults to an adult, then he will mate with her – a process that typically lasts a day but may take less if there are many more females than males in the population. After mating, the female lives for a further 30–40 days during which she lays about five eggs every day.

4.2.4 Suborder Ixodida

The suborder Ixodida contains all the ticks and all of these are parasitic (Oliver, 1989). There are three families within the suborder: the Argasidae ('soft ticks'), the Ixodida ('hard ticks'), and the Nuttalliellidae. The Nutalliellidae contains a single obscure species, *Nuttalliella numaqua* that is found in South Africa but whose main host is not known and the life cycle has yet to be determined. Although ticks can cause serious pathology, especially if they are present in large numbers, they are of principal importance as vectors of viral, bacterial, and parasitic diseases.

Ticks are generally much bigger than mites and can be seen with the naked eye. The mouthparts of ticks also include a large-toothed structure called a hypostome that the tick uses to firmly anchor itself to its host. Some ticks also secrete chemicals that act like a glue to further anchor themselves in position. Any attempt at pulling a tick from its host usually results in the ripping the tick apart and leaving the mouthparts embedded in the flesh of its victim. To extract ticks, one needs to twist and pull – although it is often simpler to leave them to drop off of their own accord. The hypostome has evolved from the coxae of the pedipalps that have fused together to form a single structure. A hypostome is also found in the mites but it is not toothed and is small and not easily seen. Ticks also differ from mites in possessing a sensory structure called the Haller's organ on the tarsal segments of the first walking legs. The Haller's organ consists of an anterior pit and a proximal capsule. The pit region detects humidity and the capsule region is used for olfaction. Ticks often wave their front pair of legs in front of them, especially when 'questing'. It is therefore probable that the Haller's organs on the tarsi are used for sensory perception in a similar manner to the receptors on the antennae in insects.

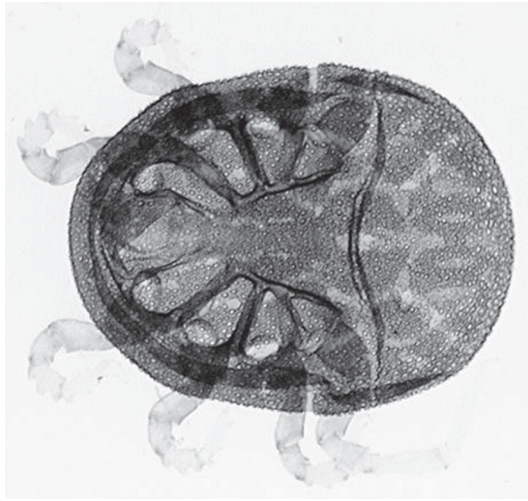


Figure 4.4 Soft tick *Ornithodorus*. Note how the mouth parts cannot be seen from above. Only two pairs of walking legs are visible on the tick's right hand side.

4.2.5 Family Argasidae

The Argasidae are often known as the 'soft ticks' because they have a leathery appearance and lack a hard scutum (protective shield) on their upper surface (Figure 4.4). Unlike the 'hard ticks' there are no obvious morphological differences between the sexes apart from the shape of the genitalia. The family contains about 170 species and they are mostly parasites of birds although some species parasitise bats and some exploit a variety of terrestrial mammals. One species, *Argas* (*Microargas*) *transversus*, is totally dependent upon the Galapagos giant tortoise (*Chelonoidis nigra*). The argasid life cycle typically comprises of the egg, larval, and anything from 2 to 7 nymphal stages before the ticks become adults. As a rule, the nymphs and adults feed quickly (30 minutes to a few hours) and then drop off their host while the larvae may remain attached for several days. Regardless of how long they remain attached, after dropping off, the larvae and nymphs usually moult to the next developmental stage while the adults produce another batch of eggs or sperm. This exploitation of numerous hosts makes the ticks potential vectors of a range of diseases. It should be noted that there are several exceptions to this generalised life cycle. For example, *Argas transversus* is unusual in spending its whole life aboard its tortoise host and even lays its eggs directly onto the tortoise.

Argas persicus *Argas persicus* is commonly known as a fowl tick although it will also feed on turkeys, pigeons, ostriches and a variety of wild birds. Hungry ticks will also bite humans although they are not important hosts. *Argas persicus* has a cosmopolitan distribution but is most common in tropical countries. The adult has a flattened oval shape that narrows towards the anterior. It has well-developed legs and the mouthparts cannot be seen from above because they are hidden by the integument. It is quite large, growing up to 10 mm in length and 6 mm in width and is yellow-brown when unfed and slate-blue after feeding.

The female mite lays batches of 20–100 eggs in crevices and the eggs hatch after about 3 weeks to release the larvae. The larvae have round bodies and 6 legs. They hunt for a suitable bird host

and usually attach underneath the wings where they remain attached for about 5 days. The larvae then drop off and hide for about 7 days during which time they moult to the first nymphal stage. There are a total of two nymphal stages during which the ticks feed and then moult after which they become adults. The nymphs and adult ticks remain hidden in crevices during the day and come out to feed at night. If large tick populations have been allowed to build up in chicken sheds, the repeated disturbance and loss of blood can leave the birds unthrifty and the anaemia may prove fatal. The ticks can also transmit fatal diseases, such as fowl spirochaetosis (*Borrelia anserina*). *Argas persicus* are notoriously resistant to starvation and can probably live for years without feeding. This is a typical trait of bird ticks since birds often only nest once or twice a year and therefore the ticks must be able to survive the long time between nesting seasons.

4.2.6 Family Ixodidae

Ticks belonging to the family Ixodidae are known as the 'hard ticks' because they have a hard scutum (chitinous shield) on the upper surface. In adult male Ixodid ticks this scutum extends to cover the whole dorsal surface while in the adult female, the nymphs and the larva, it extends only a short distance behind the head. Unlike the soft ticks, in the hard ticks the mouthparts project in front of the body and are clearly visible from above (see Colour Plate 10). Their life cycle is generally simpler too and usually consists of the egg, a 6-legged larval stage, a single nymphal stage, and then the adult stage. Ixodid ticks tend to be less active than soft ticks and they often adopt a 'sit and wait' strategy that is called 'questing'. Basically, the ticks crawl up vegetation or some other structure that will give them a prominent position and extend their front legs. When a potentially suitable host passes by the ticks then grab hold of it and then look for a place to start feeding. Feeding typically takes 4–6 days after which the tick proceeds to the next stage in its life cycle. Whether or not the tick drops off between feeding and the number and species of hosts involved varies between tick species. Ixodid ticks exhibit three different life cycle strategies.

- *One-host ticks*: The whole life cycle (apart from egg-laying) takes place on a single individual animal. The larval tick climbs aboard an animal, feeds, moults to the nymphal stage that then feeds and moults to the adult stage, and the adults then feed and mate. The adult ticks then feed and mate after which the female leaves the host animal and lays its eggs on the ground. *Rhipicephalus annulatus,* formerly known as *Boophilus annulatus* (the North American cattle tick), is a one-host tick that feeds on domestic and wild ungulates. Humans and dogs are occasionally attacked but are not thought to be suitable for the maintenance of the whole life cycle. Until the 1940s this tick was a major problem in the USA as a consequence of it transmitting 'cattle fever' (*Babesia bigemina*). The tick has now been eradicated from the USA but it still exists in Mexico. Consequently, a permanent quarantine zone is maintained at the border between the two countries to prevent the tick from being reintroduced.
- *Two-host ticks*: The larval and nymphal stage take place on a single host but the adult stage parasitises a different animal. In this case, the larva climbs aboard an animal, feeds, and moults to the nymphal stage. The nymph then feeds on the same host but having engorged it drops off, and moults to the adult stage on the ground. The adult tick then finds a suitable host on which it will feed and mate after which the female tick drops off and lays its eggs. For example, *Rhipicephalus evertsi*, the red-legged tick is a two-host tick that feeds on a wide

range of mammals including domestic species such horses, cattle, sheep, and goats. This is a common tick species in southern Africa and has also spread to several other countries and is an important vector of many diseases including *Theileria parva* and *Babesia bigemina*.

- *Three-host ticks*: This is the most common life cycle strategy among Ixodid ticks. These tick species parasitise a different animal at each stage in the life cycle. Therefore, the larva climbs aboard an animal, engorges and then drops off and moults to the nymphal stage on the ground. The nymph then climbs aboard a different animal, engorges and then drops off and moults to the adult stage which then climbs aboard another animal, engorges, drops off and then lays its eggs. The hosts utilised at each stage may belong to the same species or be different species although typically, the tick feeds on a larger host animal after each moult. This can be achieved by climbing further up the vegetation at each stage in the life cycle. *Ixodes ricinus*, the castor bean tick or sheep tick is an example of a three-host tick. It is common in Europe and many other countries. The larvae and nymphs have been recovered from a wide range of animals including birds and lizards while the adults are commonly found on sheep and dogs but also numerous other wild and domestic mammals. In common with several other ticks, *Ixodes ricinus* is a long-lived animal. The larval period occupies the first year of its life, the nymphal period lasts a further year, and the adult stage lasts for a third year. The adult female feeds only once and after she is fully engorged, she is about 1cm in length and slate-grey in colour and has all the appearance of a castor bean – from which it gets its common name. She expands to such an extent that her legs cease to be visible when viewed from above. The male, by contrast, takes several smaller blood meals and he mates with several females. *Ixodes ricinus* is an important vector of a range of parasitic, bacterial, and viral diseases including *Babesia divergens*, *Babesia bovis*, Lyme disease (*Borrelia burgdorferi*), human granulocytic anaplasmosis (also known as human granulocytic ehrlichiosis) (*Anaplasma phagocytophilum*) and tick-borne encephalitis virus.

4.2.7 Tick paralysis

In addition to acting as vectors, a number of Ixodid ticks also cause a condition known as tick paralysis. Tick paralysis has been described in a wide range of animals including humans, cats, dogs, sheep, birds, and even snakes. It occurs in many countries and has been linked to over 46 species of ticks. Australia is blessed with an unusual abundance of poisonous creatures and is also home to *Ixodes holocyclus* that has a particular propensity to cause tick paralysis. *Ixodes holocyclus* does not induce any harmful reactions in koalas, kangaroos, and bandicoots which are its natural hosts but in domestic animals and humans its attachment can prove fatal. The ticks gain no benefit from killing their host and paralysis often does not develop until after the tick has engorged. Furthermore, even for *Ixodes holocyclus*, tick paralysis is an unusual rather than invariable feature of attachment. It is therefore possible that the development of tick paralysis is heavily dependent upon a combination of individual tick and host features. All ticks inject a variety of chemicals with their saliva when they feed to facilitate the uptake of blood and to reduce the host immune response (Steen *et al*., 2006). In hosts that have not co-evolved with the tick, some of the components of tick saliva may induce a particularly harmful reaction.

In humans, tick paralysis tends to affect children more than adults although this may be a reflection of their tendency to play and roll around in fields where they are likely to be bitten by

ticks. It is a potentially fatal condition and tends to evolve slowly. It therefore differs from the typical toxicosis that follows being stung by a wasp or bitten by a snake in which the reaction is almost immediate. Tick paralysis caused by *Dermacentor andersoni* and *Dermacentor variabilis* usually improves after the tick is removed but in the case of *Ixodes holocyclus* full weakness may not occur until two or more days after the tick is removed or has dropped off after finishing feeding (Grattan-Smith *et al*., 1997). The affected person becomes increasingly unsteady and flaccid paralysis then extends symmetrically to affect the other muscles of the body and death may occur through respiratory paralysis. Tick paralysis can also be complicated by the development of inflammation of the skeletal muscles (myositis) and heart muscles (myocarditis).

Tick paralysis can be mistaken for Guillain-Barré syndrome although a distinguishing feature is that tick paralysis usually causes paralysis of the extra-ocular muscles that bring about eye movements (ophthalmoplegia). The presence of a tick still attached to the body is, of course, another good indicator. The toxin derived from *Ixodes holocyclus* has been given the name holocyclotoxin although its chemical nature is not yet fully understood. It is thought to act in a similar manner to botulinum toxin and to prevent the release of acetylcholine at skeletal neuromuscular junctions although there is also evidence of action on the autonomic nervous system and central nervous system involvement (Hall-Mendelin *et al*., 2011). Recombinant holocyclotoxin-1 binds to synaptosomes but has no toxic effect. It is therefore possible that it requires some as yet unknown co-factors in order to be fully active or that the neurotoxic effects of *Ixodes holocyclus* are due to some other unknown compound(s) (Steen *et al*., 2006).

4.3 Phylum Crustacea

The taxonomy of the Phylum Crustacea is extremely complex and frequently revised. Consequently, there is little consensus between textbooks on how the phylum should be arranged. The Crustacea currently contains over 40,000 described species and there are undoubtedly many more awaiting the attentions of taxonomists. It is an enormously diverse phylum and representatives can be found in every marine, brackish, and freshwater environment but there are only a few species which are terrestrial. There is no such thing as a typical crustacean body plan although the majority of them have a distinctive nauplius larva stage. The nauplius larva may remain within the egg or be a free-swimming member of the planktonic community. The nauplius larva has a single median eye on the front of its head and three pairs of appendages: the first and second pairs of antennae and the mandibles.

The Crustacea include many examples of unusual and fascinating parasites but these tend to afflict other aquatic invertebrates or fish and have little economic or medical importance. We will therefore only be able to mention a few examples of crustacean parasites.

4.3.1 Subclass Copepoda

The copepods are the most abundant metazoan animals in the oceans and huge numbers also occur in freshwater as well. There are in the region of 1600–1800 species of parasitic copepod species and they infest animals ranging from sponges to whales. Most (~75%) parasitic copepods belong to a single order: the Siphonostomatoida. The majority of parasitic copepod species only exploit a single animal during their life but those belonging to the family Pennellidae are unique in having a life cycle that includes both an intermediate host and a definitive host. Although most copepods are

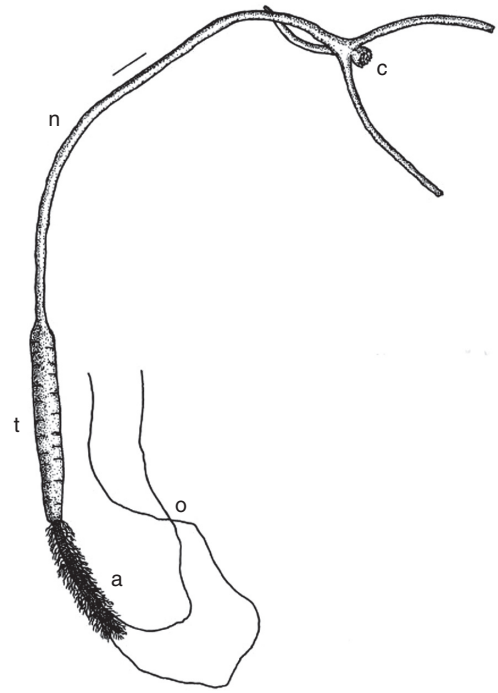


Figure 4.5 Parasitic copepod *Pennella balaenopterae*. a = abdomen; c = cephalothorax; n = neck; o = ovisacs; t = trunk. Scale bar = 1 cm. Reproduced from Abaunza *et al*., 2001. (Abaunza, P. *et al*. (2001) A contribution to the knowledge on the morphometry and the anatomical characters of Pennella balaenopterae (Copepoda, Siphonostomatoida, Pennellidae), with special reference to the buccal complex. Crustaceana 74, 193–210. Reproduced by Permission of Brill)

small (0.5–10 mm in length), one free-living species approaches a length of 2 cm and some parasitic forms are over 32 cm. An unusual relationship appears to exist between the parasitic copepod *Ommatokoita elongata* and the Greenland shark *Somniosus microcephalus*. The copepods attach to the cornea of the shark and cause lesions that could potentially lead to blindness. However, according to Berland (1961), the 3–5 cm-long pinkish white copepods might act as lures that attract char (*Salmo alpines*) close enough to be caught by the sluggish-natured shark. The largest known copepod is *Pennella balaenopterae* that parasitises whales and can grow to at least 32 cm in length. In common with several other parasitic copepod species, the body of *Pennella balaenopterae* bears little resemblance to that of free-living copepods (Figure 4.5). Overall, the body is long and thin and divided into three regions: the cephalothorax that bears two or three holdfast organs that are deeply embedded in the whale's blubber, a long thin thoracic region (~80% of the total body length) and an abdominal region that is covered in plume-like processes and terminates in two long thin ovisacs that may extend a further 40 cm beyond the tip of the abdomen.

A free-living species of copepod, *Mesocyclops longisetus*, is proving of interest to parasitologists as a biological control agent for the larvae of the mosquitoes, such as *Aedes aegypti*. *Aedes aegypti* is an important vector of dengue fever and yellow fever and is found in many parts of the world, including South and North America, the Middle East, and throughout Africa and Asia. Interestingly, not only are the copepods extremely effective at eliminating the mosquito larvae, but also the chemicals they produce attract gravid female mosquitoes to lay their eggs in water

containing the copepods. Experiments have shown that gravid female *Aedes aegypti* are more attracted to water containing the copepods or to water in which the copepods were previously held compared to distilled water (Torres-Estrada *et al*., 2001). This may increase the numbers of potential prey for the copepod and also increases their usefulness as a biological control agent. *Mesocyclops longisetus* will also prey on other species of mosquito, including malaria-transmitting anopheline mosquitoes and a number of other predatory copepod species are also being investigated for their potential as biological control agents (Pons *et al*., 2008).

4.3.2 Infra-Class Cirripedia

The Cirripedia include the familiar barnacles and they are the only sessile group of crustaceans. A large number of species among the Cirripedia are parasitic and the genus *Rhizocephala* are so highly specialised that all traces of arthropod structure have disappeared in the adult. The rhizocephalans mainly parasitise decapod crustaceans (e.g. crabs and lobsters). The female cyprid larval stage is free-living and attaches itself to the body of a suitable host. It then grows into its host by forming a root-like nutrient-absorbing structure known as the 'interna'. When she is mature, the female parasite forms an external reproductive structure called the 'externa' that emerges on the host's ventral surface. The male is free-living and after finding a female, attaches itself. It then injects cells which migrate to a special chamber in the female where they differentiate into a testis. The female is now functioning as a hermaphrodite, and fertilisation of eggs and development to the naupilar stage occur within the brood chamber. The effects of this parasitism on the decapod crustacean host include the inhibition of moulting and parasitic castration. Infestation levels can be high in some crab populations and concerns are sometimes expressed that rhizocephalan parasites might affect commercial crab fisheries (Basson, 1994). However, it has also been suggested that rhizocephalans might be used to control invasive pest crab species (Thresher *et al*., 2000).

4.3.3 Subclass Branchiura

The majority of the Branchiura are obligatory ectoparasites of fish in both freshwater and marine ecosystems although a few species parasitise amphibians. They are commonly known as 'fish lice' although this term is also applied to certain parasitic copepods (Pike, 1989) and it should be remembered that the true lice are insects. Even as adults the branchiurans retain their swimming ability and the female usually has to leave its host in order to lay her eggs on a suitable substrate. Once they have located a host, the parasites often crawl to a position behind the gill operculum or one of fins where water turbulence is less. They then use their suctorial proboscis to feed on mucus and scales or to pierce the skin and feed on blood. A distinguishing morphological feature of the branchiurans is the presence of two large sucking discs on the underside of the head that are formed by modifications to the second paired mouthparts (maxillules). The discs help the parasite maintain its grip on the host. In addition, immediately behind the sucking discs is a pair of large maxillae that are also used for grip rather than feeding.

The Branchiura consists of four genera, the best known of which is the genus *Argulus* that has representative species throughout the world. Many *Argulus* species are not host-specific and some of them are of economic importance as parasites of farmed fish and high value cultured fish such as koi carp. They have simple direct life cycles in which the female *Argulus* lays its eggs on a substrate such as stones at the bottom of the water body and these hatch to release metanauplii

that actively swim in search of a suitable fish host. Once a metanaupli has found its host, it attaches and undergoes a series of moults until it becomes an adult. The sexes are separate and they usually mate on the host.

When they feed, branchiurans inflict physical damage and also induce inflammatory reactions that further contribute to irritation. In addition, damage to the fish's skin can upset its ability to regulate its ion balance and make it vulnerable to bacterial and fungal pathogens (Saurabh *et al*., 2010). The parasites may also act as vectors of viral and microbial pathogens although there is limited published information on this. Farmed fish are maintained at high population densities within confined areas and are therefore vulnerable to suffering from high parasite burdens. This can result in low growth rates and high mortalities. Fish farmers therefore tend to use a lot of pesticides (such as ivermectin) in order to reduce the abundance of branchiurans. Unfortunately, these chemicals can have an adverse effect on the surrounding ecosystem. Furthermore, if the farmed fish are maintained in sea lochs, the parasites can be transmitted to free-living salmon and sea trout with a consequent harmful impact on their population.

4.3.4 Subclass Pentastomida – tongue worms

All pentastomes are parasitic and their apparently primitive traits are derived from their parasitic lifestyle. The pentastomes are a highly unusual group of invertebrates whose taxonomic position has been a constant source of debate. For many years they were cited as examples of primitive 'proto-arthropods' along with the tardigrades and the onychophorans. However, molecular evidence has now added sufficient weight to studies on pentastome sperm morphology, embryogenesis, and cuticular fine structure to indicate that they are actually crustaceans.

Although the name 'Pentastoma' translates as 'five mouths', the pentastomes have only a single mouth. In the adult worm the mouth lacks jaws and in some species it sits at the top of a snout-like projection. The other four 'mouths' are actually two pairs of much reduced 'legs' that sometimes bear chitinous claws that the pentastome uses to cling on to its host. The pentastome cuticle is non-chitinous, highly porous, and moults periodically, at least until the full adult size is attained. Frontal glands are present in some species and are thought to produce secretions that break down host tissue and serve as an anticoagulant. Ulcerative lesions are often found where adult pentastomes have been attached.

Over 100 species of pentastome have been described and the vast majority of these are parasitic in the lungs and nasal passageways of vertebrates during their adult stage. They grow from 1–14 cm in length and are sometimes referred to as 'tongue worms' from the shape of the adult worm. The life cycle generally involves the eggs that are shed by the female worm being coughed or sneezed up or passed out with the host's faeces. The eggs are then consumed by an intermediate host that, depending upon the pentastome species, may be an insect, fish, amphibian, reptile or mammal. The eggs hatch within the gut of the intermediate host and the pentastome larva penetrates the intermediate host's gut and then undergoes an apparently random migration until it settles down and moults to the nymphal stage. There can be several nymphal stages and in some species the nymph may become sexually mature before it moults to become an adult. The intermediate host must be consumed by the definitive host before the pentastome can moult to the adult stage. After consumption, the infective nymph either penetrates the gut of the definitive host or climbs up its oesophagus and then migrates to the lungs or nasal passageways where it becomes an adult. The definitive host is usually a snake or other reptile although there are a few species in which it is a mammal, bird, or amphibian. Fish are not definitive hosts but can act as

intermediate hosts. Autoinfection can take place in which a pentastome egg hatches in the gut of the definitive host but if this occurs, the pentastome can only develop as far as the nymphal stage. However, as cannibalism is not unusual among some reptiles, it is possible that they can act as both intermediate and definitive host. *Linguatula arctica* appears to have dispensed with the need for an intermediate host. This parasite infects the nasal passages and sinuses of reindeer (*Rangifer tarandus*). This is unusual as all other adult pentastomes described to date are found in carnivores. The egg is unable to survive exposure to freezing conditions and therefore cannot survive the long Arctic winter. Instead, direct transmission is thought to take place when reindeer calves consume eggs passed by worms infecting older reindeer (Nikander and Saari, 2006).

Humans normally acquire visceral pentastomiasis through consumption of pentastome eggs. The increasing popularity of keeping exotic pets and in particular reptiles means that their owners are at an increased risk of exposure to equally exotic pathogens. There is a large and often illegal trade in animals that are collected in the wild and then marketed via the internet. These animals are highly likely to be infested with parasites and some of these, such as the pentastomes can infect humans. The eggs hatch in the intestine to release nymphs that penetrate the lining of the gut and encapsulate within the liver, lungs and other viscera – although they may reach other organs, including the eye. Usually an infection causes humans few, if any, symptoms and the parasites are only discovered during a medical examination for another complaint or at autopsy. However, heavy infestations cause abdominal pain, coughing, and night sweats, and death may occur from secondary septicaemia or pneumonia (Tappe and Büttner, 2009).

Dakubo *et al*. (2008) provide an interesting example of how close association with snakes can increase the risk of acquiring pentastome infections. Many of the clans and tribes of Ghana identify themselves with certain animal or plant 'totems'. This should not be considered as primitivism or superstition and similar practices can be found throughout the world. Some of the Ghanaian clans have the python as their totem and they will not kill or harm pythons. Indeed, pythons will be welcomed into the village and they may share a villager's sleeping mat. None of the cases of visceral pentastomiasis described by Dakubo *et al*. (2008) proved serious but it would have been interesting to know how the level of infection of the python clans compared to that of clans with different totems.

Most human cases of pentastomiasis arise from infection with *Linguatula serrata*. This species is found in many parts of the world and is an unusual pentastome in that the life cycle does not involve reptiles (Figure 4.6). The adult worm develops in the nasal passages and sinuses of dogs, wolves, and other caniids and lives for about 2 years. Heavy infestations can cause nasal discharge and constant coughing and sneezing. In countries in which there are large numbers of stray dogs, these can act as a significant reservoir of infection. For example, a survey of stray dogs in the city of Shiraz in Iran found that 76.5% of them were infected with *Linguatula serrata* (Oryan *et al*., 2008). The eggs are either coughed or sneezed out or, if swallowed, pass out with the faeces. The eggs are then acquired by the intermediate host through faecal-oral contamination of water, food, etc. The nymphs can infect virtually any mammal although they are principally recorded from cattle, goats, and sheep. After the eggs hatch in the intestine, the larvae burrow through the gut wall and give rise to nymphs that encyst in the viscera. Humans can develop visceral pentastomiasis/linguatulosis if they ingest the eggs.

Another means of acquiring pentastome infections is through the consumption of flesh containing the nymphal stage. This gives rise to a condition known as nasopharyngeal pentastomiasis/linguatulosis and is sometimes referred to as 'halzoun'. This is particularly common in parts of the Middle East and is associated with the consumption of raw or lightly cooked liver and other

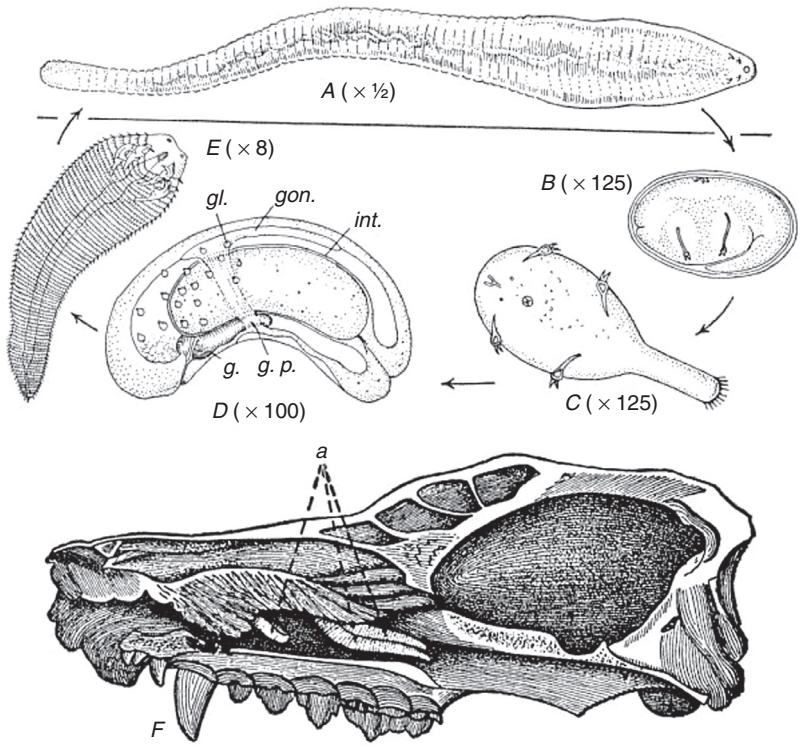


Figure 4.6 Life cycle of the pentastome *Linguatula serrata*. A = Adult female from the nasal passages of a dog; B = egg containing embryo; C = first stage larva from the viscera of a sheep; D = third stage larva after 9 weeks development; E = nymph from liver of sheep; F = skull of dog to illustrate position of adult parasites (a) in the nasal cavity. Source: Chandler and Read, 1961

visceral organs. Similarly, the consumption of snake meat is popular in parts of Asia and among 'survivalists' and is a potential source of infection if the meat is not cooked properly. The nymphs move from the gut to the nasopharyngeal region where they cause pain, inflammatory reactions, and localised swelling and these result in difficulty in swallowing and breathing.

4.4 Sub-phylum Hexapoda

The sub-phylum Hexapoda contains two Classes: the Class Entognatha and the Class Insecta. The Class Entognatha contains several orders of 6-legged wingless organisms which may or may not be true insects and whose common feature is that they do not have sufficient of the traits needed to be included in the Class Insecta. These traits are listed in Table 4.4. The Class Insecta is, thankfully, a single cohesive unit that consists of the winged insects and a few primitive wingless insects. The Hexapoda contains more than 750,000 described species and is the largest group of metazoan animals. In fact, it is three times larger than all the other animal groups combined. Hexapods are essentially terrestrial organisms and they can be found in virtually all terrestrial ecosystems. Some of them live in aquatic habitats but they are, for some reason, virtually absent from the sub-tidal

Table 4.4 Morphological features of the Class Insecta

Body divided into three regions: head, thorax, abdomen.
Three pairs of unbranched legs on the thorax. One pair of legs on each thoracic segment.
One pair of antennae.
Usually two pairs of wings carried on the thorax. The wings are absent in some primitive orders and in some of the more advanced orders the wings may be secondarily lost, or one pair has become modified so that only a single pair of wings provides propulsion.
Two pairs of maxillae on the head although one pair is fused to form the labium.
Base of mouthparts exposed and mouthparts themselves project from the head capsule.
With the exception of one primitive order, the mandibles have two points of articulation.
Gut has digestive caecae.
Separate male and female sexes.
Genital openings located on the terminal or sub-terminal abdominal segments.
Usually two or three median ocelli on the head with compound eyes to either side although these are secondarily lost in some species.
Gas exchange occurs via spiracles that open into a branching arrangement of tracheal tubules that deliver air directly to individual cells.

waters of the sea. Much of their success is undoubtedly a consequence of their wings which has facilitated dispersal, escape from predators, and the location of suitable environmental conditions in which to live and breed.

In most insects the sexes are separate and, following a courtship ritual, mating takes place by apposition of the genitals. A few species, principally among the Homoptera (aphids, whitefly, mealybugs) and Hymenoptera (wasps, ants, bees), are parthenogenetic and males are either never produced or are produced in certain generations in response to external stimuli. The female insect usually lays its eggs in a suitable location and then leaves them although in some species there are varying degrees of parental care and sociality. Primitive insect orders undergo what is called hemimetabolous development in which the eggs hatch to give rise to a nymph that looks rather like the adult insect but lacks functional genitalia and wings (if they have them). It may or may not feed on the same food and occupy the same ecological niche as the adult. The nymph goes through a series of moults and after each moult its resemblance to its parent increases. After the final moult the insect becomes an adult and it is only at this stage that its wings and genitalia are functional. Examples of insects that undergo hemimetabolous development include the human louse *Pediculus humanus* and the triatomid bugs that transmit Chagas disease. Advanced insect orders undergo holometabolous development in which an egg hatches to release a larva that looks nothing like its parent and frequently has a different diet and occupies a different ecological niche. The larva is often a simple ‘eating machine’ with a thin cuticle and limited powers of locomotion and sensory capacity. The larva undergoes a series of moults during which it simply becomes a bigger version of itself. After the final moult, the larva enters a resting pupal stage during which its body is reorganised and once this is complete, the insect emerges as a fully formed adult. Examples of insects that undergo holometabolous development include the human flea *Pulex irritans* and the flies such as mosquitoes, tsetse flies, and blowflies. With only a few rare exceptions, insects do not moult again once they have become adults and acquired their wings.

The Entognatha does not include any important parasites and they do not play an important role in the ecology of parasitic diseases that affect humans and domestic animals. We will therefore not consider them any further. By contrast, the Class Insecta includes many examples of insects

that are of parasitological importance. This is principally through the blood-feeding insects that act as vectors of diseases such as malaria and leishmaniasis or the coprophilic species that act as mechanical vectors of faecal-orally transmitted parasites such as *Entamoeba histolytica*. Unfortunately, it is beyond the scope of this book to consider the biology of insect vectors and those requiring more details on these should consult Lehane (2005), Mullen and Durden (2002), and Service (2000). There are a number of insect species that are parasitic during part or all of their lives and some of these are of considerable medical and veterinary importance. Parasitic insects are also of interest for their potential to act as biological control agents of pests of agricultural crops and also of other parasitic organisms. It should also be remembered that a great many insect species are beneficial (e.g., to pollinate crops) or do us no harm at all and they are essential for the normal functioning of many ecosystems.

4.4.1 Order Phthiraptera (lice)

The Phthiraptera is divided into two suborders: the Mallophaga, or biting lice, and the Anoplura, or sucking lice (see Colour Plates 11 and 12). They are all ectoparasitic on birds and mammals and they are relatively host-specific with most louse species feeding on a single host species or group of closely related host species. The Mallophaga have triangular-shaped heads that are broader than their thorax and biting mouthparts. They are predominantly ectoparasites of birds although there are a few species that feed on mammals. Most of them use their mandibles to chew on feathers, fur, or dander but some of them will also consume epidermal secretions or gnaw through skin/quills to obtain blood. By contrast, the head capsule of the Anoplura is narrower than their thorax and they have sucking mouthparts that they use to penetrate the skin and consume blood. The mouthparts of the Anoplura are unlike those of most other blood-sucking insects: the labrum is modified to form a snout-like process called the *haustellum* and when the louse feeds, the *haustellum* is everted. This brings into play a series of chitinous 'teeth' that are now on the outer surface and anchor the *haustellum* in the host's skin. The louse then employs three stylets to form two channels: down one of these saliva is secreted and up the other blood is sucked. The Anoplura have a more restricted host range than the Mallophaga and only parasitise certain placental (eutherian) mammals.

The Phthiraptera are all relatively small insects (0.4–10 mm) that are dorso-ventrally flattened. They are wingless and have typical hemimetabolous life cycles that consist of an egg stage, three nymphal instars and then the adult stage. The eggs are laid directly upon the host and are usually physically attached to the hairs or feathers. The nymphs resemble their parents but are smaller and lack functional genitalia. Successive generations of lice develop upon an individual host animal. Transfer between hosts takes place through physical contact although some species of Mallophaga hitchhike attached to the bodies of hippoboscid flies and other insects. As a result, theoretically there ought to be a close relationship between the lice found on parent hosts and their offspring and closely related species of louse should be found on closely related species of host. However, although there are instances in which this occurs, it does not occur as commonly as might be expected. Even among the Anoplura, which diversified at about the same time as the mammals, there is considerable conflict between the mammalian and anopluran phylogenies. This is probably as a consequence of extinctions and host-switching events over the millennia (Light *et al*., 2010).

The Phthiraptera have separate sexes and the male genitalia are unusually large and can extend to half the length of the abdomen. They lack dorsal ocelli and their compound eyes are reduced in size or entirely lacking. This is to be expected as lice spend their whole life on the skin of their

host and have no requirement for vision to find their food or to avoid predators. Those Mallophaga species that parasitise mammals have tarsi that are modified to grasp hairs but those found on birds usually have unmodified 1- or 2-segmented tarsi. By contrast, the 1-segmented tarsi of the Anoplura, particularly those of the mid-legs and hind-legs, are modified to form tibio-tarsal claws adapted for grasping hairs.

The Mallophaga are primarily of veterinary importance and many species have been transmitted around the world with domestic animals. Important species include the 'cattle biting louse', *Bovicola bovis*, that is typically found on the top of the head and feeds by chewing on the skin and scurf at the base of the hairs. The louse population can grow rapidly and causes the affected animal considerable irritation. This results in rubbing and scratching that in turn leads to hair loss, skin damage, secondary infections, and loss of productivity. The chicken body louse, *Menacanthus stramineus*, is a major pest of poultry around the world. Its yellow coloration also gives it its other common name 'the yellow body louse'. It is highly pathogenic and can cause mortalities among young birds. It causes severe inflammation and irritation especially around the vent where it lays its eggs but also around the head and neck. The population can increase very quickly, especially in caged laying hens and a single bird may be afflicted by tens of thousands of lice. *Menacanthus stramineus* can be naturally infected with eastern equine encephalomyelitis virus, but like most Mallophaga, it is not considered to be an important vector of disease.

The Anoplura includes numerous examples of species of medical and veterinary importance. The two best-known examples are, of course, the human head louse *Pediculus humanus capitis* and the human body louse *Pediculus humanus humanus*. Both species are common cosmopolitan parasites of humans. The body louse is thought to have evolved from the head louse at about the time humans started to wear clothing. The body louse has the smallest insect genome sequenced to date (108 Mb) that encodes for 10,773 protein coding genes and 57 micro RNAs. Its small size is predominantly through losing genes involved in environmental sensing and those that code for detoxification enzymes. This is to be expected in a parasite that lives permanently upon its host and relies on it to provide food, shelter from the environment and dispersal. Like other animals that live entirely on blood, body lice and head lice are reliant on a symbiotic relationship with microorganisms to provide certain essential nutrients. In the case of head lice and body lice, they contain an endosymbiotic bacterium *Riesia pediculicola* within a mycetome. This bacterium provides the lice with pantothenic acid (vitamin B5). *Riesia pediculicola* also has an unusually small genome that encodes less than 600 genes. These do not include antibiotic resistance genes and it may therefore prove possible to develop new means of controlling lice by targeting their symbiont (Kirkness *et al*., 2010).

The head louse generally lays its eggs singly upon scalp hairs and they are commonly referred to as 'nits'. The eggs hatch after about 7–10 days and the nymphal stages last a further 7–10 days. The adults live for about 2–4 weeks and during that time the female louse lays between 50–150 eggs. Unlike the body louse, the head lice do not usually lay eggs on clothing and the sharing of earphones, hats, scarves, chair head rests, or pillows is not thought to be a significant means of transmission. The life cycle of the body louse is very similar to that of the head louse, but they often lay their eggs on clothing – particularly along the seams. Depending upon the temperature, eggs laid on clothing can remain infective for up to 4 weeks but they cannot survive for more than a month and therefore nit-infested clothing that has not been worn for a long time is not infective. The adults of both head lice and body lice are unable to survive off their host's body for more than about 48 hours. Transmission takes place through close physical contact and, in the case of body lice, the sharing of clothes. Poor people living in cold climates often wear multiple

layers of clothes that are not changed and washed regularly. As a consequence they can suffer from exceptionally high louse infestations. Louse infestations are also a problem for people living in crowded, squalid conditions such as squatter camps, refugee camps, and prisoners in primitive jails. According to the Old Testament (Exodus 8:16; King James translation), the third plague to afflict the Egyptians were lice that were brought forth from the 'dust of the land'. However, these lice were said to afflict 'man and beast' which is unusual since most lice are host-specific and those that afflict domestic animals do not infect humans and vice versa. It should be noted that most natural history terms in the Scriptures are controversial and some commentators consider the original Hebrew would be more appropriately translated as maggots, gnats, mosquitoes, or sandflies.

Head lice and body lice feed several times every day and in the process they cause irritation and inflammation. The constant itching can become debilitating and the affected person literally feels 'lousy'. Heavily infested people may harbour several hundred body lice and their skin becomes thickened and deeply pigmented. This condition is sometimes called 'vagabond's disease'. Head lice have not been implicated in the transmission of disease although the scratching they cause may result in secondary skin infections. Body lice are vectors of *Rickettsia prowazeki* (louse-borne epidemic typhus), *Borrelia recurrentis* (louse-borne epidemic relapsing fever), and *Bartonella quintana* (trench fever). Louse-borne typhus is fatal for the louse that transmits it and although it is not always fatal in humans, the mortality rate can reach over 60% in malnourished populations. Louse-borne typhus is, not surprisingly, a common disease wherever lice are able to flourish and it often accompanies wars. For example, an examination of a mass grave containing the remains of some of Napoleon's soldiers who died during the retreat from Moscow in 1812 found that many of them were infested with lice and suffered from louse-borne epidemic typhus and trench fever (Raoult *et al*., 2006). Similarly, much of the literature of the First World War contains vivid descriptions of louse-infested clothing and several million people died of typhus across Europe at the end of the war. Fortunately, the discovery of the insecticidal properties of DDT prevented similar mortalities due to louse-borne typhus during the Second World War.

Pthirus pubis, also known as the crab louse from its appearance and the pubic louse from its normal dwelling place, is a fascinating creature (Figure 4.7). Ever romantic, the French refer to

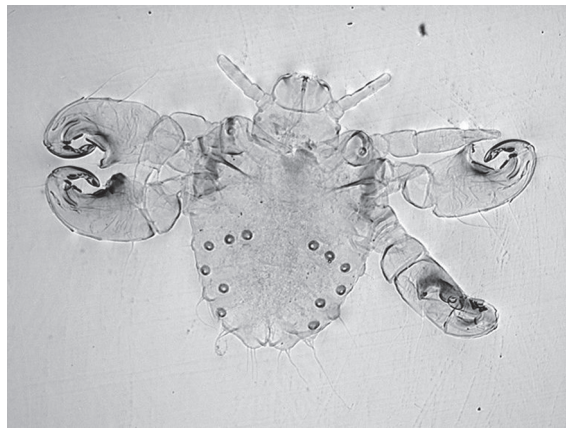


Figure 4.7 Human crab louse, *Phthirus pubis* (Order Phthiraptera). The tibio-tarsal claws are adapted for clinging to coarse hairs. The three thoracic segments are fused (as in other Anoplura). The stylet-like mouthparts can be seen within the squat head capsule.

it as '*papillon d'amour*' although it is doubtful if those who are infected look on it with any affection. It is a small louse that normally only grows to about 2 mm in length. The body is almost as broad as it is long and the middle and hind legs end in large claws that are used for grasping onto coarse hairs. The life cycle takes about 17–25 days from egg-laying to moulting to the adult stage. The female louse glues her eggs to coarse body hairs and several may be attached to a single hair. The eggs take 6–8 days to hatch and there are three nymphal stages that take a total of about 10–17 days to complete.

Crab lice are normally found on pubic hairs but may also attach to other regions of the body where coarse, sparsely growing hairs are found such as the eyebrows, and beard, moustache and chest hair in men. They are normally transmitted through sexual intercourse although transmission may also occur through other forms of close physical contact such as between parents and children who share beds. The crab louse causes inflammation and this together with the localised bleeding results in the formation of purple or blue-grey coloured spots (macules) at the feeding site. This results in irritation and scratching but crab lice are not considered to be vectors of disease. Crab louse infestations often correlate with infections with other sexually transmitted diseases but this is a reflection of sexual behaviour rather than an actual link. In common with other sexually transmitted infections, there is an enormous amount of ignorance about how one acquires crab lice and also what the best means of control are. For example, a survey of US College students found that many of them thought that crab lice could be caught by sharing living space with an infected person or using a toilet seat immediately after them and that crab lice could be effectively treated using antibiotics (Anderson and Chaney, 2009). None of this is true. Crab lice are very slow-moving and grasp firmly onto their host's hair and they are unlikely to become dislodged and need time to transfer between hosts. In common with head lice and body lice, pubic lice cannot survive for long off their host and those that are found on furniture, etc. are probably unwell or dying. Crab lice are best treated using insecticidal creams or lotions.

There are numerous species of Anoplura that are of veterinary significance. These include the delightfully named but distinctly unpleasant 'little blue cattle louse', *Solenopotes capillatus*. This louse has a world-wide distribution and is one of the commonest lice found on cattle. It is normally found on the face and jaw region and when large numbers congregate around the eyes they can give the appearance that the affected animal is wearing spectacles. As with most other lice, it causes pathology mainly through irritation that leads to disturbance, scratching and secondary infections.

The 'hog louse', *Haematopinus suis*, is one of the largest of the Anoplura and adults can grow to 5 mm in length. It is the only louse found on pigs and is a common and cosmopolitan species. The nymphs are found mainly on the ears and head region while the adults have a more widespread distribution and are also found on the neck, jowls, back and flanks. *Haematopinus suis* is normally transmitted by direct physical contact. However, they are capable of surviving for up to 3–4 days off their host so it is possible that transmission may also occur via bedding if pigs are moved into pens that have not been cleaned. *Haematopinus suis* nymphs have also been found attached to the housefly *Musca domestica* and it is therefore dispersal by phoresis may also occur.

Low intensity infections with *Haematopinus suis* are seldom a serious problem but large numbers of lice, cause constant irritation and an affected animal may rub off virtually all the hair from its body, and its skin becomes raw and broken. Heavy infestations of young piglets may result in sufficient blood loss to cause anaemia and death. *Haematopinus suis* is also considered to be a potential vector of the bacterium *Mycoplasma suis* (previously known as *Eperythrozoon suis*) that causes porcine infectious anaemia. However, more work is needed to confirm the extent to which this occurs.

4.4.2 Order Siphonaptera (fleas)

The fleas are an unusual group of insects whose relationship with the other insect orders is still uncertain. They are all rather small (1–10 mm) wingless insects that are parasitic on birds and mammals during their adult stage. Of the 2500 or so species described to date, over 70% are parasitic on rodents. They all feed on blood and have mouthparts that are modified for piercing and sucking. Adult fleas remain permanently on their host but usually move around upon it and feed periodically. However, so-called 'stick-tight fleas' such as the rabbit flea, *Spilopsyllus cuniculi*, tend to remain attached for long periods of time after firmly anchoring themselves in place with their long mouthparts. Movement of adult fleas between hosts occurs when there is close physical contact. The sexes are separate and male fleas are alleged to have the most complex genitalia in the Animal kingdom. Fleas lack wings and this is usually cited as an example of adaptation to parasitism since these could potentially make it more difficult to move about on their host. Wings could also be considered non-essential since the fleas' hosts act as their means of dispersal. However, some workers suggest that the Siphonaptera evolved from the Boreidae which is a family of wingless insects within the order Mecoptera (Whiting *et al*., 2008). If this is true, the fleas' wingless condition may be an ancestral condition and not a consequence of the parasitic lifestyle. The extant species of Boreidae are unusual in being mostly found at high altitude, they are active during winter and feed on plant material. By contrast, all other members of the Mecoptera are winged and most of them are either predatory or feed on dead invertebrates. There are, however, considerable morphological differences between the head and mouthparts of the Boreidae and the Siphonaptera and the suggestion that the two might be sister groups remains controversial.

Fleas lack compound eyes although they usually have clusters of ocelli. They therefore have limited visual capabilities but can detect changes in light intensity. Unlike other groups of insects, their body is laterally compressed and this adaption enables them to crawl between the hairs and feathers of their host. Many flea species have arrangements of backward-pointing bristles and rows of hardened (sclerotinised) spines called *ctenidia* in various parts of their body that are thought to enmesh with the hairs/feathers of their host and thereby make them difficult to extract (Figure 4.8).

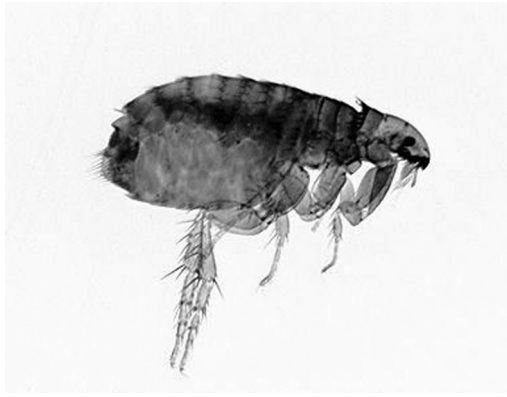


Figure 4.8 Cat flea *Ctenocephalides felis* (female). Note well-developed hind legs and backward-pointing spines that interlock with the host's hairs, making it difficult to pick them out of the pelage

Fleas have a sensory structure called a *sensillum* (*pygidium*) on the dorsal surface of their last abdominal segment that detects vibration. This *sensillum* is highly sensitive and enables the flea to detect an approaching 'predator' from changes in air currents and vibrations transmitted down hair shafts. In addition, fleas can run very quickly and their hind pair of legs are enlarged and adapted for jumping – some species can jump 30 cm in a single leap. Fleas are usually light-dark brown in coloration which makes it difficult to see them in hair/fur/feathers of their host and this, together with their shape and jumping ability, makes it very difficult to catch a flea. However, a deftly wielded moist bar of soap can prove an efficient 'flea catcher'.

Fleas are holometabolous insects whose life cycle consists of an egg, three larval instars (usually), a pupal stage and then the adult stage. The larval stage is rather worm-like and lacks legs and eyes. The larvae are not parasitic and usually feed on detritus within the host's nest or bedding. The larvae of some flea species do, however, have need a blood meal in order to complete their development. This blood is obtained by consuming the faeces of adult fleas and hungry larvae sometimes prod adult fleas to make them defecate. Having completed the larval period, the larva spins a silken cocoon within which it first enters a resting pre-pupal period and then moults to the pupa. Fleas that are parasitic on mammals will often breed throughout the year but those that are parasitic on birds only breed during their host's nesting season. After completing their pupal development, the adult fleas do not emerge until they detect mechanical stimuli because this indicates the return of a suitable host to the nest. This explains why houses that have been unoccupied for months, or even years, suddenly become 'alive with fleas' when a new owner moves in.

Most fleas are associated with a particular host species but this is seldom a highly specific relationship and a hungry flea is liable to feed on any warm-blooded animal. For example, in addition to the so-called human flea (*Pulex irritans*), about 20 other flea species have been recorded feeding on humans. However, a flea's fecundity often declines if it is not able to feed on its 'preferred host species'. This catholic diet can have serious implications for the transmission of zoonotic diseases. For example, bubonic plague (*Yersinia pestis*) is essentially a disease of rodents but when the rodents die, the fleas will leave their host's dead body as it cools and crawl and jump in search of alternative food. The catastrophic outbreaks of bubonic plague in Europe in earlier centuries were associated with the deaths of large numbers of rats and the consequent movement of fleas onto humans.

The human flea (*Pulex irritans*) is becoming uncommon in industrialised countries, and in the UK and Europe its place is being taken by the cat flea (*Ctenocephalides felis*). *Pulex irritans* remains common in many developing countries and is a potentially important vector of diseases. For example, in a survey of 12 villages in Tanzania, Laudisoit *et al*. (2007) found *Pulex irritans* in 72.4% of houses and, unlike the other flea species that were present, its density was positively correlated with higher frequency of bubonic plague. Although it is called the human flea, *Pulex irritans* is also commonly found on sheep, cattle, goats, pigs and even chickens. The dog flea, *Ctenocephalides canis*, has also been replaced by *Ctenocepahlides felis* in many regions so the most common flea found on dogs is now usually the 'cat flea'. In towns and cities, feral pigeons (*Columba livia*) often nest on or near to dwellings and humans are sometimes attacked by the pigeon flea (*Ceratophyllus columbae*) which migrates from deserted pigeon nests and into homes.

Although some flea species act as vectors of bubonic plague (*Yersinia pestis*) and murine typhus (*Rickettsia typhi*), the list of other pathogens of medical and veterinary importance for which fleas are the principal vectors is actually rather short. Flea bites can prove intensely irritating and in sensitive individuals and domestic animals they induce flea-bite dermatitis. Fleas have a reputation for preferring to bite some people more than others and women are often said to be bitten more

frequently than men. However, there is limited experimental evidence of differential attractiveness and many of the reported differences are probably more a consequence of some people reacting more severely to flea bites. Breathing in aerosolised flea faeces and cuticular components can also induce allergic asthmatic reactions. In addition to acting as vectors of disease, fleas are intermediate hosts for the tapeworms *Dipylidium caninum*, *Hymenolepis diminuta* and *Hymenolepis nana*. For humans, especially in developed countries, the psychological consequences of flea bites can be extremely upsetting. Although we are often willing to 'accept' mosquito bites, flea bites carry a certain stigma and are considered evidence of being dirty and uncivilised.

Box 4.1 Tungiasis

Tungiasis refers to infection with fleas belonging to the genus *Tunga*. These unusual fleas are commonly known as 'sand fleas', 'jiggers', or 'chigoes'. There are several species of *Tunga* but the best known is *Tunga penetrans* that was initially restricted to tropical America. However, in 1872, it found its way to West Africa in ballast sand being shipped from Brazil to Angola. It has since then colonised much of Sub-Saharan Africa and parts of the Middle East. Although *Tunga penetrans* was transported to Bombay and Karachi in 1899, it failed to spread in the way that it did in Africa.

Tunga penetrans is a very small flea, the adults being about 1 mm in size and it is typically found in sandy soil that is shaded by vegetation and in the earth floor of huts. After the adults have emerged, they latch onto a wide range of mammals and usually commence feeding on the feet. Much of the pathology is caused by the female flea that, after mating, burrows into the soft skin between the toes and under the toenails although other body regions may also be infected if the human (or animal) was lying on the ground. In animals that snuffle along the ground as they walk, such as pigs and dogs, the muzzle may be infected. Unlike many other flea species, the female *Tunga* retain their eggs within their body and as a consequence their abdomen swells enormously. Within 8–10 days the abdomen of a female *Tunga penetrans* becomes an almost spherical 6mm in diameter so that the thorax and head look like a tennis ball sitting on top of a football (Figure 4.9). The posterior two abdominal segments do not swell and they project

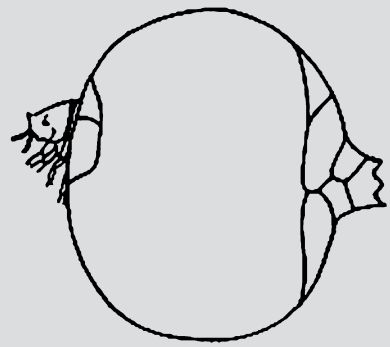


Figure 4.9 Engorged *Tunga penetrans* Source: Chandler and Read, 1961

out of the host's body – and it is through these that the eggs are ultimately expelled. The eggs subsequently hatch and the larval stages are free-living but unlike other Siphonaptera, there are only two larval instars.

The flea's feeding causes an inflammatory reaction and its increase in body size puts pressure on the surrounding tissues. Together, this results in intense pain and where large numbers of fleas are present, they can effectively 'honeycomb' the affected region and cripple the host. Obviously, this has economic consequences as it reduces the ability of people to work and the productivity of domestic animals. In the past, some military campaigns were brought to a literal standstill when so many soldiers were crippled by tungiasis that operations had to be abandoned. Tungiasis lesions are also often secondarily infected and tetanus may occur in individuals who have not been vaccinated. In poor communities in which most people walk barefoot, over 50% of the community can be infected. In addition, tungiasis is a zoonotic disease and dogs, cats, pigs, and rats can act as reservoirs of infection. The popularity of exotic holidays also means that tungiasis is increasingly being seen in returning travellers in developed countries.

4.4.3 Order Diptera (true flies)

The Diptera is one of the largest insect Orders and contains over 100,000 described species. From a parasitological perspective, it is the most important group of arthropods since the Diptera includes numerous examples of species that are vectors of viral, microbial, and parasitic disease and also those that are either facultative or obligatory parasites of humans or domestic animals.

Adult Diptera are characterised by possessing a single pair of membranous forewings that provide all the propulsion for flight. The hind wings are modified to form tiny club-shaped structures called halteres that function as gyroscopes. Most adult Diptera are active fliers and consequently have a well-developed thorax and large compound eyes. The adult flies usually feed on fluids and have mouthparts adapted for either piercing and sucking or their labium has become expanded to form two fleshy lobes that function a bit like a sponge. The Diptera exhibit holometabolous development and the legless worm-like larvae go through three or more instars depending upon the species. Fly larvae are commonly referred to as 'maggots' although many entomologists do not like to see this term used in scientific literature. The larvae exhibit a wide variety of feeding strategies and in some species this includes parasitism on vertebrates or other invertebrates. Currently, most taxonomists divide the Diptera into two suborders: the Nematocera and the Brachycera.

4.4.4 Suborder Nematocera

The Nematocera are considered to be the most primitive of the Diptera. The adult flies are characterised by having antennae that are usually longer than the combined length of the head and thorax and consist of numerous small segments. An exception to this rule is the Simuliidae (blackflies) in which the antennae are more compact but still consist of many segments. Nematoceran larvae have a well-developed head capsule that is equipped with biting mouthparts that operate in a horizontal plane like a pair of pincers. After completing its growth, the last larval instar moults to form an obtect pupa, that is as the adult fly develops, its legs and other appendages are fastened

Table 4.5 Examples of Diptera that act as vectors of parasitic diseases

Suborder	Family	Example	Common name	Parasitic disease transmitted
Nematocera	Culicidae	*Culex quinquefasciatus*	Mosquito	*Wuchereria bancrofti* (Lymphatic filariasis)
		Anopheles gambiae	Mosquito	*Plasmodium falciparum* (Malaria)
	Psychodidae	*Phlebotomus martini*	Sandfly	*Leishmania donovani* (Visceral leishmaniasis)
	Simulidae	*Simulium damnosum*	Blackfly	*Onchocerca volvulus* (River blindness)
Brachycera	Tabanidae	*Tabanus* spp.	Horsefly	*Trypanosoma evansi* (Surra)
	Muscidae	*Musca domestica*	Housefly	Faecal-oral transmitted parasites
	Glossinidae	*Glossina morsitans*	Tsetse fly	*Trypanosoma brucei* (Nagana)
	Calliphoridae	*Calliphora vicina*	Blowfly	Faecal-oral transmitted parasites

down to the rest of the body. Despite this, in some species, such as the mosquitoes, the pupa can remain mobile. The suborder Nematocera includes numerous species that are vectors of parasitic diseases but there are few that are themselves parasites of medical or veterinary importance (Table 4.5). We will therefore not consider the Nematocera any further here.

4.4.5 Suborder Brachycera

The Brachycera consists of the infraorders: Tabanomorpha, Asilomorpha, and Cyclorrhapha. The Tabanomorpha contains several families but it is the Tabanidae that are the most important from a veterinary and medical perspective. The Tabanidae are stoutly-built flies with short 'horn-like' antennae and large compound eyes that are often beautifully coloured. They are strong fliers but seldom make much noise, which means that they can approach their victim without drawing attention to themselves. Like mosquitoes, only the female tabanid feeds on blood and the male lives on nectar, honeydew and other natural sugary secretions. The male tabanid therefore lacks the rigid hypopharynx, blade-like mandibles, and recurved teeth on the maxillary laciniae that are found on the female flies. Although they might approach silently, the bite of a tabanid is painful and this means that they are usually disturbed before they have taken a full meal. However, they are also persistent and after being disturbed they quickly return to the same victim or another one nearby. Consequently, female tabanid flies make good mechanical vectors. The female tabanid fly lays up to several hundred eggs in a single mass or as several smaller masses. The larvae have a discernible head region although it is not as scleronitised as in the Nematocera and they are equipped with fang-like mandibles that operate in a vertical plane. Depending upon the species, tabanid larvae may be aquatic or develop in the soil and they go though up to 13 moults. Some of them are predacious on other invertebrates and cannibalism can be an important factor influencing larval density and dispersal.

The Asilomorpha contains the robber flies that are voracious predators of other insects but are not important as parasites of humans and domestic animals or as vectors of disease. The infraorder does, however, include several species of the bee-fly *Thyridanthrax* (Family Bombyliidae) that are parasitoids of tsetse fly pupae. These can have a significant impact on the tsetse fly population but it has not proved possible to exploit them as biological control agents (Laird, 1977). Flies belonging to the family Acroceridae often have remarkably small heads in relation to their body size while the larvae are endoparasitoids of spiders. The female fly lays eggs in masses at the end of twigs or scattered upon the ground and after hatching the first instar larvae search for a spider to parasitise. The larvae are able to crawl along spider webs and can jump up to 6 mm by bending and suddenly straightening their body. After locating a spider, the larva climbs up one of its legs and then penetrates its body wall. It then slowly consumes the tissues so that the spider does not die until the larva has completed its development.

The Cyclorrhapha is also known as the Muscomorpha and contains a number of species that are parasitic on humans and domestic animals. It also includes the unusual Nycteribiidae and Streblidae that are highly specialised parasites of bats. Adult cyclorrhaphan flies usually have well-developed compound eyes and their antenna consist of three short segments, on the last of which there is a feather-like sensory structure called the '*arista*'. Cyclorrhaphan flies typically lay their eggs singly or in batches and there are three larval instars. The larvae are usually bullet-shaped and lack a head capsule (Figure 4.10). The larvae lack compound eyes but have lateral ocelli (stemmata) that in some species are arranged in complex arrays. The larvae are equipped with an internal cephalopharyngeal skeleton that includes a pair of mouth hooks. The cephalopharyngeal skeleton is attached to the exoskeleton by a series of powerful muscles that swing it back and forth (see Colour Plate 13). In the process of moving the cephalopharyngeal skeleton, the mouth hooks are also moved back and forth and this drags food into the mouth or enables them

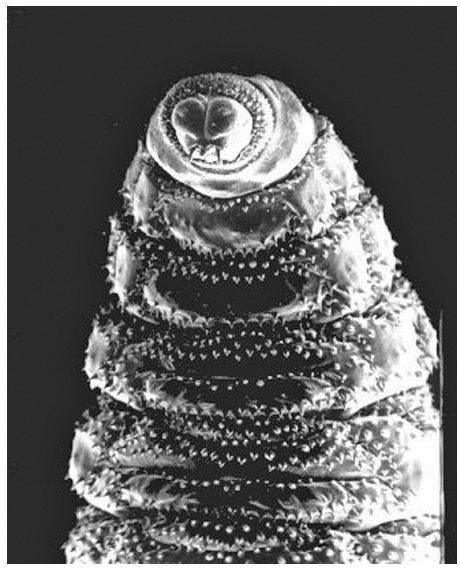


Figure 4.10 Scanning electron micrograph of third instar larva of *Wohlfahrtia magnifica*. Note the well-developed body spines

to be employed as 'grappling hooks' to facilitate movement. After completing its third instar the larva forms a coarctate pupa. This involves the larva moulting but the cast exoskeleton is retained and becomes a puparium. The cast exoskeleton is then sclerotinised which results in it becoming hardened and darkened. The puparium acts as a protective covering within which the pupa develops. The term 'Cyclorrhapha' translates as 'circular seam' because the puparium has a weak point running around it close to the anterior end. When the adult fly is ready to emerge, it exerts pressure at this point and the puparium breaks open to release the fly. Many cyclorrhaphan flies (i.e. those belonging to the Schizophora) employ an inflatable bag-like structure called the *ptilinum* to facilitate their escape. The *ptilinum* is located at the front of the head and the fly pumps air into it to inflate it. Once the fly has emerged, the *ptilinum* is deflated and retracted into the head behind the ptilinal suture never to be used again.

Box 4.2 Myiasis

The development of Diptera larvae within a living vertebrate animal is known as myiasis. The species causing myiasis can be broadly categorised as those that are facultative parasites and those that are obligatory parasites. Facultative species are those in which the larva can develop as a detritivore living in a dead animal or as a parasite of a living animal. These species are often associated with wound myiasis in which larvae develop in lesions on an animal while it is still alive. Obligatory species are those in which larval development can only take place in a living animal. Those species that are facultative parasites often show little host specificity. For example, *Lucilia sericata* will cause myiasis in virtually any warm-blooded animal. By contrast, those species that are obligatory parasites are often more host-specific although there are exceptions. For example, *Oestrus ovis* is usually only able to complete its growth in sheep and other oviids while *Cochliomyia hominivorax* will infect most mammals.

An alternative means of myiasis classification is based on the part of the body afflicted or an association. For example, fly larvae found in the superficial layers of the skin might be classed as a cutaneous myiasis or as a wound myiasis if they invaded an existing wound (see Colour Plate 28). Among those species that cause cutaneous myiasis, we can also distinguish between species upon their ability to initiate a myiasis. Primary species are those that initiate a myiasis through inducing a wound or establishing an infection through invading via one of the body orifices. Secondary species are those that do not initiate an infection but invade a wound once it is already infested with fly larvae. Primary species will also lay their eggs on a wound that is already infested with fly larvae.

As its name suggests, wound myiasis results from the infection of a wound with fly larvae. The wound does not have to be large and some flies will lay their eggs in a lesion as small as a tick bite. In addition, some fly species are highly sensitive to the odour of blood and can detect a wound from long distances. Farm animals often carry wounds from a variety of causes. For example, surplus males are usually castrated, cattle are often dehorned, and sheep often receive nicks when they are clipped or from catching themselves on barbed wire. Farm animals, like their wild counterparts, are also frequently wounded when in conflict with one another as they seek to establish their place in the flock or herd. For example, head wounds are common in rams. Wounds that are caused by infections such as foot rot in sheep or digital dermatitis in milking cows are also potential sites of infestation. Similarly, leech bites, tabanid fly bites, and lesions

caused by rubbing and scratching in response to lice infestations can all be exploited by the flies that cause wound myiasis. The umbilicus of new born mammals is also often infested.

Apart from the botfly infestations, myiasis in humans is usually associated with personal neglect or incapacity and tends to occur among those who are mentally or physically incapable of looking after themselves. Filth and wounds give off smells that attract various species of flies and these then lay their eggs on the skin or soiled clothing. One of the more famous historical personages thought to have suffered from myiasis is Herod the Great (73 BC–4 AD). He is reputed to have died a protracted and miserable death during which his genitals were said to have 'putrefied and brought forth worms'. Anything white and wriggly tends to be called a 'worm' and therefore it has been suggested that among other things he may have suffered from myiasis of the genitalia subsequent to a bacterial or other infection (Retief, 2005). Another noted sufferer of myiasis was St Symeon Stylites (c.390–459) who voluntarily spent twenty years of his life chained to the top of a stone column not far from Aleppo in Syria. Like many of his counterparts he believed that squalor was a sign of holiness and neither washed or changed his clothes for years. One of the many stories about him are of his gently returning the maggots that fell from his rags and encouraging them to feed because they were 'God's creatures and a manifestation of his will'. Presumably, these fly larvae were actually feeding upon his encrusted filth rather than his flesh since St Symeon lived to a comparatively old age.

Wound myiasis is a potential nosocomial infection in hospital patients if care is not taken to exclude flies and change dressings regularly. Any skin lesion such as a post-operative scar, diabetic ulcer and even psoriasis can become infected (Dagci, 2008). Oral myiasis can occur in which the fly larvae will invade the periodontal pockets and then be observed crawling across the surface of the gums. These cases are relatively rare and usually involve patients with poor oral hygiene, a habit of mouth breathing, and mental impairment (Rossi-Schneider *et al*., 2007; Tamizi *et al*., 2008). Tumour lesions such as squamous cell carcinomas can present a particular problem because in the end stage these are associated with bleeding that may prove fatal. Health care workers therefore tend to minimise changing dressings and wound treatments when their patients reach this point. However, the carcinomas can become infected with fly larvae and this causes considerable psychological torment to the patient who has to cope with their body being consumed by maggots even as they must come to terms with their own impending death (Sesterhenn *et al*., 2009).

4.4.6 Family Calliphoridae

The Calliphoridae contains over 1000 species and includes species with a diverse array of lifestyles. However, those of medical and veterinary importance belong to the groups that are commonly known as the blowflies and the screwworm flies. The term 'blowfly' comes from the fact that one of the several meanings for the word 'blow' is to describe a mass of fly eggs. A piece of meat that has fly eggs on it is said to be 'flyblown' and the fly that leaves those eggs is therefore referred to as a 'blowfly'. The term 'screwworm' is derived from the way in which the larvae of these flies burrow deeply into the flesh of their host and it appears as if they are screwing down into the tissues. The adults of blowfly and screwworm species are generally harmless as they feed on nectar, plant sap, and fluid decaying organic matter. Some of them will also feed on

bodily secretions (e.g. tears) and blood oozing from wounds but they lack piercing and sucking mouthparts. These species can be a considerable nuisance and cause disturbance and may also act as mechanical vectors of faecal-orally transmitted pathogens.

The word 'blowfly' is a generic term and is applied to both species that are capable of causing myiasis and those that are detritivores or parasites of invertebrates. Among the most important blowfly species that cause cutaneous and wound myiasis are *Lucilia sericata*, *Lucilia cuprina*, *Calliphora vicina*, and *Protophormia terrae-novae*. The *Lucilia* species are commonly known as greenbottles as a consequence of their shiny green thorax and abdomen. The *Calliphora* species tend to be called bluebottles because of their dark blue coloration while *Protophormia* species are called blackbottles because they are usually extremely dark. However, such terms are not to be recommended as they invariably lead to confusion. The life cycle of those species causing myiasis is relatively similar although the duration of the different stages varies. The female fly lays her eggs in large masses that can contain over 100 eggs upon the host, either at the edges of a wound or upon soiled skin. The eggs hatch after a few hours and the larva starts to feed. If there is an existing wound, the larvae will move into this and extend it but if one is not present, they may initiate a wound by inducing an inflammatory reaction that weakens the skin. The larvae do not have strong enough mouthparts to penetrate healthy unbroken skin. There are three larval instars and once the larva has reached optimal size, it ceases feeding and drops off. It then voids its gut and crawls across the ground until it reaches a suitable site where it digs into the soil. The larva then contracts and undergoes pupariation – although some species (e.g. *Calliphora vicina*) may undergo a resting diapause stage at this point. After the pupa within the puparium has completed its development, the adult fly emerges, crawls up to the soil surface, expands its wings and flies off. The whole life cycle from egg hatch to adult emergence typically takes about 3–4 weeks but is heavily influenced by environmental factors.

Box 4.3 Blowfly strike in sheep

Although it is called a blowfly strike, this is a generic term and other species of fly may also be responsible such as sarcophagid flies or screwworm flies. Before the introduction of insecticidal sheep dips, blowfly strike was a cause of considerable suffering wherever sheep were raised. Even today, anybody who rears sheep has to keep a careful watch on the flock during the 'fly season'. Unless an infection can be halted before it has developed too far, a sheep can be literally 'eaten alive' by successive waves of thousands of fly larvae although the animal often succumbs to septicaemia before vital organs are affected.

Sheep are usually first 'struck' around their rear end where the wool becomes soiled with faeces and urine (see Colour Plate 14). This is known as 'breech strike'. Gastrointestinal parasites that induce diarrhoea will increase a sheep's susceptibility to breech strike by increasing the soiling of the wool. Fatty-tailed sheep such as the Awassi and other breeds that are popular in the Middle East and Central Asia are also particularly vulnerable to blowfly strike because of the soiling that occurs under their tails. Allegedly, some farmers allow the rear end of these sheep to become heavily soiled because it increases the live weight at sale.

Blowfly strike typically begins when a primary fly species such as *Lucilia sericata* or *Lucilia cuprina* is attracted by the smell given off by the soiled wool. Bacteria contribute to fleece rot and the odours released by species such as *Pseudomonas aeruginosa* stimulate blowfly oviposition

(Emmens and Murray, 1983). After landing, the female fly lays her eggs and once these hatch, the first instar larvae move down to the surface of the skin where they commence feeding on bacteria, detritus, and dead skin tissues. In the process, the larvae release proteolytic secretions and these are absorbed across the sheep's skin where they initiate an inflammatory reaction. As a consequence, the surface of the skin becomes raw and inflamed and releases watery exudates. At this point, the skin no longer presents an effective barrier and the larvae start to feed on the underlying tissues. Because there are usually numerous larvae present, an open wound is soon formed and then extended. This is attractive to both secondary and primary flies and they arrive and lay their eggs. In addition, when they move from the faeces-contaminated wool onto the wound, the larvae transport bacteria that results in the wound becoming septic and toxaemia may develop.

The screwworms include the important species *Chrysomya bezziana* – the Old World Screwworm – and *Cochliomyia hominivorax* – the New World Screwworm. As their common names suggest, *Chrysomya bezziana* is predominantly found in Asia and Africa while *Cochliomyia hominivorax* is found in South America.

4.4.7 Genus *Chrysomya*

There are several species of *Chrysomya*, some of which are obligatory parasites during their larval stage while others are either facultative parasites or are detritivores. The most important species is *Chrysomya bezziana* in which the larvae are obligatory parasites of warm-blooded vertebrates. This species is found throughout parts of India, Asia, the Middle East, Africa and also New Guinea. The adult fly is bright metallic green or blue and 8–10 mm in length and superficially resembles the blowfly *Lucilia*. The adult flies are not often seen in the wild because they are not attracted to dead animals or the sorts of baits that attract blowflies. The female quickly lays batches of approximately 100–300 eggs at the site of a wound or on skin that is contaminated with blood. The eggs hatch after 18–24 hours and the first instar larvae feed on blood and discharges emanating from the wound. After 12–18 hours the larvae moult to the second instar and move into the wound and start to extend it. After a further 12 or so hours the larvae moult to the third instar and burrow deep into the wound so that only their posterior spiracles may be visible. The third instar larvae feed communally and voraciously and grow to about 18 mm in length over the course of 3–4 days. Once they are mature, the larvae then emerge from the wound, drop to the ground and burrow into the soil where they pupariate. The adult fly emerges after 7–9 days although the development period may last several weeks in cool weather.

4.4.8 Genus *Cochliomyia*

The genus *Cochliomyia* contains a single species, *Cochliomyia hominivorax*. The term '*hominivorax*' translates as 'man-eater' and for very good reasons. The first published report on the fly was written by the French surgeon Dr Charles Coquerel in 1858. He described treating five men who were parasitised by the fly in Cayenne (French Guiana, South America) that was at the time a hellish penal colony. The flies had laid eggs in the nostrils of the men and after these hatched,

hundreds of maggots proceeded to eat into the men's heads. Despite many attempts at flushing the larvae out, three of the men subsequently died.

Adult *Cochliomyia hominivorax* are about 15 mm in length and have a bright greenish blue metallic coloration, a yellowish face and bright orange-red compound eyes. The females mate only once while the males mate several times and this is one of the reasons the fly can be successfully controlled by the release of sterile males (FAO, 1992). After mating, the female fly lays her eggs in batches of 10–500 at the edges of wounds so that the eggs overlap the wound. She lays 4–8 batches of eggs over a lifespan that is typically 7–10 days but can extend to over a month. The larvae hatch after 11–24 hours and move into the wound. The larvae feed communally and burrow much deeper into wounds than most fly species that cause wound myiasis and may be found 10 cm or so below the surface. In addition, their body is armed with backward-pointing spines that make it difficult to remove them. The wounds usually become secondarily infected with bacteria and the wounds are then said to give off a characteristic odour that is highly attractive to other female *Cochliomyia hominivorax*. As a consequence, the wounds rapidly become deep and extended. The larvae take about 4–8 days to reach the end of the third instar and at this point they drop off and pupariate in the soil. The adult flies emerge after 7–54 days depending upon the temperature. The whole life cycle from egg laying to adult emergence can therefore take as little as 21 days under ideal conditions.

Cochliomyia hominivorax is predominantly a parasite of wild and domestic animals although human infections do arise occasionally. It used to be a major problem in parts of North America and Mexico where it was responsible for annual losses amounting to millions of pounds. For example, in 1976, the cost of surveillance, treatment and control of *Cochliomyia hominivorax* was estimated to amount to US$ 300 million per annum in just the state of Texas. Since then, the success of the sterile male release programme to control *Cochliomyia hominivorax* has eliminated the fly from North America, Mexico, and some South American countries. In the late 1980s an outbreak of *Cochliomyia hominivorax* occurred in Libya through the import of infected cattle. The flies spread rapidly and there were fears that the species would establish itself in neighbouring countries with consequent devastating impact on their economies. At the time Libya and the USA were politically hostile to one another but they cooperated to mount a sterile male release control programme that eliminated the fly before it could become a major problem (FAO, 1992).

4.4.9 Genus *Auchmeromyia*

The Congo floor maggot, *Auchmeromyia senegalensis* (also known as *Auchmeromyia luteola*) is sometimes stated as a specific parasite of humans. However, there are several reports of it attacking warthogs, pigs, and hyenas. The larva feeds on blood but spends the daylight hours hiding in the soil and crevices and emerges at night to feed on sleeping humans. Presumably, the maggot reverses its activity cycle when parasitising nocturnal animal hosts. In the colonial era it was a common problem in what was then the French Congo. This region has since become divided up into the Republic of Congo, The Central African Republic, and Gabon. Nowadays *Auchmeromyia senegalensis* is seldom found because the villagers increasingly sleep on beds rather than mats on the floor. As a consequence, the larva's meal is now out of reach. However, another social change means that another group of people who were not previously at risk are acting as hosts. The Pygmies who live in the Chailu region of the Congo traditionally led a nomadic existence in the jungles and were not afflicted by *Auchmeromyia senegalensis*. This may have been because

they moved too frequently for the flies to establish a population at their camp sites. However, the Pygmies have started to become sedentary and live in primitive camps on the outskirts of Bantu villages. The Pygmies tend to sleep on mats on the floor and are attacked by the maggots while the Bantu villagers are protected as they sleep on beds (Noireau, 1992).

4.4.10 Genus *Cordylobia*

The Tumbu fly, *Cordylobia anthropophaga*, is found in Sub-Saharan Africa although there has also been a report of a child being infected in the Asir region of Saudi Arabia so it may be more widespread. The adult female is a stout yellow-brown fly and 8–12 mm in length. She lays her eggs on the ground, especially in sandy regions and if the soil is contaminated with urine or faeces. The larvae then hatch and they wait until a suitable host passes by. Despite the fearsome name '*anthropophaga*', humans are but one of their potential hosts, and dogs and many other mammals are commonly parasitised. The larvae quickly burrow into the skin and each larva forms an individual boil-like lesion within which it develops. The boils typically form on the feet and lower limbs but other areas of the body may be affected. Obviously, wearing shoes and long clothing minimises the risks of becoming infected. The larva takes about 7–15 days to become a mature third instar by which time it is 12–28 mm in length. The thick-bodied larva feeds head down and has recurved spines that make it difficult to remove. The boil that develops around the larva has a hole at its centre through which the larva is able to breathe. When it is mature, the larva manoeuvres out of the boil, drops to the ground and pupariates in the soil. The adult fly then emerges after 3–4 weeks.

The Tumbu fly is notorious for laying its eggs on washing that is hung out to dry. The fly does not lay its eggs on washing that is in full sunlight and favours that which is in the shade and still has a smell of urine – such as underwear or nappies. When the clothes are next worn, the larvae then hatch in response to the heat of the skin. Many people consider ironing one's underwear to be a waste of time but in parts of Africa it can make good sense because it kills any Tumbu fly larvae or eggs that are attached. There are many possibly apocryphal stories of servants who intentionally did not iron the washing of employers who were miserly or ill-treated them.

4.4.11 Family Sarcophagidae

Flies belonging to the family Sarcophagidae are commonly known as 'Flesh Flies' because some of them are attracted to meat and dead animals. Unlike the blowflies, the sarcophagids are larviparous. That is, the eggs hatch inside the uterus of the female and she deposits first instar larvae directly onto their food source. Adult sarcophagids are handsome grey coloured flies with red eyes. Their thorax usually bears black longitudinal stripes and their abdomen has a chequered or tessellated pattern. Their tarsi have large pluvilii (pads) that can give them the appearance of having big feet.

The family Sarcophagidae is divided into two subfamilies: the Miltogramminae and the Sarcophaginae, and together they contain over 2000 described species. Most of the Miltogramminae are parasitoids of other invertebrates although some species are detritivores and a few cause myiasis in vertebrates. The Sarcophaginae contains a number of species whose larvae develop on dead animals and some are either facultative or obligatory parasites of vertebrates.

One of the most commonly encountered sarcophagid flies causing myiasis is the wonderfully named *Wohlfahrtia magnifica*. This fly belongs to the Miltogramminae and is widely distributed in Eastern Europe, the Mediterranean region, and the Middle East. It is a relatively large fly that can grow up to 14 mm in length and its larvae are obligate parasites of warm-blooded animals. The female fly deposits her larvae on wounds or at moist body orifices and the larvae then quickly burrow into their host. There are reports of nosocomial infections of unconscious patients occurring through flies depositing larvae in intubation tubes (Yazar *et al*., 2005). The larvae burrow deep into the subcutaneous tissue and cause liquefaction and necrosis of the surrounding flesh. The larvae go through three instars and the mature third instars start to leave their host after about 5–7 days. The mature larvae drop to the ground and pupariate in the soil.

Unless treated promptly, infestations with *Wohlfahrtia magnifica* will lead to serious pathology and death may occur within 1–2 weeks. The female fly can deposit 120–170 first instar larvae and even on their own these will cause considerable damage. However, infested wounds soon attract other *Wohlfahrtia* as well as blowflies. This means that an infested wound is rapidly extended, and septicaemia can develop.

4.4.12 Family Oestridae

This family contains the so-called warble flies and botflies. The term 'warble' as a verb to describe a style of singing dates back to the 1300s with roots indicating whirling motion. However, 'warble' subsequently also became a noun to describe a raised swelling. One suggestion is that in this case it was derived from an old Swedish word for a boil '*varbulde*' (*var* = pus; *bulde* = swelling). The term 'bot' was being used in the English language to describe a parasite or maggot in the 1500s but how it was derived is not known. Certainly, the same term was also being used elsewhere in Europe at the time, for example, a Dutch word for a liver fluke was *leberbot*. The Oestridae is a relatively small family that contains about 150 or so species. They are all obligate endoparasites of mammals during the larval stage and the adults lack fully-functional mouthparts, although a few species are thought to be able to imbibe water or other fluids (Colwell *et al*., 2006). Many species are host-specific and are capable of infesting only a single host species or group of closely related species. Therefore, although the family includes several species of economic importance, it also includes some that are in serious danger of extinction. For example, the population of rhinoceroses and many other large mammals has declined so badly in recent years that those animals that are dependent upon them are also being pushed to the brink of extinction. It is highly unlikely that the public could ever be convinced of the need to preserve parasites but all species have a role to play in the ecosystem and their loss may have unforeseen consequences.

Subfamily Oestrinae The larvae of flies belonging to this subfamily are usually found in the nasal cavities of their host and they are therefore often referred to as nasal botflies. There are a variety of genera and some of them have fascinating life cycles. For example, the larvae of *Gedoelstia hässleri*, which is a parasite of large African antelopes such as the Blue/Common Wildebeest (*Connochaetes taurinus*), undergo an apparently unnecessarily complicated migratory pathway. The female fly places her larvae into the eyes of a suitable host and these then penetrate the tissues and get into the blood system. The first instar larvae are very small (0.7–0.9 mm in length) and make their way via the bloodstream to the subdural space – this is a thin layer that is found between the *dura mater* and the *arachnoid mater* (two of the three meninges that surround

the brain and spinal cord). The larvae then migrate through the cribiform plate and ethmoid bone to reach the nasal cavity where they moult to the second instar and then the final (third) instar. The closely related *Gedoelstia cristata*, which also parasitises various African antelopes, shows a similar migratory pattern. After the larvae are deposited in the eye of their host, they enter the blood circulation, pass through the heart and are transported to the lungs. The larvae then penetrate the alveoli, climb the bronchi and trachea and make their way to the nasal cavities where they moult to the second instar. In their normal hosts, *Gedoelstia hässleri* and *Gedoelstia cristata* appear to cause little harm but in domestic sheep and cattle (in which they are unable to develop to maturity), they cause a condition known as '*uitpeuloog*' (bulging eye disease) that results in glaucoma and in severe cases the eyeball may rupture. The migrating larvae can also induce inflammatory reactions within veins that can lead to the formation of thrombi (clots) and the localised softening or loss of brain tissue (encephalomalacia).

Oestrus ovis The sheep nasal fly, *Oestrus ovis* is the most important member of the subfamily Oestrinae and it is found throughout the world wherever sheep are reared. The adult fly is about 12 mm in length and has a stout body with a large stumpy head. It has a dark grey coloration with black spots on the abdomen and thorax and a covering of short brown hairs. The mouthparts are non-functional and they are unable to sting but the approach of a fly causes sheep to panic and they attempt to protect their nostrils by pushing their noses into the ground or huddling together so that their noses are pushed into one another's fleece. The force of being expelled through the female fly's ovo-larvipositor causes the eggs to hatch and therefore she deposits first instar larvae rather than eggs. She typically places up to 25 larvae in the nostrils of sheep and goats. There are also accounts of them being deposited in the nostrils and eyes of humans. *Oestrus ovis* larvae are usually unable to complete their development in humans although they may cause irritation, inflammation, and pain. Infections of the human eye (opthalmomyiasis) result in conjunctivitis and photophobia but the larva normally dies as a first instar larva and it does not invade the body of the eye. In their normal host, the larvae crawl into the nasal passageways where they moult to the second instar and these larvae then continue to the sinuses where they moult to the final third instar stage (see Colour Plate 15). The larvae feed on nasal secretions and irritate the lining of the nasal mucosa with their sharp mouth hooks. Although they do not feed on blood they may cause bleeding from the nose (epistaxis). The larvae become large thick-bodied wedge-shaped maggots up to 3 cm in length. When they are mature, the larvae move to the nasal passageways and are then sneezed out after which they pupariate in the soil. The duration of the different life cycle stages is heavily dependent upon the environmental conditions. The adult flies are active on hot sunny days and in warm climates there may be up to three generations a year. By contrast, in colder climates, the first instar larvae cease growing during the autumn/winter and resume their development when the air temperature increases. The ability to suspend their development (hypobiosis) is thought to be an important part of the insect's life cycle and can also be induced by other stressors such as crowding and the host immune response (Colwell *et al*., 2006).

The disturbance caused by adult *Oestrus ovis* can lower productivity but it is the fly larvae that cause most harm to sheep. Heavy infections can induce a condition known as 'false gid' in which the afflicted animal becomes unthrifty and exhibits a lack of co-ordination and staggers around in circles. More normally, the animal develops a nasal discharge, constantly sneezes and rubs its nose against objects as though attempting to relieve an irritation. Inflammatory reactions to the physical irritation of the larvae and also to their excretory-secretory products results in rhinitis

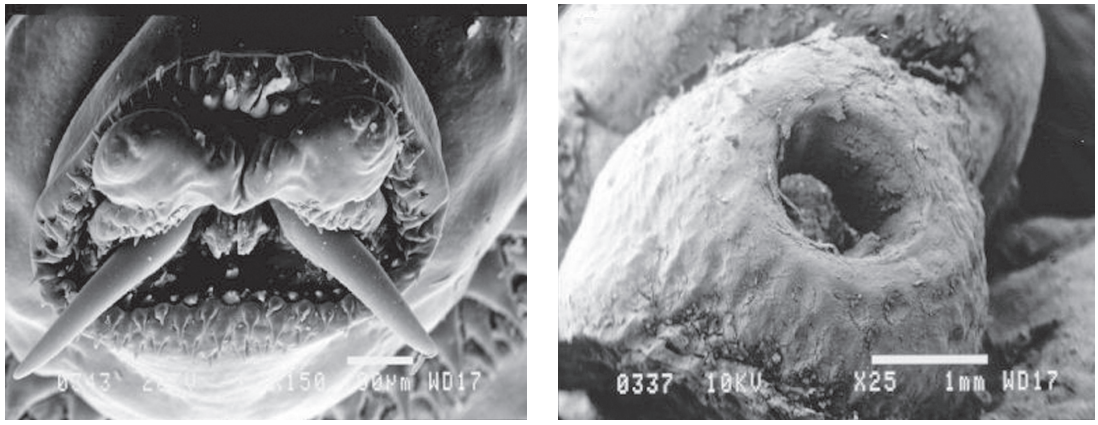
and sinusitis. Unlike the migrating larvae of *Hypoderma*, the larvae of *Oestrus ovis* appear to promote an inflammatory response so that they can feed on the resultant exudates. In serious cases of *Oestrus ovis* infection, secondary bacterial infections may develop that result in potentially fatal pneumonia. It has also been suggested that the larvae may activate viruses such as orf (a zoonotic parapox virus) and the *maedi-visna* virus (Angulo-Valadez *et al*., 2010). *Maedi-visna* virus is a lentivirus that is usually present as a subclinical infection but when activated, it can induce fatal disease characterised by dyspnoea (*maedi*; breathing difficulty) or neurological (*visna*) signs. In some breeds of sheep *Oestrus ovis* infection has been linked to the development of tumours (Bergeaud *et al*., 1994). This may also be related to virus activation but there is currently very limited published information on this and the evidence of an association is not strong (Dorchies *et al*., 2003).

4.4.13 Subfamily Gasterophilinae

This subfamily contains the well-known horse botflies *Gasterophilus intestinalis*, *Gasterophilus nasalis, Gasterophilus pecorum*, and *Gasterophilus haemorrhoidalis* that infect horses, donkeys, and asses. In older literature the genus name '*Gasterophilus*' is often spelt '*Gastrophilus*'. Other Gasterophilinae infect wild equids such as zebra while some species are specific to elephants and rhinoceroses but in all cases the final instar larvae are found within the gastrointestinal tract – usually the stomach. Appropriately enough, the rhinoceros stomach botfly *Gyrostigma rhinocerontis* (previously *Gyrostigma pavesii*) is reputedly the largest fly in Africa and its body can be up to 35 mm in length while the larvae can grow to 40 mm.

Adult *Gasterophilus* are usually 10–15 mm in length and covered in dense brown/yellowish hairs so that they bear a superficial resemblance to bumble bees. The adults are strong fliers but they lack functional mouthparts and therefore, like many other adult oestrid flies, their lifespan is probably quite short. The female fly has a long stout ovipositor which she uses to glue eggs to hairs or to the skin on specific regions of the host animal. On horses, *Gasterophilus intestinalis* usually lays its eggs on hairs on the fetlocks of the forelegs but may also attach them as high up as the scapular region, *Gasterophilus nasalis* lays its eggs underneath the chin while *Gasterophilus haemorrhoidalis* lays its eggs on the lips. An exception is *Gasterophilus pecorum* that lays its eggs on vegetation that is subsequently consumed by the host. Egg-laying flies disturb horses and they attempt to protect the region the flies are aiming for. In some species, such as *Gasterophilus nasalis* the eggs hatch spontaneously while those of other species, such as *Gasterophilus intestinalis* have to be licked by the host.

The first instar larvae either migrate to the mouth or the eggs hatch in the mouth after being consumed. The larvae then undergo a species-specific series of movements and development within the buccal cavity before re-locating to specific locations in the gastrointestinal tract (Cowell *et al*., 2006). Ultimately, third instar larvae of both *Gasterophilus pecorum* and *Gasterophilus intestinalis* attach to the mucosa of the stomach although *Gasterophilus intestinalis* tends to be restricted to the junction of the glandular and non-glandular regions. The third instar larvae of *Gasterophilus nasalis* attach to the lining of the duodenum near the pylorus while, as the name suggests, the larvae of *Gasterophilus haemorrhoidalis* complete their development attached to the lining of the rectum. The larvae take about 10–12 months to develop to maturity after which they detach and are then passed out with the faeces in the Spring. The larvae pupariate in the soil and the adults emerge after 3–5 weeks.



(a) (b)

Figure 4.11 Scanning electron micrographs of *Gasterophilus intestinalis* (a) anterior of larva showing curved mouthhooks; (b) sites of attachment in the stomach of a donkey

The third instar larvae are usually found clustered together in dense groups. They are barrel-shaped, grow to around 16–20 mm in length, and attach to the mucosa of the gut using large, sharply pointed, sickle-shaped mouth hooks (Figure 4.11a). In many species the second and third instar larvae are coloured various shades of dark red although those of *Gasterophilus nasalis* tend be light yellow. This coloration is owing to the presence of haemoglobin molecules that facilitate the uptake, transport, and storage of oxygen. In *Gasterophilus intestinalis* larvae, the haemoglobin occurs as an intracellular molecule in a variety of cell types during the early stages of larval development but is subsequently restricted to the tracheal cells. A further adaption to their lifestyle is that their posterior spiracles are sunk into a pouch that opens when they are above fluid level.

The migration of the first instar gasterophilid larvae in the gums/palate/tongue causes bleeding, pain, inflammation and secondary bacterial infections. There is some debate whether final instar gasterophilid larvae feed on host tissue/secretions or act as so-called 'kleptoparasites' that obtain their nutrition from ingesting the host's digesta. However, the attachment of the larvae induces inflammatory reactions that result in ulceration, localised fibrosis and the loss of functional mucosal tissue (Figure 4.11b). *Gasterophilus pecorum* has the most pathogenic larvae because they attach to the walls of the pharynx and the oesophagus for up 10 months and it is only the older third instar larvae that migrate to the stomach. Larvae within the oesophagus induce inflammatory reactions that result in localised swelling. Consequently, the presence of large numbers of larvae or an excessive inflammatory reaction to them can result in the oesophagus becoming sufficiently constricted to cause choking and difficulty in swallowing – and this can prove fatal.

4.4.14 Subfamily Hypodermatinae

This subfamily includes the well-known 'warble flies' because the raised nodule-like swellings caused by the third instar larvae are sometimes referred to as 'warbles' (see Colour Plate 16). There are two species of warble fly of importance in cattle: *Hypoderma bovis* and *Hypoderma*

lineatum. Their life cycle begins when the female fly attaches her eggs to a cow. The flies have non-functional mouthparts and are unable to bite but they induce panic in cattle. Frightened animals rush blindly away bucking their hind legs and with their tails in the air. This behaviour is known as 'gadding' and the animals can injure themselves by colliding with fences, gates, and barbed wire. Even if the cattle are not injured, the disturbance reduces feeding and hence affects live-weight gain and milk production. According to Gansser (1956), the flies do not produce a characteristic buzzing while in flight although this does not preclude them from emitting a high pitched noise that is audible to cattle but not humans. Clearly there must be a distinctive feature of an approaching warble fly that distinguishes it from the many hundreds of other flies that plague cattle during the summer months.

Female *Hypoderma bovis* attach single eggs to hairs on the legs below the hocks while *Hypoderma lineatum* attach rows of six or more eggs to hairs on the legs above the hocks and on the lower parts of the body. Female *Hypoderma bovis* therefore have to make more frequent approaches in order to lay their eggs and their repetitive darting flight around the feet of cattle may explain why they are more prone to causing gadding than *Hypoderma lineatum*. After hatching, the first instar larvae of both species crawl down the hairs and penetrate the hair follicles. The larvae of the *Hypoderma bovis* migrate along nerves until they reach the epidural fat that surrounds the spinal cord. By contrast, the larvae of *Hypoderma lineatum* migrate through the muscles and connective tissue until they reach the submucosa of the oesophagus. Both species reach their respective destinations in the late Autumn and they remain there overwinter during which time they moult to the second instar. In the Spring, the larvae resume their migrations and both species ultimately reach the skin underneath the host animal's back. Here they moult to form barrel-shaped larvae that can grow to about 28 mm in length (see Colour Plate 17). The larvae cut a breathing hole through the host's skin through which they breathe and when they are mature they manoeuvre their way out, drop to the floor and pupariate in the soil. The adults emerge after about 3–5 weeks although those of *Hypoderma lineatum* are usually active about a month before those of *Hypoderma bovis*.

The host immune reaction against the third instar larvae is responsible for the formation of the warble because it results in swelling with fluid accumulation. It is impossible to pull a larva out of its 'warble' but one can sometimes squeeze them out by exerting pressure from below – rather like squeezing a spot. When the third instar larvae cut their breathing holes/emerge, the damage they cause cannot be repaired. Consequently, the hide of a heavily infected animal is ruined. In addition, the inflammatory reaction against the larvae means that the surrounding flesh is unfit for human consumption and has to be cut away. The first and second instar larvae do not usually induce serious pathology although if the larvae of *Hypoderma bovis* die while they are overwintering, the chemicals they release can induce an inflammatory reaction that results in nerve damage and paralysis.

Hypoderma bovis and *Hypoderma lineatum* are virtually extinct in Great Britain following a successful eradication campaign. The eradication campaign began in 1978 at which time it was estimated that about 38% of the cattle were infected and losses to the livestock industry amounted to about £13 million per annum. Legislation was passed (Warble Fly [England and Wales] Order 1982 and the Warble Fly [Scotland] Order 1982) that made the warble fly a notifiable disease. Anyone who had an animal that they knew was infected or suspected was infected had to first report it to a Ministry Divisional Veterinary Officer before attempting any treatment. If the infestation was confirmed, then all cattle on the farm over 12 weeks of age had to be treated. Warbles become obvious in the Spring and treatment at this time kills the larvae before they

can become adults. Further treatment was also required in the Autumn (before the larvae had time to reach their winter resting sites) to prevent infections developing to the 'warble' stage. In addition, cattle on surrounding farms also had to be treated regardless of whether or not they had warbles. These measures were so successful that by 1986 less than 0.007% of inspected cattle were infected.

4.4.15 Subfamily Cuterebrinae

The members of this subfamily are sometimes referred to as the 'New World skin bot flies' because they are restricted to the North and South America. However, some taxonomists feel that the subfamily should include the genera *Neocuterebra* and *Ruttenia* that are parasites of African elephants and this would make the common name inappropriate. Members of the genus *Cuterebra* are often host-specific and most are parasites of rodents and rabbits. Typically, they lay their eggs near to burrows or other sites of host activity and the eggs hatch in response to the heat of the passing host. The first instar larvae then quickly attach to the host's hair and make their way to its mouth or nose. The larvae then penetrate the mucosa and spend about 7 days wandering around the pharyngeal region. The larvae then migrate sub-dermally through the host's connective tissue until they reach a species-specific site where they stop, cut an air hole in overlying skin and develop into the final instar. The larvae of some species of *Cuterebra* can reach up to 30 mm in length which is exceptionally large in relation to the size of their host. After reaching maturity, the larva forces its way out through the skin and pupariates in the soil. The various species of *Cuterebra* are of little medical or veterinary importance although cats and dogs are sometimes infected and heavy infestations can prove fatal. The most important member of the subfamily Cuterebrinae is *Dermatobia hominis* although its lifestyle, lack of host specificity and development distinguish it from other members of the group.

Dermatobia hominis *Dermatobia hominis* is found throughout South America and also the islands of Trinidad. Although it is often called the 'human bot fly', it will infect a wide range of large mammals and it is also the only species among the Oestridae that naturally parasitises carnivores. However, *Dermatobia hominis* is most important as a pest of cattle and in 1976 the annual losses to cattle production it caused in Brazil and Central America were estimated to be US$ 200 million per annum.

The fascinating thing about this fly is that the female does not lay its eggs directly onto its host. Instead, the female captures flies that are likely to frequent cattle or other mammals. She usually chooses insects that are in the vicinity of the host animal and glues up to 25 eggs to their abdomen or thorax. Day-flying mosquitoes are often exploited but other flies such as *Musca* spp. and *Stomoxys calcitrans* may also be used. There is even a report of ticks being exploited. The female fly then releases her 'vector' and when this next alights on a mammal the rise in temperature stimulates the eggs to hatch. The first instar larvae then drop onto the host and quickly burrow into its skin. If the mosquito or *Stomoxys* had fed, then the larvae may make use of the puncture wound. The larvae do not migrate and develop slowly at the site of their initial invasion over the next 3 months. A raised nodule forms around the developing larva with a central breathing hole. The structure can become inflamed and resemble a boil. These lesions may be secondarily

infected by bacteria and also attract the attentions of screwworms and other flies that cause wound myiasis. Once it is mature, the larva emerges, drops to the ground and pupariates in the soil.

The larva is pear-shaped and its curved spines make it very difficult to pull out of the skin. However, covering the breathing hole with petroleum jelly or similar substance can usually stimulate the larva to move out or at least reposition itself so that it can be extracted more easily. Some reports suggest using bacon fat but it is the effectiveness of the material at blocking the breathing hole rather than tempting the larva with a tasty morsel that will encourage it to leave the skin. In humans, infestations are usually found on the head, so wearing a jungle hat can protect against mosquito bites and hence *Dermatobia hominis* infections in this region. However, other body parts can be infected and Passos *et al*. (2004) describe a case in which a larva developed in the penis of a man who routinely wore shorts without any underwear.

4.4.16 Family Streblidae

The Streblidae are all obligatory ectoparasites of bats (Chiroptera) and are primarily found in the tropics and especially on bats that roost communally in caves. They are sensitive to low temperature and are unable to survive on hibernating bats. Bats go into hibernation when the winter temperature drops to $+10^{\circ}$C and during hibernation their body temperature falls to slightly above ambient temperature – and average temperatures of below $+10^{\circ}$C are too low for streblids. Approximately 240 streblid species have been described and they are typically small, 1.5–2.5 mm in length but some species can grow to 5 mm. Although they are totally reliant upon their host for food, most species are instantly recognisable as Diptera and about 80% of species have retained functional wings. Nevertheless, their compound eyes are poorly developed or absent and their antennae are extremely small. The wings are folded back like pleats over the abdomen when not in use so that they do not interfere with movement through their host's pelage. All species feed on blood and have piercing and sucking mouthparts. The extent to which Streblid and Nycteribiid flies harm their host is not known and while bats have their share of trypanosome and other blood parasites, it is not known whether these flies are able to act as vectors. It is also an intriguing question how and why two families of specialist dipteran parasites of bats should have evolved and the extent to which competition between species exists for a remarkably specialised niche.

The eggs hatch within the female's uterus and the larva is nourished by the secretions of two milk glands in a similar manner to the larvae of tsetse fly. One larva develops at a time and once it has reached maturity, the female fly leaves its host and attaches the larva to a nearby surface. The female fly then returns to its host and the larva pupariates.

Female streblids belonging to the genus *Ascodipteron* are somewhat unusual in that although they are initially winged, once they have found a suitable host they undergo a series of morphological changes. This is very rare among adult insects. First, the female fly locates a suitable site on the host's body and then she uses her well-developed mouthparts to pierce and cut through the skin and into the underlying dermis. During this process, she sheds her wings and legs and ultimately only the posterior of her abdomen protrudes from the bat's skin. The abdomen then swells until the head and thorax are embedded within it. Because female *Ascodipteron* are permanently attached to their host, they have to drop their larvae onto the floor of the bat roost rather than attach them to the surrounding surfaces. Presumably, they also have a limited ability to influence mate choice – the male flies retain their mobility.

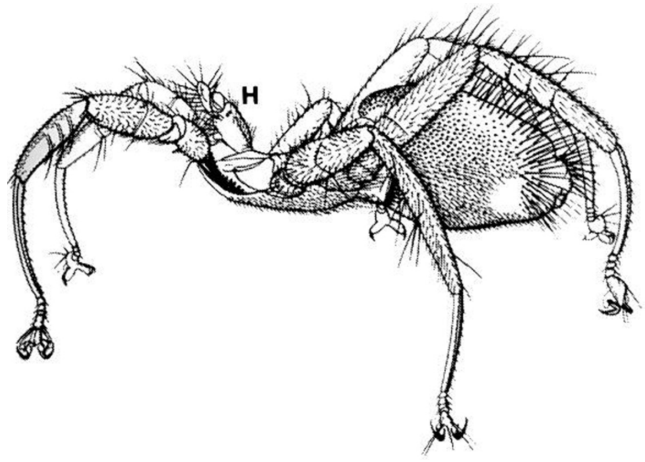


Figure 4.12 Adult Nycteribiid. These wingless flies are parasitic on bats. H = head. Note the long legs that end in large hooked claws. Source: Oldroyd, 1964. (Reproduced with permission of W. W. Norton & Company, Inc)

4.4.17 Family Nycteribiidae

Flies belonging to this family are all highly modified blood-feeding obligatory ectoparasites of bats. They are relatively small flies and are typically 1.5–5.0 mm in length. Bats are well known for their species diversity and there is a similar level of diversity among the Nycteribiidae: to date, 951 species of bats and 274 species of Nyceteribiidae have been described. Species diversity among the Nycteribiidae is promoted by their lack of mobility and they spend their whole life on a single host (ter Hofstede *et al*., 2004). The adult flies lack wings but have retained their halteres. Because they lack wings the thorax muscles have atrophied and the thorax is compressed and reduced in size (Figure 4.12). Their eyes are either absent or reduced but they have well-developed antennae and their legs are long and end in grasping claws that enable them to maintain a firm grip. The flies can therefore move easily and quickly about their host. When at rest the head projects upwards and appears to perch on top of the thorax a bit like the gun turret on a tank. Consequently, the insect has to rotate its head forward and downwards so that it can exploit its piercing and sucking mouthparts.

The reproduction of Nycteribiidae is very similar to that of the Streblidae in that the eggs hatch within the female's uterus and the larva is nourished by the secretions of two milk glands. One larva develops at a time and once it has reached maturity, the female leaves her host and attaches the larva to a nearby surface. She then scuttles back to the host while the larva pupariates.

Questions

1. List three morphological features that could be used to distinguish between a mite (Acari) and a louse (Phthiraptera).
2. What is a hypostome and in what organism would you find one?
3. What is a pentastome and where might you expect to find one?

4. Briefly explain the problems that branchiuran fish lice can pose to commercial fish farms.
5. Why are human lice infections often a problem during wars?
6. With the aid of named examples distinguish between the terms ‘one-host tick’, ‘two-host tick’, and ‘three-host tick’.
7. Distinguish between the terms tungiasis and myiasis.
8. Name three Dipteran vectors of parasitic diseases and the diseases they transmit.
9. The species names of *Cochliomyia hominivorax* and *Cordylobia anthropophaga* indicate that they are ‘man-eaters’. Briefly explain how they differ in their approach to ‘man-eating’.
10. The larvae of *Dermatobia hominis* live underneath the skin surface of humans and a range of other mammals. How do they get there?

5

Parasite transmission

5.1 Introduction

Living as a parasite within or upon another organism offers numerous advantages but it also poses two fundamental problems. The first is that all life is mortal and therefore either the parasite and its progeny have to be able to move between hosts in order to ensure the survival of subsequent generations. Assuming that a means of transfer is available, the second major problem is identifying a suitable host. In particular, those parasites that live within the body of their host usually only have 'one chance' once they are committed to the invasion process. This means that they must be able to identify a potential host with a high degree of certainty otherwise the consequences would be fatal to the parasite. Some permanent ectoparasites, such as the human body louse *Pediculus humanus corporis* are able to move between hosts at several life cycle stages, including the adult stage. However, for the majority of parasites, including many ectoparasites, there is a single transmission stage in the life cycle. This stage is resistant to environmental conditions and expresses features that enable it to recognise and invade the new host.

The simplest means of moving between hosts is to sit and wait for the host to find you. This can be seen at its most basic in contaminative transmission in which the parasite's involvement consists of surviving until ingested by a suitable host. This can be a high-risk strategy but it is obviously an effective one since it is exhibited by numerous protozoan and helminth parasites. These parasites usually produce vast numbers of cysts (protozoa) or eggs (helminths) because most will never be ingested by a suitable host. This is very energy-demanding and wasteful, but this is not a problem for the parasite since the majority of the parasite's energy needs are met by the host. Despite this, many parasites have evolved mechanisms which increase the chances of finding a suitable host. These can include actively searching for a suitable host, modifying host behaviour, and employing the services of an intermediate host, paratenic host or vector. Some parasites can be transmitted in a variety of different ways. For example, although the protozoan *Entamoeba histolytica* is normally transmitted in contaminated food or water, it can also be acquired through the actions of a mechanical vector, during sexual intercourse, or from medical procedures. The transmission of parasites is also affected by numerous variables such as natural environmental factors (e.g. weather), man-made factors (e.g. pollution, cultural and agricultural practices), and biological factors (e.g. behaviour of the host, pathogens that affect the parasite). Many of these factors are interlinked and therefore the transmission of parasitic disease is influenced by numerous biotic and abiotic factors.

Parasitology: An Integrated Approach, First Edition. Alan Gunn and Sarah J. Pitt.

5.2 Contaminative transmission

Contaminative transmission often occurs when a parasitic infection is acquired through consumption of either food or water that is contaminated with the infective stage. It can also occur through the infective stage being breathed in or otherwise accidentally ingested. Contaminative transmission is therefore an essentially passive process in which parasite and host meet through chance and therefore its success is heavily influenced by the abundance of the infective stage(s) of the parasite. Sometimes contamination can occur in the most unexpected ways. For example, in 2007, there was an outbreak of Chagas disease among children attending a school in Venezuela who had consumed guava juice containing the metacyclic trypanosomes of *Trypanosoma cruzi* (Alarcón de Noya *et al*., 2010; Clayton, 2010). Chagas disease is normally transmitted by blood-feeding reduviid bugs so how it could be transmitted via guava juice is at first sight a mystery. However, the bugs feed on a variety of animals which can be infected with *Trypanosoma cruzi*. It is thought that infected reduviid bugs defecated on the guavas and the juice prepared from them was not properly sterilised before being drunk by the children. This raises concerns because there is considerable interest in developing the market for açaí palm collected in the Amazonian forest.

Contaminative transmission is a common strategy among gastrointestinal parasites in which the infective stage is voided in the faeces of one host and subsequently contaminates the food or water of another, which may or may not be the same species. This is often referred to as faecal-oral contamination and the means by which this occurs are best remembered by the mantra of the four 'f-words': 'faeces, food, fingers, flies'. For human parasites, contamination often results from faeces being used as a fertiliser (night soil) for salad vegetables. The use of unsuitably composted human faeces as agricultural fertiliser in Europe, Asia and South America also contributes heavily to the spread of contaminatively transmitted parasitic infections.

Alternatively, transmission might be effected by the host when it cleans itself or grooms another animal and ingests cysts or eggs contaminating the body. For parasites of humans this may occur through people not washing their hands properly after going to the toilet or handling domestic animals, hence the reference to fingers. In developed countries, food hygiene laws are rigorously enforced to reduce the possibility of contaminative transmission but in developing countries, different standards can apply. In a survey of food handlers in Abeokuta in Nigeria, Idowu and Rowland (2006) found that 98.7% of street vendors and 92% of school food vendors were infected with one or more species of faecal-orally transmissible parasites. Furthermore, few of the vendors admitted to washing their hands after defecation and those who did wash seldom used soap as this was looked on as an unnecessary expense. Most of those surveyed considered being infected with parasitic worms to be 'normal' and attempts at getting rid of them to be a wasted effort. Lack of personal hygiene is partly the reason why the prevalence of gastrointestinal parasites is often higher in children. For the same reason, faecal-orally transmitted parasites can also be a problem among those who are senile or otherwise mentally impaired. Many dung-frequenting flies (e.g. *Musca domestica*) and other insects (e.g. cockroaches) can act as mechanical vectors when they pick up parasite infective stages (particularly on their legs) while feeding on faeces and then transport them to the food or body of the parasite's host. Water is also a major means by which faecal-oral transmission can take place. This may be through faeces contaminating drinking water or water used to wash food. Needless to say, faecal-orally transmitted parasites are a common problem among people living in poverty who lack sanitation and access to safe drinking water. Polluted water supplies, such as wells, ditches and springs are common sources of

infection for a variety of gastrointestinal parasites, such as *Entamoeba histolytica*. Similarly, poorly maintained plumbing has led to transmission via cracked mains water supplies. This is not restricted to developing countries. For example, the tap water supply of the city of St Petersburg in Russia is a notorious source of giardiasis, so much so that visitors are advised to consume only bottled water, which means not just for drinking, but for cleaning their teeth as well. Also, there are occasional outbreaks of *Cryptosporidium parvum* infection from the mains water in the UK, usually as a result of a pipeline cracking, poor general maintenance or inadequate treatment of sewage.

Box 5.1 Geohelminths and geophagy

Helminth parasites whose infective stages are found in the soil are sometimes referred to as 'geohelminths'. This therefore includes species such as *Trichuris trichura* and *Ascaris lumbricoides* in which the eggs are passively ingested, and hookworms such as *Ancylostoma duodenale* in which the eggs hatch in the soil and the larvae actively invade the host by burrowing through the skin or are consumed in water or food. Geophagy is the term used to describe the voluntary consumption of soil or earth. It is remarkably common and has been described in most societies. It is a behavioural trait that goes under the umbrella term 'pica'. Pica is the Latin word for a magpie (*Pica pica*) – a bird that has a reputation for collecting bright objects for no obvious reason. The trait is most common in children and women, especially during the later stages of pregnancy. Dr Sera L.Young (2011) has written a fascinating book on the subject. In addition to earth, chalk, charcoal, cooking starch and ice are other commonly consumed materials. The common factor being that they are 'crunchy' and serve no obvious metabolic purpose. Geophagy is also common among primates and many other mammals as well as birds (Krishnamani and Mahaney, 2000). It has often been suggested that infection with hookworms stimulates geophagy in humans as an adaptation to replace lost iron. This is usually supported by the observation that during the 1800s and early 1900s geophagy was often observed among poor people living in the southern states of USA, among whom hookworm was a common affliction. However, there is no clear link between geophagy and iron status and the consumption of excessive amounts of some earths can be harmful and interfere with the absorption of essential nutrients, including iron (Young, 2011). It might be expected that this behaviour would pose an increased risk of contracting so-called 'geohelminth' infections. Although there are reports that this is the case (e.g. Geissler *et al*., 1998), other studies have failed to confirm this (e.g. Young *et al*., 2007). The lack of a clear link is mainly because those who consume earth do not do so indiscriminately. Usually particular sources of earth are chosen such as from termite mounds or from the walls of huts where there is a lower chance of parasite transmission stages being found, though in many cases this is probably not a conscious factor guiding choice. In addition, the earth is often dried or baked in an oven before it is consumed. Finally, of course, the infective stages of geohelminths vary in their ability to withstand environmental stress.

Most herbivorous animals do not eat food that is contaminated with faeces if they can avoid it, though they may find this impossible when the stocking density is high or when food is limited (e.g. during a drought). Some parasites have therefore evolved mechanisms which enable them to leave the faeces and contaminate the surrounding 'clean' vegetation. For example, after a

proglottid of the beef tapeworm *Taenia saginata* is shed with its host's faeces, it crawls away into the undergrowth, dispersing eggs onto the vegetation as it moves, these then await ingestion by a bovine host. The cattle lungworm (nematode) *Dictyocaulus viviparus* has evolved a more dramatic means of escape from the dung pile. In this species, the parasite eggs hatch shortly before or immediately after they are shed with the faeces and then develop to the infective third stage larvae. At the same time the fungus *Pilobolus* is also developing on the faecal pat and the nematode reaches its infective stage just as the fungus forms its fruiting bodies, that are known as sporangiophores. In order to colonise new dung pats the fungus relies on its sporangia being consumed by a herbivore (e.g. cow, sheep or horse): the sporangia pass harmlessly through the animal's gut and then germinate when passed with the dung. The globular sporangiophores of *Pilobolus* develop at the top of long 'stalks' and when they are ripe they 'explode', thereby propelling the sporangia up to 2 metres from the dung pat. The infective larvae of *Dictyocaulus viviparus* climb to the top of the ripe sporangiophores and when these 'explode', they too are catapulted into the surrounding vegetation. Once on 'clean' vegetation, the larvae wait until they are eaten by a suitable host and they are then able to continue with the next stage of their life cycle. By contrast, there are some animals that feed on faeces ('coprophiles') and the infective stages of the parasites of these animals remain within the faeces. For example, the proglottids of the pig tapeworm *Taenia solium*, unlike those of *Taenia saginata*, remain immobile within the faeces they are shed in. This is because the next stage in the life cycle (usually) depends upon the eggs being consumed by a pig and pigs will eat human faeces.

The 'sit and wait' strategy can mean a lot of waiting and therefore the transmission stage is usually capable of withstanding environmental extremes and surviving for months or even years. The eggs and cysts often have thick walls to protect against water loss and environmental extremes as well as attack from fungi and bacteria. They are also usually provided with plenty of metabolic reserves and have a low metabolic rate. In some nematode species the larvae hatch from the egg and then develop to the infective stage and this is adapted to survive environmental conditions etc. For example, the infective third-stage larvae of *Dictyocaulus viviparus* do not feed (they rely on stored reserves) and retain the second-stage cuticle as a protective 'coat'. The eggs of the nematode *Ascaris lumbricoides* are notorious for their ability to survive in situations that would kill the majority of creatures. For example, they can embryonate in 10% formalin, 7% glacial acetic acid, and 1% hydrochloric acid (Yoshida, 1920). Although the eggs of some parasite species, such as *Ascaris suum* and *Trichuris suis*, remain infective for at least 6 years under experimental conditions, it is likely that even robust species such as these survive for much shorter periods in real life (Larsen and Roepstorff, 1999).

Faeces is the natural home of countless bacteria, fungi and invertebrates and these, together with the effects of the weather, ensure that faeces is quickly broken down. In addition, birds that feed on the dung invertebrate fauna will tear the dung apart in search of their prey. This means that even those parasite cysts and eggs that do not have an 'exit strategy' soon become dispersed into the soil and from here they may be washed into streams, rivers, lakes, or ponds. Alternatively, faeces may be deposited directly into the water. Cattle often defecate when they wade into streams or ponds to drink and humans living on the banks of rivers will defecate into the water if there is no sanitation. Even in developed countries it is not unusual for untreated sewage to be discharged into the sea or large bodies of water either as a matter of routine or following flooding. In parts of Asia it has been common practice to add human faeces to pools containing farmed fish as a cheap food. Consequently, many sources of water can become contaminated with a wide range of faecal-orally transmitted parasites.

Box 5.2 Contaminative transmission of *Cyclospora* spp.

High levels of *Cyclospora* spp. contamination (3.75–11.6%) have been found on coriander and other herbs sold in markets in Viet Nam (Tram *et al*., 2008). Herbs and salad vegetables are a potentially 'good' source of infection because they are often consumed raw and the opportunity for contamination can occur at many levels. For example, plants may be contaminated when they are growing through the use of human faeces as a fertiliser or faecally contaminated irrigation water. The hands of the person harvesting the plants and/or the containers used to collect and transport them may be contaminated and on market stalls the plants are usually kept fresh by being sprinkled with water – which may also be contaminated. In developing countries with poor levels of sanitation, contact with soil is an important factor in the transmission of *Cyclospora cayetanensis* (Chacín-Bonilla *et al*., 2006), since flies and other invertebrates can also be expected to pick up and transmit the oocyst to food. The globalisation of the food supply may help to spread the parasite within and between distant countries and the rise in air travel has probably helped spread the parasite to non-endemic regions (Chacín-Bonilla, 2008).

5.3 Transmission associated with reproduction

5.3.1 Sexual transmission

Sexual intercourse between animals is an exceptionally dangerous activity for all sorts of reasons. For example, during intercourse the participants become vulnerable to attack because they focus upon one another rather than their surroundings and their ability to move and defend themselves is limited. Consequently, the physical act is usually extremely brief. Sexual intercourse also presents an ideal opportunity for disease transmission because two hosts are in close physical contact. Furthermore, intromittent sex and the exchange of body fluids mean that endoparasitic and intracellular species can be transmitted without being exposed to the environment (and therefore the need for special transmission stages in the life cycle). Sexual transmission does, however, pose difficulties of its own. One of these is that it is dependent upon the sexual behaviour of its host. If the host breeding season is short and the animals are not promiscuous, then there are few transmission opportunities for the parasite. For example, the male honeybee (*Apis mellifera*) only mates once and then dies. In addition, as already mentioned, the sexual act may be brief and therefore the parasite must be in a position to move quickly when the opportunity arises. Furthermore, because sexual reproduction is vital to the host and the risks of sexually transmitted infections (STIs) so real, most animals have well-developed immune mechanisms to combat pathogens that attempt to establish themselves via the reproductive organs. For some parasite species, sexual transmission is the principal means of moving between hosts but, for many others, copulation is just another transmission opportunity.

Transmission during sexual intercourse usually occurs through one of the following ways: actively moving between the bodies, transmission within the reproductive fluids, and transmission through tissue damage caused during copulation. Although transmission can occur in both

directions, the male tends to be the one transmitting the infection and the consequences of the infection are often worse for the female.

Whenever two or more animals come into physical contact, it presents an opportunity for ectoparasites to move between hosts. For social animals, contact may be a regular occurrence but other animals live solitary lives and only come into contact with one another when they need to reproduce. Adaptations for exploiting the act of copulation (as distinct from other forms of contact) are more likely to evolve, the more frequently such acts take place. For example, the crab louse *Phthirus pubis* (see Figure 4.7 on page 157) is adapted to clinging onto the thick coarse hairs around human genitalia and in the process of evolution it has gained grasping strength but lost the ability to move quickly. Although crab lice will attach to other regions of the body where there are coarse hairs (e.g. eyebrows, beards), they are primarily transmitted during sexual intercourse when the genital hairs are in close contact. By contrast, the human head louse *Pediculus humanus capitatis* (see Colour Plate 12) is also adapted to grasping onto hair but it has retained sufficient mobility to move between hosts during brief moments of physical contact. Therefore, although the head louse could be transmitted between people at the time of copulation, it should not be considered an STI. The current popularity among both men and women for waxing their genital regions is reputedly reducing the crab louse population but there is little scientific information on this.

Most sexually transmitted parasites are protozoa, the best known of which are *Trichomonas vaginalis* and *Trichomonas foetus*. *Trichomonas vaginalis* cannot form cysts and although the parasites can survive for up to 24 hours outside the body (in urine, semen, and water) contaminative transmission – such as through shared towels or toilet seats – is not a common occurrence. The parasite infects both men and women and is typically transmitted within the genital secretions. Because men are often asymptomatic, it can be difficult to persuade them to be tested or treated for the infection. However, unless this is done, they will constantly re-infect their sexual partner(s). *Trichomonas foetus* was once of major economic importance in the cattle industry around the world and especially on dairy farms. Even though bulls are not particularly harmed by the parasite because it is transmitted sexually, they are a source of infection and therefore cannot be used for breeding purposes. It is extremely difficult to completely cure an animal of the parasite and therefore because stud bulls can be worth tens or even hundreds of thousands of pounds, their loss can have serious financial implications. The increasing use of artificial insemination has resulted in a decline in the prevalence of *Trichomonas foetus* infections although it remains an important cause of disease in some countries.

One nematode species that is transmitted during copulation is *Mehdinema alii* which infects the ‘decorated’ or ‘Indian house cricket’, *Gryllodes sigillatus* (Luong *et al*., 2000). The adult worms live within the hind gut of the male cricket and form infective *dauer* larvae that migrate to the insect’s genital chamber. The larvae are passed to the female during mating and she in turn can then transmit these to other males. Although the nematodes may subsequently reach maturity in the female crickets, this is not thought to happen frequently and the male is the principal host. This species is very much an exception and very few helminths are transmitted when their hosts engage in sexual reproduction.

Human sexual behaviour encompasses a remarkable variety of activities that provide the opportunity for the transmission of parasites that might not otherwise be considered STIs. For example, the protozoan *Entamoeba gingivalis* normally inhabits the mouth but it has also been recovered from the uterus – a circumstance the reporting authors conjectured arose from an unfortunate combination of oral sex, an intrauterine device and an existing bacterial infection (Clark and Diamond, 1992). Oro-anal intercourse (anilingus) facilitates the transmission of those

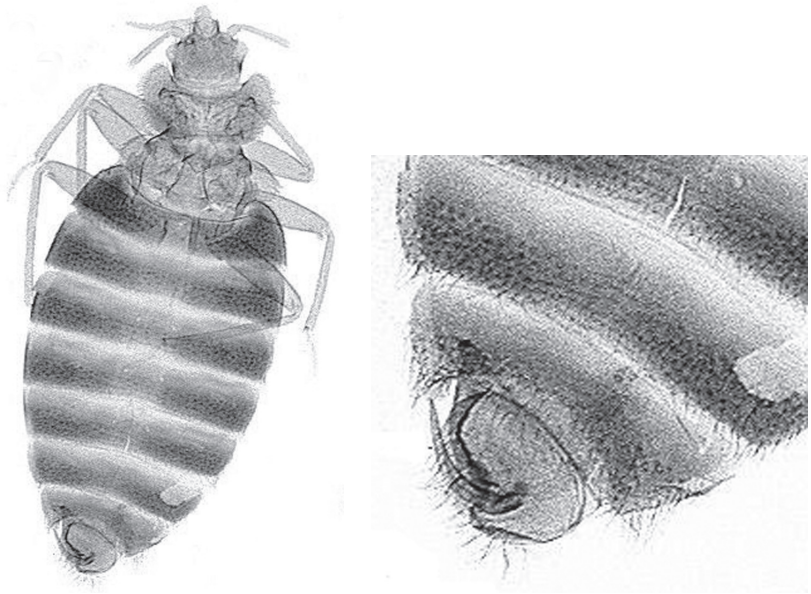


Figure 5.1 Adult male bedbug *Cimex lectularis* (a) with close-up of the aedeagus (b)

gastrointestinal parasites that produce cysts or eggs that are immediately infective, such as *Entamoeba histolytica*. In addition, protozoan trophozoites may also be transmitted in this way although they are not usually considered a 'transmission stage'. Anal intercourse can also facilitate the transmission of pathogens between sexual partners and lead to parasites colonising regions of the body they would not normally be found. This is partly through faecal contamination of the genitalia but also because anal intercourse can damage the lining of the rectum and the resulting bleeding facilitates the transmission of blood and tissue-dwelling parasites. Physical damage to the genital area or existing lesions caused by other STIs are associated with increased likelihood of transmission of an infection between partners. Humans have sexual relations with other animals far more frequently than many of us would like to consider (Munro and Thrushfield, 2001). This activity increases the chances for the transmission of zoonotic infections but, hardly surprisingly, there is limited published information on the topic (e.g. Ergun *et al*., 2007; Khorvash *et al*., 2008).

Humans are not the only animals to indulge in potentially risky sexual behaviour. Several invertebrates, of which the bed bug *Cimex lectularis* (Figure 5.1a, Figure 5.1b) is the best known, reproduce by a process known as traumatic insemination. In this case, the male injects his spermatozoa into the female's abdomen and these then migrate to her oviducts where they fertilise the eggs. The female has functional genitalia but these are only used for laying the eggs. The wounds that are caused to her abdomen can be infected by bacteria with potentially fatal consequences. Other bugs belonging to the family Cimicidae reproduce in the same way and they are capable of transmitting a number of bacterial and protozoan diseases, including *Trypanosoma cruzi*. It is quite possible that an infected male might transfer pathogens to an uninfected female during reproduction but there is little published information on this.

Box 5.3 *Entamoeba histolytica* as a sexually transmitted infection

Entamoeba histolytica is a gastrointestinal parasite that is normally considered to be transmitted through faecal contamination but above average rates of infection occur in high-risk groups, such as men who have sex with men and people who practise anilingus. In New York City, a study of 126 homosexual male volunteers found that 39.7% of them harboured *Entamoeba histolytica* (Kean *et al.,* 1979). This figure might have increased to 50% if multiple stool samples had been taken. It is recommended that at least three separate stool samples be analysed to confirm the presence or absence of infection. This study pre-dated the availability of tests capable of distinguishing between *Entamoeba histolytica* and *Entamoeba dispar* and therefore it is uncertain how many of these infections were actually owing to *Entamoeba histolytica*. However, in developed countries in the Asia-Pacific region, *Entamoeba histolytica* infections are becoming increasingly associated with men who have sex with men. For example, in Taiwan and Japan, invasive amoebiasis is usually associated with middle-aged men who have sex with men and a similar scenario is also becoming apparent in Australia (Ohnishi *et al*., 2004; Stark *et al*., 2008; Tsai *et al*., 2006). However, in Japan there are increasing numbers of cases being reported in female sex workers (Anon, 2007).

5.3.2 Transmission within the gametes

Gametes are unicellular structures and therefore only intracellular parasites can be transmitted within them. The majority of male gametes, especially in mammals, are very small and contain little cytoplasm, which limits the space available for any parasite. Indeed, the evolution of small male gamete size may be driven by the host's attempt to control the transmission of parasites within them (Law and Hutson, 1992). There are, of course, exceptions. For example, the males of some species of the fruit fly *Drosophila* produce sperm that are over ten times their own body length. Another major factor preventing organisms from parasitising spermatozoa is that sperm competition is intense so anything that compromises their movement would reduce their chances of fertilising the egg and thus passing on the disease to offspring. It is therefore not surprising that there are relatively few records of spermatozoa being parasitised and this is usually with viruses or rickettsia-like organisms (e.g. Afzelius *et al*., 1989). Intriguingly, the outer surfaces of the spermatozoa of soft ticks are coated with protists belonging to the genus *Alderocystis*. The relationship is thought to be symbiotic and *Alderocystis* may provide a nutritive function or prevent the sperm being damaged by bacteria (Perotti and Braig, 2011). *Alderocystis* is also found in hard ticks but has not yet been observed in direct contact with the sperm. By contrast to spermatozoa, female gametes are often large and a number of pathogenic and non-pathogenic symbionts are transmitted within them. For example, *Wolbachia*, a rickettsiel bacterium found in many invertebrates, is typically transmitted via the cytoplasm of the egg. Transmission within the gametes is an example of vertical transmission and the reproductive success of the parasite and its host are therefore closely linked.

5.3.3 Congenital transmission

Congenital transmission is an example of vertical transmission. This refers to transmission that occurs through infection of the developing egg or embryo while it is still within its mother's body or at the time of birth/egg-laying. In invertebrates it is often referred to as trans-ovarial transmission and in mammals the infection is either acquired across the placenta ('trans-placental' transmission) or through passage through the birth canal or contact with the mother around the time of the birth ('perinatal' transmission). The end result is the same in that the young are born (or hatch) already infected with the parasite. Although vertically transmitted parasites are often portrayed as being less pathogenic than those that are horizontally transmitted, since the infection occurs during an exceptionally sensitive time during the host's development, they can have fatal consequences. Indeed, the protozoan *Neospora caninum* can cause abortion epidemics in cattle.

In humans, the most common parasite associated with congenital infection is the protozoan *Toxoplasma gondii*. There are also reports of *Leishmania donovani* and *Trypanosoma cruzi* being transmitted this way, but it appears to be rare (Hermann *et al*., 2004; Meinecke *et al*., 1999). However, it is possible that the frequency of congenital Chagas disease is underestimated. This is because asymptomatic women can transmit *Trypanosoma cruzi* to their babies and the baby may not display any immediate signs of infection (Theiler *et al*., 2008).

Human congenital toxoplasmosis usually results when the mother becomes infected with *Toxoplasma gondii* for the first time during her pregnancy. It can also result from the reactivation of a dormant *Toxoplasma gondii* infection although in this case the mother is often immunosuppressed, for example, as a consequence of systemic lupus erythematosus or AIDS (e.g. Minkoff *et al*., 1997). Intriguingly, according to Kaňková *et al*. (2007a), women who are latently infected with *Toxoplasma gondii* give birth to more sons than those who are not infected. This is the first study to find a parasite influencing the sex ratio of human babies although it is relatively common occurrence in invertebrate host–parasite relationships. Whether or not the parasite is directly affecting the sex ratio of human babies or a third factor – such as changes to the immune system or to the balance of hormones – is not known. Kaňková *et al*. (2007b) found that female mice in the early phase of latent *Toxoplasma gondii* infection (i.e. gave birth 89–120 days after infection; mice usually give birth ~21 days after conception) also produced more male offspring than uninfected controls. Male mice tend to move further away from their nest than females and also tend to be more active in their search for mates. Consequently, they are more effective at dispersing the parasite and are more likely to be encountered and consumed by cats. However, they found that pregnant mice in the later phase of infection (i.e. gave birth >120 days after infection) produced more females than uninfected controls. They suggested that this was because these pregnant females would be in poorer physical condition because their infection was of longer standing. According to the Trivers–Willard hypothesis (Trivers and Willard, 1973), in species in which females are not in serious competition with one another for food or mates, it is 'better' for a female in poor condition to produce more female offspring than males. This is because even if the offspring is small and weak, if it is female, it is likely to be able to reproduce. By contrast, a male that is small and weak and has to compete with other males is unlikely to reproduce.

Congenital toxoplasmosis can also be a problem in sheep and, as in human infections, it can result in spontaneous abortion, stillbirth, or the lamb is born weak and unable to thrive (Taylor *et al*., 2007). It has been suggested that sheep may become infected as a consequence of consuming 'concentrate feed' that has somehow become contaminated with cat faeces. Sheep are usually fed

supplements before mating ('tupping') and also before lambing and this would fit in with the subsequent congenital infections. However, cats normally bury their faeces and food concentrate is stored in sacks. Although it could be argued that flies might transport oocysts between cat faeces and the sheep concentrate, sheep usually mate in the autumn and give birth in early spring, when insect activity is reduced. It has also been suggested that the *Toxoplasma gondii* might be spread as an STI between sheep. *Toxoplasma gondii* has been detected by PCR and bioassay in the semen of experimentally infected rams (Lopes *et al*., 2009) but it is uncertain whether this would result in the transmission of the infection. However, Arantes *et al*. (2009) demonstrated that *Toxoplasma gondii* present in the semen of dogs could be transmitted sexually to female dogs and thence vertically to their pups. *Toxoplasma gondii* has also been found in the semen of a number of other mammal species and therefore it is possible that sexual transmission may be more widespread than previously thought.

Transovarial transmission of parasitic infections is relatively common among invertebrates. In some situations this can have relevance to human and veterinary medicine because several diseases that are transmitted by ticks are passed between mother and offspring in this way (e.g. *Babesia bovis*). Consequently, the disease can rapidly spread through the tick population and the young ticks may be capable of transmitting an infection when they take their first blood meal.

The protozoan parasite *Histomonas meleagradis* is unusual in exploiting another parasite's reproduction to ensure its own transmission. Infected birds pass *Histomonas melegradis* trophozoites in their faeces but these are unable to survive for more than a few hours in the outside environment. Despite this, direct bird-to-bird transmission (presumably via contaminative transmission) is known to occur in turkeys (McDougald and Fuller, 2005). More typically, infection takes place by the protozoa becoming incorporated into the eggs of the common caecal nematode parasite *Heterakis gallinarum,* while still in the bird's gut. The protozoa initially parasitise the gut of the nematode but then invade and multiply within its body tissues. When the ovaries are infected, the parasites become incorporated within the developing eggs (Ruff *et al*., 2007). Male nematodes are also infected and the parasites can invade their reproductive system – it is therefore conceivable that they could be transmitted to the female at copulation (Gibbs, 2007). The parasites do not invade the spermatozoa but they could be transmitted in the secretions that accompany them. Although the protozoa continue to reproduce within the nematode eggs, they do not kill the developing larva – indeed, the eggs (and the protozoa within them) can remain infective for at least two years. Interestingly, if the eggs are consumed by earthworms, they will hatch and the second-stage larvae invade the earthworm's tissues where they become dormant (Lund *et al*., 1966). When the eggs of an infected earthworm are consumed by a suitable bird host, the nematode egg hatches or a juvenile is released in the bird's gut. The nematodes then make their way to the bird's caecum. Once there, the protozoa, which are present in the nematode gut (and reproductive tissues), are shed with its faeces and they are then free to invade the bird's tissues.

5.4 Autoinfection

Autoinfection occurs when a parasite produces infective stages that colonise the same individual host as their parents. Because the offspring remain within or upon their host, they do not have to overcome all the biological and environmental factors that normally reduce the chances of transmission. However, parasites often induce an immune reaction in their hosts that prevents or limits the ability of other parasites of the same species from establishing themselves. In addition,

the parasite must ultimately infect other hosts if it is to spread and thereby ensure its survival as a species.

In humans, the intestinal helminth *Enterobius vermicularis* (the 'pin worm') provides a good example. The female worm emerges from the host's body during the night, to lay its eggs on the skin around the anal area. Infective larvae develop inside these eggs within a few hours. The presence of the eggs causes the skin to itch, so on waking, the person scratches their bottom, which transfers the parasite to their fingers very effectively. Therefore if the person puts their fingers straight into their mouth before washing their hands, they re-infect themselves. Pin worm infections are commonly found in children because they are less likely to consider hand hygiene before sucking their fingers. Furthermore, they are more likely than adults to suffer frequent autoinfection.

For other helminth parasites, however, the life cycle limits their ability to autoinfect their host. For example, many nematode parasites (e.g. *Ascaris lumbricoides*) produce eggs that do not reach their infective stage until after they are shed in the faeces while others require a period of larval development in an intermediate host or vector (e.g. *Wuchereria bancrofti*). In these species, autoinfection is not possible. A notable exception is the nematode *Strongyloides stercoralis* which infects humans. This worm has an unusually complex life cycle that involves free-living and parasitic cycles with adult reproduction taking place in both of them. In humans, the adult female worm lives in the small intestine – there are no males in the parasitic part of the life cycle. She produces eggs that hatch soon after they are laid and the larvae can develop so rapidly that they reach the infective third stage before they are shed with the faeces. These infective larvae can penetrate the gut and become disseminated throughout the body. Some of these larvae complete their migration through the body and return to the small intestine where they mature into adult females that give rise to further rounds of autoinfection. Serious 'disseminated strongyloidiasis' is most commonly seen in patients with compromised immune systems (e.g. as a result of AIDS) and can prove fatal. Autoinfection may also account for the length of time a person can be infected with this parasite. For example, men who were infected with *Strongyloides stercoralis* while they were Japanese prisoners-of-war during the Second World War are at risk of developing disseminated strongyloidiasis. In many cases, after the war, these men returned to their home country where there was no risk of infection and for decades they were unaware that they harboured the parasite within them. However, as they became increasingly elderly and their immune system was weakened as a consequence of disease or drug treatment, they became susceptible to developing disseminated strongyloidiasis (Viney and Lok, 2007). It should be noted that some of the larvae produced by the parasitic female worms are voided with the host's faeces and develop into free-living male and female worms.

5.5 Nosocomial transmission

Nosocomial infections are those that are acquired in hospital or as a result of a medical procedure. Hospitals can be unhealthy places as they contain lots of sick people within a very confined space, it is therefore not unusual for patients to acquire an infectious disease while being treated for a different condition. The dangers posed by hospital acquired bacterial (e.g. MRSA methicillin resistant *Staphylococcus aureus*) and viral (e.g. norovirus) infections are well known, but some parasites can also cause problems. For example, unless hygiene is rigorously enforced, there is the potential for the contaminative transmission of a range of parasitic diseases – particularly gastrointestinal parasites. This can be a major concern in countries where a high proportion of the

patients are infected with parasites before they arrive at the hospital and they become a source of infection to others. There is also the potential for the transmission of ectoparasites such as lice and mites (see Colour Plate 18). For example, even in a developed country such as Switzerland, nosocomial transmission of scabies (*Sarcoptes scabei*) has been reported as a problem (Jeanneret *et al*., 2007).

The re-use of needles and poor sterilisation of hospital equipment can also result in the transmission of a number of parasitic diseases. A bizarre event occurred in a clinic in Colorado in 1980, when an epidemic of amoebiasis occurred among patients who had undergone colonic irrigation with a contaminated enema machine. Ten of the patients had to have colectomies and seven of them died. Blood transfusions and organ transplants are a potential source of transmission for parasitic infections such as malaria, leishmaniasis, and Chagas disease (Lettau, 1991). It is therefore important that blood and tissues from donors are screened for parasitic infections before they are used to treat recipients. Because people travel so extensively, one cannot assume that because a disease is not endemic in a country that a blood or organ donor would not be infected with an 'exotic' parasite. For example, vector-borne Chagas disease is rare in the USA, but in 2007 a new blood-screening test revealed that a significant number of blood donors were antibody positive for *Trypanosoma cruzi* and their blood could have been a source of infection if used for a transfusion (Theiler *et al*., 2008).

5.6 Active parasite transmission

In some species of parasite there is a free-living infective stage that actively searches for and invades its host. Schistosomes have two free-living infective stages in their life cycle. The schistosome egg hatches to release a free-living stage, the miracidium, which searches for and invades the snail intermediate host. After a series of developmental stages in the snail, another free-living stage, the cercaria, is released. The cercaria searches for and invades the final host within which it becomes an adult worm. These different stages of the life cycle are marked by dramatic changes in gene expression patterns. For example, the cercaria stage is characterised by an up-regulation of genes responsible for energy production, movement (e.g. actin), and the protease enzymes that play a part in the invasion process (Jolly *et al*., 2007).

Actively searching for a host requires there to be a reliably high density of potential hosts. This is because searching is more energy-demanding than 'sitting and waiting' and in the transmission stage of a parasite's life cycle it does not usually feed. Therefore, once the infective stage runs out of energy, the parasite loses the capacity to invade and will die. For example, the schistosome miracidium can survive for only a few hours after hatching; in contrast the infective stage of some hookworm species may survive for 1–2 weeks under ideal conditions. This transmission strategy also requires a warm, moist or aquatic environment to avoid the danger of desiccation and exposure to UV light.

The infective stage must have some means of identifying a suitable host. Schistosome miracidia are attracted by components of the mucus of their snail intermediate host while their cercariae respond to the thermal gradient created by their warm-blooded host. The host identification capabilities of schistosomes are not particularly sophisticated and both the miracidia and cercariae will attempt to invade unsuitable hosts. For example, there is a report of *Schistosoma mansoni* miracidia attacking tadpoles (Barbosa and Carneiro, 1965). It has been suggested that it might be possible to control schistosomes using 'decoy snails' that the miracidia invade but within which

they are unable to develop. This could be done either by promoting the growth and reproduction in an existing snail species in the relevant area, or introducing a new one (e.g. Johnson and Thieltges, 2010). However, the decoy might have to be present in very large numbers to be effective (Babiker *et al*., 1984).

Both the miracidia and the cercariae of schistosomes have glands containing digestive enzymes and other chemicals and they physically and chemically force their way into their host. However, having gained entry, they then require the correct environment to continue their development and without this they die. For humans, paddling or swimming in water sources in some areas carries the risk of being invaded by the cercariae of schistosome species that usually parasitise ducks and other aquatic birds. These cercariae die within people shortly after invasion but the helminths and their secretions trigger an immune reaction that is manifested as a rash, popularly known as 'swimmer's itch'. This rash may persist for up to a fortnight and in sensitive individuals can give rise to fever (Skírnisson *et al*., 2009). The surface tension provided by a slowly drying film of water facilitates invasion by cercariae. Consequently, one way of reducing the risk of swimmer's itch is to rapidly dry oneself after bathing in schistosome-infested waters.

The infective stage larvae of hookworms are attracted by factors such as temperature, carbon dioxide, humidity, and certain chemical components in the skin (e.g. Granzer and Haas, 1991). They invade their host using a combination of enzymatic and chemical secretions and physically forcing their way into the body. In humans, invasion with human-adapted hookworm species usually results in no reaction or the formation of a rash at the site of invasion. However, invasion with non-human adapted species (e.g. *Ancylostoma caninum*) can give rise to a condition known as cutaneous *larva migrans* in which vivid red serpentine tracks underneath the skin indicate the path of the larvae.

5.7 Hosts and vectors

5.7.1 Paratenic hosts

As indicated in Chapter 1, paratenic hosts are not usually essential for the transmission of a parasite but they do improve the chances of it taking place. For example, the larvae of some *Ancylostoma* species (e.g. *Ancylostoma tubaeformae, Ancylostoma braziliense*) sometimes invade rodents but they are unable to complete their development. The invading larvae enter arrested development (hypobiosis) within the tissues of the rodent and are able to infect a dog or cat should it consume the infected rodent (Norris, 1971). The transmission cycle can theoretically become even more complicated since the infectious third stage larvae are capable of surviving for a prolonged time in insects and a rodent can become infected if it consumes an infected insect (Little, 1961).

In the case of the mermithid nematode *Pheromermis vesparum*, the paratenic host plays a much more important role in the parasite's life cycle (Molloy *et al*., 1999). Like most mermithid nematodes, the larval stages develop within their insect host, which in this case are social wasps (Hymenoptera: *Vespidae*). Once they have completed their development, they physically cut their way out of their host, usually killing it in the process. This emergence takes place when the adult wasp is next to a stream or pond. Some mermithid nematodes cause a change in the behaviour of their hosts which results in them being attracted to and falling into bodies of water. The larvae of *Pheromermis vesparum* then enter the water, moult to the adult stage, mate and the female worm starts to release eggs. If the eggs are consumed by the larval stages of aquatic insects such

as caddis flies (Trichoptera) and stone flies (Plecoptera), they will remain within them even after the insect has moulted to their adult stage. The nematode's life cycle is completed when the adult aquatic insect is captured by an adult wasp and fed to a wasp larva. The mernithid eggs hatch in the wasp larvae and begin to develop. The mermithid larvae develop slowly within their host such that the wasp is able to complete its own development to adulthood and be capable of flight. The aquatic insects are therefore acting as a paratenic host, effecting the transmission of the eggs to the wasp host and also facilitating the dispersal of the nematode when they themselves emerge and disperse from the pond or stream.

5.7.2 Intermediate hosts

Many parasites have complex life cycles involving two or more species of host within which different developmental stages occur. The intermediate hosts are those within which some (or all) of a parasite's immature (non-sexual) stages occur. The intermediate host is therefore essential, since without it the parasite would be unable to complete its life cycle. The absence of a suitable intermediate host can therefore prevent a parasite from extending its geographical range and thus the targeting of the intermediate host may prove to be the most effective control strategy.

In some cases, such as Schistosomes and the liver fluke *Fasciola hepatica*, the parasite leaves the intermediate host after a period of development. Many parasite species, however, rely upon the intermediate host being consumed by the next host in its life cycle: this may be another intermediate host or the definitive host. In this situation, transmission is determined by 'predator–prey' dynamics. Being parasitised can harm the host's health and thereby increase the chances of it being captured and consumed. For example, the host may become weak and therefore less able to detect or avoid a predator. This is a 'by-product' of being parasitised and cannot be considered a specific adaptation on the part of the pathogen to increasing the chances of transmission. However, parasites sometimes do induce changes in the morphology, physiology or behaviour of their hosts that appear to have evolved specifically to enhance the chances of transmission taking place but it is not always easy to distinguish instances of this. For example, the coenurus (bladderworm) of the tapeworm *Taenia multiceps* causes a condition that is colloquially known as 'gid' in which infected sheep exhibit a characteristic high stepping gait and walk around in circles (Figure 5.2 and

Figure 5.2 *Taenia multiceps*: sheep expressing 'gid'

see Colour Plate 19). Such sheep would be easy for dogs, the tapeworm's final host, to catch. 'Gid' results from the parasite invading the brain and forming a large fluid-filled coenurus that causes pressure atrophy and localised blockage of the blood supply, thereby bringing about the death of brain cells. One could suggest that this is an evolutionary adaptation to enhance transmission, although it could also simply be a fortuitous consequence of the coenurus developing in the brain.

5.7.3 Vectors

Some parasites use another animal, called a vector, to physically transmit them between hosts and these play an important part in the parasite's epidemiology. Consequently, controlling the vectors usually plays a large part in combating these parasites. Excellent comprehensive reviews of the roles invertebrates play in vector-assisted transmission of parasites are provided by Lehane (2005) and Mullen and Durden (2002).

In some cases the parasite–vector relationship is a loose association in which the vector acquires the parasite by accident and carries it to a point at which it can infect the host. For example, when the infective stage of the parasite is transported from faeces and deposited on the host's food. This can therefore be looked on as a form of contaminative transmission and the animal doing the transporting is referred to as a mechanical vector or mechanical transmission host. Importantly, the parasite does not undergo development within a mechanical vector and is usually carried upon its outer body surface or passes through its gut. A mechanical vector is therefore distinct from a paratenic host in which the parasite invades the host's tissues.

Flies (e.g. the housefly *Musca domestica* and the blowfly *Calliphora vomitoria*) and cockroaches (e.g. *Periplaneta americana*) are important mechanical vectors of a variety of gastrointestinal infections, including parasites such as *Entamoeba histolytica*. Their bristly appendages can easily carry protozoan cysts or helminth eggs from a stool to the host's food. Flies may also ingest the parasites, so their habit of vomiting and defecating while feeding is another means of transmission. In many rural areas it is not unusual for dogs, chickens and pigs to wander about farmyards and villages, moving freely in and out of buildings. Chickens will root through faeces looking for invertebrates and undigested plant material, while pigs are corprophagic. Dogs will also eat faeces if they are hungry, and village dogs usually are. Therefore, unless there is good sanitation, chickens, dogs, and pigs will consume human faeces as well as that of other animals. They could also acquire faecal contamination on their outer body surface. Dogs can act as mechanical transmission hosts for a number of human gastrointestinal parasites, such as *Ascaris lumbricoides* (Traub *et al*., 2002). Although a dog might ingest cysts such as those of *Entamoeba histolytica* through consumption of faeces, it is not known whether the parasite can survive and remain infective following passage through a canine intestinal tract. Similarly, unembryonated eggs of *Ascaris suum*, *Trichuris suis*, and *Oesophagostomum dentatum* eggs can travel through the guts of pigs and chickens and thereby be further dispersed around the local environment (Olsen *et al*., 2001).

Blood-dwelling parasites face a particular problem in moving between hosts and the majority of them employ a vector, usually blood-sucking arthropods such as mosquitoes, reduviid bugs, or leeches. In many of these cases the parasite passes through a series of developmental stages in its vector. In this situation the vector is acting as both transmission agent and intermediate host (e.g. *Plasmodium falciparum* in the mosquito *Anopheles gambiae*). It is usually a very specific relationship and even strains of the same species of vector may differ in their ability to transmit

a particular parasite. For example, a range of factors influence the tsetse fly: trypanosome relationship including the strain of the trypanosome, the species and sex of the tsetse fly (infections establish more readily in male flies than females), and the presence of and strain of rickettsia-like symbionts in the fly (Geiger *et al*., 2005; Maudlin and Ellis, 1985).

The effectiveness of a vector as a transmission agent is also heavily determined by its feeding behaviour: that is, what it feeds on, where it feeds, and when it feeds. Vectors that preferentially feed upon humans are said to be 'anthropophilic' while those that prefer to feed on other animals are classed as 'zoophilic'. Obviously, anthropophilic species are more likely to be important vectors of human parasitic diseases. If the vector is willing to enter buildings, it is said to be 'endophilic', and this also increases the chances of it transmitting an infection to humans. Although some blood-feeding invertebrates are relatively catholic in their choice of victim, most have a restricted host-range. Even within a single species, different strains often show different host preferences. For example, some strains of the bed bug *Cimex lectularis* prefer humans and others prefer rabbits. This is important because the parasite is relying on the vector to make the 'correct host choice'. Unless the parasite has a wide host range, if it is injected into the 'wrong host', it will die. Similarly, not all hosts of the same species are equally attractive: whether or not a human is bitten by mosquitoes can depend upon numerous variables including sex, age, blood group, pregnancy, physical health, and smoking habits (Lehane, 2005). Obviously, if the vector does not bite a particular host, then it will not transmit any parasites to it. This can all change if the invertebrate is starving. The flea *Xenopsylla cheopis* normally feeds on rats and in the process can transmit bubonic plague (*Pasteurella pestis*). Plague is fatal to rats and once their population declines the fleas start to feed on humans and in the process initiate a human plague outbreak. *Leishmania* spp. promastigotes move to the head and mouth parts of infected sandflies, thus making it physically difficult for the vector to take and digest a complete blood meal. This means that an infected sandfly bites the mammalian host more frequently than an uninfected insect would – thus enhancing the opportunities for transmission of the parasite. Sometimes a parasite exhibits behaviour patterns that increase the likelihood of it being transmitted by a particular vector. For example, over much of its range, the microfilariae of the nematode *Wuchereria bancrofti* are only found in the peripheral circulation of its human host late at night – usually between the hours of 10 p.m. and 2 a.m. This is probably related to this being the peak of feeding activity of its principal mosquito vector(s) and outside this time interval the microfilaria retreat to the pulmonary capillaries and other blood vessels deep within the body. However, in some areas the microfilariae either do not exhibit periodicity or there is a peak during daylight hours and in these situations the microfilariae are always present within the peripheral circulation. This is probably related to the biting behaviour of the local mosquito species or strains.

An interesting example of the mechanisms determining how a parasite may become associated with a particular vector is beginning to be uncovered in the relationships of *Leishmania* and sandflies. When small numbers of *Leishmania major* promastigotes are artificially inoculated into a suitable vertebrate host, they fail to establish an infection. In contrast, the same numbers of promastigotes, when injected along with salivary extracts from sandflies, are able to successfully establish an infection. The reason is that although *Leishmania* infects macrophages, these can kill *Leishmania* by producing superoxide (O_2^-), nitric oxide (NO) and other toxic metabolites. The macrophages are stimulated to produce superoxide, etc. by gamma interferon (IFN-γ) produced by activated parasite-specific T cells. The T cells are activated by the macrophages presenting them with parasite antigens. Sandfly saliva contains a substance known as *Leishmania*-enhancing factor that inhibits the IFN-γ production of superoxide and other cytotoxic molecules. Sandflies

inject saliva into the skin of the mammalian host during feeding to facilitate their location of a suitable blood vessel and prevent clotting. *Leishmania*-enhancing factor also inhibits the ability of the macrophages to present antigens to the T cells thereby decreasing the specific production of IFN-γ. The *Leishmania*-enhancing factor appears to inhibit the immune response of the host just long enough to allow the parasite to become established. The *Leishmania*-enhancing factor therefore appears to be important for the establishment of *Leishmania* in a new host. It has not been found in any other blood-feeding insect and is probably a factor in the co-evolution of *Leishmania* and their sandfly vectors.

Sandfly saliva also includes vasodilators and anticoagulants and these, along with other chemicals, may contribute to the recruitment of neutrophils and macrophages (potential host cells) to the bite site (Teixeira *et al*., 2005). Neutrophils are the most numerous of the mononuclear phagocytes and are found within both the circulating blood and the tissues. The *Leishmania* parasites invade neutrophils, develop within them and either kill them directly, cause them to be ingested by macrophages, or induce apoptosis (programmed cell death). The *Leishmania* may be ingested by macrophages while they are still inside neutrophils or after being released (e.g. following apoptosis). Although neutrophils are probably involved in the early stages of infection, their importance in the establishment of the parasite within macrophages is uncertain and may vary between species/strains of *Leishmania* (Novais *et al*., 2009; Ritter *et al*., 2009). *Leishmania* can also invade dendritic cells but it is not certain whether this is relevant to the development of the disease.

5.8 Host factors

5.8.1 Host identification

Most parasites infect only a single host species or a group of closely related species and therefore they need to identify their hosts reliably. Vector-transmitted parasites effectively delegate host-identification to their vector, while those that are transmitted when their host is consumed depend upon the specificity of a predator–prey relationship. Those parasites that actively search for and invade their host or are transmitted by contamination have a more active involvement in the transmission process since they can reject potential hosts in favour of waiting for a more suitable one to turn up. For contaminatively transmitted gastrointestinal parasites the conditions experienced in the gastrointestinal tract determine whether or not excystment (protozoa) or egg hatching (helminths) takes place. Factors include temperature, oxygen reduction potential, carbon dioxide, and composition of the bile. For example, digestive enzymes in the gut of mice digest away the outer layers of the eggs of the tapeworm *Hymenolepis diminuta* and stimulate the hexacanth embryo within to begin secreting its own enzymes (Holmes and Fairweather, 1982). In some helminth species (e.g. *Trichuris muris*), interactions between the parasite eggs and gut bacteria are important in stimulating hatching (Hayes *et al*., 2010). The gut microbial flora is incredibly complex and the composition varies between individuals. It is therefore possible that further research will identify a role for bacteria in the hatching of other parasite species and it may also help to explain why some people are more resistant (or susceptible) to certain parasites than others.

These stimuli are not always totally reliable and it is not unusual for eggs to hatch in unsuitable hosts in which the parasite is unable to develop to maturity. For example, the eggs of the nematode *Toxocara canis* will hatch in the human gut and the larvae migrate through the body just as they

would in dogs, which are the natural host. However, in humans, the larvae are not able to complete their development by returning to the small intestine and transforming into adults.

Starvation usually induces blood-feeding invertebrates to feed on hosts that they would normally ignore. One would therefore expect that starvation might affect host-choice in parasites that actively invade their host but there is limited information on this.

5.8.2 The influence of host behaviour on parasite transmission

Unless an infection is vertically transmitted, the only way an animal can become infected with a parasite is by coming into contact with its infective stage. Consequently, anything that increases or decreases this contact affects the chances of parasite transmission. One of the most important factors that determine host: parasite contact is host behaviour. There is evidence that hosts will modify their behaviour to avoid exposure to parasites and some parasites modify their host's behaviour to increase the chances of transmission.

Hosts typically become infected while undertaking their normal activities (e.g. eating, drinking) although the manner in which they do these things can increase or decrease their risks of infection. Although there is some evidence of predators avoiding parasitised prey, there is often no selective pressure for them to do so (Lafferty, 1992). Indeed, for predators, parasitised prey is often easier to catch and subdue and the energetic costs of becoming parasitised may not be high. For example, rodents infected with *Toxoplasma gondii* are easier for cats to catch and the parasite usually has little serious impact on the cats. Humans have the option of cooking their food and provided this is carried out at a high enough temperature and for long enough, it will kill parasite infective stages. However, in many cultures certain foods are considered best eaten raw and this can increase the risk of contracting parasitic diseases. For example, the recent worldwide popularity of Japanese sushi and sashimi cuisine – which includes raw fish – poses a risk of becoming infected with a number of infectious diseases including the nematode *Anisakis* spp. (Nawa *et al*., 2005). Raw meat, in particular beef and pork, is popular in many continental European countries with the consequent risks of contracting *Trichinella spiralis* and tapeworm infections. For example, the cyclist Laurent Fignon, winner of the Tour de France in 1983 and 1984 put his poor performance in the 1988 race down to a tapeworm he acquired through consuming raw meat. Among the many weird diets being promulgated on the web is the 'raw meat diet' on the basis that our health would improve if we ate as our ancestors did in the Stone Age. It goes without saying that this also provides an opportunity for acquiring a wide range of parasites.

Defecation is something that all animals do and is fundamental to life. Animals vary widely in their defecatory habits and there are big differences in human societies in their attitudes towards defecation and the subsequent disposal of faeces (Lewin, 1999). The study of defecation is therefore a fascinating topic but one that is also sadly neglected. It is, however, important to engage with the subject because it has major implications for the transmission of many parasitic diseases. Some animals defecate indiscriminately (e.g. cows) and this can lead to widespread contamination of the environment. For migratory animals or those with a large range, this does not matter since they are unlikely to come into contact with the contaminated areas, but it can be a big problem when these animals are constrained within a limited space – such as on farms or within zoos. Some animals defecate in a restricted area or construct latrines or dung piles. For example, horses often defecate in one corner of a field while both black and white rhinos (*Diceros bicornis* and *Ceratotherium simum*) defecate in a particular spot until they have built a dung pile that can grow

to over a metre in height. This behaviour reduces the faecal contamination of the surrounding area but depending upon where and how they are constructed may not reduce the risk of contaminative transmission. Individual deposits of faeces can dry out rapidly and break up, thereby resulting in the death of any parasite eggs, larvae or cysts present, through a combination of desiccation and exposure to UV light. By contrast, large accumulations of faeces present a reduced surface area to volume ratio and therefore remain moist for longer and facilitate the survival of the parasite transmission stages. In North America, racoons (*Procyon lotor*) construct latrines on logs or at the base of trees. The racoons are often infested with the nematode *Baylisascaris procyonis* and its eggs are therefore found in high densities around the racoon latrines. Numerous mammals and birds can act as intermediate hosts for this parasite within which it causes visceral *larva migrans* and potentially fatal damage to the central nervous system. Because the racoon latrines are constructed on the 'runs' of many small mammals, they increase the chances of them becoming infected (Page *et al*., 1998).

Box 5.4 Babycare and *Strongyloides fuelleborni kellyi* infection

Strongyloides fuelleborni kellyi has a very restricted distribution but in parts of New Guinea the prevalence in children aged 3–5 years old can reach 100%, while in adults the infection rate is 15–20%. It is especially pathogenic in babies of around 2 months of age and causes a potentially fatal condition known as 'swollen belly syndrome'. The afflicted children can pass enormous numbers of eggs in their faeces – figures as high as 300,000 eggs ml^{-1} have been reported – and they typically present with a distended abdomen and suffer from respiratory distress. It is intriguing question of how young babies become infected with such a large burden of nematodes. One possibility is that the nematodes are transmitted to the baby through the mother's milk: trans-mammary infection is known to occur in other species of *Strongyloides*. Another possible source of the worms is through exposure to large numbers of infectious third-stage larvae. The local practice of mothers is to place their babies in string bags that are then kept slung around the body. The bags are lined with banana leaves and other vegetation but these are not changed very often and therefore become heavily contaminated with faeces. The fact that so many eggs of *Strongyloides fuelleborni kellyi* are found in the faeces suggests that few if any of these hatch in the gut and therefore auto-infection via the gut or perianal region is unlikely. However, the eggs hatch soon after they are passed with the faeces and rapidly develop to the infectious third stage and this, coupled with the poor hygiene, facilitates the reinfection of the child (Ashford *et al*., 1992).

Among the many fascinating accounts of parasitic diseases related by Desowitz (1987), there is one concerning the transmission of hookworms in a Bengali village. The village had no sanitation and everyone defecated on a nearby patch of land. This land was heavily contaminated with faeces and most villagers walked barefoot. It might therefore have been expected that hookworm infection levels would have been high, with larvae infecting people as they went to the toilet. However, this was not the case – although it was found that men had higher worm burdens than women. The low burdens were partly a consequence of the villagers defecating quickly and then washing themselves – as proscribed by their religion. The infective larvae (see Figure 3.15 on page 124) in previously deposited faeces therefore had very little time to locate their host and would also have

to invade deeply enough to avoid being washed off the skin surface. The men tended to defecate in the morning while the ground was still moist – and thus facilitating the movement of the infective larvae – while the women tended to defecate in the afternoon when it was hot and dry and therefore less suitable for the parasite. Furthermore, the men's morning faeces were rapidly dried out thereby killing many of the parasite eggs and larvae. By contrast, the women's faeces were exposed to the sun for less time and the eggs would hatch and the larvae reach the infective stage during the night – and therefore be in a 'pole position' to infect the men the following morning.

Even if sanitation is provided, it does not mean that it will be used, maintained, or the waste disposed of safely. Even within developed countries such as the UK, public toilets are often poorly maintained and the notoriously inadequate provision of sanitation at events such as pop concerts encourages people to urinate and defecate wherever they can find sufficient privacy. It should not, therefore, be surprising that in developing countries it can be difficult to persuade people to fund, use, and maintain toilet facilities.

The advent of cheap air travel has meant that millions of people are moving rapidly between countries every year. In the process they take with them their existing infections and acquire new ones which they then transport across the globe. For example, the movement of people from malaria-endemic to malaria-free countries is a constant source of worry. The fact that there are about 2000 cases of malaria diagnosed each year in the UK despite the fact that the disease has not been present in the general population for many years indicates the scale of the problem. In addition, parasite vectors (possibly already infected) can also be transmitted between countries within aircraft either by flying into the cabin while it is on the runway or as a 'contaminant' among agricultural produce, etc. Once arrived at their destination, these 'stowaways' have been known to transmit infections to people who have never been abroad. For example, there are several confirmed cases of so-called 'airport malaria' in the UK and Northern Europe where malaria is no longer endemic (Martens and Hall, 2000). There are also legitimate concerns that vectors or intermediate hosts transported between countries might subsequently establish themselves and thereby enable the parasite to set up a transmission cycle as well.

The popularity of extreme sports and adventure holidays can also put people at risk of contracting parasites that they might not ordinarily come into contact with. For example, jumping or diving into water enhances the risk of infection with the protozoan *Naegleria fowleri*. This is because the activity may damage the mucus membranes lining the nose, which facilitates the entry of the parasite into the body. Cases of infection have been described from all age groups although the majority occur in younger individuals: this is probably a reflection of the fact that they are more likely to indulge in water sports than in any immunological reason. Most cases occur during the warmer months of the year which probably reflects the fact that this is the time of year most people will be swimming outdoors and also that *Naegleria fowleri* is a thermophile that grows best at high temperatures. This protozoan reproduces rapidly at temperatures above 30°C and can survive at 45°C. This has led to the suggestion that global warming may result in an increase in the number of human infections and more cases being reported in countries in which it has not previously been a problem (Cogo *et al*., 2004).

5.9 Co-transmission and interactions between infectious agents

Interactions, including transmission events, between hosts and parasites do not happen in isolation of their environment (Lello *et al*., 2004; 2008). The susceptibility to infection and subsequent

pathology in the host can be affected by interactions between different species microorganisms inside it. Individuals harbour a wide range of microorganisms and so it is clear that when passing one particular type of organism to another host, representatives of several species might follow suit. For example, the Bengali villagers in the example mentioned previously could easily be transmitting and acquiring protozoan, bacterial and viral diseases from the uncovered faecal depositions, even though the account concentrated on helminths. Similarly, arthropod vectors can be capable of carrying more than one organism pathogenic to the animal(s) they feed on, which would then be passed on while the insect was having a meal. In some cases, the association between the organisms is minimal and there is little obvious effect on the host. In other situations, the organisms compete with each other, to the detriment of at least one of them. There are also instances where biochemical and immunological interactions between organisms during co-infection cause altered and often serious pathology in the host. There are many potential examples of co-transmission and co-infection involving protozoa and helminths with other parasites or other types of microorganism. A few examples follow to illustrate some of the points, but there are plenty of others.

Some invertebrate species are hosts for more than one species of parasite, which could lead to the mammalian host being infected with more than one infection via the same route and at the same time (co-infection). A documented example of this is when *Anopheles* mosquitoes transmit both malaria parasites and filarial worms. It is theoretically possible for an individual mosquito to carry either or both parasites and therefore there are a number of ways in which a human infected with malaria and bancroftian filariasis at the same time could have acquired the infections. The most likely scenario is that a person is first bitten by a mosquito carrying *Plasmodium falciparum* sporozoites and then some time later has the misfortune to encounter a different mosquito infected with *Wuchereria bancrofti* microfilariae or vice versa. The mosquito could also be carrying both parasites and pass them on to a human during a blood meal, although it would be virtually impossible to prove either way. There is some evidence that being infected with one of these parasites causes physical or physiological disturbance within the mosquito, making it more susceptible to infection with the other (Manguin *et al*., 2010). Thus co-infection of the human host is a possibility; however, this concomitant infection may also affect the fitness of the mosquito, meaning it cannot fly as far or live as long as others. Surveys of insect populations in endemic area tend to find only small proportions of them carrying both parasites. For example, Muturi *et al*. (2006) studied populations of *Anopheles gambiae* and *Anopheles funestus* in Kenya, where these species are the main vectors of *Plasmodium falciparum* and *Wuchereria bancrofti* and found $<2\%$ were carrying both. Similar low rates of dual infection are reported in humans (Muturi *et al*., 2006; Manguin *et al*., 2010). Although the interactions between species of parasites within the human host have not been investigated extensively, it has been suggested that the presence of filariae can have an adverse effect on the development of the *Plasmodium,* resulting in lower parasitaemia. This could in turn be detrimental to the transmission of the parasites, particularly the protozoan. If there is a relatively low concentration of all life cycle stages in the host's blood, the chances of male and female and gametocytes being present in sufficient concentrations to ensure their uptake by a feeding female *Anopheles* are reduced.

A rather unfortunate example of co-infection was reported involving *Schistosoma mansoni* and Hepatitis C virus in parts of Egypt. During the 1950s, the Egyptian Ministry of Health began a concerted campaign to treat cases of schistosomiais in an attempt to reduce the transmission of the disease. Stool samples were collected from people in endemic areas and anyone with *Schistosoma* spp. ova was given the standard treatment, which was tartar emetic administered

intravenously. At the time, there was a world-wide lack of awareness about blood-borne diseases and to maximise resources, people were treated *en masse*, with healthcare workers re-using needles between patients. This policy of mass treatment by injection continued for about 30 years, until the oral drug praziquantel became available in the 1980s. It subsequently transpired that the practice of using one needle to treat more than one patient led to the widespread transmission of blood-borne viruses, in particular Hepatitis C (HCV). The prevalence of HCV infection in Egypt in the general population, as indicated by the number of people testing positive for antibodies to the virus, is reported to be somewhere between 14% (Strickland, 2006) and 19% (Harrison *et al*., 2009) compared to <1% in the UK. Epidemiological studies have shown a clear link between being given intravenous treatment for schistosomiais and developing HCV in Egypt. This is indicated by the high prevalence of infection and the demographics of the population currently affected by HCV. It is more common in middle-aged males, who would have been small boys playing near infected water – and thus highly likely to have been carrying schistosomes – in the 1950s and 1960s. Transmission of *Schistosoma haematobium* and Hepatitis B appear to have been better controlled due to earlier implementation of appropriate prevention strategies (Strickland, 2006), but a significant number of patients in Egypt are co-infected with *Schistosoma mansoni* and HCV. It is a particularly tragic co-incidence that both organisms cause chronic liver damage. Furthermore, interactions between the virus and the parasite, particularly at the level of the induced immune response appear to contribute to co-infected patients suffering more serious disease.

Pigs are commonly infected with *Toxoplasma gondii* and eating poorly cooked pork is thought to be a major source of human infections. The parasite seldom causes serious disease in pigs but there is now concern that co-infections of *Toxoplasma gondii* with porcine circovirus-2 infections (PCV-2) could result in the development of systemic toxoplasmosis (Klein *et al*., 2010). PCV-2 is currently a major cause of disease in pigs in many parts of the world and causes potentially fatal pneumonia, enteritis, and abortion. It also causes immuno-suppression and is commonly associated with co-infections with other viruses and also bacteria.

Acute toxoplasmosis is relatively uncommon in dogs and is usually associated with young animals suffering from canine distemper, which is caused by Canine Distemper Virus (Ahmed *et al*., 1983). Toxoplasmosis in dogs therefore differs from neosporosis in which serious disease is usually a consequence of a primary infection. Care is needed when interpreting literature that pre-dates the identification of *Neospora caninum* and ascribes a disease condition in dogs to *Toxoplasma gondii*. Canine distemper virus (CDV) is a highly contagious and potentially fatal disease that affects not only dogs but a number of other mammals including lions, racoons, ferrets, and seals. It suppresses the immune system and causes fever, vomiting, diarrhoea, and nervous symptoms such as convulsions and paralysis (Beineke *et al*., 2009). CDV is a member of the morbillivirus group that also includes measles virus, rinderpest virus, and phocine distemper virus. CDV is spread via aerosol droplets and contact with secretions and body fluids. Canine distemper is now uncommon among domestic dogs in developed countries owing to the availability of an effective vaccine. However, CDV has not been eradicated and it is common in developing countries and can have serious consequences for wild animals. For example, massive losses among some seal populations have been attributed to CDV (e.g. Kennedy *et al*., 2000). Seals, like other mammals, can be infected with *Toxoplasma gondii* (Dubey *et al*., 2003) but it is not known whether co-infections with CDV are common or whether they contribute to the severity of the disease.

Burkitt's Lymphoma (BL) is an unusual facial tumour (see Colour Plate 20) which usually occurs in children living in areas which are holoendemic for malaria. This disease was first described in Uganda and is more common in East Africa, though high rates are also reported in Malawi (Orem, *et al*., 2007) and also Papua New Guinea (Haque and Crawford, 2009). The Epstein-Barr virus (EBV) was first isolated and characterised by researchers who were investigating the causes of BL. The virus is a gamma herpes virus which is transmitted in saliva and which infects B lymphoctyes. In common with other herpes viruses, after the primary infection, EBV persists in the body for life. It is associated with glandular fever among teenagers and young adults in Western Europe. However, it is usually acquired much earlier in life in many other parts of the world and infection is often asymptomatic, meaning that most African children over the age of 2 carry EBV.

The tumour cells in Burkitt's Lymphoma are B lymphocytes which transform to be malignant due to a specific chromosomal translocation between chromosome 8 and usually chromosome 14, 2 or 22, with the first being the most common. This translocation moves the c-myc gene in chromosome 8 to be under the regulatory control of genes coding for Ig heavy (chromosome 14) or light chains and to become an oncogene which is expressed constituitively.

There is an observable association with co-infection with *Plasmodium* spp. and EBV and Burkitt's Lymphoma (Rochford *et al*., 2005). The incidence of the tumour is found to decrease in areas where malaria control programmes have been successful and is less likely to occur among children who move away from holoendemic regions (Orem *et al*., 2007). Also, children with sickle cell trait and other haemoglobinopathies, which afford some protection against malaria infection, are less prone to developing Burkitt's Lymphoma. The *Plasmodium* infection somehow facilitates the development of the tumour, but the exact mechanism of the interaction has not been fully elucidated. It has been suggested that because malaria stimulates B cell activity in the human host and there are more EBV infected B cells which increases the chances of the c-myc oncogene transformation. Malaria also suppresses T cells and the immune mechanisms whereby EBV infected cells would be noticed and eliminated, which again would encourage transformed cells to proliferate (Haque and Crawford, 2009).

The interaction, though specific, is not the only route to the malignant transformation in Burkitt's Lymphoma. In recent years, cases of Burkitt's Lymphoma which are not associated with *Plasmodium* infection or EBV either have been reported; these are referred to as 'sporadic Burkitt's Lymphoma' as opposed to the 'endemic Burkitt's Lymphoma' discussed above. In 'sporadic Burkitt's Lymphoma' the tumours are more common in the abdomen rather than the face, but they also express the *c-myc* oncogene transformation (Haque and Crawford, 2009; Orem *et al*., 2007). There appears to be a particular association for sporadic Burkitt's Lymphoma with the immuno-suppression caused by HIV in AIDS (Orem *et al*., 2007).

5.10 How religion can influence parasite transmission

There are many faiths in this world and religion plays a major role in the day-to-day lives of millions of people. Religious practices can influence what people eat and drink, their interactions with animals, how they conduct sexual relationships, and where and how they dispose of their dead. Religion can therefore be an important factor in the epidemiology of parasitic infections. Most religions include strict instructions over what foods can be eaten, when it is eaten, and how it should be prepared. For example, the consumption of pork is forbidden in the Jewish

religion, Islam and Ethiopian Orthodox Christianity. The ban on eating pork was initially made in the Old Testament (Leviticus 11: 7–8; Deuteronomy 14: 8) and is also found in the Quran (2: 173; 16: 115). The pig was, and is, considered to be an unclean animal by people practising these religions – a view that is probably not helped by its coprophagic tendencies. By contrast, the Hindu religion forbids the consumption of beef because cows are considered to be sacred. Refraining from consuming pork prevents infection by *Trichinella* spp. and *Taenia solium*, while not eating beef protects against acquiring infection with the beef tapeworm *Taenia saginata*. When people feel vulnerable to disease transmission, they show increased aversion to those who are obviously disabled and to animals thought to be associated with disease (Prokop *et al*., 2010) but whether or not the religious lawmakers made an informed decision on the basis of disease prevention is debateable. The consumption of virtually any animal (and unwashed vegetables) carries the risk of disease transmission and according to Leviticus, hares, swans and owls (among many others) are ranked alongside pigs as being 'unclean'. The association of pigs with being unclean is a feature of Near East and Middle Eastern religions. By contrast, many other faiths and cultures with equal risks of exposure to parasitic disease consider pigs to be highly valuable and therefore have much closer relationships with them. For example, the pig is one of the signs in the Chinese Zodiac and people born under its sign are considered hard-working and intelligent. The Romans valued pigs and 'Porcus' was considered an esteemed name. Among some Melanesian peoples it is believed that humans were initially fashioned in the shape of pigs but the god Qat subsequently beat the pigs down into their current four-legged state (Codrington, 1881). Pigs are so highly valued by some Melanesian peoples that in the past women were willing to suckle piglets to help them grow (see Colour Plate 2).

During the three-day Feast of Sacrifice ('Īd al-Adha), the majority of Muslims who can afford to do so sacrifice a sheep, goat, camel, cow or other bovid to commemorate the prophet Abraham's willingness to sacrifice his son Isma'il. In many countries that have a sizeable Muslim population the numbers of animals required vastly exceeds the local supply. Consequently, huge numbers of animals are imported from far and near. This has caused problems in parts of North Africa and south-western Asia where farmers are encouraged to overstock their grazing land. Furthermore, the vagaries of the Islamic lunar calendar mean that the requirement for animals does not always match the cycles of plant growth and animal reproduction. Similarly, large numbers of sheep are shipped thousands of miles from Australia to the Middle East and elsewhere in the Islamic world and this has raised animal welfare concerns (Brooke, 1987). Although some countries have attempted to ensure that the animals are killed for free by trained slaughtermen or butchers in licensed abattoirs, many animals are killed in backyards or at the side of the road. Far more animals are killed than it is possible to consume and all too frequently blood and offal are simply poured down drains, thrown onto rubbish heaps or, at best, buried under a thin layer of soil. Needless to say, domestic and feral dogs as well as rats eat much of that which is thrown away and there can be an explosion in the carrion insect population (Zaidi and Chen, 2011). The movement of large numbers of animals between countries over a short period of time, their confinement in small spaces, the absence of meat inspection, the lack of safe disposal of offal, and the abundance of insects capable of acting as mechanical vectors provide ideal opportunities for the spread of animal and zoonotic diseases. However, there is surprisingly little published information on the extent to which the Feast of Sacrifice influences the spread of parasites that one might expect to be enhanced such as *Chrysomya bezziana* and *Echinococcus granulosus* (e.g. Latif *et al*., 2010). However, there are increasing concerns about the transmission of microbial pathogens

such as anthrax (*Bacillus anthracis*) and viruses such as Rift Valley Fever (e.g. Sheriff and Osgood, 2010).

Most faiths require that the dead be disposed of by burial or burning. Consequently, although man is the intermediate host for several parasite species, we are normally 'dead-end hosts' because we are seldom preyed on, and after death our bodies are removed from the disease transmission chain. The Parsees and some Tibetan Buddhist sects arrange for their dead to be consumed by vultures but these are not thought to play a significant role in the transmission of diseases. By contrast, a traditional belief among the Nandi tribe in Kenya is that unless a person's dead body is eaten by a hyena, his *mukuledo* (the personality aspect of the soul) cannot reach the spirit land and is destined to roam forever as a ghost on earth (Hollis, 1909). Similarly, the Turkana, another Kenyan tribe, do not have a tradition of burying their dead and corpses are left in the bush where the dogs soon sniff them out. These practices contribute to the transmission of hydatid disease caused by *Echinococcus granulosus* in which humans are one of the intermediate hosts and the adult tapeworm develops in the intestine of dogs and other caniids.

5.11 The influence of war on parasite transmission

Warfare can be considered an aspect of human behaviour that impacts on parasite transmission. Although we tend to focus on the consequences of warfare on the transmission of human disease, it often also leads to widespread alteration and pollution of the environment which can have consequences for the transmission of other animal parasites. Pollution can enhance parasite transmission by weakening the host's immune system or increasing the host's exposure to the infective stages of parasites. However, there is limited information on pollution caused directly by warfare. For example, there remains considerable controversy over the levels and possible effects of depleted uranium from the use of munitions during the invasion of Iraq in 2003. Sheep and goats are particularly vulnerable to ingesting depleted uranium because they crop vegetation close to the ground and will ingest large amounts of soil in the process. It is therefore somewhat surprising that Al-Kinani (2006) found no evidence of meat or milk being contaminated with depleted uranium from farms in the Basra region. There is little published information on whether there has been any change in the incidence of parasitic diseases in domestic livestock in the regions of Iraq most affected by the recent military activities.

Wars and economic crises also lead to the collapse of the health infrastructure and the mass migration of people between areas with quite different incidences of particular infections. For example, recent problems have caused migration of susceptible individuals to leishmaniasis-endemic areas, in Sudan, Afghanistan and Pakistan. In the Kurram Agency in the north-west frontier province of Pakistan, cutaneous leishmaniasis was seldom seen until 2002 when approximately 5000 new cases were recorded. This was attributed to the influx of large numbers of Afghan refugees. Similarly, within Afghanistan itself, large numbers of new cases of cutaneous leishmaniasis have been recorded in Kabul as people return to the city now that it has become (comparatively) more peaceful. With an influx of susceptible people, a lack of effective control measures to reduce the abundance of the sandfly vector, the health infrastructure in a parlous state, and inadequate medical supplies, it is hardly surprising that the disease has become an important emerging infection. Leishmaniasis has also become a significant problem for foreign troops serving in Iraq and they can carry the infection with them when they return home (Aronson, 2008).

Box 5.5 Has successful anti-rabies control resulted in an increased risk of *Echinococcus multilocularis* infection in Europe?

In parts of Germany, Switzerland, and several other European countries there was a dramatic increase in the red fox population between the 1990s and the early years of the new millennium. The reason for the increase is probably a result of a combination of factors but one of these is almost certainly the success of anti-rabies control programmes. In the past, rabies was an important cause of fox mortality but the risk of the disease being transmitted to humans and domestic animals led to the introduction of widespread control programmes in which baits laced with anti-rabies vaccine were employed. In addition, foxes are changing their behaviour patterns and they are becoming increasingly urbanised; many large towns and cities in Europe now support thriving fox populations. Unfortunately, the rise in the fox population has often been accompanied by an increasing prevalence of their infection with *Echinococcus multilocularis*. For example, in Lower Saxony in Northern Germany the prevalence of *Echinococcus multilocularis* in foxes rose from 12% in 1991 to 20% in 2005 (Berke *et al*., 2008). Similar surveys have shown 20% of foxes in Stuttgart harbour *Echinococcus multilocularis* as do 40% of the foxes in Zurich with 400–500 worms being an average worm burden (Lucius and Bilger 1995). There is therefore concern that foxes will contaminate the environment with tapeworm eggs and this could lead to an increase in the prevalence of *Echinococcus multilocularis* infections in humans (Schweiger *et al*., 2007).

5.12 The influence of parasites on host behaviour

Although many parasites cause changes in the behaviour of their host, most of these are 'non-adaptive' and confer no advantage to either the parasite or its host (Poulin, 2000). These changes result from one or more of the following: impairment of vision, hearing or other sensory systems, physical damage to the central nervous system and/or altering the levels of neurotransmitters, physical damage to the endocrine system and/or altering the levels of hormones, causing starvation through interfering with feeding or drinking or removal of metabolic resources. In some cases, however, the changes in host behaviour appear to be adaptive and promote the chances of parasite transmission. For example, the larval stage (plerocercoid) of the tapeworm *Schistocephalus solidus* lives in the peritoneal cavity of three-spined sticklebacks (*Gasterosteus aculeatus*). These must be consumed by fish-eating birds such as Arctic terns (*Sterna paradisaea*) in order for the parasite to complete its life cycle and develop into an adult. The plerocercoid grows to an enormous size, which compromises the movement of the fish host and increases its oxygen demand. Consequently, infected fish tend to swim close to the surface of the water where the oxygen tension is higher. In addition, the infected fish exhibit a reduced fright response when attacked and either fail to react to the shadow of a predator approaching from above or quickly return to their former position after being startled. Uninfected fish, by contrast, quickly retreat and hide among underwater vegetation in response to a shadow passing over the surface of the water and do not swim in open water for some time after being startled. Infected fish are therefore much easier for the definitive bird host to catch (Barber *et al.,* 2000).

Another interesting example of how a parasite appears to modify a host's behaviour is that of the protozoan *Toxoplasma gondii*. This parasite infects most warm-blooded animals but its natural transmission cycle is probably between rodents and cats. The asexual stages encyst within the nervous tissue of its intermediate hosts but the parasitaemia often reaches exceptionally high levels in rats and mice and this causes changes in behaviour that have been called 'suicidal'. In particular, the rodents become more active and lose their normal fear of new objects (neophobia). Consequently, they are more likely to be caught in live traps than those which are uninfected. Even more importantly, the infected rodents lose their aversion to the smell of cats and even become attracted to their odour (Webster, 2001; 2007). Rats and mice infected with *Toxoplasma gondii* are therefore much more likely to be caught by cats – which are the only hosts within which the parasite reproduces sexually. Precisely how *Toxoplasma gondii* causes these changes in behaviour is not known and may involve a combination of killing infected brain cells, neuromodulatory changes and immunologically mediated pathology. *Toxoplasma gondii* also induces behavioural changes in other animals although whether or not they can be considered adaptive is uncertain. For example, southern sea otters (*Enhydra lutris nereis*) suffering from toxoplasmic encephalitis are more likely to be attacked and killed by sharks (Kreuder *et al*., 2003). This may be because they display aberrant behaviour that attracts the attention of sharks or because they are less able to avoid them when they do attack. There are no records of *Toxoplasma gondii* infecting sharks or fish. Fish such as anchovies can filter out *Toxoplasma gondii* oocysts from the surrounding water but these do not hatch and are passed through the gut. The fish can, however, act as transport hosts if they are then eaten by marine mammals, such as otters or dolphins, before the oocysts are defecated.

Box 5.6 The effect of *Toxoplasma gondii* on human behaviour

In humans, infection with *Toxoplasma gondii* has been linked with a variety of behavioural changes although it is difficult to generalise because men and women respond differently to infection. For example, personality profile tests indicate that infected men tend to be jealous and to disregard rules while infected women are trusting and conscientious (Lafferty, 2006). *Toxoplasma gondii* infection also affects hormone titres although again the effects differ between men and women. For example, infected men tend to have higher testosterone levels than those who are uninfected while infected women have lower testosterone levels than those who are infected. People who are infected with *Toxoplasma gondii* are more likely to be involved in serious road accidents (Flegr *et al*., 2009; Yereli *et al*., 2006) and it is tempting to suggest that this may be related to behavioural changes caused by the parasite. A statistical correlation does not necessarily indicate 'cause and effect' but as traffic accidents are responsible for millions of deaths each year (on a global basis) it is a potentially worthwhile area of research to be pursued. The development of schizophrenia has also been linked to infection with *Toxoplasma gondii* although the evidence for this is far from proven (e.g. Krause *et al*., 2010; Yolken *et al*., 2009; Yuksel *et al*., 2010). Schizophrenia is a lifelong neuropsychiatric condition that is characterised by emotional and memory problems, difficulties in expressing oneself and controlling one's thoughts and some patients become delusional and hear voices. What causes schizophrenia remains uncertain but it probably includes a combination of genetic susceptibility and agents that disturb brain chemistry such as infectious agents. Interestingly, experiments have shown that some of the drugs that are used to alleviate the symptoms of schizophrenia (e.g. haloperidol and valproic acid) also

prevent the replication of *Toxoplasma gondii* (Webster and McConeky, 2010). Nowadays, humans are dead-end hosts for *Toxoplasma gondii* and therefore its effects on our behaviour cannot be considered adaptive. However, this may not have always been the case. Our primitive ancestors would have been prey for large felines, such as sabre-toothed cats, and it is possible that there was once a *Toxoplasma gondii* transmission cycle between the two. This could have been enhanced by the parasite, making our ancestors more likely to take risks or place themselves in harm's way, although this is, of course, pure speculation.

5.13 Environmental factors

5.13.1 Natural environmental variables

The environment plays a major role in parasite transmission either directly, by affecting the survival of the transmission stage (e.g. cysts, eggs, or free-living larvae) or indirectly, by affecting the distribution and survival of the host (intermediate or definitive) or vector. For example, parasite cysts and eggs usually die quickly if they are exposed to dry conditions especially if this is coupled with exposure to UV radiation. By contrast, moist conditions favour their survival and facilitate the movement of hookworm larvae. Countries with tropical climates often experience sudden torrential downpours that rapidly overwhelm the ability of the drainage system, if there is one, to cope with the arrival of so much water. This is further exacerbated by the continued use of open sewers and their overflow leads to widespread contamination with raw untreated sewage. This increases the risk of contaminatively transmitted parasites. Although there is a tendency to consider environmental factors individually, in reality they act in concert and can include variables such as soil or water chemistry as well as atmospheric properties. A good review of how environmental factors affect the transmission of parasites of domestic livestock is provided by Stromberg (1997) although the scenarios he discusses could equally be applied to many parasites of medical importance.

5.13.2 Pollution

Pollution can occur naturally, for example as a result of volcanic eruptions but it is more commonly associated with human activity. The effects of a pollutant upon an animal are highly case dependent and are affected by a vast range of abiotic and biotic variables. Therefore, the presence of a pollutant in the environment does not mean that it is biologically available: for example, it might be bound to clay minerals and cannot be absorbed (Table 5.1). Similarly, the effect of a pollutant upon disease transmission will depend upon a complex interplay between the parasite, the host, the pollutant, and other environmental factors. For example, see Morley *et al*. (2003) for a discussion of how pollution can affect the transmission of digenean trematode larvae. The mechanisms by which a pollutant might enhance parasite transmission are summarised in Table 5.2. However, this list could equally be reversed to say how the pollutant might reduce the chances of transmission by having a deleterious effect on the parasite. The effect of a pollutant on other organisms also needs to be considered. For example, if a pollutant kills the predator, pathogen or competitor of a vector or intermediate host, then its population may rise.

Table 5.1 Biological and abiotic factors that influence the toxicity of a pollutant to an animal

Biological factors	Abiotic factors
Species of animal	Type of pollutant
Genetic constitution	Concentration
Age	Distribution
Gender	Environmental variables (e.g. temperature, pH, soil and water characteristics, etc.)
Health	Presence and concentration of other pollutants
Nutritional status	
Pre-existing disease	
Reproductive status (e.g. pregnancy/gravid)	

Table 5.2 How a pollutant can increase the chances of parasite transmission

Pollutant is poisonous to the host but not the parasite. The pollutant could therefore weaken the host's immune system and makes it more susceptible to the parasite. The increased parasitaemia could increase the chances of transmission (e.g. more infective stages released into the environment/more chance of vector 'picking up' the parasite).
Pollutant is poisonous to both the host and the parasite and the outcome will depend upon which suffers the most harmful effect.
Pollutant is beneficial to the parasite's vector or intermediate host, thereby increasing the number of opportunities for transmission.
Pollutant is beneficial for the survival of the parasite's infective stage, thereby enhancing the chances of transmission.
Presence of the pollutant increases the likelihood of the parasite and host coming into contact.

Theoretically, parasites with simple life cycles should predominate in disturbed habitats such as those affected by pollution because fewer species are required to ensure the life cycle is completed. There is some evidence for this (e.g. Diamant *et al*., 1999) and some researchers have even suggested that parasite species diversity could be used as a pollution indicator (Broeg *et al*., 1999). Similarly, some parasites accumulate toxins, such as heavy metals, and it has therefore been suggested that they could be used as indicators of the risk of bioaccumulation within the local environment (Sures *et al*., 2004).

Box 5.7 Sewage effluent, *Toxoplasma gondii* and marine sentinel species

It has been proposed that marine mammals are excellent 'sentinel species' that could be used as indicators of the health of the oceans and coastal environments (Bossart, 2006). This is because they tend to live for many years, feed at higher trophic levels, and their 'public profile' means that they are more likely to be observed with dead or dying animals being reported. In addition,

many species have high fat reserves in which lipophilic man-made chemicals (e.g. DDT) become sequestered. The decline in the population of a number of marine mammal species has been attributed to disease and human activities are thought to have contributed to the spread and severity of these diseases. For example, in the USA the southern sea otter (*Enhydra lutris nereis)* population remains low despite federal protection. Infectious diseases are estimated to account for an unusually high 38.5% of mortalities. In one seroprevalence study, *Toxoplasma gondii* was identified in 52% of freshly dead beachcast otters and 38% of live otters sampled along the Californian coastline (Conrad *et al*., 2005). Of course, being seropositive is not the same as suffering clinical symptoms but autopsies have indicated that toxoplasmic encephalitis was responsible for 16.2% of the otter deaths (Kreuder *et al*., 2003).

It is not unusual for cat owners to dispose of the waste from their pet's litter tray down the toilet and flushable litter can be bought that reduces the risk of blocking the pipes. It is therefore possible that *Toxoplasma gondii* oocysts are surviving passage through the sewage system and being transported into the marine environment where they ultimately cause infections of marine mammals (and possibly birds as well). It is presumed that the sea otters become infected from oocysts that are washed into the coastal environment in this way, although 60% carry *Toxoplasma gondii* with a novel genotype – designated Type X (Conrad *et al*., 2005). Whether or not the type X genotype is also common among mainland animals is not yet known. It is possible that the otters become infected through ingesting oocysts in the seawater (e.g. while grooming) –especially if they are particularly susceptible to the disease. However, it is more likely that they consume filter feeding prey that have concentrated the oocysts within their tissues. For example, under laboratory conditions both eastern oysters (*Crassostrea virginica*) and anchovies (*Engraulis mordax*) concentrate *Toxoplasma gondii* oocysts from seawater (Lindsay *et al*., 2001; Massie *et al*., 2010). Although the circumstantial evidence is compelling, as of 2011, *Toxoplasma gondii* oocysts (unlike the cysts of *Giardia* and *Cryptosporidium*) have not been identified in any seawater and marine invertebrates or fish in the wild (Fayer *et al*., 2004).

5.13.3 Global warming

Most scientists now agree that global warming is a genuine phenomenon although there remains considerable disagreement as to the cause, the consequences, and the speed with which environmental change will occur. Although there are regional differences, there has been in an increase in average temperatures by about 0.8°C since 1880. Although this may sound like a small rise, it is sufficient to increase mosquito abundance by up to 100% in some circumstances (Pascual *et al*., 2006). In addition, some experts consider the recent increase in extreme weather events such as tropical storms, droughts, heatwaves, and flooding are related to changing weather patterns brought on by global warming. As we have seen, environmental factors can have a major impact on the transmission of many parasitic diseases and there are concerns that some of them could increase their range or become more problematic. For example, tropical storms can create widespread flooding. As the flood recedes, the resulting pools of dirty water can provide suitable environments for the breeding of mosquitoes and other vectors as well as aquatic snail intermediate hosts. Serious storms can also destroy infrastructure, disrupt healthcare services, damage homes and sewage systems, and create homeless people or refugees which together facilitate

the transmission of contaminatively transmitted parasites. Environmental change is particularly noticeable in the Arctic regions and this is already having consequences for parasite: host dynamics among the wild animals that live there. For example, in the Canadian Arctic, climate change is facilitating the transmission of nematode parasites of musk oxen (*Ovibos moschatus*) and this could have deleterious effects on their health (Kutz *et al*., 2005).

Box 5.8 A perfect storm: did global warming contribute to disease in African lions?

In recent years there have been sudden crashes in the lion (*Panthera leo*) population in some parts of Africa. For example, in 1994, the lion population in the Serengeti National Park declined by about 33% and in 2001 there was a similar population crash in the Ngorogoro Crater region. The deaths were initially attributed to outbreaks of canine distemper virus that was acquired (probably indirectly) from domestic or feral dogs (e.g. Roelke-Parker *et al*., 1996). However, subsequent analysis of lion serum samples taken before and after the population declines indicated that several outbreaks of CDV had taken place without any noticeable effects on the lion population. This suggested that the lions were acquiring CDV on a regular basis and the population crashes were therefore unlikely to result from sudden exposure of an immunologically naïve population to the disease. It is now thought that the lions were killed by massive exposure to *Babesia* parasites (Munson *et al*., 2008). This was brought about by changes in the climate, possibly resulting from global warming, that caused unusually prolonged droughts. The lack of rain resulted in the death of vegetation and consequently many of the buffalo and other herbivores on which the lions preyed died of starvation. When the rains eventually restarted, the surviving herbivores were in poor health but the conditions were ideal for the ticks that fed upon them. Furthermore, the ticks were able to feed and reproduce more successfully owing to the weakened state of their host's immune system. Most *Babesia* species are relatively host-specific and the lions are unlikely to have been infected with those present in their prey. However, in feeding on buffalo, they would be exposed to unusually large numbers of ticks (e.g. *Rhipicephalus appendiculatus*) and these are capable of feeding on lions and transmitting lion babesias such as *Babesia leo* and *Babesia felis*. Babesiosis is seldom a serious condition in lions but in this case they would be in a naturally weak state owing to the lack of prey and this, combined with infection with CDV, probably reduced the immune system to a point at which a disease that is not normally fatal resulted in mass mortalities. It is not known whether *Toxoplasma gondii* may also have been involved but it is capable of causing fatal disease in lions (Ocholi *et al*., 1989).

Much of the focus on the impact of global warming on parasitic disease has been based on the potential of malaria to spread to new countries and become a more serious problem within those where it already exists. In particular, a rise in temperature and increased rainfall are thought to make the environment more suitable for the mosquito vectors. Computer models have been developed that support this conjecture (e.g. Tanser *et al*., 2003) but there are so many factors that influence the transmission of malaria that their validity has been strongly questioned (e.g. Reiter *et al*., 2004; Reiter, 2008). In addition, it is difficult to disentangle global and local influences on climate change. For example, changes in land use, such as deforestation can result in local environmental and climate changes that increase the breeding of mosquitoes and the consequent

transmission of malaria (Pascual *et al*., 2006; Patz and Olson, 2006). Societal changes also influence the transmission of malaria. For example, malaria was common in parts of the UK and Northern Europe up until the early 1900s but has since disappeared. The decline in malaria was not related to changes in the climate or the disappearance of the mosquito vector and these countries remain theoretically vulnerable to the establishment of the disease (Kuhn *et al*., 2003). However, despite the arrival of thousands of people infected with malaria into the UK every year, the disease shows no signs of re-establishing itself here. This is, in part, a result of the improved standards of living and the quality of the health service which mean that people with symptomatic malaria are quickly identified and treated and therefore do not become a source of infection for the mosquitoes (Lafferty, 2009).

Questions

1. Briefly describe what is meant by the term ‘contaminative transmission’ and give three examples of parasites that are transmitted in this way.
2. How does a mechanical vector differ from an intermediate host?
3. Explain why a mosquito may be a good vector of human parasitic diseases under laboratory conditions but might have little involvement in disease transmission in the field.
4. What is a nosocomial infection? Give two examples of parasites that can be acquired as nosocomial infections.
5. Why are humans considered to be ‘dead-end hosts’ for certain parasitic infections?
6. Briefly discuss an example of how a parasite manipulates its host’s behaviour to increase the chances of transmission.
7. Give four reasons why wars usually increase the spread of parasitic diseases.
8. Briefly discuss three ways in which pollution might increase the transmission of parasitic diseases.
9. Why might global warming result in an increase in the spread of malaria?
10. Why is global warming unlikely to result in malaria becoming endemic in the UK?

6

Immune reactions to parasitic infections

6.1 Introduction

Immunology is the study of the means by which an organism distinguishes 'self' from 'non-self' and thereby identifies and removes viruses, bacteria, parasites, inanimate materials, the organism's own dead or dying cells and any other potentially harmful substance. The immune mechanisms a host uses against a parasite therefore determine whether or not the parasite is able to establish itself, whether it is able to grow and reproduce, the progression of the infection, the host's subsequent response to other pathogens, and whether or not it is possible to design an effective vaccine against the parasite. The immune reaction to an infection is also important in determining the amount of damage (pathology) it inflicts. For example, a strong immune reaction may remove the parasite and therefore reduce pathology or it may cause serious harm to the host and even increase its vulnerability to other pathogens. Within this chapter we will consider the immune mechanisms and in Chapter 7 there will be a more in-depth consideration of how the immune response both limits and contributes to the pathology associated with parasitic diseases.

Different types of immunity can be recognised. At the most basic level is innate or non-specific immunity, which consists of structural and physiological features that prevent an invading organism gaining entry to the body (Delves *et al*., 2006; Medzhitov, 2010). As its name suggests, the innate response does not distinguish between potential pathogens and all invading organisms experience the same level of challenge. This is the only form of immunity found in invertebrates. It provides an immediate response to an invading pathogen but it does not confer any long-lasting protection. Adaptive immunity, also referred to as acquired or specific immunity, is restricted to vertebrates and is triggered by a response to specific antigens. Aspects of the innate immune response are immediately active or fully mounted within a short time after the immune challenge but in adaptive immunity there is a longer time gap between recognition of the antigens and production of an immune response. Another difference is that an effective adaptive immune response can provide long-term protection against a particular microorganism. Immunology is a fast-moving and complicated subject but we have attempted to keep things simple while still including examples of some of the most recent advances. Inevitably, some of the explanations of how the immune system operates contained in this chapter are not complete and will be superseded or modified within a short time of publication: the science of immunology should be considered as 'work-in-progress' rather than a series of 'absolute truths'. Those requiring a more in-depth coverage of

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vertebrate immunity, and in particular that of humans, are advised to consult Delves *et al*. (2006) while the immunology of animals of veterinary importance is covered by Tizard (2008).

6.2 Invertebrate immunity

The outer body surface of invertebrates provides protection from both biotic and abiotic factors. For example, nemerteans and molluscs secrete mucus that prevents potential pathogens and predators from making contact with the body wall while nematodes and arthropods are protected by their thick cuticle. In the case of arthropods, the cuticle can extend to line the foregut and hind gut while in insects the midgut epithelial cells are protected by the peritrophic membrane. Should these outer defences be breached, invertebrates have a range of innate immune mechanisms that can kill or isolate potential pathogens (Table 6.1). These mechanisms depend upon pattern recognition receptors such as Toll-like receptors (see later) detecting the presence of common cell surface antigens. The pattern recognition receptors are attached to blood cells or other cell types. Invertebrates are not, however, able to mount responses to specific antigens and neither do they form antibodies. There is a great diversity of invertebrates and therefore it should not come as a surprise that there are also considerable differences in their immune response to invading pathogens. The general information given below is therefore not applicable to all invertebrates. Further details on invertebrate immunity can be found in Söderhäll (2011); Iwanaga and Lee (2005) and Gillespie and Kanost (1997).

Invertebrates do not have a closed circulatory system, that is, they lack the network of veins, arteries etc that are found in vertebrates. In addition, many of them have hydrostatic exoskeletons, i.e. they owe their body shape to the pressure of fluid within their coelom (fluid-filled body cavity). Consequently, if their body wall is punctured, the organism can shrivel like a punctured balloon as fluid leaked through the wound – and this would be fatal. Many arthropods therefore have coagulation systems that immediately seal off wounds and this can also trap pathogens that attempt to physically penetrate into the body (Cerenius and Söderhäll, 2011). Once within the body, the pathogen is met with a range of physiological responses. If the pathogen is detected by the phagocytic cells that float freely within the haemolymph, it is phagocytosed and then digested, provided it is small enough. If it is too large for this, numerous phagocytes converge on the invader until it is completely surrounded in a process called encapsulation (see Colour Plate 22). This is accompanied by activation of the pro-phenoloxidase cascade at the site of invasion or on the surface of the pathogen. This results in the formation of melanin (melanisation) and reactive oxygen

Table 6.1 Principal invertebrate immune mechanisms, after Iwanga and Lee, 2005

Haemolymph coagulation entraps the invader
Invader is phagocytosed or encapsulated by blood cells
Activation of the pro-phenoloxidase cascade results in melanisation that isolates and/or kills the invader
Production of reactive oxygen radicals kills the invader
Lectin-complement system kills the invader
Agglutinin-lectin system agglutinates the invader
Activation of Toll-like receptor pathways and/ or Imd-Relish pathways results in the production of antimicrobial peptides

species such as superoxide (O_2^-) and hydroxyl radicals (·OH). The reactive oxygen species are toxic while melanisation strengthens the capsule/nodule around the pathogen and probably also restricts oxygen and nutrient availability.

Invertebrates produce a range of antimicrobial peptides and proteins such as cecropins and defensins in response to the activation of Toll-like receptor pathways and Imd-Relish pathways. The gene '*Toll*' was initially identified in the fruit fly *Drosophila* in which it is required for the development of patterning. Subsequently, it was also found to bring about the production of the anti-fungal protein drosomycin. It is now known that Toll-like receptors are present in numerous organisms, including mammals. Toll-like receptors are proteins that are located within membranes and have both an intracellular domain and an extracellular domain. The intracellular domain resembles that of mammalian interleukin-1 receptors. Organisms vary in their number of Toll-like receptor genes but they are absent from platyhelminths. It is thought that Toll-like receptor genes initially evolved in the Cnidaria (jellyfish, etc.) but were subsequently lost by the Platyhelminthes. It was probably only later in evolution, following the development of the Coelomate animals that Toll-like receptors became involved with mediating innate immune responses (Zheng *et al*., 2005).

Defensins are produced by most animal phyla but cecropins have a more restricted distribution. Although an initial report stated that cecropins are produced by pigs it subsequently transpired that these actually originated from *Ascaris suum* nematodes living in the intestines of the pigs (Pillai *et al*., 2005). Some antimicrobial proteins kill bacteria by interacting with and destroying their cell walls, but it is not known whether they all act in this way. In addition to acting on bacteria, some antimicrobial proteins also have antiparasitic activity but it is not known how they exert this effect. The presence and levels of these antimicrobial proteins may, at least in part, determine the effectiveness of a strain or species of invertebrate to act as a vector or intermediate host. For example, the stable fly *Stomoxys calcitrans* coexists with tsetse flies over parts of Africa but despite sharing the same hosts and having similar physiologies, the stable flies only act as mechanical vectors for trypanosomes such as *Trypanosoma evansi*. In most cases in which *Stomoxys calcitrans* is allowed to feed on animals heavily infected with *Trypanosoma brucei*, the parasites fail to establish an infection in the flies and in those in which it is successful, all the parasites die within 24 hours (Taylor, 1930). One possible reason for this is that stable flies produce an antimicrobial protein, stomoxyn, which is lethal for trypanosomes but is not found in tsetse flies (Boulanger *et al*., 2006).

It has been suggested that some vector-borne parasitic diseases could be controlled by releasing genetically engineering vectors that express or over-express particular antimicrobial proteins and are therefore refractory to infection. For example, the bird malaria *Plasmodium gallinaceum* is unable to develop in *Aedes aegypti* mosquitoes that are genetically engineered to over-express defensin-A and cecropin-A (Kokoza *et al*., 2010). However, the mass-release of genetically engineered vectors of pathogenic diseases has ethical considerations and would undoubtedly raise public concern (e.g. Anon, 2011).

There is little information on the immune responses of nematodes and platyhelminths to pathogens. There are only six coelomocytes within the pseudocoelom (body cavity) of the free-living nematode *Caenorhabditis elegans* and therefore phagocytosis and encapsulation are unlikely to be a part of the nematode's innate immune mechanisms (Engelmann and Pujol, 2011). *Caenorhabditis elegans* expresses a range of antimicrobial proteins such as defensins as well as producing reactive oxygen species in response to pathogen challenge. Defensins have also been identified in *Ascaris suum* as well as cecropin-P1 and three other cecropins while cecropin-P1-like

sequences are present in the genome of *Ascaris lumbricoides* and *Toxocara canis* (Minaba *et al*., 2009; Pillai *et al*., 2005).

6.3 Vertebrate immunity

6.3.1 Innate immunity

The vertebrate immune system is often considered as two distinct branches – that is the innate and adaptive components – although in practice they work together and influence one another (Table 6.2). For example, natural killer cells (NK cells) are a subset of the lymphocytes that are

Table 6.2 The sequence of major immune responses that occur in a typical mammal following parasite invasion. The actual times taken for individual reactions to take place after pathogen invasion are very case-dependent. Modified from Schmid-Hempel (2008)

Time after invasion	Type of immune response	Nature of immune response
Minutes-Hours	Innate immune response	Recognition by leukocytes Non-specific phagocytosis (neutrophils, macrophages) Cytokine release (e.g. alpha tumour necrosis factor [TNFα], interleukin-1, interleukin-6, interleukin-8, and others) Release of toxic chemicals (e.g. hydroxyl radicals, nitric oxide) Complement activation (alternative pathway)
Minutes-Hours	Early induced response, inflammation	Recruitment of neutrophils, monocytes, natural killer cells, etc. Differentiation to effector cells Cytokine release Release of toxic chemicals (e.g. hydroxyl radicals, nitric oxide) Complement activation
Days-Weeks	Adaptive immune response	Transport of antigen to lymph nodes Antigen presentation Recognition by B- and T-lymphocytes Production and release of antibodies Lymphocyte maturation Clonal expansion Differentiation to effector cells (cytotoxic T lymphocytes etc) Activation of natural killer cells Major histocompatibility Complex-1 expression (MHC-1)
Months-Years	Protective Immunity	B-cells differentiate into memory cells

usually considered to be part of the initial innate immune response. However, NK cells also exhibit adaptive processes and are important in shaping the adaptive immune response.

Innate immunity can be divided into physical, microbial and physiological aspects. Physical barriers, such as the skin and the mucus that is secreted over the epithelium of the gut and respiratory tract, prevent many infectious agents from gaining access to the body. The natural microbial flora that live externally upon the body surfaces and within the intestinal and genital tracts also play a major role in both preventing and facilitating infections. The resident microbial flora help prevent invasion through a combination of competition and producing antimicrobial substances. Indeed, the successful treatment of some diseases depends upon the restoration of a normal microbial flora (Reid *et al*., 2011). For example, in mice, the composition of the gut bacteria flora influences whether or not the protozoan parasite *Giardia* is able to establish itself (Singer and Nash, 2000).

Physiological defence mechanisms are exerted through the secretion of chemicals onto the outer body surface and also when an invading organism has breached the outermost physical barriers and gained entry into the body. They include soluble chemical substances such as defensins that are found in the lungs and gastrointestinal tract, leukocytes, the complement system, acute phase proteins, and the acute inflammatory response. Leukocytes are located within tissues (e.g. tissue macrophages, dendritic cells) and circulate within the blood (e.g. eosinophils, neutrophils, natural killer cells and lymphokine-activated killer cells). These cells are able to recognise invading organisms and kill them by either phagocytosing and then digesting them or releasing noxious chemicals such as hydroxyl radicals (·OH) and antibiotic peptides that kill them.

Recognition is possible because leukocytes have pattern recognition receptors that enable them to recognise unique 'pathogen-associated molecular patterns' (PAMPS). Several categories of pattern-recognition receptors have been described although they can be broadly divided into those that function as 'signallers' and those that promote endocytosis. For example, among the 'signalling' pattern recognition receptors are the membrane-bound Toll-like receptors and the cytoplasmic NOD-like receptors. For example, if the Toll-like receptors on a macrophage are stimulated, then it will secrete inflammatory cytokines. There are a variety of Toll-like receptors and each one is primed to recognise a panel of internal and external stimuli. To date, 10 Toll-like receptors have been characterised from humans and 13 from mice. Toll-like receptor 4 (TLR4) responds to lipopolysaccharide, and when stimulated, it initiates a signalling cascade that results in the production of proinflammatory cytokines and the differentiation of T helper 1 cells (see later). Similar cascades are initiated by the other Toll-like receptors, and, in addition, there is expression of co-stimulatory molecules such as CD14, CD40, CD80, CD86 and major histocompatibility complex II. CD14 is expressed on the membranes of macrophages, neutrophils and dendritic cells and also exists in a soluble form. Like Toll-like receptor-4, CD14 detects bacterial lipopolysaccharide and is therefore involved in the immune response to *Wolbachia* bacteria (Taylor, 2002). Among the endocytic pattern recognition receptors are the mannose receptors on the surface of macrophages and dendritic cells that initiate phagocytosis via the complement system. Although pattern recognition receptors are clearly important for the detection and removal of pathogens, some parasites are able to subvert or exploit them for their own purposes. For example, *Leishmania donovani* exploits the mannose receptor to gain entry to macrophages (Wilson and Pearson, 1986).

Like the dendrites associated with nerve cells, dendritic cells are named for their wispy processes that gives them a tree-like appearance. However, dendritic cells form part of the mammalian immune system and have no role in nervous transmission. There are four types of

dendritic cell: Langerhans cells (in the skin), interstitial dendritic cells, myeloid cells, and lymphoid dendritic cells. Immature dendritic cells can recognise invading pathogens and phagocytose them after which they transform into mature dendritic cells, process their captured material and move to the secondary lymphoid tissues (spleen and lymph nodes). Once there, they present antigens and non-specific stimulatory signals to naïve T helper cells, T killer cells and B lymphocytes, thereby activating them. For example, within the skin the Langerhans cells constantly monitor antigens as these pass through the *stratum corneum*. Interestingly, both *Leishmania* and HIV-1 compete for the same membrane receptor (DC-specific ICAM-3-grabbing nonintegrin [DC-SIGN]) in order to gain entry into dendritic cells (Caparrós *et al*., 2005).

The complement system is a triggered multi-component enzyme cascade found in the plasma. Once activated, the complement system initiates a wide range of actions. For example, components of the cascade attract phagocytes to invading microorganisms, increase the permeability of capillaries and mediate the acute inflammatory response. Activation of the innate immune response also brings about an increase in the levels of acute phase proteins such as C-reactive protein within the circulation which binds to invading microorganisms and dead cells. The act of binding activates the complement cascade and the target being opsonised – and this stimulates phagocytes to engulf it. *Leishmania donovani* is able to exploit C-reactive protein to gain entrance to the macrophages and it also stimulates the transformation from the promastigote to amastigote stage. Lipophosphoglycan on the cell membrane of the promastigotes binds to C-reactive protein and this increases their uptake into macrophages in a process mediated by their mannose receptors but without activating the cells (Bodman-Smith *et al*., 2002).

Box 6.1 Innate immunity to trypanosome infection

There are numerous species of animal trypanosomes and they are found in many parts of the world (including the UK) but only a few species are capable of infecting humans. This is because humans naturally express apolipoprotein L-1 (apoL1) in their serum which has trypanolytic activity (Pays and Vanhollebeke, 2009). The structural similarity of apolipoprotein L-1 to certain other regulatory molecules indicates that it was probably originally involved in normal apoptosis mechanisms (programmed cell death) and subsequently evolved a role in the immune response against pathogens. In susceptible trypanosome species, apolipoprotein L-1 creates anionic pores in the membrane of the parasite's lysosomes. Because there is a higher ion concentration inside the lysosomes compared to the surrounding cytoplasm, water flows through these pores into the lysosomes as a consequence of osmosis. This causes the lysosomes to swell uncontrollably and burst. The subsequent release of lysosomal enzymes causes the lysis and death of the trypanosome. It is thought that *Trypanosoma brucei rhodesiense* is able to infect humans because it expresses a VSG-like protein that is coded for by the serum resistance-associated (SRA) gene and is able to neutralise the effects of apolipoprotein L-1 (Gibson, 2005; Pérez-Morga *et al*., 2005). The SRA gene is not found in *Trypanosoma brucei gambiense* and it is therefore uncertain how parasites of this species are able to resist the effects of apolipoprotein L-1.

Trypanolytic activity is thought to have evolved during the course of human evolution in Africa where the ground-dwelling early hominids were continually exposed to tsetse flies and other biting flies and therefore challenged by a variety of trypanosome parasites (de Raadt, 2005). Indeed, Lambrecht (1985) has suggested susceptibility to trypanosome infections may have had

an important role in the evolution of early hominids. Many African primates express trypanolytic activity although, surprisingly, it is absent among our nearest living relatives, the chimpanzees (*Pan troglodytes*). One suggestion for this is that chimpanzees are less exposed to the bites of tsetse flies because they spend much of their time living in trees rather than on the ground. In support of this hypothesis is the fact that gorillas (*Gorilla gorilla*), which spend most of their lives on the ground, do express apolipoprotein L-1 (Poelvoorde *et al*., 2004). However, the theory starts to fall apart when one considers that chimpanzees actually spend a lot of time on the ground – sometimes over 60% – and usually travel between resting and feeding sites on the ground rather than by swinging through the trees (Doran and Hunt, 1996). One consequence of the absence of trypanolytic activity in chimpanzees is that they, unlike humans, can be experimentally infected with *Trypanosoma brucei brucei* (Baker and Taylor, 1971).

6.3.2 Adaptive immunity

Two types of adaptive immunity can be identified: passive and active. Passive adaptive immunity occurs when an animal receives antibodies (immunoglobulins) from another organism. In mammals this typically occurs when the developing foetus receives antibodies from its mother across the placenta and within colostrum. The levels of these antibodies subsequently decline over a period of weeks or months and the conferred immunity is lost. The idea is to allow the neonate to survive challenge from infections in the first few months of life while it is very vulnerable to disease and before its own immune system is fully functional. Active adaptive immunity occurs when the animal mounts its own immune response to challenge by either live pathogens – as in an infection – or to dead or disabled pathogens – as in a vaccine.

Whether it is passive or active, adaptive immunity represents a specific response to specific antigens and is primarily mediated by lymphocytes. In humans there are three types of lymphocytes (Table 6.3) and these have a variety of functions. All of the lymphocytes produce cytokines although the composition varies between cell types. There is a complex cross-regulation between the cytokines and the cytokines themselves affect the activity of both immune and non-immune cells as well as, in some cases, acting directly upon pathogens.

At a molecular level, lymphocytes are distinguished by their membrane receptors, each lymphocyte carrying the receptor for a particular ligand (antigen). Binding of a ligand to the receptor stimulates the lymphocyte to divide and hence the numbers of lymphocytes possessing this particular receptor increases in a process called clonal expansion. Most of the cells are ‘effector cells’

Table 6.3 Classification of lymphocytes

Lymphocytes	Response to stimulation
B lymphocytes	Develop into B cells that secrete antibodies (immunoglobulins)
T lymphocytes	Regulate other immune cells or kill virus-infected cells
Natural Killer (NK) lymphocytes	Kill virus-infected cells and tumour cells

Table 6.4 Classification, distribution, and function of mammalian immunoglobulins

Immunoglobulin	Distribution and functions
IgG	Major (~75%) immunoglobulin in plasma and extravascular spaces in adult humans; associated with secondary immune response; transmitted to foetus via the placenta and colostrum, activates complement, binds to Fc receptors
IgA	Second highest level in serum; present in external secretions such as intestinal mucus, saliva, tears, and colostrums; does not activate complement; binds to Fc receptors on some cells
IgE	Least common immunoglobulin. Involved in allergic reactions: induce mast cells to release histamine and other chemical mediators; does not activate complement; binds to Fc receptors on eosinophils
IgM	Third highest level in serum; activate complement; first immunoglobulin made by foetus; first class of antibody raised in primary infection; react to blood group antigens; binds to Fc receptors
IgD	Fourth highest level in serum; present on surface of B lymphocytes; does not activate complement; role(s) uncertain although can activate basophils and mast cells

that perform the job they are designed to do and then die after a few days. Some of them, however, become 'memory cells': these are long-lived cells and their subsequent stimulation enables rapid clonal expansion in which effector cells and memory cells can be formed more quickly than in the initial (primary) immune response. It is these memory cells that enable a protective secondary immune response to be mounted against subsequent infections by a particular pathogen and the aim of vaccination is to induce this without exposing the person or animal to the wild type infection.

B lymphocytes are produced in the bone marrow and when activated they form plasma cells (effector cells) that secrete antibodies into the surrounding plasma. This gives rise to what is called 'humoral immunity'. These antibodies are glycoproteins that are also referred to as immunoglobulins or, collectively, gamma globulins (γ globulins). In mammals, immunoglobulins are divided into five classes (Table 6.4) and these in turn are divided into subclasses. Teleost (bony) fish lack immunoglobulin A (IgA) but produce their own immunoglobulin that is referred to as either IgT (trout or teleost) or IgZ (zebra fish). Immunoglobulin T, like IgA, is secreted into the intestine where it controls the composition of the gut microbe community. Immunoglobulins that are secreted are known as soluble immunoglobulins and these lack a trans-membrane region. Immunoglobulins that are bound to the membrane surface of lymphocytes are referred to as membrane- or surface immunoglobulins. The basic immunoglobulin monomer is a Y-shaped molecule the stem of which is the Fc region (Fragment, crystallisable region) and the two arms are referred as the Fab regions (Fragment, antigen-binding region). The structure of the Fc region varies between the different classes of immunoglobulins although within a class it is constant. The Fc region binds to specific receptors (Fc receptors) and other immune molecules, such as those in the complement cascade, thereby initiating a variety of physiological reactions. For example, if the Fc receptor is situated on a phagocyte, then the binding of an immunoglobulin with the appropriate Fc characteristics will initiate phagocytosis. By contrast, if the Fc receptor is on a cytotoxic cell, then the binding of the immunoglobulin to the Fc receptor will cause the cell to be destroyed.

Antibodies are not themselves harmful to invading pathogens but they initiate a variety of physiological processes that result in their removal from the body (Table 6.5).

Table 6.5 Mechanisms by which immunoglobulins bring about the removal of pathogens and toxins

Soluble immunoglobulins attach to antigens and thereby make them recognisable to phagocytic cells as objects that should be phagocytosed. This process is sometimes called opsonisation.
Soluble immunoglobulins bind to antigens and cause them to 'clump': this effectively 'increases the size of the target' and thereby increases the chances of phagocytosis.
Immunoglobulins bind to certain toxins and thereby render them non-toxic.
Following the binding of an antigen to an immunoglobulin, the Fc end of the immunoglobulin activates the complement cascade.
IgE is bound to the surface of mast cells and the attachment of an antigen to the IgE induces the mast cell to release histamine and other chemicals that mediate the inflammatory response.
The binding of an antigen to an immunoglobulin brings about the activation of antibody-dependent immune cells via Fc binding. For example, this can result in the activation of B cells and the production of antibodies or the initiation of phagocytosis by macrophages.

6.3.3 Cell-mediated immunity

Antibodies are only effective against extracellular pathogens because once the pathogen has invaded a cell, its antigens are no longer accessible. Intracellular pathogens are therefore dealt with by another branch of the immune system called cell-mediated immunity in which the T lymphocytes play a central role. The T lymphocytes have receptors on their outer cell membrane called T cell receptors that that bind to cells that display antigens as part of a major histocompatibility complex (MHC) on their cell membrane. Major histocompatibility complexes are a family of membrane proteins that are found in most vertebrates. The MHC complexes act as signals to the T lymphocytes by taking fragments of the cell's own proteins as well as those of any invading pathogen and displaying these on the cell surface. Two types of MHC complex are found in humans. Class I MHC proteins are found in all nucleated cells and they interact with Cytotoxic T cells and Natural Killer Cells. Antigens derived from intracellular pathogens that are presented by MHC class I proteins are detected by Cytotoxic T cells and Natural Killer Cells and these then kill the infected cells. They accomplish this by secreting the cytolytic protein perforin that forms a pore in the target cell membrane and the serine protease granzyme B that then enters the target cell through the pore where it induces apoptosis. Cytotoxic T cells carry a surface glycoprotein called CD8 and these are sometimes referred to as CD8+ T cells. Class II MHC proteins are found predominantly on B-lymphocytes, macrophages, and dendritic cells and they interact with T helper lymphocytes (T_h cells). Mature T helper cells express a surface glycoprotein called CD4 and they are therefore sometimes referred to as CD4+ T helper cells. The T helper cells do not directly kill antigen-presenting cells but instead produce a variety of cytokines that influence the behaviour of other immune cells. There are three types of T helper cell which have different roles and they cross-regulate one another (Table 6.6). The Th17 cells have recently been acknowledged as a distinct group of CD4+ T cells and are very important in the initiation and regulation of inflammation (Korn *et al*., 2009). Interleukin 17 receptors and interleukin 22 receptors are found on numerous cell types and therefore their stimulation results in a powerful tissue response. A further level of control is provided by regulatory (or suppressor) T cells (T_{reg}), these cells are known by the abbreviation, CD4+CD25+FoxP3+ T_{reg} cells. These regulatory T cells suppress the activation of other immune cells and are therefore very important in preventing autoimmune conditions. There is increasing evidence that they are involved in the immune response to

Table 6.6 T helper lymphocytes and some of their functions

	T helper cell 1 (T_h1)	T helper cell 2 (T_h2)	T helper cell 17 (T_h17)
Main cell type interaction with	Macrophages	B lymphocytes	Interleukin-17 receptors and interleukin-22 receptors are widely distributed on a variety of cell types They include T cells, B cells, vascular endothelial cells, fibroblasts, and several other cell types.
Cytokines released	Interferon gamma (IFN γ) Tumour Necrosis Factor (TNF), Interleukin-3, Interleukin-10	Interleukin-3, Interleukin-4 Interleukin-5 Interleukin-6 Interleukin-9 Interleukin-10 Interleukin-13 Interleukin-25 Interleukin-33	Interleukin-17 Interleukin-17F Interleukin-6 Interleukin-21 Interleukin-22 Tumour Necrosis Factor (TNF)
Main effect	Stimulation of cellular immunity through promoting phagocytosis by macrophages and the production of Cytotoxic T cells and Natural Killer (NK) cells.	Stimulation of humoral immunity through promoting the proliferation of B lymphocytes and antibody production.	Induce tissue inflammation (e.g. through recruitment and activation of neutrophils.
Other effects	IFN-γ stimulates dendritic cells and macrophages to release interleukin-12. Interleukin-12 stimulates T_h1 cells to release IFN-γ in a positive feedback loop. IFN-γ inhibits the production of interleukin-4 and thereby reduces the T_h2 response.	Interleukin-4 stimulates the production of itself and other T_h2 cytokines. Interleukin 10 inhibits macrophages from presenting antigens, producing cytokines and killing pathogens using nitric oxide (NO). Interleukin-13 is important in the expulsion of gut helminths and the response to schistosome eggs.	Stimulate target cells to produce antimicrobial substances (e.g. β defensin-2 and β defensin-3). Mediates protection against specific pathogens such as fungus *Candida albicans*.

parasites and that parasites may in turn influence the activity of T_{reg} cells to improve their chances of survival (Taylor *et al*., 2009).

6.4 Innate immunity to parasitic infection

6.4.1 Physical factors

The physical integrity of the outer body surfaces and the lining of the gastrointestinal tract, lungs, etc. are sufficient to prevent many potential pathogens from causing infections. For example, the

first instar larvae of the New World screwworm fly, *Cochliomyia hominivorax*, are unable to penetrate sound skin. Consequently, the female flies lay their eggs on pre-existing wounds and, after hatching, the larvae invade the wound and extend it and this attracts further flies to lay their eggs on the wound. All animals therefore have mechanisms for rapidly sealing and repairing wounds before these can be exploited as entry portals. Similarly, within the gastrointestinal tract, the cytokine interleukin-10 is important for the maintenance of the integrity of the gut epithelium. If the levels of interleukin-10 fall too low, then the epithelium becomes more permeable and this facilitates invasion by parasites such as *Entamoeba histolytica* (Mortimer and Chadee, 2010).

6.4.2 Chemical and microbial factors

Secretions over the outer and inner body surfaces protect the host through being either toxic and/or preventing the parasite from making contact with the body. Sometimes these secretions are produced all the time but they may be produced in response to specific stimuli. For example, in mice, the mucin Muc5ac is produced in the lungs but not in the intestine. However, mice infected with the gut nematode *Trichuris muris* exhibit a dramatic rise in the level of Muc5ac in their caecum. Genetically engineered mice that are unable to express Muc5ac cannot expel *Trichuris muris* and remain vulnerable to infection despite being able to mount a strong Th2 type immune response (Hasnain *et al*., 2011).

Microbes are often found within bodily secretions and they can either contribute to the protective effect or facilitate the parasite's invasion. For example, the constant secretion of mucus can make it difficult for invading pathogens to make contact with the underlying epithelial cells. The highly acidic nature of the stomach contents is sufficient to kill many organisms and most gastrointestinal parasites pass through this region rather than dwell here on a permanent basis. Within the vagina, colonisation by the protozoan parasite *Trichomonas vaginalis* is affected by the pH of the vaginal fluid. The normal vaginal pH is relatively acidic (pH4.5) while *Trichomonas vaginalis* grows better in a more alkaline environment (Petrin *et al*., 1998). The acidic conditions are maintained by a combination of oestrogen levels which promote the secretion of lactic acid and the resident bacterial flora which secrete lactic acid as a waste product. The bacterium *Lactobacillus acidophilus* is an important member of the vaginal flora (and also yoghurts) and contributes to the lactic acid present in vaginal secretions. However, the relationship between vaginal lactobacilli and the establishment of *Trichomonas vaginalis* is uncertain. Vaginal lactobacilli bacteria also influence the vaginal immune system and there is an inverse relationship between the levels of the cytokines interleukin-1b, interleukin-6, and interleukin-8 and the population of these bacteria (Nader-Macias *et al*., 2010). By contrast, *Trichomonas vaginalis* stimulates the production of interleukin-8 by neutrophils which results in the recruitment of inflammatory cells and the promotion of the inflammatory response (Ryu *et al*., 2004). Therefore, by reducing the levels of interleukin-8, the lactobacilli might be expected to interfere with the immune response against *Trichomonas vaginalis*. However, they may also modulate the inflammatory response and thereby reduce the potential for an excessive inflammatory response which would cause localised damage to the vaginal tract. It should also be borne in mind that our original conceptions that the normal vaginal microbial flora is composed of relatively few species of bacteria are incorrect. Molecular culture-independent analyses of the vaginal microbiome have revealed that there is an enormous diversity of microorganisms present and considerable variations in its composition, even among healthy women (Kim *et al*., 2009).

6.4.3 The acute inflammatory response

In order to invade its host a parasite has to first attach to and then physically penetrate its host's body – even if this is only to insert its feeding apparatus. In the process, the parasite advertises its presence through the production of specific chemicals and causing cell damage. It is at this point that the parasite engenders an initial acute inflammatory response – this can be divided into a series of stages:

1. Leukocytes detect the presence of the invader or cell damage and are stimulated to phagoctyose the debris and foreign material. They also release cytokines and other inflammatory mediators such as tumour necrosis factor, interleukin-1, and interleukin-6.
2. The surrounding capillaries are stimulated to dilate and become more permeable to proteins and fluid. This facilitates the movement of immune cells, proteins, etc. into the damaged area where they mediate the defensive response. The damaged area therefore becomes semi-liquid and oedema can result.
3. Leukocytes are stimulated to migrate to the site of the infection. Neutrophils are the main mediators of the acute immune response.
4. Mast cells are stimulated to release immune mediators that amplify the response.
5. If effective, the foreign material is localised and stopped from spreading further into the body and the leukocytes clear the infection through a combination of phagocytosis and secretion of inflammatory cytokines.
6. However, if excessive amounts of cytokines are released, these can enter the general circulation and affect organs distant from the site of invasion. The rise in cytokine titres in the circulation results in a systemic inflammatory response: for example, interleukin-1 is involved in the development of fever and anorexia while interleukin-6 stimulates hepatocytes in the liver to release acute phase proteins.
7. If the infection is cleared, the inflammatory response subsides and tissue repair commences. However, if the object or organism that induced the response remains, then the acute phase is followed by chronic inflammation which is characterised by the formation of granulation tissue and fibrous scar formation.

Many parasitic protozoa have adhesin molecules on their cell surface that enable them to attach to their host cells. For example, in *Entamoeba histolytica* these include molecules such as lipophosphoglycan (LPG) and lipopeptidophosphoglycans (LPPG) that are bound the parasite cell membrane by glycophosphatidylinositol (GPI) anchors. These may also be secreted into the surrounding region and, together with their GPI anchor, adhesins can be important for both the parasite's virulence and also the host's ability to mount an immune response. For example, the GPI anchor and the membrane molecules such as glycoinositol phospholipid, lipophosphoglycans and lipopeptidophosphoglycans are recognised by Toll-like receptor-2 and Toll-like receptor-4 and these initiate an inflammatory response. In the case of *Entamoeba histolytica* this results in the recruitment of large numbers of neutrophils to the affected region (Ropert and Gazzinelli, 2000; Wong-Baeza *et al*., 2010). Although inflammation can be protective, it may also result in localised tissue damage and during the initial stages of invasion by *Entamoeba histolytica* this is

manifested as a bout of abdominal cramping and diarrhoea that is symptomatic of amoebic dysentery. Neutrophils kill invading organisms by a combination of phagocytosis and releasing toxic substances such as reactive oxygen species. They are capable of killing *Entamoeba histolytica* but the toxic chemicals they release also damage the gut epithelium – and this may facilitate parasite invasion (Mortimer and Chadee, 2010). The end result is therefore a balancing act between the killing of invading *Entamoeba histolytica* and the parasite's abundance and ability to invade (virulence). As we have seen, numerous other factors can influence this balance. For example, although the neutrophils can kill the parasite trophozoites, the trophozoites are also capable of killing the neutrophils to an extent that varies between parasite strains. It has been calculated that a single trophozoite of a highly virulent strain of the parasite can destroy several thousand neutrophils (Guerrant *et al*., 1981). Activation of Toll-like receptors is probably an important first step in the immune response to many parasitic protozoa infections. For example, glycophospatidylinositol anchors and glycoinositolphospholipids derived from *Trypanosoma cruzi* activate Toll-like receptor-2 pathways to initiate changes in macrophage function (Campos *et al*., 2001). Similarly, one of the main causes of the pathology associated with malaria is thought to result from the excessive immune response triggered by glycosylphosphatidylinositol anchors acting via Toll-like receptor-2 pathways (Nebl *et al*., 2005).

Another important adhesin molecule found on the cell surface of the trophozoites of *Entamoeba histolytica* is a lectin known as Gal/GalNAc-lectin because it recognises galactose (Gal) and N-acetylgalactosamine (GalNAc), which are common components of the mucosa and epithelial cells. Gal/GalNAc-lectin induces the secretion of IgA from plasma cells in the lamina propria that can prevent parasite adherence. Indeed, experimental trials indicate that vaccination with *Entamoeba histolytica* Gal/GalNAc lectin can generate a protective immune response against the parasite (Houpt *et al*., 2004). The secretory IgA, however, also down-regulates the inflammatory response and influences host-gut microbial flora interactions. In addition, the parasite secretes cysteine proteases that break up the IgA and render it inactive.

Apart from membrane components, a variety of other substances can also stimulate the acute inflammatory response. For example, the DNA of *Entamoeba histolytica* can stimulate an acute inflammatory response by activating Toll-like receptor-9 on macrophages which stimulates them to secrete tumour necrosis factor (Ivory *et al*., 2008). Chitin fragments can stimulate an inflammatory response through activating Toll-like receptor-2 pathways that then induce macrophages to produce the proinflammatory cytokine interleukin-17 (Da Silva *et al*., 2008). Chitin is a major component of arthropod cuticle, the cuticle of nematodes and the cell walls of fungi and therefore this may be an important feature of the immune response to these organisms. The microsporidians *Encephalitozoon cuniculi* and *Encephalitozoon intestinalis* induce an inflammatory response via Toll-like receptor-2 pathways that leads to the production of tumour necrosis factor and interleukin-8 (Fischer *et al*., 2008). The spores of microsporidia contain chitin and also glycosylphosphatidylinositol anchors but it is as yet uncertain which, if either, of these molecules is responsible for stimulating the pattern recognition receptors.

Sometimes the parasite does not induce the inflammatory response directly. For example, although ivermectin is effective at treating human filariasis, it can result in harmful side effects. This is because when the dead and dying worms release their symbiotic *Wolbachia* bacteria, these trigger a systemic inflammatory response. This is because the bacteria induce an inflammatory reaction through stimulating Toll-like receptor-4 and CD14 pattern recognition receptors. The bacteria are also released by live worms and stimulation of the pattern recognition receptors results in the recruitment of neutrophils (Taylor, 2002).

6.4.4 Cell-mediated immunity

The nature of the immune response to parasitic helminths is somewhat different to that raised against protozoa because the latter can be ingested by phagocytes and some of them are intracellular. By contrast, helminths are too large to be phagocytosed and they are almost all extracellular parasites (the larvae of *Trichinella spiralis* that live in large striated muscle cells is an obvious exception). In the intracellular parasite *Leishmania major* and the extracellular parasite *Entamoeba histolytica* a Th1 response is protective while a Th2 response is favourable to the parasite (Ehrchen *et al*., 2010; Mortimer and Chadee, 2010). The Th1 response promotes cellular immunity by bringing about the activation of neutrophils and macrophages and stimulating them to produce nitric oxide. By contrast, a Th2 response results in the production of cytokines that suppress the production of gamma interferon (IFN-γ). Because IFN-γ has an important role in stimulating neutrophils and macrophages, etc., its suppression effectively down-regulates the cellular immune response, which is protective against these parasites. A Th1 response is also protective against many other parasitic protozoa, such as *Toxoplasma gondii* (Jongert *et al*., 2008), *Plasmodium falciparum* (Radosevic *et al*., 2010), and *Trypanosoma cruzi* (Hoft and Eickhoff, 2002).

Helminth infections are characterised by a strong CD4+Th2 response in which there are elevated levels of interleukin-4, interleukin-5, and interleukin-13. These cytokines bring about the mobilisation and expansion of effector cells such as mast cells, eosinophils, and basophils. There are also raised levels of total and parasite-specific immunoglobulin E (IgE). Although interleukin-4 is usually thought to be crucial for initiating the Th2 response, the situation is probably more complicated and other Th2-inducing cytokines might be equally or more important – at least in some helminth infections (Maizels *et al*., 2009). An effective Th2 response is essential for clearing many helminth infections but if the response is excessive or prolonged, it can result in pathology. Some of this control is provided by the induction of regulatory T cells although some parasites are also able to exploit this means of control to establish long-lasting infections.

Box 6.2 Cross-immunity and parasite interactions

Cross-immunity, in which a parasite generates an immune response that impacts upon the establishment of another antigen-related parasite, is probably a common occurrence (Lafferty, 2010; Telfer *et al*., 2010). In addition, parasites may also be in competition with one another for niches and resources which also affect their ability to survive and reproduce within their host. For example, the ability of a parasitic protozoan to establish itself in the gut of its host is not only affected by the host's immune system but also the interactions between both the parasite and the host with the gut microbial flora and also other pathogens. It can therefore be difficult to disentangle the influence of any one factor upon a parasite. For example, *in vitro* experiments have shown that defensins released by intestinal Paneth cells can kill *Giardia duodenalis*. However, under *in vivo* conditions these antimicrobial substances also affect the intestinal microbial flora and the flora have a feedback effect on the release of defensins and also on the establishment of *Giardia*. Similarly, although immunoglobulin A (IgA) contributes to the immunity against *Giardia*, animal models have shown that mice that are unable to release IgA into their intestine are still able to eradicate the parasite (Solaymani-Mohammadi and Singer, 2010). In addition, the gut often

contains a variety of helminth parasites and there can be feedback effects between the immune responses to the protozoa and the helminths. For example, mast cells are important for the development of immunity against *Giardia* (Solaymani-Mohammadi and Singer, 2010). However, although *Trichinella spiralis* causes an increase in the numbers of intestinal mast cells, *Giardia* is able to establish more effectively in hosts that are already infected with the nematode (Von Allmen *et al*., 2006). This is probably because helminth infections engender a predominantly Th2 immune response and this either alters the gut environment or reduces the immune response against *Giardia*.

6.5 Adaptive immunity

Although many parasites induce antibody responses, the consequences of these are influenced by a wide variety of parasite and host factors such as genetics, age, health, etc. (e.g. Bourke *et al*., 2011). Host adaptive immune responses therefore vary considerably between parasites and between individuals to a particular species of parasite and range from highly protective to ineffective. For example, antibodies against *Trichomonas vaginalis* can be detected in the serum and vaginal fluids of infected women but even after repeated infections, these do not provide protective immunity (Lewis, 2010). In other cases concomitant immunity develops in which the host is unable to remove the resident parasite population but becomes resistant to newly invading parasites (Brown and Grenfell, 2001). For example, immune reactions against filarial nematode infections tend to be directed against the infective third-stage larvae rather than the adult worms (Maizels and Lawrence, 1991). In this situation, the resident parasite is able to reduce or prevent competition but remains able to reproduce and therefore act as a source of infection for other hosts. By contrast, the invasion of infective larvae of the nematode *Haemonchus contortus* precipitates the expulsion of the adult worms in a phenomenon known as 'self-cure'. Adult *Haemonchus contortus* live in the abomasum sheep; they are relatively large worms, growing up to 3 cm in length and they move about the surface of the mucosa which they pierce with their lancet-like mouthparts to feed on blood. The parasite's eggs are passed with the host faeces and these hatch and develop to the infective third stage larvae that are consumed when the sheep grazes. Heavy rain provides ideal conditions for egg hatching and larval survival and it is also associated with the sudden mass-expulsion of the adult worms. This is thought to be because large numbers of invading third-stage larvae trigger an increased IgE titre that results in the adult worms being expelled. The rise in IgE titre is associated with an immediate hypersensitivity reaction and can be prevented by the administration of antihistamine drugs (Adams, 1983). However, the invading larvae sometimes continue to develop to adulthood and there are also suggestions that the 'self-cure' reaction may actually be a result of a non-specific reaction to a constituent of freshly-growing grass rather than third-stage larvae (Taylor *et al*., 2007).

In the case of gastrointestinal helminth infections, protective immunity is usually a consequence of Th2-mediated responses although there are considerable differences between species in their effectiveness. By contrast, chronic infections with tissue helminths tend to generate a mixed Th1–Th2 immune response (Anthony *et al*., 2007; Bourke *et al*., 2011). A characteristic feature of both gastrointestinal helminth infections and tissue helminth infections is the stimulation of elevated levels of parasite-specific immunoglobulin E (IgE). Elevated titres of this antibody class

are not usually seen in other infectious diseases although they are a feature of allergic reactions. There are, however, relatively few instances in which IgE has been shown to have a protective effect. One of these is the expulsion of *Trichinella spiralis* from the intestinal lumen of rats in which the IgE is secreted from the plasma into the gut lumen via transporting epithelial cells. There is also some evidence for IgE having a protective effect against tissue-dwelling *Trichinella spiralis* in rats but in humans the role of IgE in immunity to *Trichinella spiralis* is less certain (Watanabe *et al*., 2005). Gastrointestinal helminth infections also cause elevated titres of other immunoglobulins and in particular IgG1 and IgG4, which, together with IgE, are under the control of Th2 cytokines, although the importance of these varies between species. For example, immunity to the nematode *Nematodirus battus* is associated with increased titres of IgM, IgG1, and IgA. This nematode lives in the duodenum of sheep and induces a strong protective immunity. The immunoglobulins act in concert with other immune changes and in particular the mass shedding of the villi lining the duodenum. The nematodes coil around the villi and thereby use it as a 'holdfast' – with the loss of their anchor the worms are quickly shed (Winter, 2002). The strength and longevity of the immune response mean that *Nematodirus battus* is only pathogenic in previously uninfected lambs although adults may be infected and remain a source of infection for others. Interestingly, there is not an increase in the secretion of IgA associated with infection with adult hookworms – possibly because it is destroyed by proteases released by the nematode (Loukas and Prociv, 2001). IgA is important in protecting against gut bacteria, and it is therefore possible that a decline in its levels may influence the development of these infections. For example, a hookworm (*Uncinaria* spp.) enteritis plus bacteraemia complex found in the California sea lion (*Zalophus californianus*) population in US waters has killed many seal pups (Spraker *et al*., 2007). However, although enteritis is a feature of severe hookworm infestations in humans and a range of other mammals, there is limited information on the extent to which bacteria contribute to the pathology of the helminth infection.

6.5.1 Avoiding the host immune response

Many parasites survive within their host for long periods of time – sometimes extending to five or more years. For example, adult schistosomes typically live for about 3–6 years although there are cases of people still passing eggs over 20 years after their last possible exposure to infection. Such long life spans are notable in themselves, since the majority of invertebrates have relatively short lives but they are even more remarkable in that the organisms are living in an ecosystem that is 'designed' to kill them. The reason that some parasites survive for so long is the same as the reason they are able to survive as parasites in the first place – they have evolved mechanisms which enable them to avoid the harmful consequences of the host immune response (Maizels *et al*., 2004; Maizels *et al*., 2009; Schmid-Hempel, 2009). This may be because the host does not generate an immune response or that the immune response is not protective. The mechanisms parasites employ to avoid the host immune response can be crudely divided into the same categories that a spy might use when in enemy territory (Table 6.7) and many parasites use a combination of these mechanisms.

Hide in a 'safe house' Many of the most successful protozoan parasites are intracellular parasites and are therefore protected from some aspects of the immune system. For example, *Leishmania* amastigotes reside within macrophages, *Plasmodium* trophozoites live within red blood cells, and

Table 6.7 Summary of the main mechanisms parasites employ to avoid detection by the host immune system

Means of avoiding detection	Parasite example
Hide in a 'safe house'	Intracellular parasites develop within vacuoles and therefore they are 'hidden' from some (if not all) aspects of the immune system, for example, *Leishmania* spp. amastigotes reside within parasitophorous vacuoles in macrophages.
Stay quiet and don't draw attention to yourself	The parasite encysts within tissues and either do not grow/reproduce or do so very slowly, for example, tissue cysts of *Toxoplasma gondii*.
Camouflage	The parasites acquire host antigens or manufacture the same/similar substances and therefore no longer 'appear foreign', for example, adult schistosomes.
Keep changing your disguise	The parasites constantly alter their surface coat, for example, *Trypanosoma brucei* in the vertebrate host.
Put out false information	The parasite manipulates the host immune system, for example, *Entamoeba histolytica* affects T_{reg} activity.
Neutralise any threats	The parasites produce chemicals that harm specific immune cells and/or neutralise the chemicals they produce, for example, *Entamoeba histolytica* trophozoites degrade IgG.

Toxoplasma gondii tachyzoites invade a wide variety of cell types. Many intracellular parasites reside within parasitophorous vacuoles rather than being free within the cytoplasm of their host cell. These parasites are either able to survive the fusion of lysosomes with the vacuole (e.g. *Leishmania*) or they prevent fusion taking place (e.g. *Toxoplasma gondii*). While within their vacuoles/host cell cytoplasm the parasites are protected from circulating antibodies although they remain vulnerable to cell-mediated immune responses. These intracellular parasites do, however, require mechanisms of leaving their host cell and rapidly re-infecting a new cell so as to avoid exposure to the wider immune system.

Stay quiet and don't draw attention to yourself The onset of an effective immune response stimulates some parasites to encyst within the host. Within the cysts the parasite is often protected to a large extent against the immune system and the parasite either ceases to grow or reproduces very slowly. In this way the parasite may survive for many years until either the immune system starts to fail or the host is consumed by another host in the parasite's life cycle. In the case of *Toxoplasma gondii*, the onset of a protective immune response induces the parasite to forms tissue cysts (zoitocysts) containing bradyzoites. The tissue cysts are thought to persist for life in some hosts although their presence may be sustained by periodical re-activation, transformation into tachyzoites, followed by the formation of new tissue cysts.

Camouflage Parasites can avoid the host immune response if they are not recognised as 'foreign'. This can be done by hiding 'vulnerable' antigens beneath a protective coat, capturing host antigens or manufacturing 'look-alike' molecules. This is best exemplified by schistosomes. Despite their immunologically-exposed position within blood vessels, adult schistosomes are well known for their long lifespan. This is a consequence of the larval and adult parasites expressing a range of evasion strategies, including 'camouflage'. By contrast, schistosome eggs engender a

pronounced immune response that is essential if they are to traverse the tissues between the blood vessels within which they are released and the lumen of the gut/bladder.

In schistosomes, immune evasion strategies commence immediately after the cercariae invade through the host's skin. The cercariae shed their outer surface, known as the glycocalyx (which is highly immunoreactive) and this is replaced by an inner plasma membrane and outer secreted bilayer called the membranocalyx. One of the schistosome immune evasion mechanisms is to camouflage their outer layer with host antigens. Some of these antigens are captured from the host and incorporated into the outer membrane. In the case of *Schistosoma mansoni* these include red blood cell antigens (e.g. major histocompatibility complex class I antigens), serum glycoprotein ligands, and IgG. In addition, 'host-like' molecules are manufactured by the parasite such as some resembling β2-macroglobulin. The schistosomula become progressively less susceptible to the host immune system as they mature during their migration first from the skin to the lungs and then to their final destination. This may, in part, be a consequence of the progressive acquisition and expression of host molecules although there are also ongoing developmental changes in the parasite's tegument and in its antigenic composition.

Interestingly, IgM antibodies formed against the eggs of *Schistosoma mansoni* cross-react with surface antigens on the schistosomulum. However, these do not appear to contribute to concomitant immunity, i.e. prevent further infections taking place while there is an existing infection. Instead, the antibodies appear to compete for binding sites on the schistosomula and thereby reduce the effectiveness of antibody-dependent, eosinophil-mediated killing (Dunne *et al*., 1987). IgM binding to the surface tegument has also been described in juvenile *Fasciola hepatica* in which it is thought to prevent eosinophil adhesion (Moreau and Chauvin, 2010). The strong Th2 response engendered by the eggs of *Schistosoma mansoni* and consequent down-regulation of the Th1 response may also favour the establishment of the schistosomula because a Th1 response appears to be protective against this stage (Pearce *et al*., 1991). Nevertheless, concomitant immunity is a well-known phenomenon in human schistosomiasis although it is usually only apparent in adults and the mechanism(s) by which it is brought about remain uncertain (Brown and Grenfell, 2001).

Keep changing your disguise Some parasitic protozoa are able to express a variety of surface proteins and this means that the host is presented with a constantly moving target in which having mounted an antibody response to one common parasite antigen, the parasite then expresses a new surface protein to which a new antibody response is required. This approach to evading the immune response is particularly suited to protozoa because they have the potential to proliferate extremely rapidly. Consequently, those that are killed by the immune response can be rapidly replaced by new antigenic variants. For instance, *Giardia duodenalis* delays the antibody response against it by expressing variant-specific surface proteins (VSPs) (Solaymani-Mohammadi and Singer, 2010). However, perhaps the best example of avoiding the host immune response by expressing novel antigens is exhibited by *Trypanosoma brucei*. In this parasite the surface of the trypomastigotes is covered with a layer of invariant antigens which are shielded from the external environment by a dense coat of a single variant surface glycoprotein (VSG). The host is able to recognise the VSG coat and mount an immune response against it that kills the trypanosomes. However, because the parasites have an archive of hundreds of genes coding for these VSGs, they are able to periodically alter the composition of their coat and thereby express a constantly changing, immunologically distinct challenge to the mammalian host (Berriman *et al*., 2005). This almost limitless ability to change the VSG coat also makes the development of an anti-*Trypanosoma brucei* vaccine a huge challenge.

The VSG genes are expressed at the telomeres of the chromosomes. Telomeres are regions of repetitive DNA found at the end of the chromosomes in all eukaryotes and in *Trypanosoma brucei* these consist of tandem 5′-TTAGGG-3′ (T_2AG_3) repeats (Horn *et al*., 2000). The expression of a new VSG takes place when a previously silent VSG gene is copied into one of the bloodstream form expression sites (BES), replacing the 'old' VSG gene in the process (Figueiredo and Cross, 2010; Stanne and Rudenko, 2010). There are about 15 of these bloodstream expression sites situated on the telomeres although only one of these is active at a time (McCulloch and Horn, 2009). (The metacyclic trypanosomes found in the tsetse fly have a separate class of expression sites called 'metacyclic expression sites'.) The initial stage in the process of inserting a new VSG gene into the active expression site is the formation of a DNA double-strand break in one of the 70 base pair repeats that are to be found adjacent to each VSG gene (Barry and McCulloch, 2009; Boothroyd *et al*., 2009). Double strand breaks are one of the mechanisms by which new genetic material can be inserted into a length of DNA and have been described in a variety of organisms (Alsford *et al*., 2009). In addition to the VSG gene, a number of other expression-site associated genes are also co-transcribed into the bloodstream expression site. The function of these genes has not been fully established but may include coding for specific cell surface receptors/transporters.

In the tsetse fly vector, the transformation to the procyclic form is essential for the trypanosome's survival and in the majority of cases the infection fails to establish itself. The adoption of the procyclic form is accompanied by a change in the parasite's surface coat. The variant surface glycoprotein coat (VSG) that is characteristic of the mammalian bloodstream form is shed and replaced by an invariant procyclin coat. Procyclins are proteins that contain either extensive tandem repeats of glutamic acid (E) and proline (P) or internal pentapeptide repeats (GPEET = glycine: proline: glutamic acid: glutamic acid: threonine). Not only are there changes in the trypanosome's surface coat but the procyclins are anchored to the cell membrane by different glycophosphatidylinositol (GPI) structures to the VSGs. There are only six or seven genes coding for EP procyclins (the number depends on the strain) and two genes that code for the GPEET procyclins compared to the hundreds of VSG genes. However, the procyclin coat also changes during development and it serves to protect the parasites from the tsetse fly gut hydrolase enzymes although they probably have other functions too (Ruepp *et al*., 1997; Utz *et al*., 2006).

This 'changeability' is not limited to protozoa and similar mechanisms can be found in some helminths. For example, the outer membranocalyx of adult *Schistosoma mansoni* is continually replaced and under *in vivo* conditions it has a half-life of about 5 days. This is probably important for clearing potentially harmful antibodies from the outer surface but may not be rapid enough to prevent the parasite suffering damage from the immune system. However, the parasite does have the ability to repair itself (Saunders *et al*., 1987). The 'new' membranocalyx is not antigenically identical to the one it replaces and analysis of the *Schistosoma mansoni* genome has revealed a remarkable diversity of genes encoding membrane proteins and enzymes that produce the glycans found in the membranocalyx (Berriman *et al*., 2009).

Despite the numerous immune avoidance mechanisms deployed by schistosomes, the host is still able to mount an antibody response against the parasites. However, these are not protective against the adult worms, probably because the antibodies are unable to bind with sufficient antigens for long enough to have a protective effect. The continual shedding of the membranocalyx probably helps to prevent potentially harmful antibodies accreting on the outer surface. Evidence for this comes from the observation that the drug praziquantel is only able to cure schistosome infections if the host can produce antibodies. This is because praziquantel physically disrupts the parasite's outer surface membranes and this exposes the previously hidden parasite

antigens. The antibodies then initiate immune processes that cause the death of the parasite (Brindley *et al*., 1989).

Put out false information A classic means of avoiding detection is to convince the authorities that there isn't a problem worth investigating. Parasites can achieve a similar effect by manipulating the activities of the regulatory T cells (T_{reg}) which suppress the activities of other immune cells. For example, the filarial nematode *Litomosoides sigmodontis* can maintain long-lasting infections in its mouse host by stimulating the activity of regulatory T cells. The effectiveness of this strategy becomes apparent if the numbers of these cells decline, because the immune system promptly removes the parasites (Dittrich *et al*., 2008; Taylor *et al*., 2009). Similarly, an expansion in T_{reg} numbers occurs in mice following their infection with the nematode *Strongyloides ratti*. The increase in T_{reg} activity suppresses the immune system at a critical time in the parasite's life cycle and facilitates establishment in its host, but reducing the numbers of T_{reg} cells at later stages in the infection does not improve resistance (Blankenhaus *et al*., 2011). The trophozoites of *Entamoeba histolytica* also appear to influence the activity of regulatory T cells and this in turn affects the activity of other immune cells (Mortimer and Chadee, 2010).

Some filarial nematodes, such as *Brugia malayi*, as well as the gastrointestinal nematode *Nippostrongylus brasiliensis*, produce cysteine protease inhibitors (cystatins) that suppress the ability of dendritic cells to present antigens to T cells and they thereby reduce the T cell response (Manoury *et al*., 2001). In the case of adult *Fasciola hepatica*, an as yet unidentified component of the tegument called 'tegument antigen' inhibits the phagocytic capabilities of dendritic cells as well as their capacity to prime T cells (Hamilton *et al*., 2009).

The *Leishmania* parasites exploit and manipulate the immune system in a wide variety of ways – starting with living within some of the most important immune cell types such as the macrophages and dendritic cells. Dendritic cells infected with *Leishmania major* are stimulated to produce the immune regulatory enzyme indoleamine 2,3 dioxygenase (IDO). This enzyme activates T_{reg} cells and suppresses the ability of the dendritic cells to communicate with T cells and also T cell activity towards parasite antigens. The parasites are therefore able to down-regulate both the innate and adaptive immune responses against them (Makala *et al*., 2011).

Neutralise any threats Many parasites produce chemicals that either cause the death of specific immune cells or neutralise the chemicals that they produce. For example, when the trophozoites of *Entamoeba histolytica* breach the gut epithelium and invade the body, they are suddenly exposed to new immune cells and humoral factors, including immunoglobulin G (IgG) and the complement cascade. Unfortunately, although over 95% of people infected with *Entamoeba histolytica* mount a specific IgG response against the parasite, it is not protective because the immunoglobulin is degraded by cysteine proteases secreted by the trophozoites (Tran *et al*., 1998). Similarly, although the parasites activate the complement system and this is capable of bringing about their death, some strains of *Entamoeba histolytica* express cell surface characteristics that enable them to avoid this immune mechanism. For example, lipophosphoglycans and lipopeptidephosphoglycans in the parasite cell membrane may form a physical barrier to the complement system. In addition, complement-resistant parasites express Gal/GalNAc lectin on their surface that prevents the binding of complement-components that would initiate lysis to the parasite cell membrane (Braga *et al*., 1992). A further means by which *Entamoeba histolytica* avoids the immune response is by a process known as 'receptor capping' in which parasite membrane receptors to which immune

components have bound are rapidly moved to the posterior pole of cell and then released into the surrounding medium in membrane vesicles (Espinosa-Cantellano and Martínez-Palomo, 1994).

Larval and adult *Schistosoma mansoni* avoid attack by the complement system through expressing inhibitors such as paramyosin and other proteins that prevent the activation of the complement terminal pathway (Deng *et al*., 2003). In addition, they produce serine protease that prevents complement osponisation and thereby reduce the risk of attack by neutrophils, eosinophils, and macrophages. However, they also produce serine protease inhibitors which may inhibit protease enzymes produced by neutrophils (Fishelson, 1995). However, it has also been suggested they might combine with the parasite proteases and thereby render them undetectable by the immune system (Modha *et al*., 1996). Although adult hookworms cause a localised inflammatory response that may be sufficient to explain why they change feeding sites every few hours, some authors have reported it is marked by an unusually low number of neutrophils (Loukas and Prociv, 2001). In the case of *Ancylostoma caninum* this is may be because the hookworms secrete a glycoprotein called 'neutrophil inhibitory factor' that blocks the interaction of the neutrophils and fibrinogen and prevents them secreting hydrogen peroxide (H_2O_2) (Moyle *et al*., 1994). However, in *Ancylostoma ceylanicum* infections (and some reports of *Ancylostoma caninum*), there is a heavy infiltration of neutrophils at feeding sites (Prociv, 1997).

The eosinophil chemokine eotaxin-1 attracts eosinophils to immune targets and is therefore important in the clearance of *Brugia malayi* and many other helminth infections (Simons *et al*., 2005). The hookworm *Necator americanus* secretes a metalloprotease enzyme that destroys eotaxin (Culley *et al*., 2000) and anti-eotaxin activity has also been described from metabolites produced by metacestodes of *Echinococcus multilocularis* (Mejri and Gottstein, 2009) while the nematode *Heligmosmoides polygyrus* down-regulates eotaxin although the mechanism is not certain (Rzepecka *et al*., 2007).

6.5.2 Depression of the immune system

Immune system depression may be due to deliberate medical interventions, such as following organ transplants or for cancer therapy or it may arise as a consequence of poisoning, autoimmune disease (e.g. leukaemia), or infectious agents (e.g. HIV). Parasites can, of course, also depress the immune system either through manipulating its expression or through exhausting the host's metabolic reserves. Whatever the cause, immune depression often results in the affected person or animal becoming more vulnerable to opportunistic pathogens. For example, *Babesia* is not a particularly common infection in humans, but many of those who suffer severe disease following infection have a compromised immune system; patients who have severe babesiosis have generally undergone splenectomy or suffer from immunodeficiency or AIDS. Splenectomy is a relatively common medical procedure that is performed for a variety of reasons ranging from injury following a car crash to cancer. In 1983, it was estimated that over 8000 people were undergoing splenectomy every year in France (Bonmarchand *et al*., 1983) although since then the importance of retaining the spleen wherever possible has meant that fewer such operations are being undertaken (Rose *et al*., 2000).

Depression of the host immune system by the parasite is a common feature of infection with both *Trypanosoma brucei* and *Trypanosoma congolense*. This is partly caused by the variant surface glycoproteins causing overstimulation of the host macrophages and thereby inducing them to produce excessive amounts of tumour necrosis factor (TNF) and other inflammatory

substances. The macrophages are stimulated by both the soluble VSGs that are shed by the parasites all the time and also by the membrane-fixed VSGs that are released *en masse* whenever large numbers of parasites are killed (Antoine-Moussiaux *et al*., 2009). The latter occurs when the host mounts a successful immune response against the dominant VSG form being expressed by the trypanosomes. The disease tends to take a cyclical course in which the parasites multiply until the host develops sufficient antibodies to clear the majority of parasites that express the dominant VSG form. The rise in the concentration of tumour necrosis factor to harmful levels results in suppression of the immune system, anaemia, cachexia and localised cell death (Tracey *et al*., 1988).

6.6 Immunity to malaria

Immunity to malaria will be covered separately and in some detail because it exemplifies so many aspects of the immune response to parasites. The complex life cycle of malaria parasites involves the invertebrate mosquito vector and, within the vertebrate host, two distinct cell types (hepatocytes in the liver and red blood cells) and both intracellular and extracellular stages. This means that at various stages in their development the parasites are exposed to a wide variety of very different immune mechanisms.

The various species of human malaria can only be transmitted by specific species of *Anopeheles* mosquitoes and there are also differences between strains of a single species of mosquito in terms of vector competence. This indicates that genetic factors are important in determining the ability of mosquitoes to transmit malaria. However, although a number of immune-related genes have been identified as being important, many of these are based on laboratory studies using the mouse malaria *Plasmodium bergei*. Unfortunately, genes that influence the transmission of *Plasmodium bergei* are not always relevant for the transmission of other species of *Plasmodium* (Boëte, 2005, 2009).

Once male and female *Plasmodium* gametes have fused within a mosquito's midgut, the ookinete has to invade an epithelial cell, after which a series of developments and multiplications take place that culminate in the migration of the parasite to the insect's salivary glands. Therefore, within the insect vector the parasite has both intracellular and extracellular stages and is exposed to a variety of immune responses. However, the major limiting step that determines vector competence appears to be the ability of the ookinete to invade the midgut epithelial cells and then establish itself as an oocyst (Cirimotich *et al*., 2010).The mosquito immune response is initiated via one or more of the common signalling pathways such as the Toll pathway, the IMD pathway, and the Jak/Stat pathway. These then mediate the expression of genes that control anti-parasite processes such as melanisation and the release of nitric oxide. There is therefore interest in identifying relevant pathways more precisely so that genetically engineered mosquitoes that are resistant to malaria might be used as part of a malaria control programme. However, as already mentioned, there is difficulty in translating laboratory studies to field scenarios. For example, the gene LRIM1 codes for leucine-rich repeat immunoprotein-1 has a major role in the melanisation and killing of *Plasmodium berghei* during the early stages of infection, but is not important for protection against *Plasmodium falciparum*. In addition, a mosquito's response to *Plasmodium* has to be set within the context of its ongoing response to the bacteria and other microbes that live within its gut and elsewhere in its body. The mosquito immune response to bacteria and *Plasmodium* share many of the same processes. It is therefore to be expected that gut microbes can

both facilitate and suppress the ability of malaria to establish in mosquitoes (Cirimotich *et al*., 2010). For example, *Anopheles gambiae* infected with bacteria are less susceptible to infection with *Plasmodium falciparum* than those that are bacteria-free. This may be because the bacteria up-regulate the insect's immune system and bring about the expression of antimicrobial proteins that harm the parasite (Dong *et al*., 2009). Antimicrobial proteins may, however, in some circumstances protect the parasite against pathogenic bacteria while it is in its insect host. For example, bacteria such as *Serratia marcesans* and *Enterobacter* spp., in the midgut of *Anopheles albimanus* prevent *Plasmodium vivax* from completing its development in the mosquito (Gonzalez-Ceron *et al*., 2003).

Within humans, malaria is combated by a combination of innate, humoral, and cellular immune mechanisms. The innate mechanisms provide variable levels of protection and have arisen predominantly among populations in which malaria is endemic and there is constant exposure to infection. For example, the merozoites of *Plasmodium vivax* and *Plasmodium knowlsei* can only penetrate red blood cells carrying the Duffy buffer blood group antigens Fy^a and Fy^b. Most West Africans do not express these antigens and they are therefore resistant to these species of *Plasmodium*. Similarly, the ability of *Plasmodium falciparum* to invade and develop within red blood cells is considerably reduced if these express the sickle-cell trait (genotype Hb AS) genotype. In addition, infected Hb AS genotype red blood cells tend to deform (sickle) and are then destroyed by the spleen. In cases of mild malaria, the expression of the Hb AS genotype also contributes to the development of adaptive immunity. The mechanisms by which this occurs are not yet certain but the slower development of the parasites may facilitate the development of antibodies to antigens expressed on the surface of infected red blood cells (Williams *et al*., 2005). The sickle cell trait is due to a point mutation on the gene coding for β-globin. Because of the alteration to the β-globin it forms haemoglobin that polymerises at low oxygen saturations and gives rise to deformed red blood cells. In people who are heterozygous for the gene, only about 30% of the haemoglobin is affected and they do not usually express any clinical abnormalities and they gain protection from *Plasmodium falciparum*. However, if they are homozygous for the condition, then over 80% of the haemoglobin can be affected and they are at risk of developing the potentially life-threatening condition 'sickle-cell anaemia'. Furthermore, should they become infected with *Plasmodium falciparum*, they are at an increased risk of suffering severe malaria and dying (McAuley *et al*., 2010). The blood disorder thalassemia also results from defective synthesis of globin chains of haemoglobin although its ability to protect against malaria is more uncertain. There are two basic types of thalassemia although within each there are subtypes, depending upon the number of genes affected and the nature of the alteration. α-thalassemia (alpha-thallassemia) mostly results from deletions rather than mutations on the genes coding for the α-globin chains whilst β-thalassemia (beta-thallassemia) can result from any of over 90 different mutations affecting the genes coding for the β-globin chains. There is evidence that β-thalassemia can provide some protection against malaria although this form of thalassemia is relatively uncommon in sub-Saharan Africa (Willcox *et al*., 1983). α-thallasemia is common throughout sub-Saharan Africa and although it does not prevent a person becoming infected or developing high parasitaemias those with α-thalassemia appear to be less likely to suffer from severe anaemia (Wambua *et al*., 2006). Intriguingly, people who exhibit a mutation in the gene coding for the Fc-gamma receptor-IIb that results in threonine being substituted for isoleucine at position 232 ($Fc\gamma RIIb^{T232}$) have both an increased susceptibility to the autoimmune condition *Systemic Lupus Erythematosus* (SLE) and enhanced protection against *Plasmodium falciparum*. In this case, protection is thought to be because macrophages derived from individuals homozygous for this mutation 'phagocytose malarial parasites more avidly'

(Willcocks *et al*., 2010). The Fc-gamma receptor-IIb is an immunoglobulin-G receptor that is found on a range of immune cells such as macrophages and B cells and is involved in a range of regulatory processes such as the control of phagocytosis and the production of inflammatory cytokines. If the receptor does not function properly, it can result in uncontrolled inflammation, and this is a feature of SLE. This condition is particularly common among women in South-East Asia and parts of Sub-Saharan Africa and it may be that this, like sickle cell anaemia, is because the mutation has been conserved as a consequence of the enhanced protection provided against malaria.

Although humans do develop immunity to malaria, this is never completely protective. There are two main theories of how this immunity develops. The first of these, the 'strain-specific' hypothesis, is widely accepted but a more recent suggestion, the so-called 'cross-reactive' or 'strain transcending' hypothesis is gaining increasing recognition (Doolan *et al*., 2009). The strain-specific hypothesis is based on the observation that children growing up in malaria endemic regions gradually develop immunity to the local strains of malaria as a consequence of repeated exposure. Within a geographical area the different species of malaria exist as a variety of strains and it is believed that immunity therefore develops slowly ($\sim$10–15 years) because the immune system needs repeated exposure to the various strains in order to develop and maintain sufficient memory cells to mobilise a response against future infections. This would explain the observation that a person may be relatively resistant to malaria within their own region but susceptible to malaria elsewhere. Similarly, a person who moves away from a malarious region may be susceptible to malaria when they return. By contrast, the 'cross-reactive' hypothesis has more limited supporting evidence and is currently based mainly upon observations on transmigrants (people passing through the country) in Indonesian West Papua. According to this hypothesis, protective adaptive immunity can develop rapidly in 1–2 years in previously non-immune individuals as a consequence of sudden heavy exposure. Furthermore, this immunity is not limited to the local strains (i.e. it is 'strain transcending'). The distinction between the mechanisms by which adaptive immunity develops is important because it affects the ways in which vaccines are designed and utilised.

Assuming that a person has been previously exposed to malaria, the sporozoites will be targeted by antibodies when they are injected by the bite of an infected mosquito. Under natural circumstances, this is not thought to be an important means by which protective immunity is obtained. However, under experimental conditions using vaccines containing irradiated sporozoites or pre-erythrocytic stage antigens, a substantial degree of immunity to subsequent challenge infections can be obtained (Langhorne *et al*., 2008). Vaccine studies have also indicated a role for CD8+ T cells in the destruction of sporozoites that is mediated through the production of gamma interferon (IFN-γ). The next stage in the *Plasmodium* life cycle involves penetration and replication within hepatocytes in the liver. The infected hepatocytes are also targeted by CD8+ T cells although it is not fully certain whether they exert their effect through the production of IFN-γ (Tsuji, 2010). Having left the hepatocytes, the malaria parasites invade the red blood cells and this is associated with the development of a humoral response based on polyclonal B-cell expansion. This is associated with the production of large amounts of immunoglobulins, in particular immunoglobulin G, immunologlobulin A, and immunoglobulin M. However, only a small proportion of these immunoglobulins are actually *Plasmodium*-specific. These antibodies bind to parasite antigens present on the surface of red blood cells and also the merozoites that are released when the infected red blood cells disintegrate. The antibodies exert their protective effects in a variety of ways although their relative importance is not yet clear (Langhorne *et al*., 2008). For example, they can block merozoites from invading fresh red blood cells, facilitate antibody-dependent cell

destruction through cytophilic antibodies binding to in the infected cells or merozoites thereby encouraging them to be phagocytosed by macrophages and other immune cells (especially in the spleen). The binding of antibodies to the antigens can also activate the complement cascade and thereby destroy the parasite or infected cell. In addition, the antibodies also reduce the pathology associated with malaria (see Chapter 7) by blocking the binding of infected red blood cells to the surface of blood vessels and they also inhibit tumour necrosis factor induction by malaria toxins.

Red blood cells are not nucleated and therefore their cell membranes lack major histocompatability complex class I and class II presenting molecules. It might therefore be expected that cellular immunity would play little part in the immune response against the schizont and merozoite stages of the malaria life cycle. However, CD4+ T cells are essential for the expression of protective immunity against the red blood cell stages of the malaria life cycle both as 'helper cells' for B-lymphocytes and as producers of inflammatory cytokines and activators of macrophages. Mouse models indicate that CD8+ T cells can also be activated following previous exposure to *Plasmodium* antigens and they then activate macrophages through the release of gamma interferon and induce perforin-mediated destruction of the infected cell (Good and Engwerda, 2011). However, the importance of the CD8+ T cell response in protecting against human malaria remains to be confirmed. Nevertheless, what can be certain is that the immune response against malaria involves a combination of humoral and cellular immune mechanisms and their relative importance in conferring protective immunity probably varies with the circumstances.

The gametes of *Plasmodium* are unusual in that they are the only life cycle stage that has to spend a prolonged period outside a host cell. This occurs within the gut of female mosquito – within the vertebrate host the gametocytes live within the host red blood cells. Following the blood meal the gametocytes must emerge from the blood cells, transform into gametes, fuse to form a zygote which then transforms into the ookinete stage that invades the mosquito gut epithelial cells. This complex series of events takes time and some estimates suggests that it associated with up to a 300-fold loss in abundance (Kuehn and Pradel, 2010). Consequently, it is seen as a potential target for a transmission-blocking vaccine. Within the vertebrate host the immune system has relatively little impact on the gametocytes although they can be killed by cytokines such as tumour necrosis factor. However, after the mosquito has taken its blood meal, the now exposed gametocytes can be killed by host-derived complement-mediated and antibody-mediated events (Paul *et al*., 2002).

Box 6.3 Why humans do not develop protective immunity against *Plasmodium*

Despite the fact that humans generate a variety of immune responses against all stages of the *Plasmodium* life cycle, they are never fully protected. One of the reasons for this is that the parasite expresses variant immunodominant antigens (i.e. to which much of the antibody response is generated) on the surface of the infected red blood cells. For example, the genome of *Plasmodium falciparum* includes a large number of so-called *var* genes that encode for a protein that becomes incorporated into the cell membrane of infected red blood cells. This protein, called *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1), is an important target in the development of adaptive immunity and can both activate and suppress dendritic cells, monocytes, and T cells (Langhorne *et al*., 2008). PfEMP1 has an important role in

facilitating the attachment of infected cells to the walls of blood vessels and thereby, presumably, reducing the likelihood of the parasite reaching the spleen where it would be killed. Antibodies formed against PfEMP1 can prevent infected red blood cells sticking to the walls of blood vessels. The *var* gene is present at numerous loci on different chromosomes and during the blood stages the parasite is able to switch between different antigenic variants of PfEMP1. In addition to the problems caused by antigenic variation, the development of a protective immunity is compromised by antigenic diversity. For example, another important antigen, merozoite surface protein-1 has numerous alleles, and antibodies formed against one 'version' do not necessarily recognise another (Hisaeda *et al*., 2005). Malaria also induces immuno-suppression in which the immune response to vaccines and a variety of other infectious agents is also compromised. Immuno-suppression results from a combination of processes including stimulating the production of the anti-inflammatory cytokines interleukin-10 and transforming growth factor-β and inhibiting the maturation of antigen-presenting cells. There is also evidence from mouse models that malaria could also cause immuno-suppression through inducing the activation of regulatory T cells (Hisaeda *et al*., 2004).

The difficulty of designing a vaccine against the blood stages of malaria that provides an acceptable level of protection has led to the suggestion that it would be better to focus efforts on a transmission-blocking vaccine targeting the gametocyte stage within the mosquito vector (Greenwood, 2008). This would then be employed in conjunction with other control measures such as identification and treatment of infected individuals and vaccines against the sporozoite and hepatic stages should they become available. Several candidate *Plasmodium falciparum* gametocyte antigens have been identified for vaccine targeting, of these, Pfs48/45 and Pfs230 show considerable promise. Attempts are also being made to develop vaccines that will target gametocyte-infected red blood cells while these are still in the human host. This approach has the advantage of supplementing the host's immune system that already forms antibodies against dead gametocytes that arise naturally (Sutherland, 2009).

6.7 *Schistosoma mansoni* and Hepatitis C virus interactions

In Egypt, there has been a particular problem of people acquiring Hepatitis C virus through contaminated needles used to administer the drug treatment for *Schistosoma mansoni* infection. Although this no longer occurs since the anthelmintics can now be given orally, there is a significant population of co-infected patients. Both these organisms affect the liver and people who are chronic carriers of both tend to develop serious hepatopathology. Evidence suggests that schistosome infection exacerbates the HCV, with patients having higher viral loads and more severe liver complications than those only infected with the virus (Strickland, 2006). This appears to be due to the effect that the helminth infection has on the human host's immunological response. Although the majority of patients infected with HCV seem to develop chronic infection (Harrison *et al*., 2009), some do recover. Studies have shown that this ability to resolve the infection is associated with being able to mount a strong Th-1 immune response involving CD4+ and CD8+ cells. Research suggests that the Th-1 immune response is down-regulated in chronic schistosomiasis, which could explain why patients infected with both *Schistosoma mansoni* and HCV have

been shown to be more likely to develop liver fibrosis and cirrhosis (Kamal *et al*., 2004). This immuno-suppressive effect does not appear to depend on the presence of active helminth infection since studies of blood donors with detectable levels of both anti-schistosomal and anti-HCV antibodies (indicating past infection) have demonstrated a reduced CD8+ T cell response compared to donors who tested positive for only one of these antibodies (Strickland, 2006). Another interesting effect on the immune response during co-infection was illustrated by a separate study of apparently healthy blood donors in Egypt, which found a high rate of low positive anti-HCV results in people who did not have any virological indicators of active HCV, but did have anti-schistosomal antibodies (Agha *et al*., 2006). The authors suggested that since the helminth has been shown to induce polyclonal B cell activation, these blood donors may have been producing antibodies from past infections, at a relatively low level. However, they also found autoimmune markers in some of these patients, again likely to have been induced by the *Schistosoma* infection, which they speculated may have been causing false positive results in the laboratory test (Agha *et al*., 2006).

6.8 HIV-AIDS and parasitic disease

HIV stands for 'Human Immunodeficiency Virus' and if an infection with this virus progresses, it gives rise to the condition 'Acquired Immunodeficiency Syndrome' – commonly known as 'AIDS'. Up until recently, most people infected with the virus ultimately developed AIDS and died – often from opportunistic pathogens. Although there is still no cure for HIV infection, the development of a range of anti-retroviral drugs has allowed the design of a combination treatment regimen 'highly active antiretroviral therapy' (HAART) which has enabled many infected people to live more-or-less normal lives without succumbing to either the virus or associated infections. Nevertheless, it is estimated that around 33 million people are currently infected with HIV around the world and AIDS remains a major cause of mortality in many developing countries. Parasites contribute to the acquisition of the virus, the progression of the disease, and both the morbidity and mortality associated with AIDS.

HIV is an RNA virus belonging to the Family *Retroviridae*, Genus *Lentivirus*. There are two types – HIV-1, which is found throughout the world, and HIV-2, which occurs predominantly in West Africa. In common with other lentiviruses, it is capable of remaining quiescent (latent) as a provirus within the host DNA for long periods of time but when stimulated undergoes extremely rapid multiplication. Virus replication is promoted by the activation of the infected CD4+ cells and co-infecting pathogens can contribute to this in a variety of ways (Karp and Auwaerter, 2007):

1. Co-infecting pathogens activate CD4+ T cells as part of the adaptive immune response.
2. Co-infecting pathogens activate the CD4+ cell through inducing pro-inflammatory cytokines.
3. Co-infecting pathogens activate the CD4+ cell through Toll-like receptor signalling.

The virus is only able to infect cells that carry the CD4 molecule on their cell membrane and consequently, it is restricted to the CD4+ T cells, dendritic cells, and monocytes. However, HIV-1 and HIV-2 occur as a range of variants that differ in their ability to infect different CD4+ cell types and the cell types vary in their sensitivity to the lytic effects of the virus. When activated,

the virus replicates rapidly and destroys its host cell and the progeny are released to repeat the process on other CD4+ host cells. There is some uncertainty whether or not the virus directly or indirectly contributes to the decline in CD4+ cells. Initially, it was thought that as the virus destroyed infected cells, these were replaced by increased rates of proliferation within the thymus. As the disease progressed to AIDS during the chronic phase of infection, the rates of destruction would ultimately exceed the rate of production and this was seen as the decline in the numbers of circulating CD4+ cells. However, a new model is emerging in which HIV is considered to indirectly induce the activation of large numbers of CD4+ cells which then become targets for infection. According to this model, HIV rapidly destroys the CD4+ T cells found in the intestinal mucosa and as a consequence of this, the gut bacteria and/or their products (e.g. membrane components) are translocated into the circulation. This stimulates the immune system which results in the activation and proliferation of CD4+ and CD8+ T cells – and the activated CD4+ T cells become 'targets' for HIV. Furthermore, the infection and destruction of the CD4+ cells would result in lymph node fibrosis and thereby reduce the capacity of the body to replenish the CD4+ cells in the intestinal mucosa (Brenchley *et al*., 2006). Whether or not the depletion of the CD4+ cells is a consequence of direct or indirect mechanisms, their loss results in immuno-suppression – and hence the host becomes vulnerable to a variety of other pathogens. However, the suggestion that HIV impairs the mucosal barrier of the gut as well as compromises the local immune response would explain why those who are HIV positive are so susceptible to gut pathogens.

The normal lower limit of CD4+ cells in the blood is 450–500 cells μl^{-1} and once this drops to below 400 cells μl^{-1}, the patient starts to suffer from non-specific symptoms such as diarrhoea, fever and weight loss. The transition from being infected with HIV to having clinical AIDS occurs when the number of CD4+ cells in the blood drops below 200 cells μl^{-1} (Stevens and Lowe, 2000), with a concomitant increase in the viral load. However, HIV exerts more complicated effects on the immune system than simply causing a drop in the numbers of CD4+ cells since CD4+ T cell function declines more rapidly than the numbers of CD4+cells in the circulation.

6.8.1 Parasites and the transmission of HIV

HIV is transmitted through blood and body fluids and is most commonly acquired through sexual contact. It can also be spread through contaminated needles (e.g. poor sterilisation of medical equipment and intravenous drug users who share needles), transfusions using contaminated blood, and vertically from mother to child. Although the virus can be found in the genital secretions of both men and women, sexual transmission can be further facilitated by the presence of lesions on the genitalia of one or both partners. For example, in women, infection with *Trichomonas vaginalis* causes inflammation of the genital tract that damages the surface epithelium and thereby predisposes them to HIV infection as well as increasing their chances of transmitting HIV to their sexual partner(s). It can also facilitate the acquisition of other sexually transmitted diseases such as herpes and gonorrhoea as well as the development of pelvic inflammatory disease (PID) in women (Moodley *et al*., 2002). In turn, these infections further increase the risks of contracting and transmitting HIV.

Persons who contract HIV-1 in Sub-Saharan Africa tend to progress more rapidly to AIDS than those living elsewhere in the world. The reasons for this are not known but are unlikely to be a simple consequence of different HIV-1 variants circulating within the local populations. One

suggestion is that people living in Sub-Saharan Africa are more likely to be infected with a range of parasites and in particular helminth parasites (Bentwich *et al*., 1995). These parasites cause long-lasting chronic infections that result in the activation of CD4+ cells – and HIV replicates at a higher frequency in such cells. However, the mechanism of activation is important because, depending upon the means employed, CD4+ T cell activation may result in either induction or suppression of HIV replication. Helminth infections tend to induce a dominant Th2 cell response and therefore it might be expected that through feedback mechanisms this would reduce the Th1 response – which is protective against HIV. However, the evidence for such an effect remains contradictory (Bentwich *et al*., 2008).

6.8.2 Parasite-HIV co-infections

As HIV infection progresses towards AIDS, there is a major decline in the cellular immune system and consequently many parasites that were previously unable to establish themselves become serious pathogens. These so-called 'opportunistic parasites' are a significant feature of AIDS and a major cause of both morbidity and mortality. In addition, previously latent infections can become reactivated and cause serious disease. However, the consequences of HIV infection vary considerably between parasite species. For example, human infections with *Isospora belli* are most commonly associated with persons suffering from AIDS or some other immuno-suppressive illness. Among those who are HIV positive, the prevalence of *I. belli* is usually in the region of 1–2% but may be as high as 27% (Stark *et al*., 2009). Similarly, *Cryptosporidium* and *Giardia* have 'emerged' in the past 20 or so years as a common causes of severe debilitating diarrhoea in those with HIV-AIDS. *Acanthamoeba* spp. infections usually only result in encephalitis in patients who are suffering HIV infection or are otherwise immuno-suppressed or they already have a debilitating medical condition (Denney *et al*., 1997). By contrast, many cases of *Balamuthia mandrillaris* encephalitis have arisen in otherwise healthy individuals as well as those who are immuno-suppressed (Tavaras *et al*., 2006). There is a high incidence of amoebiasis among AIDS patients (e.g. Samie *et al*., 2010) although whether this is owing to increased susceptibility as a result of a depressed immune system or because AIDS has a high incidence among men who have sex with men (who appear to have a higher rate of *Entamoeba* spp. infection anyway) is not clear from the studies (Stark *et al*., 2009). By contrast, serious infections with *Sarcocystis* do not appear to be a significant problem for immuno-compromised individuals – which is just as well as there is currently no recommended treatment. In some people, subclinical and untreated toxoplasmosis can result in the development of latent cysts containing bradyzoites in the brain. The depression of the immune system caused by AIDS allows the reactivation of the *Toxoplasma gondii* parasite, which is associated with gradual deterioration in the patient's mental state (see later).

6.8.3 Leishmania-HIV co-infections

There is an 'unholy alliance' between HIV and the protozoan parasite *Leishmania* in which co-infections result in mutual activation and enhanced pathology. This is perhaps not surprising since both HIV and Leishmania infect CD4+ T cells and both can remain as latent infections until they receive suitable stimulation. However, the effect is most commonly seen in the

development of visceral leishmaniasis-HIV co-infections rather than cutaneous leishmaniasis-HIV co-infections. Visceral leishmaniasis-HIV co-infections may arise through a primary infection with either pathogen that is facilitated by the decline in immune function caused by the one that is already resident. Alternatively, the virus may activate a latent *Leishmania* infection or the *Leishmania* may activate a latent HIV infection. The two pathogens influence one another through a wide variety of immunopathological mechanisms. For example, HIV infection disrupts normal monocyte and macrophage functions by reducing chemotaxis and intracellular killing activity which therefore facilitates the invasion and multiplication of *Leishmania*. Similarly, when *Leishmania* interacts with specific cell surface receptors on HIV-infected CD4+ lymphocytes and macrophages, it results in the activation and multiplication of the virus. The pathogens can also influence one another by affecting the Th1: Th2 balance. In leishmaniasis, a dominant Th1 profile with the production of large amounts of interleukin-2 and interferon-gamma is considered protective. Conversely, those who are otherwise immuno-competent, but mount a dominant Th2 response in which the cytokines interleukin-4, interleukin-5, and interleukin-10 predominate, are more susceptible to the parasite. A dominant Th1 profile is also protective against HIV and the progression of HIV infection to AIDS is associated with a move towards a Th2 dominance and a decline in interleukin-2 and interferon gamma production (Clerici and Shearer, 1994). Consequently, HIV infection can promote the development of leishmaniasis and leishmaniasis can promote the development of HIV. The two pathogens also influence one another through a range of other immune mechanisms and these are discussed by Olivier *et al*. (2003) and Wolday *et al*. (1999).

Box 6.4 Why HIV-*Leishmania* co-infections are becoming an increasing problem

In recent years, the geographical distributions of leishmaniasis and HIV/AIDS have started to increasingly overlap and, in consequence, there has been an increase in the incidence of HIV–*Leishmania* co-infection. This is partly as a result of leishmaniasis moving from its predominantly rural focus to urban areas while HIV/AIDS, which has been a predominantly urban disease, has started to affect rural populations more severely. For example, visceral leishmaniasis has become a serious problem in the suburbs of large cities in Brazil, Bangladesh and India. In parts of South America this has been attributed to the felling of large areas of rainforest which has led to a change in the feeding habits of the sandfly vectors, which would normally have fed principally on wild animals. For the example, the sandfly vectors of *Leishmania braziliensis* have become more endophagic and there has also been an increase in the number of cases of leishmaniasis among the (peri)domestic animals that act as reservoirs of infection. By contrast, in countries such as Ethiopia, Kenya and India, the HIV–AIDS pandemic is becoming progressively more common in rural areas.

In southern Europe there has also been an increase in *Leishmania*–HIV co-infection. In this case, the rise is predominantly a consequence of parenteral transmission of *Leishmania* via blood transfusions and, especially, needle-sharing among intravenous, HIV and *Leishmania* co-infected drug users. In southern Europe, up to 70% of all adult cases of visceral leishmaniasis are related to HIV–AIDS, (particularly HIV-1) and up to 9% of all AIDS cases suffer from newly-acquired

or reactivated visceral leishmaniasis. In Spain, visceral leishmaniasis tends to remain in a subclinical state in a high proportion of those co-infected with HIV-1, becoming symptomatic once deep immuno-suppression has developed (Pineda *et al*., 1998). These asymptomatically-infected individuals represent a potential reservoir of infection for the rest of the population.

By June 1998, cases of *Leishmania*–HIV co-infection cases had been reported from 25 countries. This figure had risen to 35 countries by 2003 although in terms of numbers, the majority of cases have remained in southern Europe (Desjeux and Alvar, 2003). This is probably a consequence of a substantial underestimation of the actual number of cases in developing countries because of problems in recognition, diagnosis and reporting of either HIV infection, or leishmaniasis, or both. Other factors may also be operating, for example, in Brazil, it has been suggested that, unlike Europe, intravenous drug users do not predominate among those exposed to HIV (Rabello *et al*., 2003). It is also expected that the incidence of *Leishmania*–HIV co-infection will continue to rise in countries such as Ethiopia and elsewhere in East Africa.

6.8.4 Malaria–HIV co-infections

For many years Sub-Saharan Africa has suffered the highest number of cases of malaria in the world and the incidence of HIV infection in many countries in that region is also devastatingly high. Cases of malaria–HIV co-infection are therefore common (Nkuo-Akenji *et al*., 2008) but despite this, the relationship between the two diseases and how they influence one another (if at all) is uncertain. This is partly because in developing countries the diagnosis of malaria is often based on clinical signs rather than specific tests. As we have seen, HIV–AIDS can itself result in fevers and it also increases susceptibility to a wide range of opportunistic infections – some of which also cause pyrexia (fever), which could be confused with malaria.

Because AIDS compromises the immune system, it might be expected that it will increase susceptibility to infection with and recrudescence of malaria. Women who grow up in malaria-endemic regions develop immunity to the local species and strain of *Plasmodium* but this immunity wanes during pregnancy. Consequently, they can suffer from pregnancy-associated malaria. HIV-1 co-infection with malaria can result in marked changes in the levels of antibodies against *Plasmodium falciparum* (Jaworowski *et al*., 2009). This may explain why the placenta of women who are HIV positive is more likely to be invaded by the malaria parasite than those who are HIV negative. This in turn can increase their chances of transmitting HIV to their child (Bhambhatt *et al*., 2003).

Malaria infection of people who have HIV results in a short-lived increase in the plasma viral load and this rises with the severity of the attack (Kublin *et al*., 2005). The rise in viraemia could conceivably increase the chances of HIV transmission although persons suffering badly from malaria are not likely to be sexually active. Indeed, the progression of HIV among populations with malaria is fairly similar to those where it is not such a problem. Consequently, it is still a matter of debate whether malaria contributes to the spread of HIV (Hewitt *et al*., 2006) and it should be remembered that many variables need to be taken into account such as HIV subtype, strain of parasite and host factors. It is therefore difficult to make direct comparisons between many of the studies.

6.8.5 Toxoplasma–HIV co-infections

Toxoplasma gondii can have serious and potentially fatal effects in people who become immunosuppressed as a consequence of HIV infection or treatment for cancer. Once their CD4+ T-lymphocyte count falls below 50 cells μl^{-1} the patient becomes at considerable risk of an existing infection becoming reactivated (although some reports suggests that the risk is already increased at 200 CD4+ cells μl^{-1}). The replication of the parasites within brain cells results in the development of encephalitis and abscess formation. The consequences of the disease depend upon the size of lesions and the part of the brain affected. Symptoms can include headaches, speech defects, confusion, seizures, and, ultimately, the condition can prove fatal. Until the widespread use of highly active antiretroviral therapy (HAART), toxoplasmic encephalitis affected up to 40% of AIDS patients and 10–30% died of the condition (Tenter *et al*., 2000). It is also now possible to prevent reactivation of dormant *Toxoplasma gondii* infections by prophylactic treatment with trimethoprim-sulfamethoxyzole (TMX-Sulfa). As a result, the rate of toxoplasmic encephalitis has declined considerably among AIDS patients although it remains a risk (e.g. Abgrall *et al*., 2001). However, in those developing countries where access to HAART is more limited, toxoplasmic encephalitis is a serious problem.

6.8.6 Microsporidia–HIV co-infections

The initial descriptions of microsporidian infections in AIDS patients occurred in 1985. Subsequently, the number of reported cases and clinical spectrum of diseases has increased remarkably. This suggests that some, if not all, of those species currently causing problems may be zoonotic infections – although the source of these is uncertain (Mathis *et al*., 2005). For example, although *Enterocytozoon bieneusi* was not described until 1985, it has now become one of the most frequently identified pathogens in the intestinal tracts of patients with HIV. Approximately 15% of the 34% of AIDS patients with chronic diarrhoea have been found to harbour *Enterocytozoon bieneusi*. In the developed world, the introduction of HAART therapy has resulted in a reduction in the number of HIV positive individuals going on to develop AIDS and there has been a corresponding reduction in the number of cases of infections with microsporidia. However, reports suggest that these parasites are being increasingly found in immuno-competent people and they are now recognised as an important cause of diarrhoeal diseases in developing countries. In Uganda, Tumwine *et al*. (2002) found that 17.4% of children attending hospital with acute diarrhoea were infected with *Enterocytozoon bieneusi* although 16.8% of the children did not have diarrhoea and were similarly infected. Although this work did not demonstrate a relationship between microsporidia infection and stunting, a subsequent investigation found that infected children tended to put on less weight than others (Mor *et al*., 2009).

Questions

1. Briefly distinguish between the terms innate and adaptive immunity.
2. Describe two immune mechanisms by which invertebrates repel invading pathogens.
3. Name three types of leukocytes and state their functions.

4. Briefly discuss three mechanisms by which immunoglobulins exert their effects.
5. Evaluate the role of *Lactobacillus acidophilus* in immunity to *Trichomonas vaginalis*.
6. Briefly discuss two mechanisms by which schistosomes avoid the vertebrate immune response.
7. How do VSG genes enable trypanosomes to avoid the host immune response?
8. With the aid of at least one example parasite, explain what is meant by the term concomitant immunity.
9. Briefly discuss two reasons why it has proved so difficult to develop a vaccine against malaria.
10. With the aid of examples, cite two ways in which parasites could increase the transmission of HIV.

7
Pathology

7.1 Introduction

Pathology is the study of the mechanisms that cause morbidity and mortality. We use the term 'pathogenesis' to describe the cellular processes and reactions that bring about the diseased state while 'pathogenicity' refers to the ability of an organism or substance to cause disease. We often talk about organisms being weakly or highly pathogenic depending upon the amount of damage they cause to their host. The term 'virulence' is also used as an indicator of a parasite's potential to cause disease and this is usually strongly associated with the organism's ability to reproduce within its host. For example, *Trypanosoma evansi* is highly virulent in horses and the inoculation of even a single parasite can prove fatal (http://www.fao.org/docrep/006/x0413e/X0413E06.htm). Pathology, like immunology, requires one to learn some basic vocabulary before it is possible to make sense of the literature. We therefore begin by introducing some of the commonest types of pathology and then discuss how parasites affect individual organs of the body. Throughout the chapter we attempt to illustrate how pathology is the end result of a complex interaction of host factors, parasite factors, co-infections, and the environment. Those requiring more detail on specific aspects of pathology should consult specialist textbooks, such as Rubin *et al*. (2008) and Stevens and Lowe (2000) for human pathology and McGavin and Zachary (2007) for veterinary pathology.

7.2 Factors that influence pathogenesis

Individual host factors that determine susceptibility or resistance to a pathogen and parasite factors that influence virulence and ability to avoid the host immune system are of crucial importance in determining the outcome of any host: parasite relationship. Consequently, for any given parasitic disease, the extent to which it is pathogenic will depend upon many interacting variables (Table 7.1).

7.2.1 Host factors that influence pathogenesis

Numerous host factors influence whether or not a person becomes infected with a parasitic disease (Chapter 4) and also their subsequent reaction to it. It is not usually possible to identify a single factor as being of fundamental importance since most of them interact to varying extents. For example, the gender of the host can affect its behaviour and therefore the likelihood of coming

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Table 7.1 Host factors and parasite factors that determine the pathology associated with parasitic diseases

Host factors	Parasite factors
Genetic constitution	Genetic constitution
Age	Growth rate
Gender	Reproduction
Underlying health	Production of harmful substances
Immune status	
Presence of co-infections	

into contact with the infectious stage of a parasite; however, immune status and therefore ability to prevent the parasite from establishing itself are also influenced by gender.

Host genetic constitution plays a major role in determining susceptibility to many parasitic diseases. For example, scabies (*Psoroptes scabei*) is found throughout the world and for most people who are immuno-competent becoming infected with the mite is an unpleasant experience but seldom has serious consequences. However, for some reason Australian Aboriginals are particularly susceptible to developing a highly debilitating and potentially fatal condition called 'crusted scabies' (Roberts *et al*., 2005). Similarly, although many people in Mexico contract amoebic dysentery, hepatic amoebiasis is primarily found in young adult males and in particular it is associated with those who express the human leucocyte antigen class II alleles HLA-DR3 (Arellano *et al*., 1991; Santi-Rocca *et al*., 2009). In Bangladesh, susceptibility to *Entamoeba histolytica* has been linked to mutations in the genes coding for the leptin-receptor: a single substitution of an arginine for a glutamine at position 223 is sufficient to increase susceptibility to amoebic dysentery in children and hepatic amoebiasis in adults (Duggal *et al*., 2011). (Leptin is best known for its role in regulating body weight but it has numerous other physiological functions, including acting as a pro-inflammatory cytokine that promotes a Th1-type response and inhibiting apoptosis.) A variety of innate immune mechanisms providing a level of protection against malaria are found in people living in some malaria-endemic regions (see Chapter 6). Sometimes it can be difficult to be certain about the relative importance of immune competence and behavioural characteristics in determining disease susceptibility. Children under the age of 15 account for about half of all reported cases of *Balamuthia* encephalitis, and while this may be related to their immune status, it could also be a reflection that children are more likely than adults to come into contact with contaminated soil or water (e.g. while playing) and tend to have poorer personal hygiene. In the USA, about half of all cases of *Balamuthia* encephalitis occur in people of Hispanic descent although Hispanics comprise only about 13% of the population (Schuster *et al*., 2004). This may be a consequence of a genetic susceptibility but could also be related to the fact that as a group Hispanics in the USA tend to be poor and involved in manual work such as farm labour and gardening that might increase their exposure to *Balamuthia*.

7.2.2 Parasite factors that influence pathogenesis

Genetic constitution influences all aspects of an organism's biology and different strains of a parasite can vary markedly in their infectiveness and the pathology they cause. For example, strains of *Entamoeba histolytica* vary in their ability to induce amoebic dysentery (Escueta-de Cadiz *et al*., 2010). In mice, Type I strains of *Toxoplasma gondii* are highly virulent and infection with

just a few tachyzoites can lead to the death of the mouse, while the Type II and Type III strains are non-lethal and cause chronic latent infections. *Toxoplasma gondii* infect macrophages, among other cell types, but can be killed by them if the parasitophorous vacuole in which they reside is disrupted. This can occur as a consequence of physiological processes that are mediated by immunity-related GTPase proteins (IRG). The virulence of the Type I genotype appears to be due, at least in part, to its ability resist the IRG-mediated disruption of their parasitophorous vacuole (Zhao *et al*., 2009). This allows the parasites to survive within even activated macrophages and thereby compromise the cells' role in the immune response and, ultimately, kill them. In addition, the parasites are able to 'abandon ship' before the macrophages are able to exert their innate 'killing mechanisms'. Under *in vitro* conditions, the tachyzoites inhabiting macrophages undergo up to seven division cycles over 2–3 days before the host cell is destroyed. However, it is now apparent that in acutely infected mice the macrophage can be destroyed after only 0–2 division cycles – this takes about 6 hours and results in rapid macrophage destruction (Tomita *et al*., 2009). The parasites are stimulated to leave their host macrophages by the arrival of other, non-infected, macrophages, and these could potentially 'switch on' the parasitised cells.

Whether or not the various strains of *Toxoplasma gondii* differ in their pathogenicity in humans and other animals is uncertain. In most mammal and bird intermediate hosts, *Toxoplasma gondii* causes subclinical infections but clinical disease does appear to be more frequent in some species than others. For example, pigeons and canaries are more likely to suffer clinical disease than other bird species (Dubey, 2002). In these species, symptoms can include loss of condition, anorexia, conjunctivitis, and encephalitis.

Most surveys of human populations to date have found that the majority of people infected with *Toxoplasma gondii* are carrying the Type II genotype, although this could simply be a reflection of this being the commonest genotype infecting the animals that people are likely to consume. However, the Type I genotype and recombinants between the Type I and Type III genotypes are thought to be responsible for most cases of clinical toxoplasmosis in humans (Dubey and Jones, 2008). For example, persons suffering from severe ocular toxoplasmosis are usually infected with the Type I genotype (Grigg *et al*., 2001; Khan *et al*., 2006). Ocular toxoplasmosis usually results from infections acquired after birth but can also result from congenital infection (Holland, 2003). In the latter case, ocular disease symptoms may not be manifest until several decades after birth (Kean *et al*., 1991). However, until more studies are done on the genotypes of *Toxoplasma gondii* found in both asymptomatic and symptomatic individuals, it is probably too soon to state that there is an obvious link between *Toxoplasma gondii* genotype and human disease.

7.3 Mechanisms by which parasites induce pathology

Typically, a parasite begins to cause damage when it invades its host and starts to feed upon it although in some species most of the pathology is associated with the parasite's reproduction. The parasite antigens and host tissue damage initiate an immune response that either suppresses or expels the parasite or may cause further pathology. A parasite may therefore cause harm directly (e.g. through removal of metabolic resources) and indirectly (e.g. through inducing a harmful immune reaction) (Table 7.2). In addition, owing to the interconnectedness of physiological processes, damage to one organ or region of the body can have severe consequences for the functioning of other organs and physiological systems. In order to develop appropriate treatment regimes for parasitic diseases, it is important to understand both the mechanism(s) by which the parasite inflicts damage on the host and the host's response to that attack.

Table 7.2 Mechanisms by which parasites cause pathology

Direct	Indirect
Consumption of host tissues	Overstimulation of immune system
Competition for nutrients	Stimulation of autoimmunity
Production of harmful secretions	Compromising immune system
Physical destruction of host tissues	Alteration of gut environment
	Alteration of homeostasis
	Increasing susceptibility to other diseases

7.3.1 Direct damage

Parasites cause direct physical damage by feeding on the host's tissues, boring through tissues during the course of their migrations, or by the physical act of growing that causes pressure atrophy and the restriction of blood supply to a region of the body. Not only are nutrients lost as a result of the damage but also there is a cost associated with the repair processes. The seriousness usually depends upon the number of parasites involved, the time interval over which the damage occurs, and the region of the body affected. Of course, if the balance is too severe, then there is a point where the relationship may no longer be advantageous to the parasite. For example, the crustacean parasite *Cymothoa exigua* destroys the tongue of its fish host, which would limit the nutrients available to the parasite but it then proceeds to act as a replacement fully functional tongue so the fish does not starve (Brusca and Gilligan, 1983). Typically, acute parasitic disease results from large numbers of parasites arriving at a sensitive location over a short time interval and may be fatal. By contrast, chronic parasitic disease is associated with fewer parasites and long-standing infections that may or may not be resolved and may or may not prove ultimately fatal. For example, acute fascioliasis in sheep results from the simultaneous migration of large numbers of immature *Fasciola hepatica* through the liver. This usually follows 2–6 weeks after the ingestion of 2000 or more infective metacercariae and death may occur suddenly without any previous symptoms (Taylor *et al*., 2007). If the sheep ingest fewer metacercaria, say, 500–1500, over a longer time interval, then they are more likely to suffer sub-acute fascioliasis. In this case the disease is manifested 6–10 weeks after infection and although it may prove fatal, the infected animal does not die so quickly. Finally, chronic fascioliasis in sheep is associated with the growth of adult flukes in the bile ducts. Although chronic fascioliasis may prove fatal, it is more commonly a cause of progressively poor health and lack of condition. Chronic fascioliasis typically results from the ingestion of 200–500 cercariae and clinical disease becomes apparent 4–5 months after infection. Chronic fascioliasis is not as dramatic as the acute disease but, as with other chronic parasitic infections, it is economically more important.

Parasites release a wide variety of enzymes and chemicals into and onto their hosts. For example, these compounds can be released to facilitate invasion, migration through the body and feeding, to overcome host immune defences and in the process of excreting waste products. These substances can cause pathology either directly through damaging cells and metabolic processes or through the less direct means of stimulating a damaging inflammatory response. For example, the infective larvae of hookworms and the cercariae of schistosomes release protease enzymes that enable them to penetrate the skin. In the process they kill host cells and engender an inflammatory response. Similarly, *Entamoeba histolytica*, *Trypanosoma brucei*, *Trypanosoma congolense*, and

Trypanosoma cruzi all release cysteine peptidases into the surrounding medium. In addition to acting on their substrates, these enzymes also stimulate an immune response that results in a localised increase in the concentration of activated host-derived pro-inflammatory cytokines. Trypanotolerant cattle have a better immune response against cysteine peptidases than susceptible breeds and consequently the enzymes have been identified as potential targets for the development of new vaccines (Pillay *et al*., 2010).

7.3.2 Indirect damage

Mounting an immune response, even an effective one, is energetically expensive. Indeed, it has often been observed that flocks of sheep selectively bred to be resistant to gastrointestinal nematode infections (as determined by expressing low faecal egg counts) tend to have lower growth rates than unselected control flocks (Williams, 2011). In addition, the immune-induced pathology is the major cause of harm associated with some parasitic infections. For example, the chronic phase of Chagas disease (caused by *Trypanosoma cruzi*) is most commonly expressed in adults and is manifested as an 'indeterminate', a 'cardiac' or a 'digestive' form. Most adults with Chagas disease have the indeterminate form and may be unaware that they are infected with the parasite. Indeed, it may be impossible to detect any abnormalities using conventional electrocardiograph procedures and radiology. Many of these people will survive in this state for years and eventually die of some other cause. In some people, however, there is slow progression to the cardiac or digestive forms of disease over a period of years. In these two forms the symptoms result primarily from the destruction of peripheral and central nervous tissues, although there can also be significant damage to the skeletal, cardiac, and smooth muscle. Much of this damage is immune-mediated, but there is some uncertainty as to how much of this results from inflammatory responses induced directly by the parasite and how much is a consequence of them engendering an autoimmune response. In the cardiac form, the patient may die suddenly without expressing any previous outward signs of disease or they might suffer from a steady decline in heart function over a prolonged period, which may also end in death. Damage to the nerves supplying the heart can result in a loss of muscle tone so it becomes 'flabby'. In addition, the size of the heart can increase (cardiomegaly) as a consequence of hypertrophy of the muscle fibres, inflammation and oedema. A characteristic feature is the formation of a 'vortical lesion' at the apex of the left ventricle. Histologically, one can see the deposition of fibrous tissue with the heart and the focal accumulation of inflammatory cells – a condition known as fibrous myocarditis (Mady and Nacruth, 1995).

Box 7.1 Autoimmunity

There is increasing evidence that parasites have been important in the evolution of the immune system and they may even be required for the system to continue to function properly. In the absence of stimulation from parasites, the body appears to have a tendency to develop allergic reactions or autoimmune diseases. Indeed, there is a significant correlation between the presence of risk alleles for both inflammatory bowel disease and Crohn's disease and what Fumagalli *et al*. (2009) refer to as 'micropathogen richness'. There is therefore considerable interest in whether it

would be possible to use parasites (or chemicals derived from them) in the treatment of allergies and/or autoimmune diseases, this is discussed in Chapter 8. A few parasites, however, rather than protecting against autoimmunity, appear to induce it. For example, some patients suffering from *Cyclospora* infection subsequently develop neurological complications such as Guillain-Barré syndrome (Richardson *et al*., 1998) and Reiter's syndrome (Connor *et al*., 2001). Guillain-Barré syndrome is an autoimmune disease that causes demyelination of axons in the peripheral nervous system (Wim Ang *et al*., 2004). There are six subtypes but the most common is acute inflammatory demyelinating polyneuropathy (AIDP) that results in progressive paralysis and although those affected may recover with treatment, it can be fatal. It is normally associated with viral (e.g. influenza) and bacterial (e.g. *Campylobacter jejuni*) infections. There are a few isolated case reports linking Guillain-Barré syndrome to other parasitic diseases, notably *Toxoplasma gondii* infection (Bossi *et al*., 1998) and *Plasmodium falciparum* malaria (Tag-Eldin *et al*., 2002). Reiter's syndrome is another autoimmune condition that follows infection; it is often associated with the bacterium *Chlamydia trachomatis* although gastrointestinal infections may also initiate the condition. It is usually manifested as inflammatory reactions in the eyes (e.g. conjunctivitis), the urinary tract (e.g. dysuria) and arthritis, particularly of the large joints. Doctors therefore often remember the condition using the mnemomic 'can't see, can't pee, can't climb a tree'. There is currently very little evidence of an association of Reiter's syndrome with other parasitic protozoa or helminths, but whether this is due to lack of suitable research is uncertain.

7.4 Types of pathology

7.4.1 Abortion and obstetric pathology

Spontaneous abortion is very common in humans – some estimates indicate that around 40% of conceptions fail to establish as recognisable pregnancies, and of those that do establish around 15% are aborted at some stage during the pregnancy. There are numerous reasons for spontaneous abortion, among which are infectious agents such as parasites. Parasites may induce abortion by crossing the placenta and infecting the developing foetus, causing damage to the placenta, and by reducing general health or inducing metabolic disorders in the mother.

Placental malaria can result in low birth weight, probably through reducing intrauterine growth rate, and there is an increase in the frequency of spontaneous abortion and premature labour during malaria epidemics (Menendez *et al*., 2000). Leishmaniasis has also been implicated in causing abortion in dogs, while *Trypanosoma evansi* has been implicated in causing abortion in a variety of domestic animals including camels, horses, and buffalos (e.g. Löhr *et al*., 1986). Membrane glycosyl-phosphatidylinositol lipids derived from *Plasmodium falciparum*, *Leishmania* and *Trypanosoma* stimulate a subset of Natural Killer Cells called Vα14NKT cells that have been implicated in inducing abortion (Ito *et al*., 2000). Vα14 NKT cells are involved in the immune responses to tumours and a variety of pathogens, allergic reactions and autoimmune diseases and upon activation produce tumour necrosis factor alpha (TNF-α). Tumour necrosis factor-α (and inflammatory cytokines) can induce placental coagulopathy (i.e. bleeding that results from defects in the clotting mechanism) and this would interfere with the supply of nutrients to the developing foetus (Poovassery *et al*., 2009).

In humans, *Toxoplasma gondii* is the parasite most closely identified with causing abortion. The parasite is able to pass across the placenta and then infect the developing foetus. The foetus is most likely (60–90%) to become infected during the third trimester of pregnancy (Remington *et al*., 2006) but fortunately this is when the consequences of a severe outcome are less. By contrast, the risk of infection during the first trimester is only 10–15% but if it does occur, there is a much greater chance that the outcome could be fatal for the developing baby. Congenital infections can result in spontaneous abortion or stillbirth and if the baby is born alive, it can suffer from a range of morphological and neurological conditions. At its worst, the baby may be born with potentially fatal birth defects such as hydrocephalus and microcephalus. Congenital infection can also result in a wide range of other disorders of the nervous system, including mental impairment, some of which may not become apparent until several years later.

In cattle, *Neospora caninum* is a significant cause of abortion in many parts of the world. Two forms of abortion have been described: epidemic abortion and endemic abortion. Epidemic abortion is where a large proportion of the herd suffer abortions within a short period of time. This is sometimes called an 'abortion storm' and is thought to be due to the cattle being subject to a sudden challenge infection: for example, if their field or food or water supply is suddenly contaminated with large numbers of infective oocysts. Where the abortion rate is above 5% per year over a period of several years, it is classed as endemic. Because the maintenance of the disease within a herd over prolonged time is thought to require periodic horizontal input, it is likely that both epidemic and endemic abortion occur in localities where the disease is common. Furthermore, *Neospora caninum* exhibits considerable genetic diversity (Al-Qassab *et al*., 2010a; Regidor-Cerrillo *et al*., 2006) and therefore the various strains might be expected to vary in their pathogenicity.

7.4.2 Anaemia

Anaemia becomes outwardly obvious with the development of pallor in the mucus membranes owing to the reduction in the numbers of circulating red blood cells. The affected person or animal becomes lethargic and the pulse rate increases because the heart pumps the blood around the body faster to compensate for its lower oxygen-carrying capacity. Depending upon the amount of blood lost and the time interval over which it occurs, anaemia can be a mild chronic condition or rapidly fatal.

The anaemia associated with parasitic disease can arise from a number of causes (Table 7.3). In the case of helminth and arthropod parasitic infections, anaemia often results from the loss of blood associated with feeding or the migration of the parasite through the body. For example, hookworms consume more blood than they require and cause wounds that continue to bleed after they have finished feeding, leading to significant blood loss in the host. Similarly, when young *Fasciola hepatica* flukes migrate through the parenchyma of the liver, they damage blood vessels and cause widespread bleeding. The tapeworm *Diphyllobothrium latum* is unusual for causing megaloblastic (pernicious) anaemia in humans. However, although approximately 40% of infected people exhibit low levels of vitamin B12, less than 2% of them actually develop anaemia. The anaemia results from the worm secreting substance(s) that cause the disassociation of vitamin B12 from host-derived Intrinsic Factor within the gut. Vitamin B12 has to bind to the Intrinsic Factor if it is to be absorbed across the gut wall. The decline in vitamin B12 levels results in the formation of large red blood cell precursor cells (megaloblasts) that give rise to unusually large

Table 7.3 Mechanisms by which parasites cause anaemia

Cause of anaemia	Example
Haemorrhage of red blood cells	*Ancylostoma duodenale*
Destruction of parasitised red blood cells	*Plasmodium falciparum*
Autoimmune destruction of uninfected red blood cells	*Trypanosoma brucei*
Deficiency of metabolites needed to produce red blood cells (e.g. lack of iron and vitamin B12)	*Diphylobothrium latum*
Aplasia (failure to produce sufficient red blood cells)	*Plasmodium falciparum*
Hypersplenism (red blood cells are sequestered in the spleen and destroyed)	*Plasmodium falciparum*

red blood cells – and these are then destroyed because they are defective. Megaloblastic anaemia is also associated with the development of neurological disease and heart conditions. The worms selectively absorb the vitamin B12 but why they have such an affinity for the vitamin is not known and megaloblastic anaemia is not a common feature of infections with other tapeworms of the genus *Diphyllobothrium* (Scholz *et al*., 2009).

Parasitic protozoa induce anaemia through a wide variety of mechanisms. In the case of *Entamoeba histolytica*, extensive ulceration of the colon and large intestine can lead to sufficient blood loss to cause anaemia. However, anaemia is more often associated with those protozoa that live within the bloodstream, sometimes within the blood cells. For example, malaria causes anaemia through the destruction of both parasitised and non-parasitised erythrocytes, the inability of the body to recycle the iron bound in haemozoin, and an inadequate erythropoietic response (formation of new red blood cells) by the bone marrow. Malaria also causes coagulopathy, i.e. it interferes with the clotting process and thereby causes susceptibility to uncontrolled bleeding. Why large numbers of non-parasitised red blood cells are destroyed in response to malaria infection is unclear but some evidence suggests that it is linked to an autoimmune response. Destruction of erythrocytes leads to an increase in blood bilirubin, a breakdown product of haemoglobin. When excretion cannot keep up with formation of bilirubin, the yellow bile colour is evident under the skin and in the whites of the eyes (jaundice). Haemozoin is taken up by circulating leucocytes and deposited in the reticuloendothelial system. In severe cases the viscera, especially liver, spleen and brain, become blackish as a result of pigment deposition. After ingesting haemozoin, macrophages suffer impairment in phagocytic activity and this compromises the cellular immune response.

In cattle, and other animals susceptible to *Trypanosoma brucei brucei*, much of the pathology caused by the parasites is associated with the development of anaemia. Indeed, a key feature of trypanotolerant breeds and species is their ability to prevent the level of parasitaemia rising to a point at which it causes severe anaemia. Trypanotolerant breeds of cattle may, however, exhibit loss in productivity if subject to serious challenge by trypanosome parasites (Taylor *et al*., 2007). There is some debate over the principal cause of the anaemia and the chances are that it is multifactoral, including variables such as host species, host and parasite genetic constitution and individual circumstances such as host age, health, and nutrition (Morrison *et al*., 2010). It is possible that the trypanosomes release substances that kill red blood cells or that parasite-derived variant surface glycoproteins (VSGs) become attached to red blood cells and thereby cause them to be identified as ‘foreign’ and therefore attacked by the host immune system. In laboratory studies, the development of anaemia in mice infected with *Trypanosoma brucei* has been linked to the

release of excessive amounts of inflammatory cytokines such as tumour necrosis factor (Magez *et al*., 1999).

7.4.3 Anorexia

Anorexia is the voluntary reduction in food intake (i.e. 'loss of appetite') although some workers prefer to use the term 'inappetence' so as to avoid confusion with the human psychological condition '*Anorexia nervosa*'. Anorexia is a feature of many infectious diseases, including those caused by parasites. At first sight, this appears to be counterintuitive because infections impose a considerable metabolic cost on their host. However, anorexia is a common response by both invertebrates and vertebrates to many viral, bacterial, protozoan, and helminth infections. It is not, however, an invariable response and there is no obvious reason why some infections induce anorexia but others do not. For example, exposure of the fruit fly *Drosophila melanogaster* to both live and dead *Listeria monocytogenes* was observed to induce anorexia but their exposure to another species of bacterium *Enterococcus faecalis* did not (Ayres and Schneider, 2009). Whether or not anorexia is induced therefore probably depends upon both host and parasite characteristics at the time of the infection. Because anorexia is such a common reaction to so many infections, it is considered to be an ancestral response that has been conserved because it benefits the host. However, it has proved difficult to ascertain what the benefits are and anorexia may take a considerable toll upon the host. For example, in the case of gastrointestinal helminth infections of domestic animals, the losses caused by anorexia may account for 40–90% of the decline in productivity (Greer, 2008).

Viruses and bacteria tend to induce anorexia shortly after infection but it does not last long, while in prototozoa and helminth infections, anorexia often does not commence until several weeks after infection and is of longer duration (Kyriazakis and Doeschl-Wilson, 2009). For example, in rainbow trout (*Oncorhynchus mykiss*), food intake is related to the density of the protozoan parasite *Cryptobia salmositica* in their blood. Chin *et al*. *(*2004) found that three weeks after initial infection, the food intake of the fish reduced significantly from 1.33 to 0.94% initial body weight and by week 4 had dropped to 0.8% body weight – this corresponded to peak parasitaemia. The physiological mechanisms that underlie anorexia are poorly understood and probably differ between hosts (Colditz, 2008). Several workers have found evidence that the immune system is involved in inducing anorexia. For example, mice infected with the nematode *Trichinella spiralis* normally exhibit anorexia but this does not occur in mice lacking CD4+ T lymphocytes or the receptor for interleukin-4 (McDermott, *et al*., 2006). Anorexia can be induced in non-infected humans and other mammals by treatment with Tumour Necrosis Factor-α (TNFα) and both TNF-α and interleukin-1 have been implicated in the development of anorexia associated with influenza. The circulating levels of TNF-α and interleukin-1 increase during malaria and it is therefore possible that these cytokines may be responsible for the onset of anorexia associated with malaria (Clark *et al*., 2008).

7.4.4 Apoptosis

Apoptosis is a form of programmed cell death found in all metazoan animals and some protozoa. It fulfils a variety of functions in processes as diverse as embryogenesis and the response to

parasitic infections. Apoptosis is controlled by a variety of both extrinsic and intrinsic pathways. The extrinsic pathways are triggered by the interaction of specific cell surface receptors with ligands. For example, the best understood pathway involves the binding of the ligand CD95 (Fas) with the 'CD95 (Fas) Receptor' to bring about the formation of the 'death-inducing signalling complex' (DISC). The death-inducing signalling complex then brings about the activation of caspase enzymes that cause cell cytolysis: for this reason, caspases are sometimes referred to as 'executioner enzymes'. The intrinsic pathways are associated with the release of cytochrome c into the cytosol following the breakdown of the outer membrane of the mitochondria. The cytochrome c then binds to a substance called 'apoptosis-activating factor' that is present in the cytosol and this then sets in train a series of reactions that result in the activation of caspase enzymes. Apoptosis is also initiated by cytotoxic T cells via other pathways such as the granzyme-B pathway and the perforin pathway. Cells that undergo apoptosis are recognised and engulfed by phagocytic cells so the breakdown products are not released into the surrounding media. A number of parasites owe at least part of their pathogenicity to their ability to induce apoptosis. For example, immediately upon making contact, the trophozoites of *Entamoeba histolytica* induce apoptosis in intestinal epithelial cells (Becker *et al*., 2010). This almost certainly contributes to the virulence of the parasites and facilitates invasion. Cerebral malaria is probably facilitated by the apoptosis of endothelial cells, which leads to the disruption of the blood–brain barrier (Pino *et al*., 2005). This programmed cell death can also be induced by metazoan parasites. For example, the cysticerci of the tapeworm *Taenia crassiceps* release a substance that induces apoptosis of germ cells within the seminiferous tubules of male mice (Zepada *et al*., 2011). Apoptosis is a normal physiological process within the testis as a means of controlling germ cell numbers but infection with *Taenia crassiceps* vastly increases its occurrence. So many cells die that large numbers of macrophages infiltrate the seminiferous tubules to dispose of the dead cells. Although sufficient germ cells die to compromise male fertility, this would not be considered an example of parasitic castration because it only occurs when a large number of cysticercoids are present. Apoptosis is also a feature of pathology induced by many other parasites including *Trypanosoma brucei*, *Trypanosoma cruzi*, and *Toxoplasma gondii* (Bienvenu *et al*., 2010; James and Green, 2004). Apart from being induced by substances released by parasites, the process can also be a feature of the host immune response. For example, if a cell infected with an intracellular parasite undergoes apoptosis, it prevents the parasite from reproducing and may kill the parasite. Intracellular parasites therefore often have physiological mechanisms that control or prevent or apoptosis of the cells they are living in. For example, *Leishmania donovani* inhibits macrophage apoptosis (Moore and Matlashewski, 1994) and *Theileria parva*-infected lymphocytes lose the ability to undergo apoptosis.

7.4.5 Calcification

Calcification refers to the extracellular deposition of calcium salts in tissues other than bone. This may occur as a consequence of metabolic disorders such as those affecting the levels of parathyroid hormone or following chronic infections when calcium salts are deposited in injured tissues. In the latter situation it is sometimes referred to as dystrophic calcification and it is stimulated by the presence of dead and decaying (necrotic) tissue. The consequences of calcification depend upon its extent and where it occurs. Within lymph nodes, calcification may have no functional consequences but if it occurs within the valves of arteries, it can impede blood flow. The tissue-dwelling cysts of larval tapeworms found in humans and other mammals often have calcium salts

deposited around them. The calcification facilitates the identification of hydatid cysts (*Echinococcus granulosus*) and the cyticerci of *Taenia solium* in human patients using imaging techniques such as ultrasound, CT (computerised tomography), and MRI scans (magnetic resonance imaging). Similarly, by cutting through specific sites and feeling for gritty deposits of calcium, meat hygiene inspectors can detect the presence of *Taenia saginata* cysts in the heart muscle of cattle or those of *Taenia solium* in the masticatory muscles of pigs. Other tissue-dwelling parasites such as *Trichinella spiralis* larvae and *Anisakis* spp. larvae can become calcified, while calcification can occur in the bile ducts and liver as a consequence of infection with *Fasciola hepatica* and *Fasciola gigantica*.

7.4.6 Cancer

Usually, once a stimulus that is causing cells to divide is withdrawn, cell division stops. Sometimes, however, the stimulus causes permanent genetic change in the affected cells and they cease to respond to the normal processes that control cell growth. These 'uncontrolled' cells are referred to as having become 'neoplastic' and they give rise to a lump or ramifying mass of cells called a neoplasm. In common language, the cells are said to have become 'cancerous'. The term cancer is derived from the Latin *cancer* which means 'a crab' because the cancerous region was once thought to spread through the body in a series of 'pincer-like' movements. There are numerous reasons why some cells become neoplastic and genetic constitution is often important in determining susceptibility and disease outcome. Similarly, there are a wide variety of neoplastic diseases which vary from the relatively benign that have a good prognosis to the highly malignant that can be rapidly fatal. The International Agency for Research on Cancer (IARC) has devised a scheme in which stimuli (agents) are grouped according to their potential to induce neoplasia in humans (http://monographs.iarc.fr/ENG/Classification/index.php). Many parasites affect cell division and differentiation through constant mechanical irritation, inducing immune responses and/or secreting noxious chemicals but relatively few of them have been implicated in inducing neoplastic diseases (Table 7.4).

One of the best-studied examples of parasite-induced neoplasia is the transformation of *Theileria parva*- infected lymphocytes in cattle into rapidly multiplying cells. This results in them behaving like lymphoblastomas (neoplastic cells) and causing the development of leukaemia-like pathology. Indeed, *Theileria parva*-induced lymphocyte transformation is a potentially useful

Table 7.4 IARC classification of the ability of agents to induce cancer in humans (as of 2010)

Group	Ability to cause neoplasia in humans	Number of agents listed in 2010	Parasite example
1	Definitely	107	*Schistosoma haematobium* *Chlonorchis sinensis* *Opisthorchis viverrini*
2a	Probably	58	
2b	Possibly	249	*Schistosoma japonicum*
3	Not classifiable	512	*Schistosoma mansoni* *Opisthorchis felineus*
4	Probably not	1	

model for studying the development of naturally-arising lymphoblastomas in humans. Tumours may form in the brain, kidneys and other organs which can then invade surrounding tissues and/or metastasise to other regions. The exact mechanism by which transformation is brought about is uncertain but *Theileria parva* induces its host lymphocyte to over-express a variety of regulatory kinases such as jun-NH_2-terminal kinase, Src family kinases, and casein kinase II (Chaussepied and Langsley, 1996). Casein kinase II is involved in a wide variety of cell functions and has also been implicated in cell transformation, the development of tumours and protection of transformed cells from apoptosis (reviewed by Duncan and Litchfield, 2008).

The transformation of a cell, whether by oncogenes or viruses, brings about the activation of the cell's apoptotic mechanisms. Consequently, the transformed cell can only survive if it is able to avoid these mechanisms. In the case of *Theileria*-infected lymphocytes, the presence of the living parasite is necessary to maintain the transformed state and should the parasite die For example, following drug treatment, the lymphocyte reverts to its normal resting phenotype and then dies through apoptosis. One mechanism by which the transformed state is maintained is because *Theileria* induces the activation of c-Jun NH_2-terminal kinases 1 and 2 (JNK1, JNK2) which bring about the phosphorylation, and thereby activation, of c-Jun. Both activation of the JNK enzyme pathway and induction of the c-Jun (that forms part of AP-1 early response transcription factor) help prevent apoptosis in the transformed lymphocytes (Lizundia *et al*., 2006). In addition, *Theileria* brings about the activation of the transcription factor NF-κB within transformed lymphocytes and this, among other things, brings about the formation of inhibitors of caspase family proteases that are involved in apoptosis (Heussler *et al*., 1999). Consequently, the death of the parasite results in a decline in the activity of the JNK enzyme pathway, the inhibition of c-Jun and NF-κB, and these events together with a decline in other anti-apoptotic proteins, makes the cell susceptible to apoptosis.

The trematode parasites *Clonorchis sinensis* and *Opisthorchis viverrini* both infect the bile ducts of humans and are recognised as important in the development of cholangicarcinoma (Young *et al*., 2010). Although rare, this is a highly malignant neoplasia of the intrahepatic bile duct and most patients die within 6 months of diagnosis. As yet, the mechanism by which the parasites induce neoplasia is not known but, as in most cancers, it is probably result of a combination of factors. In sheep, the flukes *Fasciola hepatica*, *Fasciola gigantica*, and *Dicrocoelium dendriticum* also cause serious damage to bile ducts and an inflammatory condition called hyperplastic cholangitis – which is also a feature of human infections with *Clonorchis sinensis* and *Opisthorchis viverrini* – although these ovine flukes are not usually associated with causing cancer.

There are suggestions that infection with the nematode *Anisakis simplex* can predispose a person to develop gastric cancer but the evidence for this remains limited (Audicana and Kennedy, 2008). The schistosomes that infect humans vary in their tendency to induce neoplasms although the reasons for this are uncertain. *Schistosoma haemotobium* infection has a proven association with bladder cancer and this is the major cause of cancer in Egyptian males and second most common neoplasm in Egyptian women after breast cancer (Mostafa *et al*., 1999). The mechanism by which *Schistosoma haematobium* induces neoplasia is uncertain. Although the inflammatory response to *Schistosoma haematobium* results in the formation of N-nitrosamines, reactive oxygen radicals and a variety of other toxic chemicals that can damage DNA, the same is also true of the other Schistosome species and a variety of other parasites (Mostafa *et al*., 1999). Interestingly, patients infected with *Schistosoma haematobium* tend to pass higher levels of bacteria such as *Klebsiella* spp., *Staphylococcus aureus*, and *Escherichia coli* in their urine than those who are uninfected (e.g. Adeyeba and Ojeaga, 2003). It is possible that bacteria facilitate the induction

of neoplasia through the formation of carcinogenic compounds (Mostafa *et al*., 1999) but, as yet, there is little conclusive evidence of their association with bladder cancer (Abol-enein, 2008).

7.4.7 Castration

Castration refers to the prevention of an organism's ability to reproduce and may be permanent (e.g. through the removal of the sexual organs) or temporary (e.g. transient loss of function of the sexual organs). A number of parasite species can damage the genitalia sufficient to prevent reproduction (e.g. *Wuchereria bancrofti*) but these typically involve many individual parasites. 'Parasitic castration' is restricted to specific host: parasite relationships in which a single individual parasite limits or prevents host reproduction. Most instances of parasitic castration involve parasites of invertebrates although there are a few cases of it occurring in fish and amphibians (Baudoin, 1975). The parasitic castrators tend to be very host-specific and are much larger in proportion to the size of their host than other parasites. Usually a single individual parasite weighs considerably less than 1% of its host's body mass but parasitic castrators typically weigh over 1% and may reach up to 39% (Hechinger *et al*., 2008). Most cases of parasitic castration appear to arise through the removal of nutrients by the parasite although it is possible that this is supplemented to a greater or lesser extent by the secretion of substances that interfere with the development of the host's gonads. By comparison, there are far fewer recorded instances in which the parasites physically consume the gonads or mechanically prevented them from functioning (Baudoin, 1975).

In domestic animals and humans, if castration occurs while growth is still taking place, it can lead to an increase in body size because metabolic reserves that would have been devoted to reproduction are used to enhance growth. In some parasite: host relationships, parasitic castration can lead to increased growth and this is termed 'gigantism'. Presumably, this is facilitated if the host is able to acquire and metabolise more resources than the parasite requires for its own purposes. It is most likely to occur during the early stages of infection when the host is actively growing but the parasite is still relatively small. Gigantism is most commonly associated with parasitic infections of invertebrates and it is particularly well-known in snail: trematode parasite associations (Chapuis, 2009). There are far fewer recorded instances of parasitic castration-associated gigantism in vertebrates, although three-spined sticklebacks (*Gasterosteus aculeatus*) infected with the cestode *Schistocephalus solidus* grow faster and have better body condition than those that are uninfected (Arnott *et al*., 2000). The host does not benefit from gigantism since is unable to reproduce. By contrast, the parasite benefits because its host is better able to compete for food and has a longer life span – and this would enhance the parasite's reproductive output (Ebert *et al.,* 2004). It is highly likely that parasites that induce gigantism are able to avoid or subvert the host immune system because one would expect a large and otherwise healthy individual to be capable of mounting an effective immune response against infections.

Whether or not parasitic castration leads to gigantism, castration benefits the parasite because it means that the host does not divert energy to its own reproductive efforts. However, this has a serious implication for the population dynamics of the host species, since infected individuals continue to feed and occupy space but do not leave any offspring. Consequently, where there is a high population of parasitic castrators, there is selection for early reproduction in the host species (Ebert *et al*., 2004). This ensures that the host is able to leave some offspring before the castrator completely inhibits its reproductive potential. In some instances, should the castrator die

before its host, then it is possible that the host might regain some reproductive potential provided its reproductive organs were not completely destroyed by the parasite. As might be expected, destruction or inactivity of the reproductive organs is often accompanied by physiological and behavioural changes such as feminisation in males.

An interesting example of parasitic castration of vertebrates is the relationship between the crustacean isopod parasite *Anilocra apogonae* and the five-lined cardinal fish *Cheilodipterus quinquelineatus* (Fogelman *et al*., 2009). *Anilocra apogonae* does not live in the genitalia and is actually an ectoparasite that attaches itself at a specific site immediately behind the fish host's head. Surprisingly, it does not appear to induce a localised inflammatory response or make the infected fish susceptible to secondary infections. Nevertheless, infected fish are smaller in size than those that are uninfected and that suggests that the parasitism represents a considerable metabolic burden. Infected female fish develop small gonads and produce few eggs while infected males fail to mouth-brood – which indicates that they are not successful in either mating or attracting a mate. Whether or not *Anilocra apogone* acts as a castrator through extracting so many metabolites that the fish are unable to develop their gonads – or the parasite secretes chemicals that interfere with gonad development – is uncertain.

7.4.8 Delusional parasitosis

The mistaken belief that was one is suffering from a parasitic infection has been described in many societies around the world. The affected person often refers to mites, lice, or insects crawling around under their skin. It is a recognised psychological disorder and is referred to as 'delusional parasitosis' or 'Ekbom Syndrome'. It can occur in men and women of all ages but is more common in middle-aged or elderly women (Trabert, 1995). It may be the primary psychological disturbance or secondary to another psychological condition (e.g. schizophrenia, drug abuse) or illness (e.g. dementia, brain tumours). Very often the patient persistently approaches a dermatologist before being referred to a psychiatrist. However, many patients refuse such referrals. Although it is sometimes said to be rare, a survey of 144 UK dermatologists found that 56% of them had seen at least one case of delusional parasitosis in the previous 5 years (Driscoll *et al*., 1993). The patient is often frustrated and exhausted because 'nobody believes them' and they cannot 'get rid of the parasites'. Many patients suffering from delusional parasitosis present the doctor treating them with the so-called 'matchbox-sign'. This is where the patient turns up with a small container, piece of sticky tape or other trapping device that is alleged to house the 'parasites'. They are never convinced if they are told that 'there's nothing there' or 'it's just a piece of dust, hair, etc'. There is always that a risk that a patient could harm themselves through excessive use of insecticides and other chemicals to kill the 'parasites' or through physical attempts to dig them out. The condition is very difficult to treat and has considerable adverse impact on the patients' work, family, and social life (Boggild *et al*., 2010).

7.4.9 Diarrhoea

Large volumes of fluid enter the digestive tract every day via the consumption of food and water and via the body's own secretions (e.g. saliva, pancreatic fluid). In adult humans, about 9 litres of fluid enter the small intestine every day but about 90% of this is absorbed and much of the

remainder is absorbed in the large intestine. Consequently, only about 100–200 ml of water is lost with formed faeces. In the case of diarrhoea, however, there is production of excessive watery faeces. This is a major cause of morbidity and mortality in humans and domestic animals and can be caused by a large number of infectious and non-infectious agents. Diarrhoea caused by infections such as parasites is often accompanied by fever and the consequent sweating leads to the loss of more water and electrolytes. Numerous parasites cause diarrhoea, including not only the obvious gastrointestinal parasites such as *Entamoeba histolytica*, *Giardia duodenalis*, and *Strongyloides stercoralis* but also blood parasites such as *Leishmania donovani* and *Trypanosoma brucei gambiense*. The loss of too much fluid over too short a period of time results in a drop in the blood volume (hypovolaemia) and this means that the heart's ability to keep the brain supplied with oxygen and nutrients is compromised. In addition, the losses in electrolytes (particularly sodium, potassium, chloride, and bicarbonate ions) can disturb metabolic processes. Babies and small animals are particularly vulnerable to the effects of diarrhoea because the fluid and electrolyte losses are greater as a proportion of their size compared to adults and larger animals.

There are two principal causes of diarrhoea: secretory diarrhoea and osmotic diarrhoea. Both types may occur at the same time. Secretory diarrhoea results from a continued or excessive secretion of water and electrolytes (especially chloride ions) into the small intestine that is coupled with a reduction in the absorption of sodium ions. This results in a net loss of water and electrolytes. Osmotic diarrhoea results from the presence of an osmotically active substance in the gut that causes the movement of water and electrolytes from the extracellular fluid into the gut.

The consequences of diarrhoea vary depending upon the amounts of fluids and electrolytes that are lost (Table 7.5). Isotonic dehydration is the most common form of diarrhoea-associated dehydration and it occurs when water and sodium ions are lost in the same proportion as they occur in the extracellular fluid. Consequently, there is no change in the serum sodium ion concentration, but hypovolaemia occurs owing to the loss of fluid volume. In humans, once the fluid deficit approaches 5% of body weight, the physical effects of dehydration become apparent: there is thirst, the mucous membranes start to dry out, and the heart beat increases. As the fluid deficit approaches 10%, the patient may start to lose consciousness and the signs of potentially fatal hypovolaemic

Table 7.5 Metabolic consequences of diarrhoea

Consequence of diarrhoea	Cause	Features
Isotonic dehydration	Loss of water and sodium ions are in the same proportion to their occurrence in extracellular fluid	Hypovolaemia
Hypertonic (hypernatraemic) dehydration	Loss of water exceeds the loss of sodium ions	Increase in serum sodium ion concentration Seizures may develop
Hypotonic (hyponatraemic) dehydration	Loss of sodium ions exceeds the loss of water	Decrease in sodium ion concentration. Seizures may develop
Metabolic acidosis (base-deficient acidosis)	Excessive loss of bicarbonate ions	Extracellular fluid becomes more acidic. Breathing rate increases, vomiting
Potassium depletion	Excessive loss of potassium ions	Decrease in serum potassium ion concentration. Weakness, paralysis, erratic heart beat

shock appear. Hypertonic dehydration, sometimes referred to as hypernatraemic dehydration, occurs when the losses of water and sodium ions are not in the same proportion to their occurrence in extracellular fluid: more water is lost than sodium ions. Consequently, there is a rise in serum sodium ion concentration. This results in an extreme thirst that is greater than would be expected from the level of apparent dehydration. The reverse situation occurs in hypotonic dehydration in which more sodium ions are lost than water. In this situation there is a fall in the serum sodium ion concentration, which is associated with a risk of seizures. Metabolic acidosis usually occurs when the diarrhoea is accompanied with damage to the kidneys. In this situation the kidneys lose their ability to replace the bicarbonate ions lost. This may occur due to the pathogen damaging renal function or as a consequence of hypovolaemia restricting the blood supply to the kidneys. Because the serum bicarbonate ion concentration starts to fall, there is a reduction in the blood pH. Potassium depletion, or hypokalaemia, is a common feature of diarrhoea and is particularly a problem in malnourished children who are often already potassium-deficient. Provided the loss of potassium ions is accompanied by the loss of sufficient bicarbonate ions it is possible that the symptoms of hypokalaemia will not develop. This is because the loss of bicarbonate ions brings about a reduction in the pH in the extracellular fluid as a consequence of the rise in hydrogen ion concentrations. The body attempts to buffer this by exchanging extracellular hydrogen ions with intracellular potassium ions and therefore the serum potassium ion concentration may not change, despite the loss of potassium ions in the diarrhoea.

If the serum potassium concentration declines, it can have serious consequences. Many cells, but especially the excitable cells (nerves and muscles), rely on the balance of ions between the inside and outside of their cell membranes in order to function properly. For example, disturbing the ion balance can interfere with the ability of nerve cells to repolarise after transmitting action potentials. Hypokalemia can therefore result in muscle weakness, cramping, problems with breathing, and, in extreme cases the heart beat becomes erratic, paralysis occurs and the patient may die (Williams *et al*., 2008b). Hypokalaemia is a known risk factor associated with a number of gastrointestinal parasite infections (e.g. *Isospora* spp., *Strongyloides stercoralis*) in which there are non-specific symptoms such as profuse watery diarrhoea (not usually bloody), fever, vomiting, and weight loss (e.g. Kane *et al*., 1984). When disturbances in electrolyte balance such as hypokalaemia are accompanied by hypovolaemia. the situation is potentially lethal – and this is typically what happens in AIDS patients afflicted with cryptosporidiosis (Willcox, 1997).

7.4.10 Elephantiasis

Elephantiasis refers to the gross swelling of the extremities or genitalia. Typically, it is associated with lymphoedema that accompanies infections with certain filarial nematode infections (e.g. *Wuchereria bancrofti*). The nematodes block the drainage of lymph which therefore accumulates in the blocked vessels and dependent region. Lymph is the means by which proteins and interstitial cells are transported back to the general circulation. Consequently, lymphatic oedema has much higher protein content than oedema resulting from the accumulation of serum (e.g. 'bottlejaw') and this may provide a stimulus for fibrogenesis. In elephantiasis induced by *Wuchereria bancrofti*, the overlying skin becomes thickened and warty owing to the formation of fibrotic tissue (Figure 7.1).

The term elephantiasis is also used in conjunction with other medical conditions that result in gross swelling. For example, the bacterium *Chlamydia trachomatis* serovars L1, L2, and L3 can

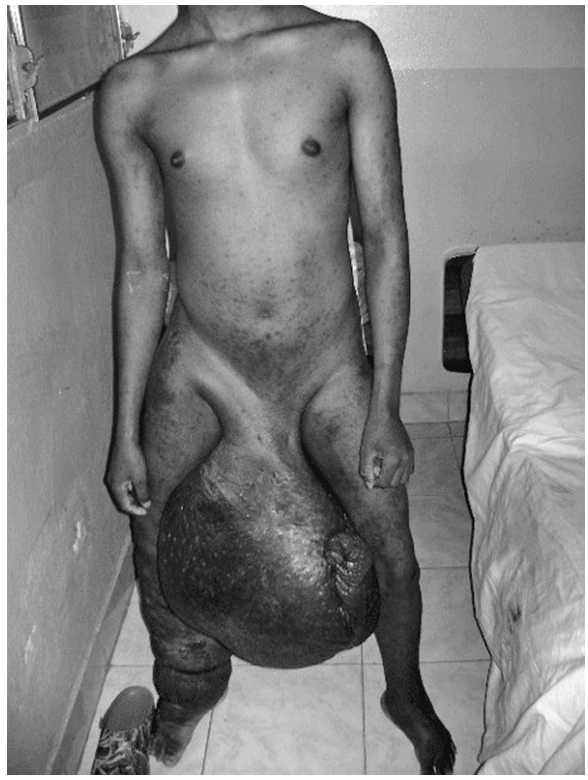

Figure 7.1 Elephantiasis of the scrotum and right leg in a 20-year-old man living in Haiti. The man became increasingly incapacitated over a five-year period and by the time this photo was taken, the scrotum weighed about 55 kg and prevented him from walking. From Jeudy, 2010

cause a condition known as *lymphogranuloma venerum* that can result in lymphatic obstruction that then results in genital elephantiasis. Podoconiosis can cause similar swelling to the feet and lower limbs as lymphatic filariasis and is known as 'endemic non-filarial elephantiasis'. In this case, however, the causative agent is not an infectious agent but the result of walking barefoot on irritant alkaline clay soils (Davey *et al*., 2007). Mineral particles within the soil enter the circulation and are phagocytosed by macrophages within the lymphatic system. Subsequently, there is an intense proliferation of macrophages, blockage of the lymphatic system and fibrosis. Unlike filarial elephantiasis, podoconiosis is usually limited to the feet and lower leg rather than the whole limb and genital involvement is rare. The congenital condition neurofibromatosis type 1 can result in extensive hypertrophy of the skin, soft tissues, and underlying skeleton. This may manifest itself in the gross enlargement of a whole extremity and is known as 'elephantiasis neuromatosa'.

7.4.11 Fever

Fever is a common non-specific reaction of the body to infectious organisms, functioning at least in part to increase the rate of metabolic reactions important in host defences. The cytokine 'tumour

necrosis factor-alpha' (TNF-α) has an important role in the development of fever. TNF-α is produced mainly by macrophages and, among a variety of effects, causes the destruction of tumours (hence the name), the migration of neutrophils and macrophages towards sites of inflammation, stimulates the killing of microbes, and acts as a pyrogen to induce fever. Clinical studies of patients naturally infected with *Plasmodium vivax* have demonstrated that there is a rise and fall in their levels of circulating TNF-α that correlate with the changes in body temperature. There is a time gap between initial infection and the onset of fevers because the first few cycles of erythrocytic schizogony do not produce enough toxins to generate a TNF-α response sufficient to raise the body temperature. The threshold that will cause a response varies between individuals and those who are immunologically naïve for malaria may exhibit fevers at very low parasitaemias (e.g. 0.001%) – by contrast, those living in malaria endemic regions may have a parasitaemia as high as 15% without developing a fever.

Patients suffering from malaria typically exhibit periodic fevers (paroxysms) every 48 or 72 hours depending on the species of *Plasmodium* responsible. Somewhat confusingly, these time intervals are given the terms 'tertian' and 'quartan' respectively. The reason is historical. The Ancient Greeks recognised the periodic nature of the fevers associated with malaria but did not know the cause and their medical writings subsequently influenced the Romans. The Romans referred to the first day of an event as day 1 and therefore 48 hours after day 1 would be day 3 and hence the term 'tertian', while 72 hours later would be day 4 and be termed 'quartan'. Intermittent fevers are also a feature of other parasitic diseases such as visceral leishmaniasis and the early stages of Human African Trypanosomiasis. It is therefore essential to obtain a definitive diagnosis if effective treatment is to be given. It should also be remembered that *Leishmania*-malaria and *Trypanosoma*-malaria co-infections occur.

Plasmodium falciparum, *Plasmodium vivax*, and *Plasmodium ovale* cause tertian malaria, i.e. the fever occurs on day 1 and day 3 while *Plasmodium malariae* causes quartan malaria in which the fever occurs on days 1 and 4. In the case of *Plasmodium knowlsei*, a new generation of merozoites is produced every 24 hours and therefore the fevers are said to be quotidian, that is, they take place daily. The time gaps are correlated with the maturation of a generation of merozoites and the rupture of the red blood cells that contain them. In *Plasmodium vivax*, the cyclic periodicity with which erythrocytic merozoites are produced is very pronounced but in *Plasmodium falciparum* it can vary between strains. Fever is probably stimulated by the waste products of the parasites released when the erythrocytes break up. Release of these malarial toxins into the bloodstream triggers a burst of TNF-α from activated macrophages. Induction of fever is among the effects of overproduction of TNF-α, and TNF-α toxicity can account for many of the other symptoms of malaria, such as nausea, muscle pain, headache, and loss of appetite.

7.4.12 Granulation and fibrosis

Granulation is a pathological term which refers to the process by which a foreign object that is proving difficult to eliminate is walled off by a surrounding mass of immune cells. The object and the cells that come to surround it are then called a granuloma (Figure 7.2). The granulation process is associated with a Th2 immune response and is marked by the arrival of large numbers of alternatively-activated macrophages. Alternatively-activated macrophages are those that are activated by interleukin-4 and interleukin-13. After arriving, the macrophages transform into non-motile epitheloid cells and these surround the target. The granuloma may also include other

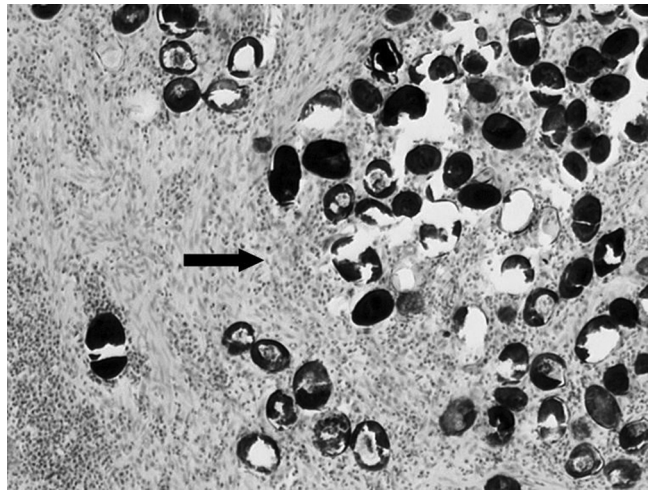

Figure 7.2 Granulomatous reaction (arrow) surrounding schistosome eggs

cell types, such as Langhans giant cells, lymphocytes, eosinophils, and fibroblasts. Inflammatory granulomatous responses are a common reaction to many parasitic infections. Examples include the granulation of liver abscesses caused by *Entamoeba histolytica* and the granulomas that form around *Schistosoma* eggs and the tissue dwelling stages of nematodes and tapeworms. Although the 'intention' of the granulomatous response is to isolate the parasite, it may not stop harmful antigens 'leaking out' and it also may be detrimental to the host. For example, the filarial nematode *Setaria tundra* induces granulomatous peritonitis in reindeer *Rangifer tarandus tarandus* which has led to the deaths of thousands of reindeer in many Arctic regions in recent years. This parasite is currently extending its range, possibly as a consequence of global warming, and is now considered a major threat to reindeer herding (Laaksonen and Oksanen, 2010). Within the liver abscess granuloma caused by *Entamoeba histolytica* trophozoites, the parasites produce secretions that prevent macrophages from functioning normally. For example, the suppressed macrophages do not respond to IFN-γ stimulation, they do not produce interleukin-12 and other cytokines, and they do not express nitric oxide synthase – and hence form nitric oxide. Consequently, the suppressed macrophages are unable to kill the trophozoites (Wang *et al*., 1994).

The schistosome species that infect humans vary in their locations and the seriousness of the disease they cause. In addition, much of our knowledge concerning the immune response to schistosomes is derived from mouse models. Consequently, it cannot be assumed that the immune response to all schistosomes in humans will be exactly the same. However, serious disease is, in most cases, associated with the immune reaction to the parasite eggs. The eggs usually pass through blood vessel walls and tissues into the urine (*Schistosoma haematobium*) or faeces (*Schistosoma mansoni* and *Schistosoma japonicum*) and are then excreted by the host. However, some eggs become trapped in the walls of the bladder and the intestine or are swept to the liver and other body tissues. The newly-laid egg of a schistosome is not immunogenic and it is only when the miracidium inside starts to develop and releases lytic and antigenic secretions that it engenders an immune reaction. This typically begins 5–6 days after being laid by the female worm. Within a previously uninfected host, the onset of egg-laying is characterised by an acute inflammatory

response that reaches a maximum after about 8 weeks and is then down-regulated as the disease progresses to the chronic stage of infection. There is a Th2 type immune response to the egg antigens in which there is enhanced production of interleukin-4, interleukin-5, interleukin-10, and interleukin-13. Interleukin-4 is the most important cytokine driving the formation of the granulomas while interleukin-10 modulates the immune response. In the absence of interleukin-10, there is an excessive Th2 response that results in more severe pathology (Burke *et al*., 2009). The levels of interleukin-4 determine the size of the granulomas and this, together with their number and location, has implications for the pathology. This is because the granulomas are many times the size of the eggs and therefore if there are numerous granulomas, they can cause swelling of the affected organ and obstruction. *Schistosoma japonicum* lays its eggs in batches and the presence of several eggs in close proximity results in the formation of large granulomas and hence serious pathology. By contrast, *Schistosoma mansoni* tends to cause less serious pathology (than *Schistosoma japonicum*) because it lays its eggs singly and therefore gives rise to smaller more dispersed granulomas. A miracidium can remain alive within its egg for at least 7 days after it is surrounded by a granuloma (Andrade, 2009). However, schistosome eggs are not 'designed' to survive unhatched for any length of time and the miracidium will ultimately die of 'old age' even if the immune response is not successful in killing it. The importance of the Th2 response, and in particular the levels of interleukin-4 in determining the pathology, has led to suggestions that tipping the Th1: Th2 balance in favour of the Th1 response might lessen pathology even if the worms remained present and continued producing eggs (James and Colley, 2001). In women, granulomas that develop within the genital tract cause serious pathology. This can be manifested as disruption to normal menstruation, preterm labour, and infertility (Nour, 2010).

In hosts chronically infected with schistosomes, the immune reaction is eventually down-regulated and this limits the granulomatous response – and hence the pathology. At this point fibrosis is initiated. Fibrosis is the formation of excessive connective tissue and is typically associated with the repair of wounds and scar formation. The immune system is involved in the development of fibrosis and interleukin-13 has a key role through activating fibrogenic pathways and up-regulating collagen production (Burke *et al*., 2009). Fibrosis is a common feature of many parasitic infections and, if severe, it can interfere with normal organ function. For example, the healing of amoebic ulcers in the gut is associated with the formation of fibrotic lesions that impair peristalsis. Similarly, fibrosis in the liver as a consequence of *Entamoeba histolytica* or schistosome infections can interfere with circulation because normal tissue is replaced by non-functioning connective tissue.

7.4.13 Hyperplasia

Cell growth is under the control of various chemical growth factors that interact with specific cell surface receptors. Stimulation of these receptors triggers various internal cell signalling pathways and influences the way in which the cell develops and divides. Anything that interferes with this process can result in altered growth patterns such as hyperplasia, hypertrophy, and metaplasia. Hyperplasia refers to an increase in the number of cells owing to an increase in the rate of cell division. This can arise from a variety of causes such as chronic irritation that gives rise to callouses on the skin and as a response to toxins (e.g. cholera toxin A1 subunit causes thyroid hyperplasia). The hyperplasia may not occur equally throughout a tissue and localised 'hot spots' can give rise to the formation of nodules between areas of normal tissue. Many infections, including parasitic

diseases, also cause hyperplasia. For example, in sheep, chronic fascioliasis (*Fasciola hepatica*) causes hyperplastic cholangitis. The epithelial cells lining the bile ducts undergo hyperplasia and are thrown into a series of folds while the term cholangitis indicates that the bile ducts are inflamed. Hyperplastic cholangitis can restrict the flow of bile through the bile ducts and in serious cases the flow may be stopped entirely. In addition, the pathological consequences of infection are exacerbated by the loss of plasma proteins that leak across the deformed mucosa. In humans, hyperplastic cholangitis is often associated with bacterial infections – the bacteria having travelled up the bile duct from the lumen of the gut. Because adult *Fasciola hepatica* interfere with the normal flow of bile and damage the lining of the bile ducts, it is therefore possible they facilitate the establishment of bacterial infections. The bacteria would then contribute to or exacerbate the development of hyperplastic cholangitis (Valero *et al*., 2006). Obviously, hyperplasia can only occur in tissues that have the capacity to divide, and is consequently not a feature of tissues comprised of non-dividing cells such as nerve cells and cardiac muscle.

7.4.14 Hypertrophy

Hypertrophy refers to an increase in cell size and functional capacity. The increase in size is brought about by an increase in protein synthesis that results in an increase in the cell's structural components. Cells that are unable to divide often respond to an increased functional demand by undergoing hypertrophy. For example, the increase in muscle bulk exhibited by body-builders is largely the result of an increase in the size of the skeletal muscle cells. Parasitic infections can also cause an increase in cell size although it is seldom beneficial to the host. For example, *Trypanosoma cruzi* induces hypertrophy of cardiac muscle cells (cardiomyocytes). Both hypertrophy and hyperplasia may occur independently or together depending upon the cell type and stimulus.

7.4.15 Inflammation and ulceration

The earliest record of the four cardinal signs of inflammation is contained within the treatise *De re Medicina* (literally 'Concerning Medical Matters') compiled by Aulus Cornelius Celsus sometime around the start of the first century AD. In Book 3 of this eight-volume treatise he discusses fever in some detail and his sentence '*Notae vero inflammationis sunt quatuor, rubor & tumor, cum colore & dolore'* continues to be repeated in medical textbooks – although usually in translation – 'Indeed the signs of inflammation are four, redness and swelling, with heat and pain' (Major, 1954). The redness and heat of the acute inflammatory response result from the increased blood supply to the affected region and the dilation of the surrounding blood vessels. The swelling results from the accumulation of fluid exudates. Pain results from a combination of chemicals released during the inflammatory process and also pressure from swelling affecting sensitive nerve endings. Tumour necrosis factor-α, interleukin-1 and several other cytokines are known to influence pain perception. In terms of pathology, there are two broad categories of inflammation: acute inflammation in response to the initial cell damage and chronic inflammation in response to a damaging stimulus that cannot be resolved. Acute inflammation is induced in response to tissue damage and the death of cells and the physiological basis is discussed in detail in Chapter 6. An acute inflammatory response is therefore commonly associated with parasites that attempt to physically penetrate their

host and when they start to feed or release harmful chemicals. For example, when the larvae of the hookworm *Ancylostoma braziliense* attempt to invade humans, they produce serpentine (wavy, snake-like) tracks underneath the skin at the dermal-epidermal boundary. The tracks extend at up to 2 cm a day and may reach 20 cm in length and 2–4 mm in width and the condition is known as *cutaneous larva migrans*. The burrowing larvae cause physical damage and release protease enzymes that initiate an acute inflammatory response in which eosinophils figure heavily. The region around the tracks can become inflamed, red, and swollen. The tracks also cause intense itching (pruritis) and can be secondarily infected with the bacteria such as *Streptococcus pyogenes* that leads to cellulitis. Cellulitis is a serious inflammation of the dermis and subcutaneous layers of the skin and should never be confused with the development of cellulite (skin dimpling) which is an obsession in the fashion media. *Cutaneous larva migrans* is also caused by a number of other parasites although the form and development of the tracks vary between species (Meinking *et al*., 2003).

Although the inflammatory response is meant to prevent pathogens from establishing themselves within their host, it can also be the means by which they gain access. For example, experimental studies indicate that, as might be expected, if the gut wall has already been damaged by pathogenic bacteria, then it becomes more vulnerable to invasion by *Entamoeba histolytica*. However, it is also apparent that amoebae which ingest bacteria such as *Shigella dysenteriae* (a common cause of severe diarrhoea) are more likely to bind to and ingest gut epithelial cells. This is because the bacteria stimulate the trophozoites to increase their production of a lectin (galactose/N-acetyl D-galactosamine-inhibitable adherence lectin – usually abbreviated to Gal/GalNAc) on the surface of their cell wall that enables them to bind to gut epithelial cells. In addition, they are also stimulated to produce more cysteine peptidase enzymes. These enzymes are released by the amoebae into the surrounding medium and they may bring about a localised increase in the concentration of activated host-derived pro-inflammatory cytokines (Galvan-Moroyoqui *et al*., 2008). This host inflammatory response is probably important in facilitating the invasion of the parasite. Because pathogenic enteric bacteria and *Entamoeba histolytica* are commonly found in poor communities that lack adequate sanitation and safe drinking water, it is possible that their interaction could be an important factor in whether or not a person develops amoebic dysentery and if so, the severity of the infection.

The progression from an acute inflammatory response to chronic inflammation is exemplified in the manner in which trophozoites of *Entamoeba histolytica* invade the liver. The parasites that reach the liver induce an acute inflammatory response that results in the influx of large number of neutrophils. As in the intestine, the neutrophils are both protective in that they kill the parasites but also a source of pathology because the inflammatory response also kills many liver cells. The parasites also experience another immune response in which invariant Natural Killer T cells (iNKT cells) are vitally important. Indeed, mice that lack the ability to mount an effective iNKT response develop larger liver abscesses in response to *Entamoeba histolytica* infection (Lotter *et al*., 2009).The iNKT cells are stimulated by lipopeptidophosphoglycan (LPPG) in the parasite cell membrane and in response they rapidly release a range of cytokines such as gamma interferon (IFN-γ) which promotes inflammation. For example, IFN-γ activates macrophages and stimulates them to produce inducible nitric oxide synthase (iNOS) and hence the secretion of nitric oxide – which is toxic to the trophozoites. Unlike many other activated iNKT cells, those that are activated by *Entamoeba histolytica* LPPG do not release interleukin 4 (IL-4) which is anti-inflammatory and promotes a Th2 response (Lotter *et al*., 2009). If the acute inflammatory response does not kill all the trophozoites, then purulent abscesses are formed. With time, a Th2 immune response starts

to predominate and a chronic inflammatory response is established. This represents a balance between ongoing tissue damage and the processes of healing and scar formation. During this process the abscesses become granulated and fibrous scar formation takes place.

7.4.16 Jaundice

Jaundice results from disruptions to the normal metabolism and excretion of bilirubin. Bilirubin is a breakdown product of the blood pigment haemoglobin and is produced in the spleen, liver, and bone marrow. If there is excessive breakdown of haemoglobin or the breakdown products cannot be excreted, then there is a rise in the levels of free or conjugated bilirubin in the circulation. This is manifested as yellowing of the skin and the whites of the eyes (sclera). An increase in the level of free bilirubin (unconjugated hyperbilirubinaemia) results from the generation of excessive amounts of bilirubin. This can occur following the destruction of large numbers of red blood cells such as happens during malaria. By contrast, conjugated bilirubin is produced by the liver cells (hepatocytes) and a rise in the levels of conjugated bilirubin in the circulation results from the secretion of bile being disrupted. It can, however, also result from a destruction of liver cells. For example, chronic fascioliasis leads to blockage of the bile ducts and thereby obstructs the flow of bile into the duodenum. Within the gut, bilirubin is converted into urobilinogen by bacteria and it is urobilinogen that imparts the brown colour to faeces. Conjugated hyperbilirubinaemia is therefore characterised by the excretion of pale or even white faeces. It also results in problems with fat metabolism because bile is important for their digestion and the urine becomes dark because conjugated bilirubin (unlike free bilirubin) is water-soluble and can therefore be excreted in the urine. It can also cause pruritis since the bile salts may be deposited in the skin where they cause intense itching.

7.4.17 Metaplasia

Metaplasia refers to the transformation of one fully differentiated tissue type into another that may be equally or more differentiated. For example, in response to chronic irritation, mucous-secreting epithelium can be replaced by squamous epithelium (squamous metaplasia) or fibrous tissue is replaced by bone (osseous metaplasia). Squamous epithelial cells are simple scale-like cells found on the outer surface of many tissues. Squamous metaplasia is often accompanied by hyperplasia so the squamous cells exhibit hyperplasia and the outer surface of the tissue is therefore thrown into a series of folds. For example, *Schistosoma haematobium* induces squamous metaplasia within the urinary bladder and this is considered to be a precancerous lesion.

7.4.18 Pressure atrophy

Atrophy occurs where there is a decrease in the size of cells and consequently a reduction in the size of the affected organ or tissue. In relation to parasitic diseases, pressure atrophy often occurs when the size of the growing parasite physically restricts the flow of blood/nerve impulses reaching a tissue or organ. Pressure atrophy is therefore a common pathological feature of parasites such as the larval stages of those tapeworms that form large cysts. For example, the

pressure exerted by the coenurus of the ovine parasite *Taenia multiceps* kills surrounding brain cells by a combination of restricting their blood supply and physically pressing them to death. Some idea of the pressure being exerted by the growing coenurus can be obtained by considering that it is sufficient to cause thinning of the overlying skull. And sheep skulls are notoriously thick! Similarly, the hydatid cysts of *Echinococcus granulosus* can grow to a large size and will cause pressure atrophy on the surrounding tissues. The consequences of this will depend upon the size of the cyst and its location. For example, in humans a large cyst growing within the liver or spleen can induce pressure atrophy on the diaphragm. Ultimately, the diaphragm may rupture and the cyst can then prolapse into the pleural cavity (von Sinner and Stridbeck, 1992).

7.4.19 Psychological disturbance

We often consider parasitic diseases solely from the physical harm they inflict. However, many of them also affect our mental well-being. This may be because they directly or indirectly affect our brain or as a consequence of the drugs used to treat them. For example, *Toxoplasma gondii* has been linked with the development of schizophrenia while the drug Lariam (mefloquine) used to treat malaria has become notorious for its side effect of inducing adverse neuropsychiatric disturbances such as anxiety and paranoia in some people (Croft, 2007). Much less appreciated are the ways in which being infected by parasites makes us feel about ourselves and the ways in which people interact with us. People who suffer from parasites often say that they feel 'unclean' and this is soon translated into a sense of unworthiness. This is because in the popular imagination, a lack of cleanliness is associated with poverty and poverty is associated with a lack of intelligence. For example, in England it has been customary to refer to someone who has done something stupid as a 'nit'. A 'nit' is also the term for the egg cases of head lice and having hair 'infested' with nits was once considered a sign of poverty – and hence stupidity (see Colour Plate 23). We now know that head lice will breed well on the heads of the cleanest children who live in the most affluent of households. However, this does not reduce the sense of anguish engendered in parents when they discover head louse infections in their children. Interestingly, there is increasing evidence that parasitic diseases can retard the mental development of schoolchildren. For example, the intense itching caused by scabies and louse infections makes it difficult to pay attention in school while gastrointestinal helminth infections reduce cognitive development through affecting the child's nutritional balance (Kvalsvig *et al*., 1991). Furthermore, for people of all ages, parasitic infections can prove painful and this makes it difficult to relax and disturbs sleep. Sleep deprivation soon leads to exhaustion, an inability to control emotions, irrationality, and confusion. Parasitic infections also result in the production of inflammatory cytokines and there is increasing evidence that the levels of some these, such as TNF-α, correlate with depressive illness (Dowlati *et al*., 2010).

Human African Trypanosomiasis is commonly known as 'sleeping sickness' because of the way it disturbs the normal patterns of waking and sleeping. This is a consequence of the disease altering the circadian secretion of the hormones prolactin, renin, growth hormone, and cortisol (Brun *et al*., 2010). In addition, the sleep process itself is disturbed. Normally, sleep consists of a series of cycles, each of which consist of five stages. During stages 1–4 our sleep becomes progressively deeper until we enter the fifth stage that is known as 'rapid eye movement' (REM). During the REM stage our brain activity increases and sleep is lighter and it is thought that this is the period during which we experience dreams. After the REM stage we return to

'stage 1 and the cycle is repeated. Interestingly, during 'sleeping sickness' the sleep pattern is altered and REM periods tend to occur at the start of the sleep cycle rather than the end (Buguet *et al*., 2005).

Stage 2 Human African Trypanosomiasis is associated with a range of psychiatric and mental disturbances. Sometimes the changes in behaviour are so subtle that they are only recognisable to friends and close family members. However, some patients can become violent, have uncontrolled sexual impulses, suffer from hallucinations, and attempt suicide. Psychotic behaviour is said to be particularly common in patients of European heritage which suggests that genetic factors are important in the way the disease progresses. Incorrect diagnosis of the condition among immigrants to Europe has resulted in patients being admitted to psychiatric clinics (Brun *et al*., 2010). Even if the patient is correctly diagnosed and successfully treated, he/she may suffer long-term mental impairment and physical disability (e.g. paralysis) as a result of the brain pathology. Another unusual feature of Human African Trypanosomiasis is disruption of sensory perception resulting in a condition known as 'hyperaesthesia' in which even a mild touch is experienced as extremely unpleasant. This may be the reason many patients complain of intolerable 'itching'.

Parasites that cause outwardly obvious deformities such as elephantiasis, cutaneous leishmaniasis, and crusted scabies frequently lead to feelings of low self-worth, and depression; affected people tend to become withdrawn and avoid contact with other members of the community. These feelings are reinforced by our ancestral tendency to avoid people who are obviously ill or different from us. Even within supposedly educated and affluent westernised societies, people who suffer physical deformities frequently experience public comment and ridicule when they venture outside their homes.

7.5 Damage to specific organs

It is not intended to provide a comprehensive coverage of the pathogenesis of parasitic diseases in all the different organs of the body because this would make this volume much too long. Indeed, a whole textbook has been written just covering parasitic infections of the human eye (Kean *et al*., 1991). Instead, we will summarise how parasites cause pathology in some of the principal organs and explain how this can result in morbidity and mortality.

7.5.1 The bladder

In humans, the most serious parasite-induced pathology that afflicts the bladder results from infection with *Schistosoma haematobium*. This parasite actually lives in the veins that serve the bladder, pelvic organs, and rectum. As is the case with other schistosome species, the adult worms cause relatively little damage but the eggs induce a dramatic immune reaction that results in the formation of granulomas and fibrosis. In the case of *Schistosoma haematobium* this gives rise to a condition known as 'urogenital schistosomiasis' although the eggs can be transported elsewhere such as the liver and lungs and cause pathology there. Within the bladder, the granulomatous reactions and deposition of fibrotic tissue can restrict the flow of urine and this in turn can give rise to secondary inflammatory reactions in the bladder, ureters, and kidneys. This makes urination painful (dysuria) and blood is often passed in the urine (haematuria). Indeed, urogenital schistosomiasis was once so common in Egypt that the passing of blood by young boys was considered

a natural event and equivalent to the onset of menstruation in girls. There is also the risk of developing cancer.

Only a few parasite species actually live in the bladder. The nematode *Capillaria plica* infects the bladder and sometimes the pelvis of the kidney of dogs and other canines. Cats are occasionally infected by *Capillaria plica* although they also have 'their own' species *Capillaria feliscati* (though this may be a synonym for *Capillaria plica*). Some taxonomists consider *Capillaria plica* and *Capillaria feliscati* should be moved from the genus *Capillaria* to form their own genus '*Pearsonema*'. Unfortunately, *Capillaria plica* is sometimes referred to as 'the bladder worm' with the consequent opportunity for causing confusion with the more general term 'bladderworm' which is commonly used to describe the larval stage of tapeworms. The adult nematodes live in the submucosa of the bladder and ureters where they induce a mild inflammatory reaction and oedema. *Capillaria plica* is not usually considered pathogenic although it can cause frequent urination and urinary incontinence and has been linked to the development of the inflammatory condition 'cystitis'. Most cases of *Capillaria plica*-associated cystitis arise in conjunction with infections of the bladder with gut bacteria such as *Escherichia coli*, *Enterobacter* spp., and *Klebsiella* spp.. The damage which the worms cause to the lining of the bladder, either directly or through inducing inflammation, probably makes it easier for the bacteria to invade. However, in heavy worm infections, cystitis can occur in the apparent absence of bacteria (Fernández-Aguilar *et al*., 2010).

7.5.2 The brain

The brain is protected from most pathogens by the blood–brain barrier. However, a number of pathogenic microorganisms are capable of breaching this barrier. Owing to the importance of the brain in controlling the rest of the body, these infections can prove rapidly fatal. For example, cerebral malaria is a common complication of infection with *Plasmodium falciparum* and may account for 10% of *Plasmodium falciparum* malaria cases admitted to hospital and 80% of such deaths. Before the introduction of HAART therapy, up to 30% of *Toxoplasma gondii* seropositive HIV-infected patients subsequently developed toxoplasmic encephalitis – usually with fatal consequences. The most common parasitic disease that affects the central nervous system is neurocysticercosis caused by larvae of the tapeworm *Taenia solium*. Neurocysticercosis causes seizures and, depending upon the location and number of the cysts, can prove fatal.

Cerebral malaria may be gradual in onset but it is more commonly sudden. A progressive headache may be followed by a coma, an uncontrollable rise in temperature to $>42^{\circ}$C, and psychotic symptoms or convulsions, especially in children. Death may ensue within a matter of hours. Initial stages of cerebral malaria are easily mistaken for a variety of other conditions, including meningitis and acute alcoholism, usually with disastrous consequences.

The sequence of events that lead to the symptoms of cerebral malaria remains controversial but it is considered that in *Plasmodium falciparum* malaria it derives from the sequestration of parasitised erythrocytes (i.e. they are effectively withdrawn from the circulation) in small blood vessels within the brain (cerebral microvasculature), where they cause obstruction, local induction of inflammatory cytokines, or both. The infected cells become 'sequestered' because the parasites produce proteins that become attached to the red blood cell membrane where they cause protrusions called 'knobs' (Nagao *et al*., 2000). Knob formation begins during the early trophozoite stage of parasite development and results in the infected red blood cell becoming 'sticky' (see Colour Plate 1). This is because proteins in the knobs undergo receptor-ligand binding to

uninfected red blood cells and also to glycoproteins such as CD36 and thrombospondin that are found on the endothelial lining of blood vessels. The endothelium is a layer of flat epithelial cells that lines the inner surface of blood vessels. Obstructing the flow of blood to specific areas of the brain would result in localised stagnant hypoxia (lack of oxygen owing to the blood flow stopping) coupled with a reduction in the supply of glucose and the rate at which waste products, such as CO_2, are removed. Because brain cells are highly metabolically active, even transient reductions in the supply of oxygen can rapidly result in their death, the consequences of which would depend upon the part of the brain affected. Because anaemia is one of the consequences of malaria, there are fewer fully-functional blood cells in the circulation, thereby reducing its oxygen-carrying capacity – this could exacerbate the effects of sequestration of red blood cells.

Tumour necrosis factor-alpha (TNF-α) is probably involved in the pathology of cerebral malaria because it up-regulates endothelial molecules such as intercellular-adhesion molecule-1 that, along with other receptors, increases the binding of infected erythocytes to the walls of blood vessels. Clinically, there is sometimes rapid recovery from profound coma without significant resultant neurological problems, implying that a reversible process contributes to the coma. This would be an unlikely occurrence if the coma resulted from stagnant hypoxia. It should be noted that some patients suffering from cerebral malaria do experience serious neurological complications upon recovery. For example, of the children who survive, 5% have permanent neurological defects, such as mental blindness. It has been suggested that excess TNF-α causes the release of nitric oxide (NO) from vascular tissues in the brain and this diffuses into the surrounding nervous tissue where it temporarily disrupts neuronal transmission (Clark and Rockett, 1996). However, experimental studies have found that nitric oxide synthase-deficient mice can still develop cerebral malaria even though they are incapable of producing nitric oxide (Favre *et al*., 1999). Alternatively, TNF-α may act directly on the mitochondria of the brain cells and interfere with their ability to undertake oxidative phosphorylation (Clark *et al*., 2006). The consequences of this would be effectively the same as the stagnant hypoxia theory because mitochondria could no longer make the ATP necessary to fuel the cell's activities and this would result in the death of the brain cells.

Plasmodium vivax malaria is usually considered to be more benign than *Plasmodium falciparum* malaria although there are reports of new forms emerging in different parts of the world that cause serious, potentially fatal disease (Genton *et al*., 2008; Tjitra *et al*., 2008). Like *Plasmodium falciparum* malaria, *Plasmodium vivax* malaria has recently been found to cause cerebral malaria and serious anaemia (Beg *et al*., 2008; Rodriguez-Morales *et al*., 2008). Not only is this of concern from a medical point of view, but it also poses a question about how the pathology is caused. This is because *Plasmodium vivax* has not previously been considered to induce sequestering of blood cells within blood vessels in the brain and therefore gives weight to the suggestion that TNF-α-induced pathology may be involved (Clark, 2009). Co-infections of *Plasmodium vivax* and *Plasmodium falciparum* are not uncommon and the disease course tends to be more severe than in single species infections (Barcus *et al*., 2008; Tjitra *et al*., 2008).

When *Trypanosoma brucei* parasites invade the brain, they cause meningoencephalitis and disrupt the normal functioning of the blood-brain barrier. This is accompanied by demyelination of nerve axons and a marked rise in the number of the astrocytes (astrocytosis). Astrocytes are glial cells that play an important role in nervous transmission but do not themselves generate or conduct action potentials. Astrocytosis is a feature of many brain pathologies and is probably connected to the repair process. The parasites release chemicals that induce apoptosis in the vascular epithelial cells and microglial cells within the brain, that is, the affected cells 'commit suicide'. This is particularly marked in the regions of the cerebellum and brain stem (Stiles *et al*., 2004). Apoptosis, or programmed cell death, is a normal part of cell ageing and organ development when

it is a controlled process. Apoptosis induced by chemicals released by pathogens such as *Trypanosoma brucei* is uncontrolled and can lead to serious pathologies. In the case of trypanosomes, procyclin and procyclin derivatives have been shown to induce apoptosis. Patients with stage 2 Human African Trypanosomiasis (sleeping sickness) often exhibit changes in behaviour, they suffer from seizures, their sleep pattern is disturbed and they become confused, disorientated and ultimately lose their ability to move, become comatose and die. The cerebellum is involved in the coordination (but not initiation) of movement, attention, mental imagery, some aspects of learning, as well as emotions and use of language. Damage to the vascular epithelium will interfere with the blood supply to the brain cells in the cerebellum (and elsewhere in the brain) and this, coupled with damage to the nerve cells themselves, could explain at least some of the symptoms observed in stage 2 Human African Trypanosomiasis. Similarly, the brain stem is responsible for the control of vital life functions, such as heart beat, blood pressure, and breathing, so damage to this region could have fatal consequences. Microglial cells engulf and destroy invading microorganisms within the central nervous system and also remove cell debris. The loss of microglial cells could therefore reduce the immune response against trypanosomes. *Trypanosoma brucei rhodesiense* Human African Trypanosomiasis begins in a similar manner but the course of the disease is usually much more rapid. As a rule, the patient develops severe fevers and swelling of the lymph glands and they lose a lot of weight. They usually die before expressing the behavioural changes, and disrupted sleeping patterns that are characteristic of late stage *Trypanosoma brucei gambiense* Human African Trypanosomiasis.

The encystment of *Toxoplasma gondii* in the brain of a healthy human seldom causes significant pathology, but if their immune system becomes compromised (e.g. due to AIDS), then the parasites start to replicate and this gives rise to focal lesions (abscesses) (see Colour Plate 24). The consequences depend upon the location of the lesions, their size, and number. The lesions destroy brain cells and also interfere with the blood supply to other regions of the brain. The patient starts to complain of headaches and then develops fever, suffers from seizures, and may die. Neurological conditions can also develop in people who are not immuno-compromised (Carme *et al*., 2009) and retinochoroiditis (inflammation of the retina and the underlying choroid layer) is a relatively common feature of *Toxoplasma gondii* infections acquired after birth (Kean *et al*., 1991). There is a report of higher *Toxoplasma* seroprevalence in epileptics than non-epileptics but there is as yet no evidence of a causal relationship (Critchley *et al*., 2008).

The cysticerci of *Taenia solium* (see Colour Plates 25, 26) can develop in various regions of the brain and spinal cord and the pathology varies somewhat dependent upon where they occur. While they are still immature, the cysticerci cause little host reaction although if they become large, they can cause pressure atrophy on the surrounding brain cells. The lack of a host immune response is because the cysticerci have a battery of immune avoidance mechanisms that subverts complement activation and cytokine production. They are even able to absorb the antibodies formed to destroy them and metabolise these as a source of amino acids. However, with time (sometimes after several years) the host immune response breaks through the parasite's defences and the cysticerci start to degenerate. At this point there is a marked inflammatory reaction against the cysticerci and mononuclear cells invade the cyst wall and penetrate into the cyst fluid. It is this host inflammatory reaction which is thought to be predominantly responsible for the seizures and other pathology associated with neurocysticercosis. The sudden onset of epilepsy in adults who have had no previous history of the condition is an indication that neurocysticercosis should be considered as a possible cause. Inflammatory reactions against cysticerci developing within the ventricles (ventricular neurocysticercosis) are accompanied by the development of ependymitis granularis and fibrillary

astrocytosis. Ependymitis granularis refers to an inflammation of the ependymal cells that causes a patchy loss of the ependymal cells that line the ventricles and maintain the flow of cerebrospinal fluid. Fibrillary astrocytes are a type of glial cell located in the white matter and they proliferate in response to injury in a similar way to fibroblasts elsewhere in the body. Their multiplication is sometimes called astrogliosis or simply gliosis. These inflammatory reactions cause the degenerating cysticerci to stick to the walls of the ventricles and this, together with the damage to the ependymal cells, interferes with the flow of cerebrospinal fluid and there is a build-up of cerebrospinal fluid in the intracranial cavity – the end result is a condition known as hydrocephalus (White, 2000). The development of hydrocephalus results in a rise in intracranial pressure that in prolonged cases may be sufficient to cause localised thinning of the bones of the skull. The rise in pressure also interferes with nervous transmission and causes the death of nerve cells – which in turn results in gliosis. The consequences depend upon the region of the brain affected and the speed and severity of the increase in intracranial pressure. Typically there are seizures, difficulty in walking, and incontinence.

Taenia solium cysticerci that start to degenerate in the basal cistern can induce a condition known as arachnoiditis. The basal cistern is a fluid-filled cavity between the *arachnoid mater* and *pia mater* situated at the back of the mid-brain. It is also known as the interpeduncular cistern. The *arachnoid mater* is one of the three meninges (protective tissue membranes) that surround the central nervous system (the other two meninges are the *pia mater* and *dura mater*). Arachnoiditis therefore refers to an inflammation of one of the meninges and it can be extremely painful and debilitating. The consequences depend upon the region of the arachnoid mater affected and the extent of the inflammatory damage. For example, movement, bladder, and bowel control can all be affected and it may even lead to impotence. The inflammatory reactions can also cause obstruction of the flow of cerebrospinal fluid and the development of hydrocephalus. Inflammatory damage to surrounding blood vessels can also occur – this is known as vasculitis – and if the veins rupture, it can result in a stroke (Kimura-Hayama *et al*., 2010).

7.5.3 The digestive system

The gastrointestinal tract extends from the lips to the anus and encompasses a variety of distinct morphological and physiological regions, each of which is associated with a particular guild of parasites. However, much of the pathology caused by parasites results from them physically damaging the walls of the tract, blocking the secretion of substances, blocking the absorption of metabolites and/or blocking the movement of food through the gastrointestinal tract. The gut contains numerous bacteria and other microbes and the pathology caused by gastrointestinal parasites is often facilitated or exacerbated by interactions with them.

Physical damage to the walls of the tract results from invasion or feeding on the cells lining the gut. For example, in parts of the Middle East a condition in humans known as ‘halzoun’ (parasitic pharyngitis) can develop, in which parasites attach to the pharynx where their feeding causes pain and bleeding as a result of physical damage and the acute inflammatory response. Some reports ascribe the condition to adult *Fasciola hepatica* acquired through eating raw or poorly cooked liver containing the parasites, while there also cases in which the developmental stages of the pentastome (Crustacea) parasite *Linguatulla serrata* have been found to be responsible (Schacher *et al*., 1969). Similarly, within the small intestine, adult hookworms cause lesions and bleeding while the invasive trophozoites of *Entamoeba histolytica* cause flask-shaped ulcers in the colon.

The pathology associated with the nematode parasite of cattle *Ostertagia ostertagi* provides an interesting example of how damage to one region of the intestinal tract can have implications for the physiology elsewhere in the body. After being ingested, the infective larvae of *Ostertagia ostertagi* exsheath and develop within the lumen of abomasal glands and once they become adults they emerge and live on the surface of the abomasum. (The abomasum is the ruminant equivalent of the human stomach and the abomasal glands perform a similar function to the gastric glands – that is they secrete mucus from mucus cells, pepsinogen from chief cells and hydrochloric acid from parietal cells.) The developing larvae damage the parietal cells and induce the mucus cells to undergo hyperplasia. The emergence of the adult worms is associated with a reduction in the secretion of hydrochloric acid and a massive rise in the secretion of the hormone gastrin into the blood. The reduced secretion of hydrochloric acid means that the pH of the abomasum rises from about pH 2 to pH 7 and therefore proteins are no longer denatured and thereby rendered easier for intestinal proteases to metabolise. In addition, pepsinogen is not converted into active pepsin, and viruses, bacteria and other potential pathogens that would normally have been killed by the low pH are now able to survive and pass on to the small intestine. The damage to the epithelium of the abomasum caused by a combination of the parasite and the acute inflammatory response also renders it more permeable, so pepsinogen leaks into the circulation and plasma proteins leak into the lumen of the gut. If the damage is extensive, there can be substantial loss of plasma proteins and this is reflected in low serum albumen levels (hypoalbuminaemia). There is some uncertainty whether the physiological changes following *Ostertagia ostertagi* infection represent an adaptation by the parasite to exploit its host or an attempt by the host to limit the damage the parasite causes. For example, by reducing the acidity of the abomasum, the parasite could be creating a less hostile environment in which to live. However, raising the levels of gastrin in the circulation not only stimulates gastric secretions but it also stimulates the formation of new parietal cells and a new mucosa (Simpson, 2000). Nevertheless, in serious infections the affected animal loses considerable amounts of protein from a combination of the losses of serum proteins and an inability to fully exploit the bacterial protein from the rumen (most microbes passing from the rumen to the abomasum would normally be killed by the acidic pH and then digested). This is further compounded by anorexia and, as a result, muscle wastage occurs because the animal has to metabolise muscle protein in order to manufacture essential serum proteins.

Food is moved from the mouth to the anus by a wave of muscular contractions called peristalsis. The smooth muscles lining the gut have their own inherent rate of contraction and this is modulated by input from the autonomic nervous system. Parasites can interfere with these muscular contractions by increasing or decreasing their force and frequency or by stopping them entirely. For example, diarrhoea results in an increase in the force and frequency of contractions. If the gut lining is seriously irritated, it can induce the formation of an intussuception in which the gut telescopes back on itself. Once the leading edge has become trapped, it is referred to as an intussusceptum and peristalsis drives this further forward (in the wrong direction). The formation of an intussusception is extremely painful and can result in a potentially fatal blockage of the gut. It can also compress the blood supply to the intussusceptum and give rise to an infarction – which is also potentially fatal. An ‘infarction’ is the term used to describe where there is blockage of the blood supply (usually the arterial supply) to a region and this leads to coagulative necrosis in the affected tissues. In domestic horses the tapeworm *Anoplocephalala perfoliata* tends to attach itself at the ileocaecal junction where it induces the formation of ulcers. These ulcers are then thought to precipitate the formation of an intussusceptum – and this is manifested as ‘colic’. Colic is one of the leading causes of premature death in domestic horses – although it should be

considered as a sign rather than a clinical diagnosis and numerous factors have been shown or been suggested to be involved in its development. Theoretically any agent that causes localised enteritis or disruption of peristalsis could initiate an intussusceptum and other parasites, such as *Parascaris equorum*, have also been implicated, while the importance of *Anoplocephala perfoliata* is far from proven (Bell and Textor, 2010). In humans, intussusceptions also usually occur at the ileocaecal junction and are much more common in children than in adults. There are several descriptions of this condition being caused by the nematode *Anisakis* spp. but parasites are not considered to be a major risk factor (Miura *et al*., 2010). Excessively forceful peristaltic contractions in the rectal region can result in rectal prolapse in which the posterior region of the rectum everts from the anus. In humans, rectal prolapse is a known risk factor associated with heavy *Trichuris trichiura* infections.

High densities of large parasites can physically slow down and block the movement of digesta through the gut (see Colour Plate 27). This usually occurs in the small intestine because this is where the largest parasites tend to live. For example, large numbers of *Ascaris lumbricoides* or adult *Taenia* tapeworms can block the small intestine in humans while *Ascaris suum* can block the intestine of pigs and *Moniezia expansa* can block the intestine of sheep. Blockage can also result from extensive scarring taking place following previous damage to the gut wall, in which smooth muscle is replaced by non-contractile connective and fibrous tissues. For example, amoebic dysentery causes ulceration of the colon and when these ulcers heal, the extensive scarring can interfere with peristalsis.

Peristalsis can also be compromised through damage to the autonomic nerves that modulate its activity. This is a particular feature of the digestive form of Chagas disease that principally manifests itself as disturbances to the normal functions of the oesophagus and colon, though other regions of the gut may also be affected. In its more extreme forms this can result in the afflicted regions swelling enormously and irreversibly to form conditions such as megaoesophagus and megacolon. The pathology in the oesophagus typically results from damage to parasympathetic ganglia in the lower oesophagus. As a consequence the muscles that the nerves innervate receive a reduced and abnormal nervous input. This results in the muscles losing their tone and becoming flabby and thereby causes problems with peristalsis and failure of the lower oesophageal sphincter. In addition, there is an increase in muscle thickness within the oesophagus and inflammatory foci form along with the deposition of fibrous tissue. Over time, the oesophagus dilates and the patient becomes unable to swallow (dysphagia). Mega-oesophagus-associated Chagas disease can afflict people of all ages and may predispose patients to oesophageal cancer. Mega-colon develops in a similar manner and results from the destruction of the myenteric plexus of the colon. As a consequence, the muscles that the nerves innervate, become flabby, fibrous tissue is laid down and inflammatory foci are formed. The colon can swell enormously, peristalsis is impaired, and the afflicted region ceases to function effectively. The condition initially manifests itself as constipation but because it is principally found in middle-aged adults (30– 60 years old), it is often not diagnosed until well developed. This is because constipation is a non-specific symptom that is common among this age group and can result from a wide range of causes. As the condition worsens, the patient often suffers from cachexia (malnutrition resulting in weight loss) that may prove fatal.

Helminth infections have been implicated as a possible risk factor in the development of acute appendicitis in humans. Adult *Ascaris lumbricoides* are well known for their migratory tendency and there are occasional reports of where the anterior of the worm has become stuck within the entrance to the appendix. The worm then irritates the appendix and induces an inflammatory reaction or dies *in situ* and the decomposing worm causes inflammation. However, there is some

uncertainty over the extent to which helminths actually initiate potentially fatal appendicitis. For example, Aydin (2007) found *Enterobius vermicularis* in 3.15% of appendectomies but in none of these had acute inflammation occurred. By contrast, da Silva *et al*. (2007) recorded *Enterobius vermicularis* in 95.8% of appendectomies and there was evidence of acute inflammation in 50% of the worm-infected appendices.

7.5.4 The genitalia

Human parasitic diseases that afflict the genitalia are not only physically debilitating and painful but they often impose extra psychological burdens. For example, genital malformations can lead to the afflicted person being openly ridiculed or excluded from society and if they are not already married, then their chances of doing so are highly unlikely. Marriage is tremendously important in many traditional societies as it confirms one's status as part of the community and is often the only opportunity for sexual relationships. For those within a sexual relationship, genital malformation may make sex painful or even physically impossible and this in turn creates psychological problems. For example, people who are severely affected by genital elephantiasis can become withdrawn from society, depressed, incapable of finding work and may commit suicide (Dreyer *et al*., 1997). If the pathology induces open wounds to the genitalia, it can also increase the chances of both contracting and spreading sexually-transmitted diseases.

The filarial nematodes *Wuchereria bancrofti* and *Onchocerca volvulus* can both induce elephantiasis of the genitalia though the condition occurs much more frequently in men than women. *Wuchereria bancrofti* is responsible for the majority of cases of filarial nematode-induced genital pathology, while *Onchocerca volvulus* is particularly associated with causing a condition known as 'hanging groin' in which folds of skin containing lymph nodes droop down from the body. In men, *Wuchereria bancrofti* is normally described as causing 'elephantiasis' but in fact it causes a variety of distinct genital pathologies including hydrocoele, chylocoele, and, of course, elephantiasis of the scrotum (Richens, 2004). Hydrocoele arises from an accumulation of pale yellow serous fluid within the scrotal sack and this causes the scrotum to swell. The development of hydrocoele is very common in areas in which *Wuchereria bancrofti* is endemic. A chylocoele is somewhat similar to a hydrocoele except that the swelling results from an accumulation of milky lymphatic fluid. Scrotal elephantiasis develops as a consequence of the accumulation of lymph within the scrotum and this process is known as lymphoedema. Because the flow of lymph is compromised, it reduces the ability of the body to respond to infections in the affected area and allows the establishment of bacterial infections. These bacteria contribute to the pathology of elephantiasis by causing acute inflammatory reactions. There are also ongoing inflammatory reactions against the adult filarial worms and their *Wolbachia* symbionts although the microfilariae may be absent during the later stages of the disease. All of this inflammatory activity gives rise to the deposition of fibrous connective tissue and granulomatous tissue within the scrotum and this causes the skin to become thickened and susceptible to surface cracking. Any cracks are then invaded by bacteria and fungi that induce further acute inflammatory attacks. Treatment to prevent microbial infections is therefore highly beneficial in reducing the pathology associated with elephantiasis. The progression of the disease is thought to be heavily influenced by prenatal exposure to *Wuchereria bancrofti* antigens and also to exposure during childhood (Dreyer *et al*., 2000).

Long-standing infections with *Schistosoma haematobium* can give rise to elephantiasis of the penis in men, but genital pathology is usually much worse in women. All parts of the female

reproductive system can be affected by the inflammatory reactions against the trapped schistosome eggs. For example, damage to the uterus can result in disturbance of menstruation while damage to the placenta may result in abortion – usually in the second trimester. If the Fallopian tubes are affected, there is an increased risk of ectopic pregnancy while ulceration of the vagina and vulva is not only painful but the open lesions increase the risk of contracting and transmitting sexually-transmitted diseases such as HIV (Richens, 2004). The damage caused by schistosomiasis may not become apparent until many years after the initial exposure. Consequently, although it is not recommended by the WHO, there is an argument to be made for travellers to be screened for the parasite if they have a history of paddling or swimming in lakes where there is a high incidence of infection among the local people. Bailey *et al*. (2011) describe two cases of serious genital pathology owing to schistosomiasis in British women that was acquired at least eight years previously, probably through swimming in Lake Malawi while they were on holiday.

Relatively few parasites specifically reside within the genitalia – although there are exceptions such as *Trichomonas vaginalis*. In women, *Trichomonas vaginalis* causes a profuse yellow discharge, irritation, and inflammation of the vulva and vagina. Petechial lesions on the cervix can be so serious that the condition is known as 'strawberry cervix'. Infection rates in men can be high but it usually causes little pathology in them. The lack of pathology in men is probably owing to the higher zinc content in the male genital tract as well as unidentified components of the secretions from the prostate gland and seminal glands, that are toxic to *Trichomonas vaginalis* (Langley *et al*., 1987). Several parasite species that normally reside elsewhere in the body can be sexually transmitted (e.g. *Entamoeba histolytica*). These species are typically associated with inflammatory reactions and are almost invariably more pathogenic in females than in males. Within humans, owing to the proximity of the vagina and anus it is not unusual for some gastrointestinal parasites to cause gynaecological problems. For example, the pinworm *Enterobius vermicularis* has been linked to the development of vulvo-vaginitis and urinary tract infections in young girls. This is probably through initiating inflammatory reactions within the genital tract and also transporting bacteria such as *Escherichia coli* from the anus that then cause genital infections (Cook, 1994).

Parasites can also affect the genitalia through pathology affecting the endocrine system. For example, Human African Trypanosomiasis can result in temporary or permanent impotence in men. It can also cause a reduction in the size of the testes and fat becomes redistributed in a similar pattern to that of women. Consequently, men can develop a condition known as gynaecomastia in which they form prominent breast tissue. Infected women may also suffer from shrinkage of the sexual organs and they can develop menstrual problems and become infertile (Kennedy, 2007).

7.5.5 The kidney

Not many species of parasites specifically reside within the kidney. Of those that do, the best known are the nematodes, *Dioctophyma renale* that infects dogs, many carnivores, and occasionally humans, and *Stephanurus dentatus* that infects pigs. *Dioctophyma renale* is a remarkably large worm and the female can grow to over 1 metre in length and 1.2 cm in width. The infective larvae penetrate the gut wall and mature within the peritoneal cavity after which they invade the kidney. Despite their large size, the worms do not always induce clinical signs since they usually only infect one of the kidneys – mostly the right one. The feeding of the worms can result in the total destruction of the parenchyma of the infected organ but most animals are capable of surviving healthily with only one functional kidney. The damage to the tissues can result in red blood

cells being found in the urine (haematuria). The loss of kidney function can also interfere with the formation and release of urine to the bladder. The consequent retention of nitrogenous waste products such as urea within the blood ('uraemia') can result in a wide variety of problems elsewhere in the body. For example, uraemia is associated with calcium deposition in the skin that causes itchiness and discoloration, inflammation of the cardiac muscles, sleepiness, nausea, anorexia, nervous disorders, seizures, coma, and may be fatal. It also interferes with cellular immunity and therefore affects the response to many infectious agents.

Stephanurus dentatus has a much narrower host range than *Dioctophyma renale* and is usually restricted to pigs. The adult worms are fairly large – although much smaller than *Dioctophyma renale*: the female grows to about 4.5 cm in length and is only about 2 mm in width. The larvae of *Stephanurus dentatus* cause serious pathology to the liver and other body organs during their migration but the adult worms are not considered particularly pathogenic. The worms are more frequently found within the peri-renal fat rather than the kidney itself and they are usually found as pairs within cysts filled with green pus that may reach up to 4 cm in diameter. They can, however, cause chronic inflammatory reactions in the ureters that result in the deposition of so much fibrotic tissue that it almost completely blocks them.

Red blood cells infected with *Plasmodium falciparum* are 'sticky' and therefore become attached to the walls of capillaries within various organs of the body – the infected cells are therefore sometimes referred as having become 'sequestered in the microvasculature'. If large numbers of infected red blood cells are sequestered within the capillaries within the kidneys, the resultant blockage of the blood flow may result in the death of the oxygen and nutrient-deprived cells. The epithelial cells of the proximal and distal tubules are particularly sensitive to a lack of oxygen and their death causes acute tubular necrosis and this is manifested as acute renal failure which can be fatal. Acute renal failure is most commonly seen in adults and is rarely seen in children suffering from malaria. It is often associated with a reduction in production of urine – this is known as 'oliguria' – and a patient may pass less than 400 ml urine in 24 hours. In extreme cases, urine production may cease – this is known as 'anuria'. However, oliguria is not always a feature of malaria-induced acute renal failure and the infection is then referred to as 'non-oliguric'. In some parts of the world there have been increases in the incidence of acute renal failure associated with malaria and it often has a poor prognosis (Mehta *et al*., 2001). Acute renal failure can also be caused by other species of malaria such as *Plasmodium vivax* and it may be presented as a feature of multiple organ failure or on its own.

Malaria can also induce acute renal failure through a variety of other mechanisms such as a decrease in the volume of blood plasma (hypovolaemia) that arises through loss of body fluids owing to sweating and decreased fluid intake. In severe cases of malaria this can give rise to a condition known as hypovolaemic shock that results in a decreased supply of blood to the kidneys and consequent acute tubular necrosis. Severe cases of malaria are also associated with the destruction of large numbers of infected red blood cells within the spleen and general circulation. The rise in levels of haemoglobin and its breakdown products in the circulation can exceed the ability of the liver to metabolise them, leading to large amounts being excreted via the kidneys. As a consequence, the urine becomes dark and in conjunction with severe fever, jaundice, and vomiting, the effect is called 'Blackwater Fever'. The condition is extremely serious and is associated with a mortality rate of around 50%. It was once thought to be linked to the use of quinine to treat malaria, but this is now not thought to be the case as it also occurs where quinine has not been used. It is possibly owing to some form of autoimmune response that results in the rapid haemolysis of large numbers of red blood cells. It is not unusual for Blackwater Fever to be accompanied with oliguria and acute renal failure.

7.5.6 The liver

In humans and many other mammals, the liver is the largest internal organ and is vitally important in regulating the chemical composition of the blood, detoxifying metabolites, metabolising carbohydrates, lipids, and proteins and synthesising bile. The liver also contains fixed phagocytes called Kupffer cells that are embedded within the wall of the sinusoids and remove pathogens, cell debris and toxins from the circulation. Damage to the liver is therefore manifested as jaundice, a failure to synthesise essential molecules, and a failure to detoxify potentially harmful molecules. Liver function tests are used to diagnose liver damage by analysing the levels of liver-specific enzymes and metabolites in circulating blood. For example, a rise in alkaline phosphatase indicates damage to the biliary (bile) system while a rise in gamma-glutamyl transpeptidase is a non-specific indicator of liver cell (hepatocyte) damage.

Although outwardly the liver appears homogenous, the different regions have different metabolic properties and therefore the consequences of liver damage depend upon which group of hepatocytes that are affected. The products of digestion, and anything else that is able to pass through the gut wall, enter the capillaries and these drain into the hepatic portal vein which in turn gives rise to capillaries that flow through the liver. Consequently, any pathogens that succeed in penetrating the gut and entering the circulation are immediately swept to the liver. Once the blood has passed through the capillaries within the liver, it enters the hepatic vein and thence enters the general circulation. This flow arrangement of mesenteric capillaries, hepatic portal vein, hepatic capillaries, hepatic vein is known as the 'hepatic portal system'. Numerous parasites cause pathology in the liver in the process of migrating through its tissues (e.g. *Fasciola hepatica*), parasitising its cells (e.g. *Plasmodium vivax*), causing pressure atrophy (e.g. hydatid cysts of *Echinococcus granuloasus*) and by inducing harmful immune reactions (e.g. eggs of *Schistosoma mansoni*).

Clinically, there are four basic manifestations of liver pathology:

1. *Acute hepatitis* in which there is sudden onset of a massive inflammatory response. People or animals suffering from acute hepatitis are often tired, there is abdominal pain and there are signs of jaundice. Acute hepatitis typically results from pathogens or toxins that cause the death of numerous liver cells over a short period of time.
2. *Chronic hepatitis* results from long-standing inflammatory responses that cannot be resolved. There is abdominal pain but in humans jaundice may not occur until a late stage in the condition and is then considered a poor prognostic indicator.
3. *Fibrosis* results from ongoing chronic inflammation that results in functional hepatocytes being replaced by non-functional fibrotic tissues. Where fibrosis occurs within the bile ducts, it leads to the blockage of the flow of bile from the liver to the duodenum. This results in the faeces becoming pale or even white as a consequence of the haemoglobin breakdown product bilirubin no longer entering the digestive tract (conjugated hyperbilirubinaemia).
4. *Cirrhosis* is the end point of liver cell destruction in which there is extensive scarring and the liver vascular architecture is disrupted. This disrupts the flow of blood through the liver and leads to an increase in the blood pressure in the portal system. This condition is known as portal hypertension and can result in the development of splenomegaly and ascites.

Acute hepatitis is a common feature of the simultaneous migration of large numbers of parasites through the liver. For example, the young flukes of *Fasciola hepatica* in sheep or the movement

of *Ascaris suum* larvae in pigs. The movement of so many parasites causes widespread cell death, bleeding, necrosis, and an acute inflammatory response. This can lead to acute liver failure which is often rapidly fatal. Chronic hepatitis arises from long-standing infections and/or the accumulation of damage over a period of time. Extensive liver damage severely compromises liver function and may lead to potentially fatal cirrhosis. However, the liver has the ability to self-repair and, providing the damage is not too severe, it may not give rise to clinical disease. For example, dogs naturally infected with *Leishmania infantum* may exhibit evidence of damage from liver-function tests and post-mortem histology but show no clinical signs of liver pathology (Rallis *et al*., 2005). This emphasises the old legal saying that 'absence of evidence is not evidence of absence'.

The liver is a major site for the synthesis of biomolecules that are then released into the circulatory system to be utilised elsewhere in the body. For example, plasma albumin and most of the plasma globulins (apart from the immunoglobulins) are manufactured in the liver. Consequently, damage to the hepatocytes compromises the production of these chemicals. For example, in sheep, sub-acute fascioliasis causes a decline in the serum albumin concentration – this is known as hypoalbuminaemia. This reduction in serum protein concentration means that there is a decline in the oncotic pressure and therefore there is a reduction in the amount of water that is dragged into the blood from the surrounding tissues. Consequently, the amount of fluid within tissues starts to rise and this is manifested as the development of oedema (fluid accumulation). In sheep and cattle a common site of fluid accumulation is in the submandibular region and this condition is commonly known as 'bottle-jaw'. Fluid accumulation within the peritoneal cavity is known as ascites (Figure 7.3).

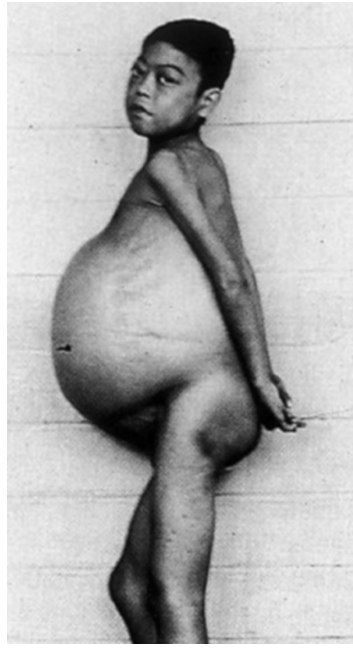

Figure 7.3 Development of ascites in a young boy as a consequence of schistosomiasis infection. Source: Kean *et al*., 1991

Adult *Schistosoma mansoni* and *Schistosoma japonicum* live in the mesenteric venules. Therefore the eggs which they release do not find their way directly to the lumen of gut and can be rapidly transported via the circulation to the liver. Within the liver the eggs become trapped in the intra-hepatic venules and here they generate a granulomatous reaction and the surrounding tissue becomes fibrotic. This is sometimes called 'pipestem fibrosis' or 'portal tract fibrosis' and it results in localised blockage of the flow of blood. As a consequence, the supply of venous blood from the spleen to the liver starts to be cut off and therefore the blood pressure within the blocked vessels rises: this gives rise to portal hypertension which in turn results in enlargement of the spleen (splenomegaly) and ascites.

The Kupffer cells found in the sinusoids are important in the pathology of several parasitic diseases. They are believed to be the portals via which *Plasmodium* sporozoites gain access to the hepatocytes (Baer *et al*., 2007). The sporozoites invade and traverse the Kupffer cells and then ultimately invade hepatocytes within which they undergo the exo-erythrocytic stages of development. The Kupffer cells are rendered insensitive to pro-inflammatory signals and the parasites induce them to undergo apoptosis. During the course of malaria the Kupffer cells become hyperplastic, probably in response to malaria toxins and the breakdown products from damaged red blood cells, and they also accumulate malaria pigment (haemozoin) granules that reduce their ability to exhibit phagocytosis. Owing to the importance of Kupffer cells in combating pathogens and clearing cell debris and toxins from the circulation, the loss of functional Kupffer cells can reduce the body's ability to deal with the malaria infection and there is also increased vulnerability to other infectious agents. In visceral leishmaniasis, the Kupffer cells, along with other mononuclear phagocytes, are invaded by the parasites. The liver becomes enlarged (hepatomegaly) owing to the accumulation of rapidly dividing parasites within the phagocytes. In addition, the enlargement and distortion of the infected cells disrupt the architecture of the liver and thereby compromise its function. In addition, leishmaniasis can also give rise to liver fibrosis and portal hypertension.

7.5.7 The lungs

Relatively few parasites are transmitted as air-borne infections and therefore most parasitic diseases arrive in the lungs via the circulation. For some of these parasite species, the lungs are merely another organ to be passed through during their migration. For example, for the gut helminths *Ascaris suum*, *Ascaris lumbricoides*, *Parascaris equorum*, *Strongyloides stercoralis*, and *Ancylostoma caninum*, the migrating larvae must break through into the lumen of the bronchioles, climb up the trachea until they reach the junction with the oesophagus and then move down the gastrointestinal tract to the small intestine where they will become adults. Small numbers of these parasites cause relatively little pathology but the movements of large numbers of parasites over a short period of time causes considerable damage, because the larvae must physically penetrate through the lung tissue. This results in focal haemorrhages and acute inflammation at the sites where the parasites damage the lungs. The destruction of the alveoli leads to the development of emphysema. Emphysema is a term for lung damage that can arise from a variety of causes and is characterised by a permanent enlargement of the air spaces distal to the terminal bronchioles and destruction of the alveoli. So-called 'generalised emphysema' does not involve the formation of scarring. However, the term 'emphysema' is also used to describe other forms of pathology in which scarring and fibrosis accompany the dilated air space. Obviously, scarring can be a feature of parasite-induced emphysema as the lungs attempt to repair the physical damage caused by the

parasites. Whatever the mechanism by which it is induced, emphysema results in breathing difficulties because of the loss of elastic recoil and the area available for gaseous exchange is reduced. Consequently, emphysema is characterised by an increase in the breathing rate in an attempt to increase the amount of carbon dioxide expelled (the rate and depth of ventilation are mainly controlled by the partial pressure of carbon dioxide in the blood). However, owing to the reduced surface area for gaseous exchange, this is not successful and affected people and animals soon become breathless and hypoxic (i.e. the oxygen supply is insufficient to meet cellular needs) when attempting to undertake exercise. In chronic cases in which worms migrate through the lungs over a prolonged period of time, there may be the formation of eosinophilic granulomas that also affect breathing through disrupting the alveoli: capillary interface and reducing the area available for gaseous exchange.

For some parasite species, the lungs are the site where they become adult and reproduce. Perhaps the best known of these are the nematode parasites known as the lungworms; these include species such as *Dictyocaulus viviparus* (cattle), *Dictyocaulus filaria* (sheep), *Aelurostrongylus abstrusus* (cats), and *Metastrongylus apri* (pigs). Humans do not generally suffer from nematode lungworm infections. A number of trematode parasites known as the lung flukes also become adults in the lungs such as *Paragonimus westermani* which is found in a wide range of mammals including humans.

The most economically important lungworm is *Dictyocaulus viviparus* and this will be used to illustrate how pathology within the lung can develop. Dictyocauliasis is primarily a disease of young cattle on their first season on grass since the initial infection generates a strong protective immunity. The severity of the disease depends upon the rate of intake of infectious larvae and develops through three distinct phases: pre-patent, patent, and post-patent. The pre-patent phase begins 8 to 25 days after the initial infection when the larvae start to leave the circulation and penetrate the alveoli. This induces bleeding and an acute inflammatory response in which inflammatory cells penetrate the lung epithelium and there is accumulation of fluid (pulmonary oedema) within the lungs and the development of interstitial emphysema. Between the blood capillaries and the alveoli is a fluid-filled 'interstitial space' or 'interstitium'. This facilitates close proximity between the two tissues with the minimal involvement of connective tissues or inflammatory cells. However, physical damage to this region caused by the parasites, coupled with the consequences of the inflammatory response, results in this delicate balance being disrupted. In addition, during the repair process, interstitial tissue replaces the normal alveoli and capillaries. The damage to alveoli and capillaries coupled with the deposition of interstitial tissue reduces lung elasticity and the area available for gas exchange – and hence emphysema develops. The development of pulmonary oedema is equally serious since it too reduces the area available for gaseous exchange and can lead to hypoxia and fatal respiratory failure. Assuming that the infection is not fatal, the migratory path of the worms is marked by the changing focus of the inflammatory response. Initially, there is alveolitis as the migrating larvae break through into the air spaces and this is then followed by bronchiolitis and bronchitis as the worms move to bronchi where they moult to become adults.

The arrival of the adult *Dictyocaulus viviparus* at the bronchi at around days 26–60 marks the start of the patent period. The worms cause epithelial cells lining the bronchi to undergo hyperplasia and produce excessive amounts of mucus which then becomes mixed with air so that the bronchi become filled with a white froth. This induces intense coughing as the infected animals attempt to clear their blocked-up lungs. In the UK, farmers know the disease as 'husk' as a consequence of the characteristic harsh coughing it causes. The coughed-up mucus results in discharges from the mouth and nose, and the animal suffers from anorexia, malnutrition and

develops a rough coat. When the worms commence reproduction, they produce eggs that hatch almost immediately to release first-stage larvae. The larvae do not feed and are coughed up and then swallowed and pass out with the faeces. However, some of the eggs and larvae are swept down into the alveoli where they induce aspiration pneumonia. This is characterised by the development of an intense inflammatory response and the alveoli become filled with inflammatory exudates.

Although much of the pathology associated with dictyocauliasis is associated with the host immune response, this usually results in the adult worms being expelled and a strong protective immunity to re-infection. This marks the onset of the post-patent period (around days 61–90) and is associated with the repair of the tissue damage. Lesions to the bronchi and bronchioles become fibrotic and therefore coughing may continue owing to impaired breathing but ultimately the tissue damage will usually be resolved and normal breathing returns. However, in about 25% of animals that suffer serious infections there is a return of clinical symptoms of respiratory distress and these may prove fatal. The main cause appears to be hyperplasia of type 2 pneumocytes (alveolar cells). There are two types of pneumocyte found in association with walls of the alveoli: type 1 and type 2. They are both epithelial cells; the type 1 pneumocytes are squamous epithelial cells that line the alveolar wall and are the principal sites of gaseous exchange. The type 2 pneumocytes (also called septal cells) are rounded or cuboidal in shape and located between type 1 pneumocytes. The type 2 pneumocytes secrete surfactants that lower alveolar surface tension and thereby reduce the likelihood of the alveoli collapsing. Hyperplasia of the type 2 pneumocytes produces a thickened layer of epithelial cells that is less permeable for gaseous exchange and it is accompanied by the development of interstitial emphysema and pulmonary oedema. This is sometimes referred to as ‘post-patent parasitic bronchitis’. It is not known what causes the type 2 pneumocytes to proliferate but it may be a response to chemicals released by dead or dying lungworms. In serious cases, squeaks and crackles can be heard in the posterior lobes of the lungs when the animal breathes and death often results within 24–96 hours of the onset of illness as a consequence of acute heart failure brought on by respiratory distress. Serious disease and death may also occur during the post-patent period as a consequence of secondary bacterial infections (e.g. *Pasteurella multocida*) established in the lesions caused by the lungworms and initiating bacterial pneumonia.

A number of parasites become established in the lungs through being swept there by the circulation and/or through growing into the lungs from surrounding tissues. For example, both *Entamoeba histolytica* and *Echinococcus granulosus* can form pulmonary infections either as a consequence of the trophozoites or oncospheres respectively reaching the lungs, via the blood or through extensions of abscess or cyst growth in the liver. *Entamoeba histolytica* establishes ulcers in the lungs in a similar manner to the liver and this is known as pulmonary amoebiasis. This can give rise to a dry cough, purulent, chocolate-coloured sputum and breathing difficulties. Most hydatid cysts develop in the liver but the lungs are the second most common site in both humans and other intermediate hosts. Pulmonary hydatid cysts principally cause problems when they become large and start to compress the surrounding lung tissue. This can lead to coughing, chest pain and haemoptysis (coughing up of blood from the respiratory tract). A growing hydatid cyst can erode the thoracic aorta and, where blood loss is severe, a patient can drown in their own blood. If the cyst ruptures into a bronchus or the pleural cavity, it is referred to as a ‘complicated cyst’ and the leakage of the cyst fluid can set up an allergic reaction. ‘Complicated’ pulmonary hydatid cysts can lead to the expectoration of cyst fluid, repetitive hemoptysis, and anaphylactic shock. Pulmonary hydatid cysts also facilitate the establishment of fungal infections such as *Aspergillus* spp. that typically colonise pre-existing lung cavities caused by tuberculosis and other diseases (Manzoor *et al*., 2008). Pulmonary *Aspergillus* infections are normally associated with

immuno-compromised people and the development of fungal hyphae inside the patient leads to necrosis, pneumonia and infarction through colonising the blood vessel walls.

7.5.8 The skin

The skin is the largest organ in the body and provides an effective barrier to most pathogens. It is also a highly complex ecosystem that contains a huge variety of micro-organisms, the composition of which varies between regions of the body and between individuals. This 'skin microbiome' has an important role to play in preventing infections and also in the development of the host's immune system (Grice and Segra, 2011). Much of the parasite-induced pathology associated with the skin is caused by ectoparasites such as leeches, mites and ticks and fly larvae causing cutaneous myiasis. Skin pathology can also result from actively invading parasite larvae (e.g. cutaneous larva migrans), skin infections such as dermal leishmaniasis, and the presence of larvae of filarial nematodes such as *Onchocerca volvulus* as they wait to be picked up by their vector (Meinking *et al*., 2003).

Box 7.2 It started with a leech bite

Where breaks occur in the skin surface, there is often secondary invasion by bacteria, fungi, and opportunistic parasites. Slesak *et al*. (2011) describe a fascinating case in which precisely this occurred to an unfortunate farmer living in village in northern Laos (see Colour Plate 28). Like many poor farmers he often walked barefoot and one day he was bitten on his left foot by a leech. Leeches are common in tropical countries such as Laos and to be bitten by a leech is an everyday experience for anybody living and working in the countryside. Unfortunately for him, however, the bite site became infected by a fungus belonging to the genus *Fonsecaea*. Species belonging to this genus are common opportunistic pathogens that can survive as saprophytes in the soil or in humans as a parasite. *Fonsecaea* and related fungi cause a condition known as 'chromoblastomycosis' in which slow-growing 'cauliflower-like growths', nodular lesions, and plaques develop. Over the course of several years these growths steadily expanded until they reached as far as his knee. Chromoblastomycosis is not painful or itchy but it is debilitating because it interferes with movement, and lesions on the skin surface can be colonised by other infectious agents. In the farmer's case, they were infected by the bacterium *Escherichia coli* and by larvae of the fly *Chrysomya bezzinana*. The farmer therefore developed cutaneous myiasis to add to his woes and even more unfortunately *Chrysomya bezziana* is a particularly aggressive deep-burrowing maggot that consumes healthy tissues as well as those that are necrotic. The lesson from this case is to always wear shoes while outdoors and to keep wounds clean!

Ectoparasites damage the skin and underlying tissues when they feed and this induces an inflammatory reaction. However, the skin exhibits numerous different inflammatory patterns and there is no typical generic response to ectoparasites. For example, in humans the skin follicle mites *Demodex folliculorum* and *Demodex brevis* have been linked with the development of a range of different skin conditions. *Demodex folliculorum* lives communally within hair follicles while *Demodex brevis* lives a solitary life within sebaceous glands. Both mite species can occur at the

same time and as many studies have not distinguished between the two we will simply refer to them as '*Demodex*'. *Demodex* mites are often found in the hair follicles of people suffering from rosacea and it is possible that the mites contribute to its development. Rosacea is an acne-like condition in which there is reddening (erythema) of the face – especially the cheeks, chin, forehead, and nose – within which papules and pustules develop. The facial dermatitis condition 'pityriasis folliculorum' that gives the skin a 'frosted appearance' has also been linked to infection with *Demodex* infection. Pityriasis folliculorum is usually described as occurring in elderly women who use heavy make-up but not much soap. However, Dominey *et al*. (1989) describe six cases in women aged between 31–46 who washed daily in soap and water and used little make-up. Other forms of dermatitis linked to *Demodex* infections include scalp folliculitis, seborrheic dermatitis, perioral dermatitis, and micropapular-pruritic dermatitis. The mechanisms by which the mites induce pathology are uncertain and may be related to blocking the hair follicles and sebaceous glands. This might be brought about by blocking the follicles and glands by their physical presence and inducing hyperplasia or immune reactions that block them. Mite faeces and cast exoskeletons could also induce a more widespread immune reaction. However, because many people who are infected with the mite show no evidence of pathology, there is still some doubt as to the role of *Demodex* in causing various forms of dermatitis. It is highly probable that a lot depends upon the relationship between *Demodex* and the skin microbiome. The mites can act as vectors for bacteria such as staphylococci that are known to be involved in the development of dermatitis and a symbiotic bacteria (*Bacillus oleronius*) found within the mite and in its faeces stimulates an inflammatory response (Lacey *et al*., 2007; Li *et al*., 2010). High incidences of *Demodex* infection are found in patients suffering from squamous cell carcinoma, basal cell carcinoma and actinic keratoses (Karaman *et al*., 2010). However, there is currently no suggestion that the mites are involved in initiating cancers and the high incidences may be a reflection of a compromised immune system or skin changes that favour the mite's growth and reproduction.

Dogs are infected with *Demodex canis* and it usually does not cause serious problems. However, it can cause 'generalised demodectic mange' which is a serious inflammatory condition in which serum, puss, and blood can be seen oozing from the skin surface. It is therefore sometimes referred to as the 'red mange'. It is thought to be linked to an inborn immunodeficiency that makes certain dogs susceptible to developing the condition. However, there is also evidence that *Demodex canis* can induce immuno-suppression (Barriga *et al*., 1992). In addition, as with other examples of *Demodex*-associated dermatitis, generalised demodectic mange usually involves secondary invasion by bacteria, especially staphylococci.

In the case of scabies, the mites (*Sarcoptes scabei*) produce short (2–15 mm long) wavy-line shaped burrows immediately underneath the surface of the skin. In humans these are typically found on the fingers, wrists, penis and scrotum. The chemicals present in the mites' saliva, faeces and eggs induce a pronounced inflammatory response in which eosinophils infiltrate both the epidermis and dermis. These eosinophils are responsible for the intense itching (pruritis) associated with scabies infections. Papules and vesicles form at the site of infection and there is localised eczema. The itching results in scratching and this results in secondary bacterial infections – particularly with streptococci and staphylococci. Scabies has also been linked as a potential risk factor for acute post-streptococcal glomerulonephritis (Scrace and Koko, 2006). In the elderly and people who are immuno-compromised, there is a risk that scabies infection may manifest itself as 'crusted scabies'. This condition is also known as 'Norwegian scabies' because the condition was first described in a group of Norwegian leprosy patients in 1848. In crusted scabies the mites multiply rapidly and induce hyperkeratotic lesions in which the surface of the skin

becomes covered in dry scales – hence the term 'crusted scabies'. The explosive increase in the mite population is thought to occur as a result of the immune system being compromised although the condition can also develop in people who are immuno-competent but are presumably genetically susceptible to this condition. The crusted lesions can spread to cover the whole body and are associated with swelling of the lymph nodes (lymphadenopathy). Crusted scabies induces a strong eosinophilic response and there are elevated levels of immunoglobulin E and interleukin-4. Interestingly, interleukin-4 stimulates the production of keratinocytes and this may therefore contribute to the hyperkeratosis. There is a high risk of secondary infections and the condition can be fatal.

In sheep, the mite *Psoroptes ovis* causes the highly pathogenic condition 'sheep scab', though it will also infect a range of other animals including rabbits and camels. Unlike *Sarcoptes scabei*, *Psoroptes ovis* does not burrow beneath the skin or feed on host tissues although it can cause equally serious pathology. *Psoroptes ovis* lives on the surface of its host's skin and feeds on the exudates and bacteria found on the skin surface. However, the mite's faeces induce a hypersensitivity reaction in sheep that causes a dramatic acute inflammatory response. Copious fluid exudates are released onto the skin surface where they form yellow crusty deposits. The inflammation results in intense itching and an infected sheep can become so distracted that it develops anorexia and incessantly rubs itself against any suitable scratching post. The combination of skin inflammation and scratching leads to wool loss over much of the body surface and there is a risk of secondary infections. The infected animal loses weight and body condition and in serious cases the sheep will die.

The adults of the filarial nematode *Onchocerca volvulus* live in the subcutaneous tissues where they cause little host reaction although they usually become encapsulated within nodules composed mainly of collagen fibres. These nodules are sometimes called 'onchocercomas' and an infected person may carry up to a 100 of them. The worms live tightly coiled within the nodules and are surrounded by either a 'fibrin lake' or solid granulation tissue. Although they are encapsulated, the worms are not safe from the body's immune system and immunoglobulins are often bound to their outer surface. For some reason, in Africa the nodules usually develop in the pelvic region although they may also be found in the spine, chest, and knees. By contrast, in Venezuela, the nodules develop predominantly in the head and neck. Regardless of where they develop, apart from causing some disfigurement, the adult worms are often said to cause little harm. However, the adult worms also release huge numbers of microfilariae and these are responsible for much of the pathology associated with onchocerciasis. These larvae migrate to superficial layers of the skin where they can be picked up by the blackfly vector – which is a surface-pool feeder. Some microfilariae, however, also find their way to the eyes, lymph nodes, and other organs of the body. While they are alive, the microfilariae do not usually induce serious pathology but once they are dead, they stimulate a dramatic inflammatory response.

Onchocerciasis results in lymphadenitis – inflammation of the lymph nodes - and eventually the nodes become enlarged and fibrotic. This disrupts the flow of lymph and can result in the development of 'hanging groin' (Connor *et al*., 1985). In African patients many of the lymph nodes contain microfilariae and lymphadenitis probably results from the inflammatory response against them. The inflammatory response against microfilariae within the skin results in dermatitis of which there are two types: hyper-reactive onchodermatitis (also called 'sowda') (Figure 7.4) and hypo-reactive onchodermatitis (Korten *et al*., 2011). The inflammatory reaction is caused by the body's immune response to antigens present in the symbiotic *Wolbachia* bacteria that are released from the bodies of the dead microfilariae. Some people are able to mount a strong Th2 biased

Figure 7.4 'Lizard skin' resulting from hyper-onchodermatitis in an 11-year-old boy. Note the areas of depigmentation on his lower legs and thickening on his thighs and penis. The child is starting to suffer from 'hanging groin' and his scrotum already hangs 20 cm from the pubis. His penis is showing evidence of elephantiasis and is 15 cm long. Source: Connor *et al*., 1985. (Connor, D.H. *et al*., Pathologic changes of human onchocerciasis: implications for future research. Reviews of Infectious Diseases, 1985, 7, 809–819, by permission of Oxford University Press)

immune response against *Onchocerca volvulus* antigens which results in the death of numerous microfilariae. However, by killing the microfilariae large quantities of *Wolbachia* antigens are released. By contrast, people who are able to tolerate the presence of large numbers of microfilariae express a less pronounced Th2 response and they also exhibit a suppressive Th1/Th3 response. Consequently, they develop less severe hypo-reactive dermatitis because fewer microfilariae are killed and therefore lower quantities of *Wolbachia* antigens are released. However, it should not be considered that people are either hyper-reactive or hypo-reactive. Many infected individuals actually exhibit a response that is at some point between these two extremes and people who are currently hyper-reactive may subsequently become hypo-reactive.

The inflammatory reaction against dead and dying *Onchocerca volvulus* causes dermatitis (onchodermatitis) and intense pruritis. This results in scratching and the risk of secondary bacterial infections. With time, the skin becomes thickened, loses its pigmentation and elasticity and it starts to crack: this is sometimes called lichenification although it is colloquially referred to as 'lizard skin'. Although it is not fatal, people suffering onchodermatitis often become isolated and develop feelings of shame and low self-esteem (Okoye and Onwuliri, 2007). Consequently, where onchodermatitis is common, it can have considerable social and economic consequences.

Microfilariae that migrate through the eye damage the cells but much of the pathology probably results from the inflammatory reactions against the dead and dying parasites. Depending upon where the damage occurs, a variety of pathologies can develop, including keratitis (inflammation of the cornea), chorioretinitis (inflammation of choroid and retina), iridocyclitis (inflammation of iris and ciliary body), and optic atrophy (loss of optic nerve fibres). In severe cases the damage to the eyes can result in blindness. Because the blackfly vectors of onchocerciasis breed in fast-flowing rivers, the disease is commonly found in villagers who live close to these rivers. Until effective control of the blackfly vector was established, many villagers near to blackfly-infested rivers became blind at an early age and the condition was therefore known as 'river blindness'.

7.5.9 The spleen

The spleen is part of the lymphatic system and represents the largest mass of lymphatic tissue in the body: in adult humans the spleen usually weighs 100–200 g. Underneath an outer layer of connective tissue can be found two types of tissue called 'white pulp' and 'red pulp' respectively. The white pulp consists mostly of lymphocytes and macrophages which are involved in the trapping and presenting of antigens. The red pulp consists of venous cords and splenic cords within which defective or worn-out circulating red blood cells, platelets, and macrophages are destroyed. In addition, the red pulp is a major site for the storage of blood platelets and during foetal development red blood cells are formed here. The spleen is therefore important for the normal functioning of the immune system and for the removal of damaged and potentially diseased cells from the circulation. Splenectomy is associated with a decreased ability to produce gamma-interferon and tumour necrosis factor-α during infections and for humans, splenectomy increases the risk of contracting opportunistic parasitic infections such as *Babesia divergens* and bacterial infections.

Enlargement of the spleen, splenomegaly, is a feature of many parasitic infections, for example, malaria, leishmaniasis, toxoplasmosis, and schistosomiasis. Splenomegaly is often accompanied by hypersplenism in which large numbers of circulating red blood cells, white blood cells, and platelets are sequestered within the spleen. In addition, many of the red blood cells are prematurely destroyed within the red pulp. This results in a condition known as pancytopenia in which there is a decline in the number of circulating red blood cells, white blood cells, and platelets. Consequently, pancytopenia results in an increase in the plasma volume (because there are fewer circulating cells), anaemia, and there is also an increased risk of bleeding and infections with encapsulated bacteria such as *Haemophilus influenzae* and *Streptococcus pneumoniae* (Ali, 2010). Pancytopenia also causes hyperplasia in the bone marrow as the body attempts to replace the lost cells.

During malaria, many infected red blood cells are destroyed in the spleen although in the process probably ten times as many uninfected cells are simultaneously destroyed and this contributes to the development of anaemia. The sequestering and destruction of red blood cells within the spleen are also thought to bring about the development of splenomegaly although there are also suggestions splenomegaly in malaria may also occur through a concomitant increase in haematopoiesis (red blood cell formation) within the spleen (Lamikanra *et al*., 2007). In endemic regions splenomegaly is usually restricted to children. The destruction of phagocytic cells by *Leishmania donovani* during visceral leishmaniasis brings about hyperplasia in the spleen as the organ attempts to replace the lost cells. This is undertaken to the detriment of the manufacture of red blood cells and leads to splenomegaly and hypersplenism. The consequent disruption of

spleen function and development of pancytopenia explain why patients suffering from visceral leishmaniasis often die of secondary infections. Splenomegaly can occur in human toxoplasmosis infection, although it is not common and is mostly found in children under 16 years old. It probably results from a proliferation of lymphoid tissues. In the case of schistosomiasis, splenomegaly usually results from portal hypertension. However, any schistosome eggs that get swept to the spleen will induce granulation and fibrosis that contributes to the development of splenomegaly. The splenic vein takes blood from the spleen, the stomach, and pancreas and then joins the superior mesenteric vein to form the hepatic portal vein. Schistosome eggs in the liver cause fibrosis which obstructs the movement of blood into the liver and therefore there is a rise in blood pressure within the hepatic portal vein (portal hypertension). Although the blood pressure rises, the blood flow is reduced or ceases (stasis) and portal hypertension induces hyperplasia within the spleen that manifests itself as splenomegaly and hypersplenism. Schistosomiasis is also associated with the development of thrombocytopenia, that is a reduction in the number of blood platelets and hence susceptibility to poor clotting and abnormal bleeding. This could be related to the development of hypersplenism associated with splenomegaly (Martins *et al*., 2010) although there is also evidence that it arises as a consequence of an autoimmune reaction in which parasite antigens cross-react with platelet antigens (Stanley *et al*., 2003).

7.6 Co-infections and pathogenesis

A recurring theme within this book is that parasites seldom act independently. The pathology caused by a parasite can increase susceptibility to other infectious agents and these in turn will affect the subsequent pathology caused by the parasite (Lello *et al*., 2004; 2008). Similarly, bacteria or viruses can facilitate the establishment of parasites and enable a parasite to cause more damage than it would do if acting on its own. For example, unless they receive effective treatment ~90% of people suffering from kala azar (*Leishmania donovani*) will die, often as a consequence of concurrent bacterial and other opportunistic infections that result in septicaemia and pneumonia. Over half of patients with visceral leishmaniasis acquire secondary microbial infections such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Haemophilus influenzae* – often while they are in hospital (i.e. they are nosocomial infections) (Andrede *et al*., 1990).

In sheep, acute fascioliasis increases susceptibility to the gram-positive bacillus *Clostridium novyi* that causes a necrotic hepatitis, often known as 'black disease'. *Clostridium novyi* is a common soil bacterium and there are various types, some of which produce toxins. Black disease is caused by *Clostridium novyi* type B that produces a toxin called α toxin. The spores are ingested with soil-contaminated food and can pass through the gut epithelium. They are then picked up by macrophages and transported to the liver and other organs. The spores require anaerobic conditions to grow and these are provided by the necrotic lesions caused by the migrating flukes. It is possible that the metacercaria may also be contaminated with the bacteria or the flukes may transport the bacteria that they pick up in the lumen of the gut with them on the surface of their tegument. In cattle, fascioliasis is associated with an increased susceptibility to bacillary haemoglobinuria due to *Clostridium haemolyticum*. *Clostridium haemolyticum* is sometimes referred to as *Clostridium novyi* type D but it is usually only recorded from sheep in experimental conditions. In a similar manner, during their migration *Ascaris* larvae can transport bacteria such as *Escherichia coli* from the gut to the lungs where the bacteria contribute to the pathology (Adedeji *et al*., 1989).

The interactions between bacteria and parasites are very important in the pathology of a number of protozoan parasites and in particular the amoebas. For example, the gum disease periodontitis is cyclical in nature, with periodical flare-ups followed by remission (Stanley, 1955). The main causative agent is the bacterium *Bacterioides gingivalis* although other microbes can also cause the condition. It has been suggested that *Entamoeba gingivalis* becomes involved in disease progression by transporting bacteria between sites of infection within the mouth (Linke *et al*., 1989). Although amoebae in general feed on bacteria, it is known that some organisms, such as *Shigella dysenteriae* can survive within vacuoles in *Entamoeba histolytica* for prolonged periods and it is therefore possible that a similar phenomenon may occur in *Entamoeba gingivalis*. Özüm *et al*. (2008) isolated *Entamoeba gingivalis* from a patient suffering from a brain abscess along with the common oral bacteria *Eikenella corrodens* and *Prevotella* spp. Whether or not all three organisms arrived in the brain at the same time or one was the initial cause of the abscess and the others arrived subsequently is difficult to say, but it highlights the close association of amoebae with bacteria in causing pathology. Necrotic periodontitis is a serious and painful disease that is often associated with HIV-1 infection. HIV-1 infection does not necessarily lead to a change in the oral bacterial flora but Lucht *et al*. (1998) found that 77% of HIV-infected patients with periodontal disease were infected with *Entamoeba gingivalis*. By contrast, they found that none of the HIV-positive individuals without periodontal disease were infected with *Entamoeba gingivalis*.

It is not known whether microbe associations contribute to the pathology of *Naegleria fowleri* but bacterial symbiont populations have been described living inside other *Naegleria* species (Walochnik *et al*., 2005). There have also been suggestions that *Naegleria fowleri* may be involved in the transmission of Legionnaires disease (caused by the bacterium *Legionella pneumophila*) but more work is needed to clarify the nature of the relationship between these two organisms (Rowbotham, 1980).

Both pathogenic and non-pathogenic *Acanthamoeba* are associated with a wide range of bacterial endosymbionts (Schmitz-Esser *et al*., 2008) and they also support several pathogenic species such as *Legionella* spp. and *Chlamydia* spp. Non-pathogenic species of *Acanthamoeba* therefore have the potential to act as transport hosts and contribute to the survival of pathogenic microbes. In addition, *Legionella* bacteria that have been in association with *Acanthamoeba* can exhibit increased antibiotic resistance. Methicillin-resistant *Staphylococcus aureus* (MRSA) have also been observed to be internalised by *Acanthamoeba*, replicate within them and to exhibit unchanged or increased antibiotic resistance afterwards (Huws *et al*., 2006; Lee *et al*., 2008). Whether or not *Acanthamoeba* species are actually important in the spread of pathogenic microbes and the development of antimicrobial resistance is not known. Horizontal gene transfer has probably occurred from bacteria to a number of parasitic protozoa species (Richards *et al*., 2003). For example, in *Entamoeba histolytica*, the genes that code for anaerobic metabolism may have originated from bacteria. Bacteria and parasitic protozoa may therefore contribute to the pathogenicity of one another to a greater extent than previously thought. There are several reports of viruses infecting *Entamoeba histolytica* trophozoites (e.g. Diamond *et al*., 1972; Mattern *et al*., 1974) but it is not known whether these contribute in any way to their epidemiology or pathogenicity.

Questions

1. State three causes of parasite-induced anaemia.
2. Explain how apoptosis can be both a normal physiological process and a parasite-induced pathology.

3. Briefly discuss the role of tumour necrosis factor-alpha (TNF-α) in the development of fever.
4. What is a granuloma and what purpose does it serve?
5. Distinguish between hyperplasia, metaplasia, and hypertrophy.
6. Briefly explain what is meant by the term hypokalaemia and why it is potentially fatal.
7. Explain the link between parasitic castration and gigantism.
8. Explain how pressure atrophy is caused and name a parasite that can induce it.
9. What evidence is there that parasites can predispose their host to cancer?
10. Distinguish between filarial elephantiasis and non-filarial elephantiasis in terms of their cause and region of the body affected.

8

The useful parasite

8.1 Introduction: the goodness of parasites?

Parasites are normally considered unwelcome guests whose presence is almost invariably harmful. Although, as we have seen in Chapter 1, defining and measuring harm can prove problematic, it can still appear perverse to consider the possibility that parasites might have their uses. The term 'the goodness of parasitism' was initially coined by David Lincicome while he was Professor of Zoology at Howard University, Washington, DC, USA. Lincicome and his co-workers performed numerous experiments using a variety of protozoan and nematode parasites of rats and mice that are summarised in a review he published in 1971 (Lincicome, 1971). He observed that the parasitised animals often performed better in terms of body weight gain, longevity, and certain physiological parameters than those that were parasite-free. He therefore felt that parasite–host relationships should not be considered to be one-way and that parasites could (sometimes) contribute benefits to their host. However, his views have never been developed and some of his conclusions could be criticised for being based on experiments involving very specific diets and low levels of parasitaemia. Nevertheless, it is becoming increasingly apparent that parasites can sometimes prove useful to their hosts (Fellous and Salvaudon, 2008; 2009) and that low levels of parasitism may be helpful for maintaining a healthy immune system. In some cases the parasite may even contribute to its host's ability to withstand harmful environmental conditions. For example, the rickettsial bacterium *Anaplasma phagocytophilum* enhances the survival of its tick vector, *Ixodes scapularis*, by inducing the expression of a gene that codes for the production of antifreeze glycoproteins (Neelakanta *et al*., 2010). Consequently, the infected ticks are better able to survive the harsh winters of the North-East and Upper Midwest of the United States of America. *Anaplasma phagocytophilum* is an intracellular parasite and causes lameness and flu-like symptoms in dogs, humans, and a range of other mammals and infections are sometimes mistaken for Lyme disease.

We have also found ways of using parasites to our advantage. For example, many parasites have evolved molecules that help them exploit their host and these may prove useful as sources of novel pharmaceutically-active substances. The effects of parasites on their hosts can also be used for the treatment of specific medical conditions and to control pest animals and plants. Parasites have even been implicated in criminal investigations and some workers have seen fit to coin the term 'forensic parasitology'.

Parasitology: An Integrated Approach, First Edition. Alan Gunn and Sarah J. Pitt.

8.2 The importance of parasites for the maintenance of a healthy immune system

Since the 1960s and 1970s there has been a rapid rise in the incidence of a wide range of allergic and autoimmune inflammatory conditions (e.g. Type 1 diabetes) in industrialised countries. For example, about 20% of children in industrialised countries currently suffer from asthma, allergic rhinitis (e.g. hay fever), or eczema (ISAAC Steering Committee, 1998). There is every indication that the high incidence of these conditions will continue to increase for many years yet to come. The reasons for these increases are not fully understood but are undoubtedly influenced by a wide variety of both genetic and environmental factors. However, one in particular, parasitic disease, is now thought to have a major role.

8.2.1 The hygiene hypothesis

The hygiene hypothesis, as it has come to be called, was initially invoked to explain why children in the UK brought up in large families tend to suffer less from eczema and hay fever (Strachan, 1989). It was suggested that in small family units there is less opportunity for transmission of infectious organisms. Furthermore, in developed countries, since the Second World War, transmission of infectious diseases has been reduced by improved sanitation, better standards of living and the widespread use of antibiotics. As a consequence, in developed countries children no longer receive the levels of immunological challenge from infectious organisms that used to be commonplace. The hygiene hypothesis proposes that when the body's immune system is no longer challenged by infectious organisms on a regular basis, it is more likely to become activated by inappropriate stimuli and therefore there is an increased susceptibility to allergies and autoimmune inflammatory diseases (Flohr *et al*., 2009). Support for this proposal comes from epidemiological surveys that indicate that autoimmune conditions such as Multiple Sclerosis and Type 1 Diabetes are rare among poor people living in Africa and Asia but common in industrialised countries (Zaccone *et al*., 2006). Although genetic constitution is an important factor in the development of autoimmune conditions, the environment is obviously involved, since people who migrate from Africa and Asia to live in developed countries have an increased susceptibility to them. Allergies and autoimmune diseases are under polygenic control and therefore it is impossible to point to any single factor that determines whether or not they develop; however, there is increasing evidence that parasitic helminth infections could have an important role to play. For example, there is an inverse relationship between the prevalence of gastrointestinal helminth infections and autoimmune conditions such as Crohn's Disease and ulcerative colitis (see later).

Parasitic helminths stimulate a Th2 type cell-mediated immune response in which the cytokines interleukin-4, interleukin-5, interleukin-10, and interleukin-13 are produced (see Chapter 6). Interleukins 4, 5, and 13 are involved in the activation, growth, and differentiation of eosinophils and also stimulate the production of immunoglobulin E (IgE) by plasma cells. By contrast, interleukin-10 tends to have an anti-inflammatory effect although it also stimulates the production and survival of B cells. There is a negative feedback relationship between the Th2 and Th1 responses, and interleukin-4 formed during the Th2 response brings about a reduction in the Th1 response. Interleukin-10 also reduces the Th1 response by indirectly inhibiting the production of gamma interferon (IFN-γ) and a number of other cytokines. There is, however, an antagonistic relationship between interleukin-10 and interleukin-4 so the two cytokines are not acting in concert. The Th1

response is principally stimulated by and directed against intracellular parasites and involves the production of gamma interferon. If the Th1 response is not controlled, it can result in damage to healthy tissues and can also lead to the development and maintenance of autoimmunity.

Helminths interact with their host's immune system in a wide variety of ways. For example, direct physical damage caused by their feeding and movement will induce an immune reaction as part of the tissue repair process. Similarly, many helminth secretions and excretions are immunogenic. The parasites may directly or indirectly influence the gut microbial flora through their feeding and secretions or excretions, which this may in turn influence the host's digestion and immunity. For example, substances with antimicrobial activity have been detected in the excretory/secretory products of *Trichuris suis* (Abner *et al*., 2001). The relationship between parasitic helminths and allergies and autoimmune diseases is therefore not one of simply promoting a Th2 response and thereby reducing the Th1 response (Liu *et al*., 2010). For example, allergic reactions and asthma are typified by an overactive Th2 response in which eosinophils contribute to the inflammation by releasing toxic chemicals that include eosinophil-derived neurotoxin (EDN). Eosinophil-derived neurotoxin has ribonuclease and antiviral activity and can be used as a physiological indicator of the severity of asthma. Helminth infections cause eosinophilia in which the proportion of eosinophils in the general circulation can increase from 2–5% of the white blood cells to as high as 40% (Reimert *et al*., 2006). However, despite this, helminth infections appear to protect against the development of allergies (Flohr *et al*., 2008; Hopkin, 2009). Some helminth species are able to survive within their hosts for prolonged periods of time and are assumed to have evolved mechanisms of modulating and avoiding the IgE-mediated response targeted against them.

Although there is increasing evidence of helminth infection providing some form of protection against developing allergies, it is not yet conclusive. An alternative explanation for the negative association between helminth infection and predisposition to allergies could be that those who are atopic are more resistant to helminths than the general population. Atopy is an IgE-mediated hypersensitivity reaction which may occur in parts of the body not in contact with the allergen. The allergens induce reactions in the skin (atopic eczema/atopic dermatitis), eyes (allergic conjunctivitis), nose (allergic rhinitis) and lungs (asthma). Indeed, there is some evidence that some of the same genes are involved in both the control of atopy and susceptibility to helminth infection (e.g. Barnes *et al*., 2005). However, much more work needs to be done using a range of genes and a variety of helminth infections.

8.2.2 Type 1 diabetes mellitus

Sometimes known as 'insulin-dependent diabetes mellitus' (IDDM) or 'juvenile diabetes', Type 1 diabetes mellitus is caused by the autoimmune destruction of the insulin-producing *beta* (β) cells in the Islets of Langerhans in the pancreas. What precipitates the autoimmune response is not known, but several genes and environmental factors are implicated. Without insulin-replacement therapy the consequences are fatal. Initially it was thought that the Th1 response caused most of the pathology and the Th2 response was protective. This view, however, is too simplistic. Although an excessive Th1 response is clearly harmful, it is the balance between the Th1 and Th2 responses that determines the onset and progression of the disease (Azar *et al*., 1999).

The incidence of type 1 diabetes mellitus has increased in recent years in many industrialised societies and this may in some way be associated with a corresponding decline in helminth

infections. Experiments with non-obese diabetic (NOD) mice have indicated that various helminths or their products can protect the mice from developing spontaneous type 1 diabetes (e.g. Hübner *et al*., 2009; Saunders *et al*., 2007). The reason for this protective effect may be related to the fact that helminths tend to cause a predominantly Th2 response and the Th1 response is reduced. However, neither the pathophysiology of type 1 diabetes mellitus nor the immune responses to helminth infections are quite that simple and many other factors are also involved. For example, helminth infections also affect the population and function of a variety of other components of the immune system such as interleukin-10, regulatory T cells, invariant natural killer cells and alternatively-activated macrophages (Liu *et al*., 2010).

Although interleukin-10 is produced by lymphocytes during the Th2 response, it is predominantly formed by monocytes and certain other immune cells including mast cells and a subset of the regulatory T cells. Interleukin-10 acts as an immunomodulator – that is, it modulates the immune response by influencing the activity of other components of the immune system. Its effects are predominantly suppressive and it prevents inflammatory responses from 'getting out of hand'. However, it can also have a stimulatory role in the immune response against some infectious agents (Asadullah *et al*., 2003). In mouse models of type 1 diabetes mellitus, interleukin-10 has a protective effect and suppresses the development of the disease. Some helminth infections induce interleukin-10 and it is possible that this may contribute to preventing the development of autoimmune conditions including type 1 diabetes mellitus. For example, children infected with *Schistosoma haematobium* produce elevated levels of interleukin-10 and this has been suggested as a reason why they are less likely to develop atopy (van den Biggelaar *et al*., 2000). Up-regulation of interleukin-10 has also been described in filarial nematode infections but there is less consistent evidence for it occurring in response to gastrointestinal nematodes.

Like interleukin-10, regulatory T cells (T_{reg}) act as a 'brake' preventing the immune system from becoming over-activated and initiating autoimmune reactions. The population and function of regulatory T cells are therefore of importance in the development of conditions such as type 1 diabetes mellitus and asthma. The filarial nematode *Litomosoides sigmodontis* induces regulatory T cell activity and this is thought to contribute to the parasite preventing the development of type 1 diabetes mellitus and allergen sensitivity in mouse model systems (Dittrich *et al*., 2008; Hübner *et al*., 2009). Interestingly, Aravindhan *et al*. (2010) report a decreased prevalence of lymphatic filariasis among diabetics in India.

Natural killer T cells (NKT) are distinct from natural killer cells and co-express both a specific ($\alpha\beta$) T cell receptor and some of the molecular markers normally found on natural killer cells. Invariant natural killer T cells (iNKT) are a subset of natural killer T cells. When stimulated, they produce a variety of immunologically-active substances, such as interleukin-4 and gamma interferon, and reductions in their numbers have been linked to the development of a number of autoimmune conditions – again including type 1 diabetes. *Schistosoma mansoni* infection in mice induces invariant natural killer T cell activity (Mallevaey *et al*., 2006) as does the injection of *Schistosoma mansoni* antigens into non-obese diabetic mice (Zaccone *et al*., 2003). The *Schistosoma mansoni* antigens protect the non-obese diabetic mice from developing diabetes and this may, at least in part, be a consequence of the increased natural killer T cell activity.

Macrophages are classically activated by gamma interferon and other Th1 type signals although some are activated by Th2 signals such as interleukin-4 and interleukin-13. These latter macrophages are referred to as 'alternatively activated macrophages' (AAMΦs) and they play an important role in the development of allergies and autoimmune disorders. Parasitic helminths induce a Th2 response and the production of interleukin-4 and interleukin-13. Consequently,

alternatively activated macrophages are also produced as part of the immune reaction against parasitic helminths (Mylonas *et al*., 2009). An elevated Th1 response is associated with the development of type 1 diabetes and the raised levels of gamma interferon brings about an increase in the numbers of classically activated macrophages which then contribute to the pathology. Helminth infections bring about elevated levels of alternatively activated macrophages and these then suppress the Th1 response and could thereby help protect against the development of type 1 diabetes (Liu *et al*., 2010).

8.2.3 Irritable bowel syndrome (IBS)

Irritable bowel syndrome (IBS) is a chronic condition involving altered bowel habits in which the patient experiences abdominal pain, constipation, and diarrhoea. The prevalence varies considerably between countries – although some of this can be put down to the way the information is recorded and the fact that many people with IBS do not seek medical help or do not receive an accurate diagnosis if they do. In Europe, Italy (12%) and the UK (12%) have the highest prevalence rates (formally and not formally diagnosed) and the Netherlands (6.2%) the lowest (Hungin *et al*., 2003). In the USA the prevalence is about 14% (Hungin *et al*., 2005). There is limited accurate information on the prevalence of IBS in developing countries but the general impression is that it is probably increasing, as people start to adopt the 'Western lifestyle'.

Some doctors consider IBS to be a psychosomatic disorder but there is strong evidence that it is linked to alterations in the gut bacterial flora and inflammatory changes (Spiller and Garsed, 2009). In some cases of IBS, the changes can be linked to taking antibiotics or to gastrointestinal infections. In the latter situation, the condition is known as post-infectious IBS. In Norway, people infected with *Giardia duodenalis* are reported to be more likely to subsequently develop diarrhoea-associated IBS (Hanevik *et al*., 2009) but in Asia, where *Giardia* is much more common among the general population, evidence of a link between the parasite and IBS is less clear (Ghoshal *et al*., 2010). The support for a relationship between *Entamoeba histolytica* infection and the development of IBS is also unclear. This is partly because many studies have not distinguished pathogenic *Entamoeba histolytica* from *Entamoeba dispar* and *Entamoeba moshkovskii* or between pathogenic and non-pathogenic strains of *Entamoeba histolytica*. The protozoan *Blastocystis hominis* has been linked to post-infectious IBS but much of the evidence to date has been contradictory. *Blastocystis hominis* is a cosmopolitan species found in both developed and developing countries and it is common in humans and a number of mammals. The taxonomic position of *Blastocystis hominis* is exceedingly uncertain and it does not fit into any of the current classifications of the protozoa (Stenzel and Boreham, 1996). Its pathogenicity is similarly mysterious, with various claims of it being pathogenic, commensal or an opportunist that becomes parasitic if the circumstances are right. A number of genotypes of *Blastocystis hominis* have been identified and these vary in their effects on their host. It is thought that this genotype variation is at least one determinant of whether or not post-infection IBS develops (Yakoob *et al*., 2010).

The role of helminth parasites in the development of IBS is uncertain. As a rule, the prevalence of IBS tends to be low in countries in which gut helminth infections are common. This is despite the fact that these countries also tend to have high levels of infections with *Giardia* and the many bacteria and viruses that cause gastrointestinal upsets. It is therefore, at first sight, surprising that people living in developing countries do not have high prevalence of post-infection IBS.

This has led to suggestions that gut helminth infections might protect against the development of IBS – provided the numbers of worms remain low. This protection might result from the helminths inducing a predominantly Th2 type immune response within the intestinal mucosa which prevents the development of a chronic inflammatory response.

8.2.4 Inflammatory bowel disease

Because their names are so similar, irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) are often conflated, but they are actually two different conditions. Inflammatory bowel disease is a generic term for a group of chronic inflammatory diseases of which Crohn's disease and ulcerative colitis are the principal forms. Crohn's disease is an autoimmune condition and can affect all parts of the gut from the mouth to the anus, although it is usually found in the region between the end of the small intestine and the beginning of the large intestine. The pathogenesis of ulcerative colitis is less certain and the disease is restricted to the colon and rectum. Both diseases are thought to result from an inappropriate inflammatory response to normal gut microbial flora rather than a specific infectious agent. Crohn's disease is typified by an elevated Th1 type inflammatory response in which large amounts of gamma interferon and tumour necrosis factor alpha (TNF-α) are produced. By contrast, elevated levels of interleukin 8 in the colon mucosa have been implicated in the development of ulcerative colitis (Li *et al*., 2009). Interleukin-8 is an inflammatory cytokine produced by circulating macrophages and a number of other cell types but not by T lymphocytes. It has a variety of functions including the attraction of migratory immune cells and the activation of neutrophil granulocytes. Interleukin-10 probably also has a key role to play in the development of ulcerative colitis although exactly how is not yet certain. For example, in patients with ulcerative colitis, the levels of interleukin-10 may be either reduced or considerably increased depending upon the stage of the disease (Louis *et al*., 2009).

Both Crohn's disease and ulcerative colitis are becoming increasingly common in developed countries but they are relatively rare in developing nations. The hygiene hypothesis has been proposed as one of the main reasons for the increasing number of people suffering from these conditions (Weinstock and Elliott, 2009; Weinstock *et al*., 2004). In rodent models of inflammatory bowel disease, rats or mice given an intra-rectal injection of trinitrobenzene sulphonic acid (TNBS) develop an inflammation in their colon (colitis) that results from a Th1 inflammatory response. However, if the rodents are infected with the nematodes *Trichinella spiralis* or *Heligmosomoides polygyrus*, the tapeworm *Hymenolepis diminuta*, or the trematode *Schistosoma mansoni*, then they are protected from developing TNBS-induced colitis (Weinstock and Elliott, 2009). This appears to be due to the interleukins produced as a consequence of the Th2 response against the helminths causing a down-regulation of the Th1 response.

8.3 The use of parasites to treat medical conditions

The fact that one disease can influence the outcome of another has been known for millennia. Although the onset of a second affliction is seldom good news for the patient, there are instances in which it can prove beneficial. Rufus of Ephesus writing in either the first century BC or the first or early second century AD (the dates aren't certain) stated that periodic fevers (by which he was

probably referring to quartan malaria) provided an excellent cure for epilepsy and melancholia. This has sometimes been cited as an early observation of the potential of malaria therapy for the treatment of mental illnesses (see later). However, he also felt quartan fever was beneficial for asthma, tetany (muscular spasms rather than the disease tetanus) and certain skin diseases. The factors linking them are that they were all generally considered to be 'autumn diseases' and caused by an imbalance in the levels of 'black bile' (Longrigg, 1998; Major, 1954). There is an anecdotal report of native Peruvian people deliberately acquiring malaria to cure themselves of mucocutaneous leishmaniasis (Delgado, 1922, cited by Chernin, 1984) but it does not appear to have been investigated further. Perhaps the most widely known story of parasites being employed for medical purposes is that of unscrupulous 'quack doctors' selling diet pills that contained tapeworm eggs. Indeed, the web is still awash with rumours, horror stories, and concerned advice about the so-called 'tapeworm diet'. The reality is that consuming tapeworm eggs would, with the exception of *Hymenolepis* (*Vampirolepis*) *nana*, not give rise to an adult tapeworm in a human. This is because after hatching, the egg of a cestode releases a hexacanth embryo that penetrates the gut of the intermediate host and encysts elsewhere in the body; the larval cestode does not develop into an adult worm until it is consumed by the definitive host (Chapter 3). Cestode eggs do not therefore as a rule hatch in the gut of their definitive host. The only exceptions (of medical importance) to this are *Taenia solium* and *Hymenolepis nana*. In the case of *Taenia solium*, humans can act as both an intermediate host and the definitive host. However, humans consuming *Taenia solium* eggs would develop cysticercosis and not an infection with adult tapeworms. So the person might develop a cysticercus in a dangerous place (such as the brain), but this would not necessarily help them to lose weight! *Hymenolepis nana* infects rodents and humans and is unusual in not requiring an intermediate host to complete its lifecycle – although tenebrionid beetles can act as intermediate hosts. After an egg of *Hymenolepis nana* hatches in the gut of a human or rodent, its hexacanth embryo burrows into one of the villi lining the small intestine and transforms into a cysticercoid. After a few days, the scolex everts and the parasite matures into an adult tapeworm. *Hymenolepis nana* is, however, a small tapeworm averaging only 4 cm in length and 1 mm in width. Although it is common in children in many parts of the world, it does not usually cause any problems unless the worm burden is exceptionally high – so again it would not be much use as a diet aid.

8.3.1 Helminth therapy

Autoimmune diseases are notoriously difficult to treat using conventional medicine and some of them condemn the patient to a lifetime of pain and disability. The fact that these conditions predominantly afflict people living in the developed world means that there is a huge market for any novel effective treatment. Epidemiological data and experimental studies using rodent models indicate that parasitic helminth infections may protect against the development of certain autoimmune conditions (see above). Consequently, it has been suggested that intentionally infecting a patient with helminth parasites or treating them with products derived from helminth parasites could, if not offer a cure, at least alleviate suffering. Infecting a patient with living parasites poses serious ethical considerations and there could be a risk that the parasites would cause harm or be transmitted to other people (McKay, 2009). Patients who are immuno-compromised as a consequence of disease (e.g. AIDS) or drug treatment (e.g. following organ transplant or cancer therapy) could be at greater risk of adverse reactions if they became infected either directly or indirectly

(i.e. they contracted the parasites from someone undergoing helminth therapy). Some workers therefore feel that it would be safer to identify the components of the parasites that might confer protection against autoimmune diseases or the precise mechanisms by which protection is obtained. Patients could then be treated with particular drugs whose safety and pharmaceutical properties were identified and the dosages controlled. However, the counter-argument is that, as has been already mentioned, helminth parasites have complex immunological relationships with their hosts and therefore it is unlikely that any protective effect will be conferred by just one or a few helminth-derived substances. In addition, autoimmune diseases are, by their nature, long-lasting conditions that tend to be better controlled rather than cured. Parasite infections can be considered to be living drug pumps that deliver a regular dose of chemicals which is more convenient for the patient than taking pills or receiving injections. However, many people have an innate disgust at the idea that there are worms living inside them.

To date, most work on helminth therapy has been done using the whipworm *Trichuris suis* and to a lesser extent the hookworm *Necator americanus*. Both of these nematodes are capable of causing serious pathology, but low worm burdens in otherwise healthy, well-nourished individuals are considered to cause little harm.

Trichuris suis is a common parasite of pigs (Figure 8.1) and found throughout the world although, surprisingly, there are no reports of natural human infections (Summers *et al*., 2005). It has been tried as treatment for inflammatory bowel disease, with some promising results (Reddy and Fried 2009; 2007) and a preliminary study indicates that it may also be useful for treating multiple sclerosis (Fleming *et al*.,2011). Natural infections in pigs and intentional infections of humans are acquired by ingesting the eggs containing the infective larvae. At 34°C it takes 19 days for an egg passed in pig faeces to develop to the infective stage and at 20°C this increases to 102 days. Consequently, even if a person harboured sexually mature *Trichuris suis* the chances of them reinfecting themselves or somebody else (or even a pig) are limited, provided basic hygiene is practised. In the closely related *Trichuris muris*, hatching is stimulated by an interaction between specific

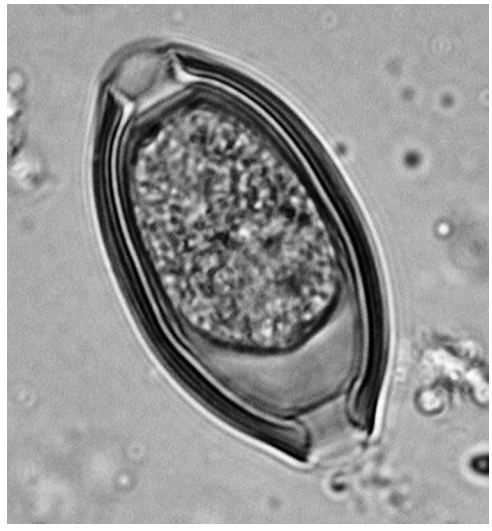

Figure 8.1 The eggs of *Trichuris* spp., are highly characteristic with 'polar plugs' at either end. This egg has recently been passed in faeces. It is not embryonated and it is therefore not infective.

receptors on the eggshell surface and *Escherichia coli* and certain other bacteria in the small intestine (Hayes *et al*., 2010) and it is likely that a similar interaction occurs in *Trichuris suis*.

After hatching, the *Trichuris suis* larvae penetrate the intestinal mucosa where they remain for several days. Subsequently the larvae move down the intestine to the caecum where they mature into adults. Some of the literature indicates that *Trichuris suis* is non-invasive (e.g. Summers *et al*., 2004) but this is not strictly true. The larvae of *Trichuris suis* invade the intestinal mucosa and in pigs the adult worms burrow their anterior region into the mucosa of the large intestine. Van Kruiningen and West (2005) raised concerns that the larvae might migrate from the intestinal mucosa and cause serious pathology elsewhere in the body but there are no reports of this actually occurring. Although *Trichuris suis* infects humans, there are contradictory reports in the literature about whether the worms are capable of reaching sexual maturity within humans and then producing viable eggs. According to Summers *et al*. (2004), *Trichuris suis* does not do this and is spontaneously eliminated within a few weeks of initial infection. However, Kradin *et al*. (2006) found a sexually mature male *Trichuris suis* in the bowel of a 16-year-old with Crohn's disease who had undergone helminth therapy.

Necator americanus has been trialled for the treatment of inflammatory bowel disease and asthma (Croese *et al*., 2006; Mortimer *et al*., 2006). As with other helminth parasites, it stimulates a Th2 inflammatory response and this, together with other modulatory effects on the immune system, is thought to ameliorate the effects of autoimmune conditions. It is a common hookworm parasite of humans and is found in many temperate and tropical parts of the world but particularly in North and South America. It can also infect dogs and pigs but humans are its principal host. The adult worms attach themselves to the mucosa of the small intestine using their cutting plates and feed on blood by slicing through the underlying blood vessels (Figure 8.2). Heavy infestations can result in anaemia but small numbers cause little damage.

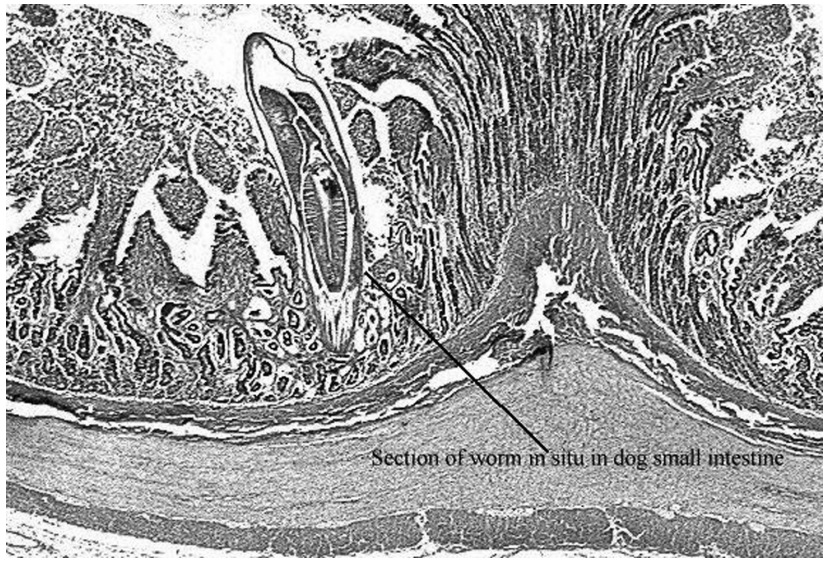

Figure 8.2 *Ancylostoma caninum*: light microscope histology slide. Longitudinal section through adult worm grasping onto the intestinal mucosa

The life cycle of *Necator americanus* resembles that of other hookworms (see Chapter 3). Because humans are the natural host for *Necator americanus*, once an infection is established, it can last for several years and during that time the infected person will be passing viable eggs into the environment. However, the eggs are not immediately infective and provided basic hygiene is undertaken, there should not be any risk of self-reinfection or the infection or transmission to others.

Box 8.1 Helminth therapy in practice

In Europe and the USA, several hundred people with inflammatory bowel disease have now been treated with *Trichuris suis* eggs and the outcomes have been mostly positive (Reddy and Fried, 2009, 2007; Summers *et al*., 2004). The eggs are sourced from pathogen-free pigs to reduce the likelihood of co-transmitting any other diseases. Because *Trichuris suis* does not tend to survive in the human intestine for more than a few weeks, patients are typically given doses of 2,500 eggs every 3 weeks for a period of time (e.g. 24 weeks) during which their condition is monitored. If there are concerns about the parasites establishing a permanent infection or causing harm, they can be easily removed using conventional anthelmintic drugs, such as albendazole. There is still a need for further double-blind studies, the determination of the optimal treatment regime, and the patient factors that affect the likely success of the treatment. In addition, because *Trichuris suis* is a cosmopolitan species, it is probable that genetic differences exist between populations. It would therefore be interesting to know whether there are strain differences which vary in the level of protection they confer.

There are few published studies on the use of *Necator americanus* to treat humans with autoimmune conditions (Reddy and Fried 2009). To date, these studies have used eggs derived from people who are already infected with the parasite. Some commentators have suggested that there is therefore a theoretical risk of transmission of human viruses, although exactly which viruses these might be has not been stated and there is no evidence that it occurs. A greater problem is securing sufficient eggs to undertake large-scale studies. The eggs are allowed to hatch and once the larvae have developed to the infective stage, they are applied in a patch to the patient's skin and allowed to invade or are inoculated under the skin surface. There is often a localised reaction at the site of invasion and some patients report abdominal pain when the worms initially establish themselves in the gut. Successful colonisation is indicated by finding hookworm eggs in the patient's faeces and the number of eggs gives a crude indication of the worm burden. Mortimer *et al*. (2006) found that a single 'dose' of 10 larvae was sufficient to have a positive effect on patients' asthma. Croese *et al*. (2006) mostly used doses of 25 larvae and re-inoculated some patients after 27–30 weeks. They found that the condition of the majority of the patients with Crohn's disease improved following infection with the worms.

Although proponents of helminth therapy consider it poses little risk to the wider community, there will always be lingering concerns. These could be alleviated somewhat if a way was found to dose patients with single-sex infections or render the worms sterile. Non-destructive sex determination of nematode eggs and larvae is probably unfeasible with current technology. There is, however, considerable expertise in the production of sterile insects in pest management (e.g. for the control of the screwworm *Cochliomyia hominivorax*) and this technology might be applied to parasitic nematodes destined for helminth therapy.

8.3.2 Larval therapy

Larval therapy – also called 'maggot therapy' and 'biosurgery' – refers to the application of fly (Diptera) larvae to wounds in order to speed up the rate at which they heal. There is a long, albeit sporadic, history of using fly larvae (maggots) to encourage wound healing though, perhaps surprisingly in this era of high-tech medicine, larval therapy is currently undergoing something of a renaissance. Australian aborigines and other native peoples around the world have used fly larvae to treat wounds for centuries. In Western medicine the first recorded observation of the potential of fly larvae to promote wound healing was made by the French surgeon Ambroise Paré (1510–1590) who noted how the wounds of soldiers injured in the Battle of Saint Quentin (1557) healed faster if they were infested with maggots (Fleischmann *et al*., 2004). The army surgeon John Forney Zacharias used fly larvae to treat wounded soldiers in the American Civil War (1861–1865) and there were several subsequent reports in the medical literature on the beneficial effects of fly larvae on wound healing (Wollina *et al*., 2000). Larval therapy was used extensively in Europe and North America in the 1930s but it virtually ceased following the discovery of penicillin and other antibiotics (Fleishmann *et al*., 2004). However, antibiotic resistance is spreading rapidly among many disease-causing bacteria and some strains are now resistant to all currently available antibiotics. Needless to say, this is causing considerable concern in hospitals throughout the world (http://www.who.int/mediacentre/factsheets/fs194/en/).

The use of fly larvae to treat wounds is counter-intuitive since wound myiasis is a serious and potentially fatal condition for humans and other animals. To intentionally apply fly larvae to an existing wound, and thereby establish a wound myiasis, could therefore be considered irresponsible. The important difference between a naturally occurring myiasis and one that is established as part of a medical intervention is that in the latter situation the fly larvae are sterile, their numbers are carefully controlled and they are removed before they can damage healthy tissue.

Larval therapy usually employs the larvae of *Lucilia sericata* (referred to as *Phaenicia sericata* in American literature) that are reared under sterile conditions. This is because *Lucilia sericata* is relatively easy to maintain in the laboratory, it has proved effective in practice and there is now a comprehensive literature on its use in larval therapy. Many other species of Diptera also cause wound myiasis but there are few if any comparative studies to determine whether any of these might prove equally useful or better than *Lucilia sericata*. The larvae of some fly species, such as *Lucilia cuprina*, are considered 'too aggressive' and therefore likely to quickly move on and damage healthy surrounding tissues. However, Tantawi *et al*. (2010) (unintentionally) used *Lucilia cuprina* successfully to treat diabetic ulcers when their *Lucilia sericata* stock cultures became contaminated with them.

Superficial and sometimes even serious skin wounds would have been a feature of everyday life for our ancestors. Consequently, our bodies, like those of other animals, are capable of remarkable levels of self-repair. This is especially true when we are young and healthy but the ability declines with age and is seriously compromised by medical conditions such as diabetes, AIDS and other immuno-suppressive illnesses. The formation of any wound results in the death of cells and is followed by an inflammatory reaction in which cell debris and, if present, any fragments of the object causing the wound are removed. During this stage, immune cells (e.g. lymphocytes, macrophages and neutrophils) produce inflammatory chemicals called cytokines such as interleukin-1α, interleukin-1β and tumour necrosis factor alpha (TNF-α). The inflammatory substances cause the blood capillaries to dilate and become more permeable and thereby allow white blood cells (leucocytes), cytokines and the chemicals responsible for the blood clotting process to reach the injured region. While the inflammatory process is ongoing, tissue

repair commences with the stage known as 'organisation' or 'proliferation' in which the blood supply is restored to the damaged region. In this stage, any blood clots are replaced with granulation tissue that contains delicate capillaries and proliferating fibroblasts (these cells secrete collagen, elastin and other proteins that provide structural stability). Finally, there is the stage of regeneration and scar formation, although the capacity for this varies between tissue types and the nature of the wound. If the stimulus causing the wound persists (e.g. microbial infection), then the events described above occur concurrently rather than sequentially and this results in chronic inflammation. For example, persons suffering from diabetes are prone to develop ulcers on their feet and legs because of their susceptibility to vascular disease and microbial infections. Similarly, those who are bedridden often develop pressure sores owing to the blood supply to affected regions of the body being restricted and these sores then become infected by bacteria and can ulcerate. In both cases, the ulcers often do not heal naturally but persist for months or even years; they may even become worse or be a source of potentially fatal septicaemia. For healing to occur, the dead and dying tissue has to be removed in a process called 'debridement' and any underlying infection controlled. Surgical debridement can be painful for the patient and is not always successful and, as we have seen, antibiotic resistance means that many drugs are no longer effective as they were once. The larvae of *Lucilia sericata* feed on bacteria and dead tissue and therefore effectively debride the wound. In the process they also eliminate the awful smell that is associated with such wounds. This is a not insignificant consideration since suffering from foul-smelling wounds is extremely unpleasant for both the patients and those who must care for them.

Box 8.2 Larval therapy in practice

A hole is cut in a hydrocolloid dressing the size and shape of the wound and this is applied to the wound site. This confines the larvae to the wound site and protects the surrounding healthy skin; 5–10 sterile first instar larvae (~2mm in length) per cm^2 of wound are placed on a sheet of semipermeable nylon mesh (e.g., LarvE™ NET, Tegapore™: 3M) and this is then laid on the surface of the wound. The hydrocolloid dressing is then covered with waterproof adhesive tape so as to confine the larvae to the wound site. After 3 days the dressings and the larvae, which have usually grown to approximately 9 mm by this time, are gently washed out of the wound. The procedure is then repeated until the wound is clean and all the necrotic tissue is removed. Alternatively, the maggots may be applied to the wound site in a Biobag (BioMonde, Germany) which contains a set number of larvae (e.g. 50 or 100) confined between two layers of a polyvinyl alcohol hydro sponge (Preus *et al*., 2004). Whichever approach is adopted, most wounds only require one to three treatments. Most patients are positive about the benefits of this treatment although a few find it psychologically repugnant. During treatment, most patients experience either a decrease or at least no increase in their level of pain beyond a 'tickling sensation' although some do find the treatment painful (Gupta, 2008; Wolff and Hansson, 2003).

Lucilia sericata larvae contribute to wound healing through physically consuming bacteria and dead tissues and by producing secretions and excretions that promote wound healing and have antibiotic properties. Bexfield *et al*. (2010) identified a range of amino acids (e.g. L-histidine) and amino acid-like compounds (e.g. L-valinol) in the secretions and excretions of *Lucilia sericata* larvae and suggested that these may contribute to wound healing by stimulating angiogenesis. It is probable that not all the blowfly larval secretions and excretions are beneficial and Elkington *et al*.

(2009) identified a secretory protein produced by *Lucilia cuprina* that had an immuno-suppressive effect through inhibiting the proliferation of T lymphocytes. Antibiotic activity probably results from the presence of specific peptides in the secretions and excretions. Among those that have been studied, lucifensin has been shown to have activity against a range of Gram-positive bacteria, although not Gram-negative bacteria (Andersen *et al*., 2010; Čeřovský *et al*., 2010). *Lucilia sericata*, like other blowfly larvae, excretes ammonia but the consequences of this in the controlled circumstances of larval therapy are uncertain. Robinson (1940) soaked gauze packs in 1–2% ammonium bicarbonate solution and applied these directly to purulent wounds and found that they promoted healing. However, in sheep suffering from blowfly strike (where there are large numbers of larvae present), the wound site becomes alkaline and unionised ammonia is absorbed into the bloodstream where it has an immuno-suppressive effect (Guerrini, 1997). The role of pH in the wound-healing process is poorly understood (Schreml *et al*., 2010). Some of the protease enzymes involved in wound healing have alkaline pH optima (e.g. matrix metalloproteinases) and keratinocytes and fibroblasts grow and migrate more effectively under slightly alkaline conditions (Sharpe *et al*., 2009; Greener *et al*., 2005). However, if the proteinases are too active, they can delay healing and mildly acidifying the wound site can reduce colonisation by bacteria (Leveen *et al*., 1973).

8.3.3 Leech therapy

Leeches have been used in both Western and Eastern medicine for over two thousand years. Indeed, the enthusiasm of Western doctors for leeches was once so great that they themselves were often referred to as 'leeches'. The horse leech, *Hirudo medicinalis*, became extinct in some regions owing to over-collection for use in human medicine. This popularity was driven by the widespread belief that a great many ailments could be cured by removing either an 'excess of blood' or blood containing 'poisons'. Leeches were applied, sometimes in large numbers, to specific parts of the body to remove the offending blood. As modern Western medicine developed, it was realised that bleeding patients whether by the application of leeches or cutting a vein usually had no therapeutic benefit. Indeed, the loss of blood could be harmful and consequently the use of leeches in Western medicine largely stopped. However, in recent years it has been shown that leeches can prove useful in the treatment of a number of medical conditions (Michalsen *et al*., 2007).

During surgery, particularly that involving the reattachment of body parts, it is usually possible to join arteries that supply blood but not the veins that take it away. This is because the veins are usually too small and fragile to be stitched together and consequently blood accumulates within the affected region. This is known as venous congestion and can lead to the patient experiencing pain and a delay in healing. In some cases the region may become necrotic and the limb has to be amputated after all. By applying leeches to the affected region, it is possible to remove the excess blood until the venous supply naturally re-establishes. Leeches inject a local anaesthetic when they bite and therefore the procedure is relatively painless and the presence of anticoagulants in their saliva ensures that the wound continues to bleed for some time after the leech is removed. An individual *Hirudo medicinalis* can extract up to 15 ml of blood during a single feeding session but it is the continued slow loss of blood after feeding that is considered most beneficial.

Leech therapy has proved particularly useful for the surgical re-attachment of the body's extremities (e.g. fingers, ears) in which problems arise because blood tends to pool within the re-attached part. Leeches have even been used after surgery to reattach a man's tongue (see Colour

Plate 29) after it was bitten off during a fight (Kim *et al*., 2007). Leech therapy can also reduce the pain and symptoms of some forms of joint osteoarthritis (Andereya *et al*., 2008; Michalsen *et al*., 2008). However, more studies are required in this particular application of leech therapy to determine how the beneficial effects are brought about and its long-term consequences.

Box 8.3 Leech therapy in practice

In Europe and North America, the horse leech *Hirudo medicinalis* is used most frequently but elsewhere in the world other species are often employed. For example, in Asia, *Hirudinaria manillensis* is commonly used and in South Africa *Aliolimnatis michaelensis* has proved to be effective (Siddall *et al*., 2007b). *Hirudo medicinalis* is a protected species in the UK and its numbers have also declined in other parts of the world as a consequence of over-collection, habitat loss, and changes in agricultural practices: the *Hirudo medicinalis* leeches used in medicine are therefore usually specially bred by commercial operatives. However, molecular studies have indicated that many of the leeches supplied as *Hirudo medicinalis* are actually *Hirudo verbana* (Siddall *et al*., 2007c).

The procedure is best undertaken in a warm, quiet environment with low light conditions. Leeches are sensitive to light and are likely to try and escape rather than feed if they are disturbed. The leech is gently grasped in a gloved hand and encouraged to attach to the appropriate site. Leeches have soft smooth bodies that are easily damaged so it is better to use a dry gauze pad to manipulate them rather than forceps. Alternatively, the leech might be placed in a small container such as the barrel of a syringe or a cupping glass and this is up-ended and held in place with tape over the place it is intended to feed. Usually the leech will attach itself but making a small incision or adding a drop of sugar solution to the chosen site can act as an extra stimulant. Surrounding the bite site with dry gauze or dressings helps prevent the leech wandering off. *Hirudo medicinalis* typically feeds for anything from 20 minutes to 2 hours after which the leech drops off. Attempting to pull a leech off while it is feeding is not recommended since, like ticks, it would leave its mouthparts embedded in the body. If the leech has to be removed it is best to stroke its head region with a cotton bud soaked in saline, vinegar or alcohol but this needs to be done carefully otherwise the leech could vomit its ingested blood into and around the wound site. After a leech has dropped off, it is killed and disposed of. Re-using leeches would carry too much of a risk for the transmission of blood-borne diseases. New leeches are applied on successive days until a functional venous blood supply has become established: this typically takes 3–7 days (Yantis, 2009).

8.3.4 Malaria therapy (malariotherapy)

Malaria therapy was initially developed to treat the disease syphilis. Syphilis is a serious disease but it is currently treatable with antibiotics and is sometimes considered to be just one sexually transmitted disease among many. It is therefore difficult to appreciate just how common syphilis once was among the general population and how infection often progressed remorselessly to insanity and an early death (Hayden, 2003). Syphilis is caused by the spirochaete bacterium *Treponema pallidum* and is usually transmitted through sexual intercourse, although it can also

cross the placenta in pregnant women and cause congenital syphilis. Syphilis gives rise to a bewildering variety of symptoms and, as a result, in the medical profession it was known as 'the great pretender'. In the later stages of the disease, it causes neurosyphilis – also referred to as 'general paresis', 'general paralysis of the insane', and '*dementia paralytica*' – in which the patient suffers from progressive dementia and loses control of their voluntary movements. Until the widespread availability of effective antibiotics in the 1940s, patients who had reached this stage of the disease were often consigned to an asylum where they usually died within 4 years of arrival.

Box 8.4 The development of malaria therapy (malariotherapy)

Julius Wagner Ritter von Jauregg (1857–1940) (usually referred to as Wagner-Jauregg) was awarded the Nobel Prize for Medicine in 1927 for his work in developing malaria therapy for the treatment of neurosyphilis. Wagner-Jauregg worked as a psychiatrist at a clinic in Vienna and noted how the mental health of some of his patients improved following illnesses that induced high fever. He decided to carry out a more in-depth series of observations and recorded that patients with neurosyphilis showed considerable improvement in their condition following bouts of fever associated with erysipelas and tuberculosis. He tried various means of inducing fever in his patients but these had little effect on their mental well-being. He ascribed this to the fevers not raising the body temperature high enough. He thought that malaria would be the ideal means by which this could be achieved but lacked a source of the infection. Eventually, in 1917, a soldier was admitted to his ward suffering from shell-shock who had caught benign tertian malaria (*Plasmodium vivax*) while serving in the Crimea. He took blood from the soldier and injected it into nine patients suffering from neurosyphilis. After they had experienced at least seven episodes of fever he treated them with quinine. Six of the patients subsequently improved so much that they were able to leave the clinic and return to work. They were not actually cured of syphilis and four of them suffered relapses in later years but it should be remembered that their prognosis at the time of their treatment was exceedingly bleak. Wagner-Jauregg was encouraged to continue with his studies and publish his findings which were met with considerable enthusiasm. Malaria therapy (sometimes referred to as 'malariotherapy') was quickly adopted in Europe and around the world and became the principal means of treating neurosyphilis. Indeed, malaria therapy continued to be used in specific cases in the USA until the 1960s and in the UK in the 1970s.

Malaria therapy entered the news briefly in the 1990s when Dr Henry Heimlich suggested that it could be used to cure Lyme disease, cancer, and AIDS (e.g. Heimlich, 1990; Heimlich *et al*., 1997). Dr Heimlich was famous for developing the 'Heimlich manoeuvre' for treating choking and drowning (although the procedure is no longer recommended). He was a master of self-publicity but held some unorthodox views that made him unpopular with many in the medical profession. Denied the opportunity to undertake work on this theory in the USA, he, together with his co-worker Edward Patrick, set up operations in China, Ethiopia, and Gabon. However, the World Health Organisation was less than impressed and a report published by the Commission on Macroeconomics and Health stated that their work was an example of 'unscrupulous and opportune research which exploits the want of a given population' (Bhutta, 2002).

Plasmodium ovale came to be the most frequently used species of *Plasmodium* for malaria therapy because it induces a high fever but seldom causes serious pathology. However, in the early years of development all the other species were employed, including *Plasmodium falciparum*. Needless to say, the procedure was not without its risks and some people treated this way suffered harmful effects from the malaria and a few died. Those treated with *Plasmodium vivax* or *Plasmodium ovale* would also have been subject to recurrent infection due to reactivation of liver hypnozoites if not treated adequately. Even so-called benign forms of malaria can induce serious pathology and the people being infected with it were already weakened by syphilis. However, the risks posed by malaria had to be counter-balanced by the almost certainty of imminent, unpleasant death from syphilis. Nevertheless, deliberately infecting someone with a known pathogen does raise ethical issues and malaria therapy has always been controversial.

Initially, transmission was undertaken by withdrawing 2–5 ml blood from the vein of an infected donor and injecting this subcutaneously into the recipient. Subsequently, other approaches were adopted including intravenous or intramuscular injections, and mosquitoes (Nicole, 1943). The recipient was allowed to undergo a series of fevers after which they were treated with quinine. The results were rather variable and were affected by the level of fever induced and the severity of the patient's syphilitic symptoms. As a general rule, about 30% of patients experienced full remission, 20% partial remission and the remaining 50% showed little or no improvement (Whitrow, 1990). Patients whose neurosyphilis was at an advanced stage and had already experienced irreparable brain damage might be given several extra years of otherwise good physical health but would not recover their mental faculties.

The physiological and immunological basis of how malaria therapy worked is still not known. Patients who experienced the most frequent fevers and the highest temperatures tended to have the best outcomes, but temperature alone is unlikely to have been the sole factor. Although *Treponema pallidum* lacks a heat-shock response and in culture all the spirochaetes are killed if maintained at 41°C for 24 hours (Porcella and Schwan, 2001), this is considerably more than a patient would experience during their episodes of malaria. In addition, other fevers that induce lower temperatures than malaria can also improve the condition of patients with neurosyphilis (Chernin, 1984). It is possible that the immune reaction against malaria also harmed the spirochaetes.

There is clearly some immunological link between the two organisms, possibly related to pathological mechanisms. Patients with *Treponema pallidum* infection produce high titres of antibody to a protein used in laboratory tests called cardiolipin. It is thought that a similar protein is released by cells in the body in response to the detrimental effects of the bacterium and it may also be contained within the treponeme itself. This causes the production of anti-cardiolipin antibodies, which although not specific, are taken as a marker of active syphilis infection and used as the basis of the VDRL (Venereal Disease Research Laboratory) and the RPR (Rapid Plasmin Reagin) tests (also sometimes called the 'Wasserman reaction'). Interestingly this anti-cardiolipin antibody has also been noted in patients with other infections, including viral diseases as well as trypanosomiasis and malaria. In these cases, the antibody is produced at a lower level than during syphilis and only lasts for about 6 months after the acute infection. This means that if the VDRL or RPR tests are used to determine whether a person might have syphilis, then the laboratory needs to be given any information about recent history of malaria to help them identify any possible false-positive results. In all cases a specific anti-*Treponema pallidum* antibody test would also be carried out, so a correct diagnosis should be assured.

8.4 Parasites as sources of novel pharmaceutically-active compounds

Parasites produce a variety of secretions and excretions and some of these include compounds with potentially useful pharmacological properties. Between the 1930s and 1950s there was considerable interest in both Russia and America in the potential of extracts derived from *Trypanosoma cruzi* to treat certain forms of cancer (Krementsov, 2009). The results of these studies were ambiguous, with some workers claiming remarkable effects and others finding none at all or even harmful toxicity. This is hardly surprising considering the political problems that limited co-operation between workers in the two countries, the logistical problems of preparing the *Trypanosoma cruzi* extracts, and the genetic diversity of *Trypanosoma cruzi*. Although products based on *Trypanosoma cruzi* extracts were eventually marketed, the active ingredients were never identified and interest in this line of research ultimately ceased.

There has been much more success in developing pharmacologically useful products from blood-feeding parasites. Any organism that feeds on mammalian blood has to overcome the problem of blood clotting which could lead to them becoming stuck to their victim or their mouthparts being blocked. When a blood vessel is injured, whether by a needle or the mouthparts of a hookworm, a sequence of physiological processes are initiated which, if able to continue to completion, would stop the loss of blood. Together, these processes are known as haemostasis and can be divided into three interlinked events: (1) vasoconstriction; (2) the formation of a plug of platelets; and (3) the production of a web of fibrin threads that coats and stabilises the platelet plug.

The formation of a platelet plug commences when circulating blood platelets are exposed to von Willebrand factor, collagen and other platelet-activating factors contained in the connective tissue that underlies the surface epithelium of blood vessels. Once activated, the platelets release the contents of their secretory granules (these include adenosine diphosphate, serotonin and the prostaglandin thromboxane A_2) into the surrounding plasma and they activate more platelets. Activated platelets change their shape from spherical to stellate (star-shaped) and become 'sticky' so they become stuck to the collagen and also to one another. The activated platelets also bind to the soluble plasma protein fibrinogen and this then cross-links with glycoproteins to form insoluble fibrin that binds the platelets together and stabilise them so they form a secure plug.

Fibrin formation (i.e. coagulation) involves a complex sequence of reactions that are divided into the contact activation pathway (intrinsic pathway) and the tissue factor pathway (extrinsic pathway). Both pathways involve enzymes and glycoprotein cofactors which, once activated, catalyse the subsequent reaction until finally fibrin monomers become cross-linked to form insoluble fibrin polymers and a clot starts to form. Each of the steps in the pathways is under the control of a specific enzyme or glycoprotein that is known as a coagulation factor. Most of the enzymes are serine proteases but factor XIII is a transglutaminase and factor V and factor VIII are glycoproteins.

Most blood-feeding (haematophagous) organisms produce chemicals that inhibit platelet aggregation and one or more of the serine protease enzymes (coagulation factors) involved in the tissue factor pathway. For example, the leech *Hirudo medicinalis* produces a protein called 'calin' that interferes with platelet aggregation and a polypeptide called hirudin that interferes with fibrin formation. Calin prevents the formation of a platelet plug by interfering with the interaction between platelets and collagen (Deckmyn *et al*., 1995). Another compound, 'hirudin' is produced in a variety of isoforms and complexes with α-thrombin (coagulation factor IIa) and

there by prevents it from catalysing the conversion of fibrinogen to fibrin (Markwardt, 1970). Similarly, the hookworm *Ancylostoma caninum* produces a protein called HPI-1 (Del Valle *et al*., 2003) that prevents platelets from aggregating together via fibrinogen and also a range of specific inhibitors of coagulation factors VIIa and Xa (Lee and Vlasuk, 2003; Mieszczanek *et al*., 2004; Stanssens *et al*., 1996). Further details of anticoagulants produced by parasites are reviewed by Ledizet *et al*. (2005).

Chemicals that interfere with blood clotting are of enormous interest for their potential for treating thrombotic diseases, that is, those in which a thrombosis (blood clot) forms within a blood vessel and thereby reduces or stops blood circulation in the affected region, such as stroke, myocardial infarction, and deep vein thrombosis. Hirudin is currently employed to treat conditions as varied as topical bruising and heart disease. It has the advantages of low toxicity, it does not interfere with any coagulation factors apart from α-thrombin or any other physiological processes, it does not appear to stimulate an immune response, and is eliminated completely via the kidneys (Stamenova *et al*., 2001). Nematode anticoagulant peptides derived from *Ancylostoma caninum* have been used successfully to prevent thrombus formation following surgery but further clinical trials are needed to determine fully their effectiveness and safety (Lee *et al*., 2001).

Viral haemorrhagic fevers such as those caused by Ebola virus and Marburg virus are characterised by the development of disseminated intravascular coagulation (DIC). This develops as a consequence of over-activation of the blood coagulation cascade which leads to the formation of an excess of thrombin and the production of small blood clots in blood vessels throughout the body. These small blood clots disrupt the blood flow to organs such as the kidneys and consume so many platelets and coagulation factors that the normal clotting process is disrupted and the patient suffers from abnormal bleeding in the gastrointestinal tract, lungs, and skin. Geisbert *et al*. (2003) found that macaque monkeys injected with a potentially lethal dose of Ebola virus did not develop the disease or expressed only mild symptoms if they were subsequently treated with recombinant nematode anticoagulant protein c2 (rNAPc2). NAPc2 is one of the anticoagulant proteins produced by *Ancylostoma caninum* and exerts its effect by inhibiting the factor VIIa/tissue complex through a unique mechanism. NAPc2 initially interacts with coagulation factor Xa and the resultant binary complex then inhibits factor VIIa of the factor VIIa/tissue factor complex. rNAPc2 inhibits angiogenesis and both primary and secondary metastatic tumour growth in mice. Interestingly, another recombinant nematode anticoagulant protein derived from *Ancylostoma caninum*, rNAP5, that specifically inhibits coagulation factor Xa has no effect on tumour growth (Hembrough *et al*., 2003).

8.5 Parasites as biological control agents

Biological control is the use of an organism to control the population of another organism and a good review is provided by Bale *et al*. (2008). The targets are pests which may be plants, invertebrates or vertebrates and the control agents are predators, pathogens, parasitoids, and parasites. Biological control can be highly effective, particularly in closed environments such as greenhouses, and may be integrated into other control practices. The characteristics of a good control agent, whether it is chemical or biological, are that it should be specific to the pest, not harm non-target organisms, rapidly eliminate the pest from the place it is causing a problem, have a long-lasting effect on pest reduction, and not give rise to resistance. Chemical control agents often suffer from a lack of specificity, harm other organisms, and tend to develop resistance problems

within a short period of time. Parasites are often specific to their hosts, there is seldom a problem with resistance developing, and they may leave eggs or cysts that ensure control of subsequent generations of the target pest. However, as a general rule, parasites do not cause epidemics and they give rise to chronic disease. Consequently, although a parasite may cause illness, an infected pest could still be growing where it is not wanted, consuming a crop or causing or transmitting a disease. Parasites can also be difficult to rear in large enough numbers to use on a commercial scale and there can be problems in spreading them in the environment so that they are able to infect the pest. Therefore, although there are several examples of parasites providing exceptionally good control of the target pest species, they are not used as extensively as other pathogens such as viruses, bacteria, fungi or parasitoids.

There are basically three ways of practising biological control: importation (classical), conservation, and augmentation. When organisms are accidentally or intentionally imported into a new country, they often leave their natural enemies behind and if there are no local control agents, they reproduce rapidly and become pests. Classical biological control aims to exert control on the introduced species by exposing the pest to its original control agent or a suitable alternative. Conservation biological control is a long-term strategy that involves manipulating the environment so as to encourage the growth and survival of the natural enemies. As its name suggests, augmentation biological control involves increasing the abundance of the control agent. This is often done by artificially rearing large numbers of the control agent and then releasing them at an appropriate time and place. The population of the control agent is therefore increased to a level considerably above that which would occur naturally in order to ensure a rapid decline in the pest population. This method, therefore, requires constant human intervention and is essentially using the control agent in a similar manner to pesticides.

An organism may leave its predators behind when it is introduced into a new country but its parasites often travel within or upon it. If the parasite finds a suitable vector or intermediate host (or does not require one), then it may continue to be transmitted and may even exploit new host species. Consequently, there are few examples of parasites being used in classical biological control, due to the accompanying risks to other animals or plants. There are similarly few examples of specific conservation practices being adopted to improve the survival of parasites in the environment. Indeed, any attempt to improve the survival and transmission of the parasite of a pest runs the risk of also enhancing the transmission of the parasites of other organisms. Most examples in which parasites have been used as biological control agents therefore fall into the category of augmentation.

Many of the parasites used as biological control agents are nematodes, although there has been some success using microsporidia (now classified as fungi) for the control of locusts (Henry and Oma, 1981) and mosquitoes (Koella *et al*., 2009). Nematodes have proved particularly useful for the control of certain insect pests and also of slugs and snails. Those species that infect insects, such as *Heterorhabditis* and *Steinernema*, are often referred to as 'entomopathogenic nematodes' and they are used to control a variety of insect pests, such as vine weevils (*Otiorhynchus* spp.) and 'leatherjackets' (Tipulidae), in many parts of the world (Grewal *et al*., 2005).

8.5.1 Life cycle of the entomopathogenic nematodes *Heterorhabditis* and *Steinernema*

When the free-living infective third-stage larvae of *Heterorhabditis* and *Steinernema* locate a suitable insect host, they invade it via its mouth, anus, or spiracles; *Heterorhabditis* larvae are also

capable of penetrating via the intersegmental membranes. The infective stage is sometimes referred to as a '*dauer*' larva and it retains the second-stage cuticle as a protective sheath. *Dauer* is a German word that is usually translated as 'duration' or 'period'. The term '*dauer*' larva is applied to third-stage nematode larvae that have entered a period of arrested development. This state is associated with dispersal and an increased ability to withstand harmful environmental conditions. *Dauer* larvae are also formed by non-parasitic nematode species such as *Caenorhabditis elegans* in response to crowding, starvation and other adverse circumstances. The *dauer* larvae disperse by attaching to other animals. The formation of *dauer* larvae has therefore been suggested as one of the first stages in the evolution of parasitism in nematodes (Ogawa *et al*., 2009).

In common with several other entomopathogenic nematode species belonging to the order Rhabdita, *Heterorhabditis* and *Steinernema* have unusual symbiotic relationships with bacteria that contribute to their pathogenicity. Once within the insect, the *dauer* larvae penetrate the insect's haemocoel and there they regurgitate bacteria that are stored within the anterior region of their gut. The bacteria ejected by the nematodes rapidly multiply within the insect and this, together with the toxins they produce, causes the death of the parasitised insect within 24–48 hours of becoming infected. In addition to killing the host insect, the symbiotic bacteria also produce antibiotic substances that prevent the corpse being colonised by other bacteria species and also feeding deterrents that make it unpalatable to ants (Zhou *et al*., 2002). The nematodes are not harmed by the bacteria toxins and they feed upon the bacteria that multiply within the corpse.

Heterorhabditis nematodes develop into self-fertilising hermaphrodites while in *Steinernema* the sexes are separate. Depending upon the size of the host and the number of nematodes, they may go through two or three generations within the dead insect. Rhabditid nematodes, such as *Heterorhabditis* and *Steinernema*, have an unusual style of reproduction called *endotokia matricida* in which the eggs hatch within the mother's uterus and the young then eat her alive. The onset of *endotakia matricida* is delayed if the food supply is plentiful but once initiated, the food supply does not affect its duration (Johnigk and Ehlers, 1999). After the first-stage larvae hatch within their mother's uterus, they commence feeding on her tissues, killing her in the process and eventually leave her body when they reach the third stage. If food is abundant, the larvae will then continue their development to adulthood and reproduce within the body of the insect to form a second generation. If, however, the food supply is limited, the nematodes will emerge from their mother's body as *dauer* larvae which leave the insect corpse *en masse* and enter the soil. The *dauer* larvae remain within the soil and are incapable of developing further until they have infected a suitable host. In some nematode species the *dauer* larvae employ a 'sit and wait' strategy while in others the *dauer* larvae actively search for suitable hosts using chemical cues.

Box 8.5 Rhabditid nematode-bacteria relationships

Different species of symbiotic bacteria are associated with different Rhabditid nematode species. This may be a single-species relationship or an association with two or more closely related bacteria species. For example, *Heterorhabditis bacteriophora* has an association with the bacteria *Photorhabdus luminescens* and *Photorhabdus temperata*. The bacteria associated with entomopathogenic nematodes are essential for both the worm's survival and the insect host's death. Entomopathogenic nematodes lacking bacteria are unable to develop and do not kill their host insect (Ciche *et al*., 2006). By contrast, the slug parasite *Phasmarhabditis hermaphrodita* is able

to form associations with assemblages of bacteria without affecting its pathogenicity (Rae *et al*., 2010). This may (at least partly) explain why *Phasmarhabditis hermaphrodita* also differs from the entomopathogenic nematodes in being a facultative parasite that can survive as a saprophyte feeding on slug faeces and as a parasite of living slugs.

The bacteria associating with entomopathogenic nematodes are unable to survive on their own in the soil and must form a symbiotic relationship with the nematodes. Initially the bacteria attach themselves by their fimbriae to the posterior region of the intestine of a developing female worm. Fimbriae are projections from the cell wall of bacteria and have a variety of functions but are particularly important in attachment and invasion. Within the nematode gut the bacteria form a biofilm and then invade the cells of the rectal gland. During the development of the nematode, the rectal glands lyse and the bacteria are released into the mother nematode's body cavity where they are ingested by the larvae growing within her. The bacteria initially invade the cells of the pharyngeal-intestinal valve of the larvae and then move to the lumen of the intestine. Although the bacteria are essential for the survival of the nematode, the association is not without its costs and *dauer* larvae that are bacteria-free survive longer than those which are infected (Chapuis, 2009).

8.6 Parasites as forensic indicators

Forensic biology is the use of biology to answer legal questions. There is a tendency to focus on crimes such as murder but in reality the legal cases in which the knowledge of biology is important range from drug trafficking to wound analysis and from the illegal trade in protected species to sexual assault (Gunn, 2009). Given that most organisms, including humans, are infected with parasites, they might be expected to prove useful forensic indicators. In fact, there are few instances of this although this is most probably because their full potential has not yet been exploited.

When someone dies suddenly and unexpectedly, there needs to be a thorough investigation to determine the cause. Although some parasitic diseases can be fatal, they are seldom of rapid onset. One notable exception to this is cerebral malaria and there are several cases in the forensic literature in which it was unexpected but subsequently found to be the cause of death (e.g. Chappuis *et al*., 2003; Menezes *et al*., 2010). There are few reports of parasites being used intentionally to harm or kill people. One of the most commonly cited cases where it did occur is that of Eric Kranz who in 1970 deliberately contaminated the food of his student roommates with *Ascaris* eggs. Kranz had been in dispute with his roommates and during one altercation he said that he would infect them with parasites. This was dismissed as an idle threat but when his roommates were hospitalised with respiratory distress, he fled. The doctors treating them had no reason to suspect that the cause was ascariasis and therefore gave them antibiotics, which of course had no effect. It was only when larvae were detected in the sputum they coughed up that the cause was established and an effective treatment regime instigated. There was clearly circumstantial evidence that the *Ascaris* infections were not acquired by chance and Kranz was subsequently arrested and charged with attempted murder. Kranz was a post-graduate student at MacDonald College, Quebec, Canada, studying parasitology and had stolen the *Ascaris* eggs from a laboratory at the college (Budowle *et al*., 2005). The fact that this is such an isolated case suggests that it is only

when there is the rare coincidence of a parasitologist with a homicidal grudge that parasites are likely to be used for nefarious purposes. However, it could also indicate the problem of distinguishing malicious from accidental parasite transmission. If Kranz had not made his initial threats and then disappeared when his victims fell ill, he might have escaped prosecution. For example, in South Africa, it is alleged that women sometimes gain revenge on unfaithful men by lacing their drink with *Taenia solium* eggs (Kriel and Joubert, 1996; Kriel, 1997). *Taenia solium* is very common in South Africa and unless the intended target found a whole proglottid at the bottom of his beer glass, it would be difficult to prove the source of the infection. The extent to which this is a common crime or an urban myth is uncertain, as is the ability of *Taenia solium* eggs to survive in alcoholic beverages.

Parasites may, in some circumstances, link victim and assailant together. For example, crab lice (*Phthirus pubis*) are normally only transmitted through sexual contact and it is possible to isolate human DNA from within their gut (Lord *et al*., 1998). Consequently, the finding of crab lice on the body of 'person X' containing the DNA of 'person Y' indicates that persons X and Y must have had sexual contact. This could be useful where there is a claim of sexual assault but no semen or other evidence of an association between the two people. The fact that parasites are hard to rear artificially in the laboratory and do not tend to cause epidemic disease means that they are not considered suitable for biological warfare or bioterrorism. However, during the Second World War, the Japanese developed 'bombs' containing fleas infected with plague bacilli (Harris, 2002).

In addition to determining the cause of death, one of the most important questions to be answered in any case of 'suspicious death' is when the person (or animal) died. This is usually determined from physiological changes, the state of decay, and the development of blowfly larvae or other invertebrates upon the body (Gunn, 2009). Various ectoparasites could also be added to this list of forensic indicators. For example, as the corpse starts to cool down, lice (e.g. *Pediculus humanus corporis*) start to leave the body. The presence of these lice on the clothing or moving off the body is therefore an immediate indicator that the person or animal died only a short time previously. Those ectoparasites that are unable to leave the dead body, such as the skin follicle mites *Demodex folliculorum* and *Demodex brevis*, eventually die of starvation, or succumb to the chemicals produced during the decay process. Whether or not these mites are still alive has therefore been suggested as an indicator of the time since host death. *Demodex folliculorum* and *Demodex brevis* are cosmopolitan parasites and in humans they have been recovered in over 50% of the study population (Desch, 2009). However, Ozdemir *et al*. (2003) were unable to find an obvious relationship between time of death and mite survival. Similarly, the factors that affect mite survival after host death still need to be elucidated.

Box 8.6 The use of parasitoid wasps to determine the minimum time since death

The parasitoid wasp *Nasonia vitripennis* lays its eggs in the pupae of a range of blowfly species using her ovipositor to bore a hole through the puparium and then lays her eggs on top of the developing pupa. This occurs 24–30 hours after the pupa has formed, i.e. the point at which the blowfly third instar larval cuticle separates from the pupal cuticle and forms the puparium.

After hatching, the wasp larvae feed on the fly pupa, killing it in the process. The wasps pupate within their host's pupaium and the adults chew their way out after 10–50 days, depending on the temperature. Because *Nasonia vitripennis* only lays its eggs during a very restricted time period, recording when the adult wasps emerge can be used in forensic science to determine the post-mortem interval (Grassberger and Frank, 2003). This is because if one knows how long the wasps take to develop, it can be calculated when they laid their eggs and on that date the pupa would be 24–30 hours old, from which point it would be possible to determine when the blowfly eggs were laid and therefore approximate the date on which the person died.

Many forensic investigations require the determination of the provenance of a person (or animal or plant) who (which) may be living or dead. In this scenario, any associated organisms that have specific environmental requirements or have a restricted geographical distribution can provide an indication of past movements. Many parasites fit both these criteria and therefore have forensic potential. For example, the illegal trade in wildlife and exotic flora is worth millions of pounds per year and often involves organised crime. One particular problem is where disputes arise whether an animal or plant was collected from the wild (and therefore its trade is illegal) or was captive-bred (and could therefore be traded subject to the local legislation). Organisms collected from the wild usually harbour parasites, which are often absent from captive bred species owing to medical treatment or the absence of a suitable vector or intermediate host. For example, wild komodo dragons (*Varanus komodoensis*) usually harbour hemogregarine parasites but these are absent in captive animals (Gillespie *et al*., 2000). In some instances, an animal may be protected in one country/region but can be hunted and collected in another. Parasites might prove useful indicators in such cases. For example, Criscione *et al*. *(*2006) found that the genotype of parasites infecting trout (*Oncorhynchus mykiss*) provided a better indication of the source population than the host's own genotype. In human forensic cases there is increasing interest in the potential of viruses as indicators of where a person grew up (Ikegaya *et al*., 2007). This can be useful in situations in which unidentified bodies are found and when a person claims a certain nationality but there is no documentation. The presence of parasites and their genotype might similarly provide evidence of an association with a particular country or region but as yet there are no published studies in which this has been done in a forensic context.

Questions

1. Briefly explain the basis of the 'hygiene hypothesis'.
2. Why is stimulation of a Th2 immune response thought to protect against autoimmune conditions?
3. Discuss the arguments for and against the role of parasites in the development of post-infectious irritable bowel syndrome.
4. Why, with one exception, would consuming pills containing tapeworm eggs not give you an adult tapeworm?
5. How are leeches useful in plastic surgery?
6. Why might a maggot-infested wound heal faster than one that is not maggot-infested?

7. Why do some people suggest that consuming the eggs of *Trichuris suis* could alleviate the symptoms of Crohn's disease?
8. Briefly explain how malaria therapy was used to treat syphilis.
9. What is a '*dauer* larva'? Explain the function of *dauer* larvae in the life cycle of entomopathogenic nematodes.
10. How might parasites be used to determine whether an animal was captive bred or collected from the wild?

9

Identification of protozoan and helminth parasites

9.1 Introduction

All practical parasitology depends upon correct identification. It is like hearing a noise in the house at night and wondering whether it is just the furniture creaking, the pet cat or a burglar. First, you need to know whether anything is there, then what it is. Having done that, you will be able decide what to do about it: if there is nothing there, then the noise can be ignored, if it is the cat, it is also not a problem (at least no more than usual), but if it is a burglar then something needs to be done to get rid of him. In this chapter we will explain how diagnostic tests can prove useful in a wide range of situations and the different approaches to identifying and quantifying parasites. Obviously, we will not attempt to provide practical instructions of how to identify specific parasites and those readers requiring more detail on parasites of medical importance should consult Cheesbrough (2005), Garcia (2009) or the more comprehensive Garcia (2007). The now dated but admirably practical MAFF Manual of Veterinary Parasitological Laboratory Techniques is still useful for those wishing to study parasites of domestic animals (Anon, 1971). The DPDx-CDC Parasitology Diagnostic Web Site (http://dpd.cdc.gov/dpdx) is also a good source of information along with the UK NEQAS Parasitology scheme site at www.btinternet.com/~ukneqas.parasitologyscheme.

9.2 The importance of correct identification

Many parasitic diseases cause non-specific clinical signs and symptoms such as fever, diarrhoea, weight loss and lassitude. Consequently a doctor or vet would seldom be able to give an immediate diagnosis from an examination of a patient or animal without requesting further tests. Contrary to popular perceptions, doctors and vets seldom perform diagnostic tests themselves. Instead, they send samples to laboratories where professionals undertake the identification and they then inform the doctor or vet of the results. Once the cause of the disease has been identified, it is possible to begin appropriate treatment and thereby prevent the condition becoming worse. Drugs are often expensive and always carry the risk of inducing harmful side effects so it is important to ensure that they are only used where appropriate and at the correct dose. For example, in a patient with symptoms of diarrhoea, it would be necessary to determine whether the causative agent was a bacterium, virus or parasite through examination of a faecal specimen. In a case

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where cysts or trophozoites of the protozoan *Entamoeba* spp. were found in the sample, then it would be necessary to establish the species of the isolate. This is because *Entamoeba coli* is a harmless commensal while *Entamoeba histolytica* is a potentially dangerous parasite (although strains differ in their pathogenicity). Once treatment has commenced, diagnostic techniques are useful for monitoring its success and can confirm when a cure is achieved.

Correct identification is important in epidemiological studies and in monitoring the effectiveness of control programmes. For example, these programmes can identify animals and people who are infected with parasites but are not expressing clinical symptoms. Such people or animals can be sources of infection for others in the population and they may also be vulnerable to developing diseases later. Similarly, correct identification can determine whether a control programme for a particular disease is required, can detect potential sources of infection, and help to decide whether a control programme is delivering the required results. In the latter instance, identification techniques would also be used to identify parasites within invertebrates that are acting as vectors or intermediate hosts. For example, the effectiveness of a programme to control the filarial nematode *Brugia malayi* through targeting its *Anopheles* vector would be enhanced by examining mosquitoes gathered in the area for the presence of the infective filarial worms.

Some diseases are classed as 'notifiable' and it is a legal requirement to inform the relevant authorities of their presence so that control measures can be instigated (Table 9.1). This is clearly a very serious matter and it is therefore essential that any diagnosis is accurate and timely. For example, in England and Wales, malaria in humans must be notified to the health authorities and notification of warble fly infections in cattle must be sent to the government agency dealing with animal health. The system of notification of infectious diseases in the UK is a very important part of surveillance. If cases of the disease are reported to a central authority, it becomes clear at an early stage whether it is an isolated, sporadic case or whether there is an outbreak with a particular pattern of dispersal. There are two main reasons why certain diseases are classed as 'notifiable'. One is when the disease is being controlled through a vaccination programme. If there are suddenly a lot of cases of that infection, then it would indicate a problem with the vaccine or that the appropriate people or animals had not been receiving the vaccine. The other reason is when it is an unusual and serious infection that could potentially establish itself or spread in the country. Malaria is a notifiable disease in the UK because mosquitoes capable of spreading the disease are present in the country and malaria used to exist there until relatively recently. By contrast, Human African Trypanosomiasis is an equally serious disease but it is not classed as 'notifiable'. This is because tsetse flies are not found in the UK and therefore Human African Trypanosomiasis could

Table 9.1 Instances in which correct identification is important

Patient management: determination of most appropriate therapy, drug dosage, and duration of treatment
Prevention of long-term complications by identifying an infection before it becomes serious
Identification of asymptomatic individuals
Identification of human and animal reservoirs of infection
Identification of contamination of food and the environment with transmission stages
Identification of vectors and intermediate hosts
Surveillance and epidemiological studies
Monitoring the need for or success of control measures by assessing parasite prevalence in host and vector/intermediate hosts
Identification of genetic markers for drug resistance and pathogenicity in the parasite population

not spread among the population. With the advent of molecular techniques, it is now possible to identify parasites not only to species level but also to determine their genetic characteristics. Consequently, one can now monitor parasite populations for their susceptibility or resistance to particular drugs and the presence of genes that are linked to pathogenicity.

9.3 Properties of an ideal diagnostic test

The properties of an ideal parasite diagnostic test are no different from those of any other test for a medical or veterinary condition and are listed in Table 9.2. Obviously, the test must be accurate and the results must be unambiguous, otherwise inappropriate treatment may be given or a control programme may be compromised. This is particularly important where pathogenic and non-pathogenic species live in the same environment. For example, as already mentioned, it is important to distinguish between *Entamoeba coli* and *Entamoeba histolytica*. Similarly, if the test cannot distinguish between a parasite of wild animals and a very similar parasite of humans, it can result in a costly and misdirected control programme. For example, there are many *Plasmodium* species that infect wild birds and mammals and these are transmitted by a range of different mosquitoes. If a test were unable to distinguish between mosquitoes transmitting bird malaria and mosquitoes transmitting human malaria, a great deal of energy would be spent combating the wrong mosquitoes. The test must be sensitive because parasites may be present in low numbers within the sample being analysed. Low parasite abundance in a blood, tissue or faecal sample does not always mean that the parasite is present in low numbers throughout the body. Similarly, even small numbers of the infectious stages of some parasites can cause potentially fatal diseases.

The ideal test must be simple because whenever anything becomes complicated, there is a greater opportunity for errors to occur. Simple tests also require less training and may be suitable for automation. The diagnostic test should also be safe for the person performing the assay and not use potentially hazardous chemicals. It should also be harmless for the patient and not cause discomfort. For example, tests that necessitate the collection of cerebro-spinal fluid (e.g. some tests for Human African Trypanosomiasis) require medical supervision, can be unpleasant for the

Table 9.2 Properties of an ideal parasite diagnostic test

Tests for a clinically useful marker
Gives reliable results, i.e. scientifically accurate and clinically credible
Gives reproducible results, i.e. if the sample is re-tested the results are the same each time
Has high sensitivity and high specificity
Easily interpreted
Quick and simple to perform
Safe to both patient and operator
Does not cause patient discomfort
Does not require unstable chemicals
Does not require delicate instruments
Cost effective
Reagents are readily available

patient and carry risks of causing nerve damage (see Colour Plate 30). This is particularly the case in developing countries in which the shortage of equipment sometimes results in the re-use of needles that then become blunt. If the test involves a painful procedure, there is a reduced chance of the patient turning up for follow-up investigations. Tests that require unstable chemicals and delicate instruments are seldom suitable for use in developing countries outside major cities. Cost is a major factor in any diagnostic test, just as it is in drug design and parasite control. The definition of affordability will depend upon the target population and the number of tests that are required: an 'expensive' test that is only undertaken a few times every month may be considered affordable while a modestly-priced test that has to be undertaken many times every day can quickly become a serious burden on resources. Within industrialised countries labour costs are a major factor in laboratory budgets and there is a move to replace manual tests with those that can be undertaken by machines. Consequently, simple tests such as blood smear analysis are becoming expensive when performed by hand, although the equipment and chemicals used are relatively cheap. By contrast, manual tests remain affordable in developing countries where labour is cheap. When considering cost, the overall expense to the health or veterinary care service should be taken into account. For example, if a sophisticated and high priced test provides an answer quickly enough to reduce the number of nights the patient has to stay in hospital or allows them to be treated with a cheaper drug, then it can be considered 'cost effective'.

The test must be quick because this ensures that concerns are dealt with promptly. This is particularly important in situations in which the patient could die within hours unless the correct treatment is given, such as where cerebral malaria or amoebic encephalitis is suspected. Most parasitic diseases are, however, chronic and the patient would not die if treatment were delayed. Nevertheless, a long gap between taking samples and diagnosing the infection can mean that the infected person or animal suffers more pathology and is a source of infection for others or vectors or intermediate hosts. It is also important to provide a quick diagnosis to ensure patient compliance. Whenever people are requested to 'come back in a fortnight', it is certain that a proportion will not, and the longer the time gap, the less chance there is of them returning.

It is impossible to develop a diagnostic test that fulfils all the above criteria. For example, a test may be accurate, sensitive, and quick but is too expensive. Alternatively, it may be accurate, sensitive, and uses cheap reagents but so complicated that it can only be undertaken by highly trained individuals and takes a long time to perform. As in all aspects of life, the test used in a particular situation is often a compromise between competing requirements. It may also have to be chosen from the list approved by the regulatory authorities. Laboratories are not 'free agents' and must abide by regulatory and financial constraints. All countries have guidelines about which tests are approved for the diagnosis of particular diseases. However, having regulations and enforcing regulations are two different things and in some developing countries, the diagnosis of parasitic diseases is sadly compromised by poor quality diagnostics. This not only affects parasite treatment but also has a 'knock-on effect' on many aspects of parasite control.

There is a bewildering array of laboratory techniques which can be applied to parasite identification, but it is helpful to think of them in two main categories. The first is the traditional approach of looking at the whole organism at a specific life cycle stage and classifying it according to morphological characteristics. The second is to assay for a defined part of the organism such as cell surface antigen, enzyme or piece of its genetic sequence. There are also some diagnostic tests which detect the immune response to the parasite infection. However, these tests tend to be of limited value, because, although they are technically straightforward, the results produced are often difficult to interpret.

9.4 Isolation of parasites

As described in Chapters 2 and 3, parasitic protozoa and helminths have more complex life cycles than most other microorganisms. Even the simpler protozoa with a single host usually exist in two morphological forms, while helminths can go through many different stages as they grow and develop into adult worms. Laboratory identification of a particular parasite requires an appreciation of its life cycle, so that the most appropriate specimen can be collected from a potential host or from the environment at the most suitable time. Host and vector invertebrates also have multi-staged life cycles and may interact with protozoa, helminths or mammalian hosts during some or all of these stages. Consequently, diagnostic tests may be necessary to identify parasites from their vectors and intermediate hosts.

Wherever possible, it is always preferable to begin the identification process by observing the whole organism. The 'traditional techniques' involved in macroscopic and microscopic examination of samples and the identification of any parasites present, using key morphological features, have changed very little since they were originally developed in the late nineteenth and early twentieth centuries. Methods for sample preparation and protocols for preparing stains are thus similar in many part of the world and therefore standardised.

The first step in identification of a parasite can be macroscopic examination by eye or using a low resolution (dissecting) microscope. If the parasite is large, this can provide enough information to determine the species to a satisfactory level (though if it is important to further classify by strain or subspecies, then further work might be required). For example, observations of whole adult tapeworms or their proglottids, fly larvae from a case of myiasis or scabies mites can be sufficient to identify the organism.

However, many pathogenic parasites are too small to be seen without the aid of a microscope in some or all of their life cycle stages. This means that samples of fluid or tissue must be taken from the infected host and examined. An understanding of the life cycle and the effect of the pathogen on the host helps to decide which specimen to collect for laboratory investigation and the expected morphological appearance of any parasites which might be present. Chemical stains are often used to show up the parasite and highlight the internal structure of the cells, which helps to identify them.

A wide range of protozoa and helminths can be detected in the peripheral blood of infected hosts by light microscopy. Blood films are prepared from whole blood – either from a small incision (e.g. finger prick in humans) or venous blood collected in a bottle containing anti-coagulant. The films are made on glass slides and then stained, before examination under the microscope. In diagnostic parasitology, it is usual to prepare 'thick' and 'thin' films from a blood sample. A larger volume of blood is used to make a thick film and the cells are not fixed, which causes red blood cells to lyse and release any organisms present, which is useful for making the initial decision about whether a parasite is present in the host or not. However, this lack of fixation means that the morphology of the parasite can be distorted, which is why the fixed thin film is more useful for the full identification of species.

Fixed and stained slide preparations can also be made, in a similar way, from skin and tissue biopsies, bone marrow aspirates, lymphatic samples and other body fluids, depending on the pathology and life cycle of the parasite in question within the host. Considerable time and skill are often required to detect and identify parasites in stained films. Nevertheless, for most situations, direct observation of the organism in this way this remains the 'gold standard' test method against which other assays are evaluated.

Protozoan cysts and helminth eggs and larvae from intestinal parasites can be detected in faeces (or urine for some species) of infected hosts. In some situations other forms can be found as well. For example, trophozoites of protozoa can be excreted, particularly during acute stages of the illness, and after treatment adult worms are passed out in faeces. The simplest way of preparing a stool or urine sample for microscopic examination is to make a direct 'wet preparation'. If there is a high concentration of parasites in the sample, this can be sufficient to provide a qualitative result. To detect trophozoites of protozoa such as *Entamoeba* and *Giardia*, a 'warm' stool is the most useful specimen. This is, as the name implies, a faecal sample taken straight to the laboratory within minutes of the patient passing it, as it easier to see the motile, but transparent trophozoites as they move around in the wet preparation. Similarly, to diagnose the sexually transmitted protozoan *Trichomonas vaginalis*, wet saline preparations are made from fresh (preferably less than 1 hour old) genital swabs. The live flagellate trophozoites can easily be seen moving around in the sample. If the slide needs to be preserved for examination, then it can be fixed in alcohol and treated with a suitable stain, such as Giemsa.

Most intestinal parasites can be identified from non-motile stages such as protozoan cysts or helminth eggs. Consequently, it is generally not necessary to have a totally fresh faecal specimen and a sample up to a day old can be suitable for examination. It is usual to employ a method to concentrate the sample before making a saline preparation, to maximise the opportunity to detect parasites, which might be present in relatively low numbers. A number of methods have been developed, but in clinical diagnosis the formal-ether concentration technique is the most commonly used. Specific preparation methods are also used in surveys (usually for helminths) when attempting to quantify the concentration of eggs, which relates to worm burden, such as the Kato-Katz method (Cheesbrough, 2005).

In some species of parasite, the shedding of eggs or cysts is intermittent. The abundance of eggs and cysts within a faecal sample collected at a random time might therefore be so low that they cannot be found – even though the helminth or protozoan is present and causing obvious symptoms in the host. For example, in the case of human schistosomiasis, asking the patient to carry out some vigorous exercise, such as running up and down stairs, helps to dislodge the ova resulting in a higher egg concentration in the subsequent faecal or urine sample. In patients with clinically symptomatic and severe giardiasis, it may not be possible to find *Giardia* cysts even when samples are taken on consecutive days at different times. In this situation a 'string test' can be carried out.

Box 9.1 *Giardia* string test (Entero-Test)

Although it is commonly known as the '*Giardia* string test' this technique can also be used to detect the nematode *Strongyloides stercoralis* (Leighton and MacSween, 1990) and obtain samples of duodenal fluid for microbial or physiological analysis (Figure 9.1). The Entero-Test consists of a gelatine capsule within which is a weight attached by a slipping mechanism to a long length ('string') of nylon line. One end of the nylon line protrudes from the capsule and this is taped to the patient's cheek. The patient then swallows the capsule and as it passes down the gut the nylon line unravels behind it. Within the stomach the gelatine capsule dissolves and the normal gut contractions carry the weighted line to the duodenum. After about 4–6 hours the weight is released and the nylon line is gently pulled back up the oesophagus. The weight continues down

the gut and is passed out with the faeces. The presence of yellow-green bile on the end of the line indicates that it has reached the duodenum while a low pH indicates that it never left the stomach. Mucus and fluid attached to the end of the line should be examined straight away for the presence of moving *Giardia* trophozoites/*Strongyloides stercoralis* although stained permanent mounts can also be made. The attached material can also be analysed for bacteria such as *Salmonella* spp. using standard procedures. The test requires the patient to remain in the surgery for several hours and they can have difficulty swallowing the capsule and may experience retching or vomiting as it is removed. It is therefore not used for the routine testing for *Giardia* unless the disease is clearly suspected but cysts and trophozoites cannot be detected in the faeces. The Entero-Test can also be used with dogs and other domestic animals although it tends to be employed as a research tool rather than for routine diagnosis.

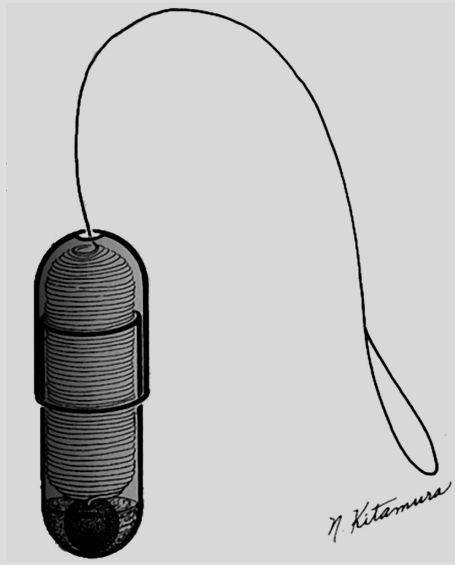

Figure 9.1 The Entero-Test capsule used to sample duodenal fluid. Source: Garcia *et al*., 2007

Another interesting method of collecting parasites to take to the laboratory is used when diagnosing human pinworm (*Enterobius vermicularis*) infection. The adult nematodes lay their eggs on the skin surface in the perianal area and the peak time for egg laying is during the night. Since the area is itchy, people commonly reinfect themselves by scratching their bottom and then putting their fingers in their mouth before washing them – a habit which is common in, but not restricted to, small children! The best way to obtain a sample from someone with suspected pinworm infection is to put a piece of clear adhesive tape on the skin in the perianal area, in the morning, before that part of the body has been washed. The tape is peeled off carefully and then, in the laboratory, put face down onto a microscope slide; this can then be examined at $\times 10$ and $\times 40$ for *Enterobius vermicularis* eggs. Other more specialised techniques applied to faecal parasites include the

agar plate method for isolation and subsequent detailed examination of *Stronyloides* and related helminth species and the *Schistosoma* hatching test to determine viability of eggs (Garcia, 2009).

9.5 Identification from gross morphology

Blood and tissue smears need to be stained before they can be examined. Parasitic protozoa can be observed at ×40 although it usually requires ×100 oil immersion to be able to see their diagnostic features. In faecal samples, helminth eggs tend to be large enough to be detected at ×10 magnification under a light microscope and examined for key identifying features at ×40 (Figure 9.2). For example, even small helminth eggs such as those of the nematode *Capillaria phillipinensis* are 45 μm × 21μm while most eggs are much larger than this and those of the trematode *Fasciola gigantica* are 190 μm × 90μm. In contrast, protozoan cysts found in faeces are smaller and mostly transparent, so they can be harder to find. In wet preparations, the addition of a drop of iodine is commonly used to highlight internal structures within the cysts. However, the addition of iodine does not enhance the recognition of helminth eggs, since the stain simply covers the thick walls, obscuring internal structures. Also, the ova of some helminth species, such as *Trichuris* and *Taenia*, already have natural brown coloration.

Wet saline preparations dry out within about 15 minutes, but it is sometimes necessary to keep the slide for longer and use additional stains, in which case it can be fixed in alcohol for a few minutes before further processing. Slides which need to be kept for further examination and future reference are stained in a permanent stain such as Trichrome stain.

Cryptosporidium cysts are small (5–7 μm in length) and often present in low concentration in faeces, so to enhance detection, the modified Ziehl-Neelson stain can be applied to a fixed faecal smear (Garcia, 2009). After staining, the cysts appear as distinctive pink, oval-shaped structures. This draws the microscopist's eye when there only are few per microscopic field and makes them easier to see. Another widely available method is to treat the fixed faecal smear with fluorescent labelled antibodies raised against *Cryptosporidium* cyst surface antigens; the reagent is often used in combination with anti-*Giardia* antibodies. Similarly, microsporidia cysts shed in faeces are extremely small (1–4 μm, depending on species) and detecting them by light microscopy is difficult. Therefore modifications to established staining protocols, such as Field's or Trichrome stain, have been developed. More reliable diagnosis and identification of species, where that is necessary, can be achieved using transmission electron microscopy (Garcia, 2009). However, transmission electron microscopes are expensive, they require skilled operators, sample preparation is slow and uses toxic chemicals and the process of examination is time-consuming. Consequently, it is not usually considered suitable for routine diagnosis.

The techniques used in preparation of samples and examination for parasites by microscopy have been in use for around 150 years (albeit with some refinements and variations to suit local conditions and resources). This means that their reliability, reproducibility and also limitations are well documented. Most of the light microscope procedures outlined above are relatively inexpensive to carry out, since they use standard laboratory equipment and reagents. To identify parasites from their morphological characteristics does require skill and training, but with over a century of collective experience among parasitologists, it is not usually difficult to find someone with the expertise willing to teach others. However, light microscopy suffers from problems of sensitivity, resolution, and specificity (Table 9.3). In the case of *Leishmania* spp. in blood and tissue samples, all three problems apply. The parasites are very small (2–4 μm) and can be hard to distinguish

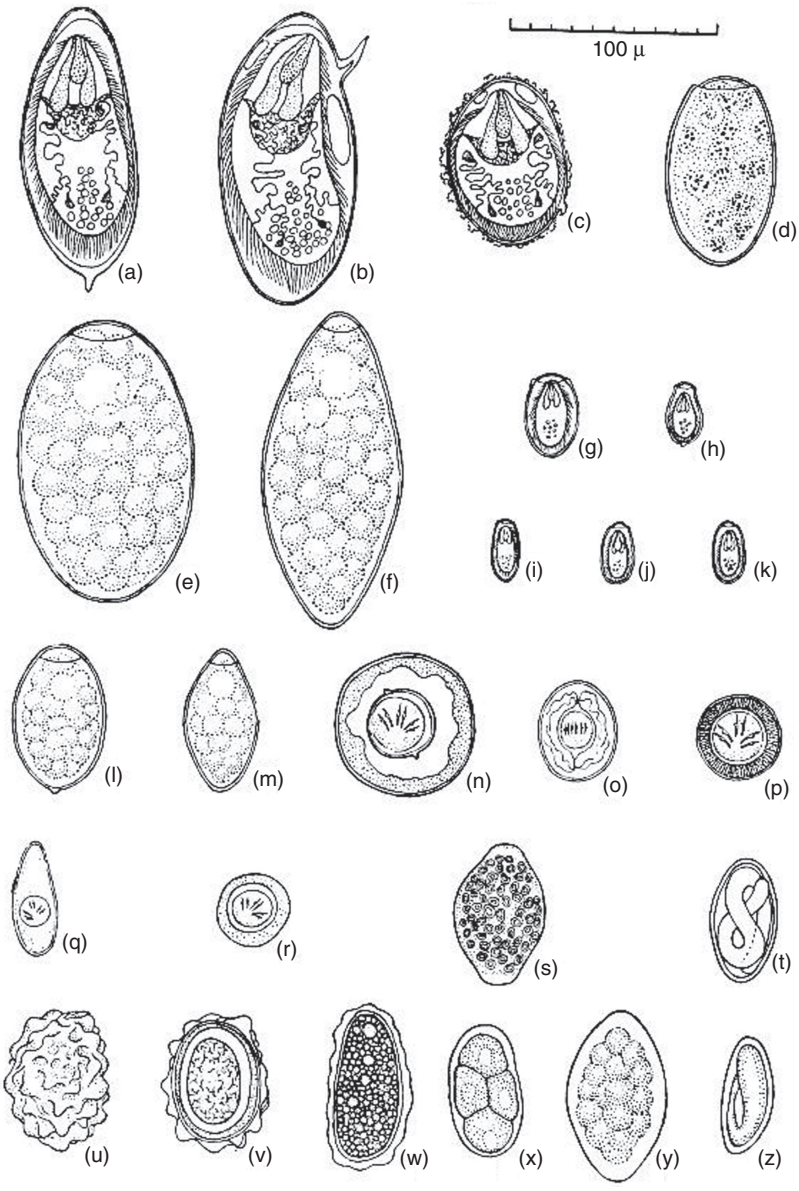

Figure 9.2 Helminth eggs drawn to scale. Trematodes: (a) = *Schistosoma haematobium*; (b) = *Schistosoma mansoni*; (c) = *Schistosoma japonicum*; (d) = *Paragonimus westermanni*; (e) = *Fasciolopsis buski*; (f) = *Gastrodiscoides hominis*; (g) = *Dicrocoelium dendriticum*; (h) = *Clonorchis sinensis*; (i) = *Opisthorchis felineus*; (j) = *Heterophyes heterophyes*; (k) = *Metagonimus yokogawai*

Cestodes: (l) = *Diphyllobothrium latum*; (m) = *Spirometra mansonoides*; (n) = *Hymenolepis diminuta*; (o) = *Hymenolepis nana*; (p) = *Taenia* spp., or *Echinococcus* spp. (species are indistinguishable on the basis of morphology); (q) = *Raillietina madagascariensis;* (r) = *Diplylidium caninum*

Nematodes: (s) = *Dioctophyma renale*; (t) = *Gonglyonema* spp.; (u) = *Ascaris lumbricoides* (surface view); (v) = *Ascaris lumbricoides* (optical section); (w) = *Ascaris lumbricoides* (unfertilised); (x) = *Necator americanus*; (y) = *Trichostrongylus* spp.; (z) = *Enterobius vermicularis*. Source: Chandler and Read, 1961

Table 9.3 Limitations of traditional light microscope techniques for parasite identification

Limitation	Example
Sensitivity	Where parasite abundance in the sample is low, they might not be detected. For example, in *Plasmodium malariae*, erythrocytic blood stages are often present in very small numbers in peripheral blood and could be missed on a blood slide. Similarly, when a patient is infected with *Entamoeba histolytica* but not suffering from acute dysentery, cysts might be excreted at low levels and the sensitivity of testing may be as low as around 60% (Stark *et al*., 2008).
Resolution	When parasites are very small (e.g. *Cryptosporidium*), it can be difficult to locate them and identify diagnostic features. This problem is exacerbated by low concentration in the sample.
Specificity	Closely related parasites share many common morphological traits and are difficult to distinguish from one another. There are no observable morphological differences between some species. The eggs of *Taenia saginata* and *Taenia solium* are the same size, shape, colour and structure and it is impossible to determine species from microscopic examination of the eggs alone. Similarly, cysts of the pathogen *Entamoeba histolytica* and its non-pathogenic relatives *Entamoeba dispar* and *Entamoeba moshkovski* are indistinguishable by microscopy.
Operative skill	To be effective the technique depends upon skilled and highly trained operatives.
Quality microscopes	To identify parasitic protozoa a well-maintained high quality microscope is required that is equipped with an oil immersion lens.

from platelets and artefacts in stained slides. They tend to be present in low numbers and it is not possible to identify *Leishmania* to species level solely on the basis of morphology.

9.5.1 Morphological identification of *Entamoeba*

Trophozoites of *Entamoeba* spp. may be visible in a 'warm stool' during the acute stage of an infection. Determination of species can be difficult, due to the fact that all amoebae naturally vary in shape quite considerably. Active *Entamoeba histolytica* trophozoites ingest red blood cells which the non-pathogenic species do not, so if these are observed, it can aid in identification. Viable parasites can be grown in culture which would allow further investigation (Fotedar *et al*., 2007), but this is not used in routine laboratory diagnosis.

Cysts may be found in a saline smear prepared from the faeces of an infected person. Concentration of the sample, for example, using the formal-ether method, before preparing the smear improves the sensitivity of this test (Fotedar *et al*., 2007). Cysts are spherical and transparent, so an iodine stain is used to enhance their detection and highlight the internal structures, which aid identification. Cysts of *Entamoeba coli* are 15–25 μm in diameter and contain up to eight nuclei. In contrast, *Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii* cysts are smaller (10–15 μm) and contain between one and four nuclei. Cysts also contain chromatin bodies which comprise ribosomal material, but these are more noticeable in immature cysts and less prominent in *Entamoeba coli* than in the other species (Garcia, 2009). Permanent stains such as Trichrome stain are applied to highlight chromatin bodies. Thus, a skilled microscopist should be able to determine whether the cyst is from *Entamoeba coli* or one of the other three species. However,

when a smaller cyst is detected, it is very important to know the exact species, in order to apply the appropriate treatment and management of the patient. This requires more specific testing.

9.5.2 Morphological identification of *Plasmodium* and *Babesia*

The genera *Plasmodium* and *Babesia* contain some of the most important parasites in human and veterinary medicine. However, the different species vary in their pathogenicity and require very different treatments and control measures. The 'gold standard' method in identification of these parasites is microscopic examination of thick and thin blood films. On staining with Giemsa, Field's or a similar stain, the parasite's nuclear material appears dark pink to red and the cytoplasm will show up as blue. If parasites are present in the peripheral blood sample, they should be visible at $\times 100$ magnification, but accurate detection and identification often requires considerable skill. In malaria diagnosis in humans, both thick and thin films are usually prepared. The thick film is useful for determining the presence or absence of *Plasmodium* spp. parasites in the patient's blood and for assessing the level of parasitaemia. However, careful examination of the thin film is more help when attempting to identify the species accurately. The most common life cycle stage seen in the early stages of acute infections is the ring form trophozoite. When the blood sample is taken while the patient's parasitaemia is quite high, it is usually relatively easy to spot infected red cells, as there will be several of them all looking similar per microscopic field (e.g. 2% parasitaemia translates into an average of 2 infected cells per 100 red blood cells examined). However, where the concentration of parasites in the blood is lower, for example, at a very early stage in the schizogony cycle or in response to effective treatment, it is sometimes hard to discriminate between cells infected with *Plasmodium* spp. parasites and other features, such as unfortunately positioned platelets. Related apicomplexan parasites also infect red blood cells and can have a similar appearance to malaria parasites.

Differentiation of *Plasmodium* species in blood films depends on observation of a range of key features. For example, high parasitaemia at the early trophozoite stage including multiple infections of red blood cells is characteristic of *Plasmodium falciparum*. In contrast, during *Plasmodium malariae* infection, low parasitaemia is typical. *Plasmodium ovale* changes the shape of infected red cells, so that they tend to be oval, with uneven edges. *Plasmodium vivax* trophozites become enlarged and amoeboid in appearance, increasing the size and shape of infected red cells, which can develop to be as large as white blood cells. As the infection progresses, stains reveal dots ('stippling') in infected red cells, which also give an indication of the species. *Plasmodium falciparum* infection is associated with blue Maurer's dots (or clefts), while in *Plasmodium vivax* and *Plasmodium ovale*, pink Schuffner's dots are seen. It is worth noting that *Plasmodium knowlesi* which is increasingly being reported as a zoonosis in South East Asia is morphologically virtually identical to *Plasmodium malariae* (Cheesbrough, 2005).

Humans occasionally acquire *Babesia* infections, notably *Babesia microti* (also called *Theileria microti*), *Babesia divergens* and *Babesia bovis* and these can be confused with *Plasmodium* spp., particularly in the early stages of infection. However, *Babesia* trophozoites have thicker cytoplasm and are in a more ovoid formation rather than a ring shape. Infected red blood cells do not become enlarged and there is no stippling. As it matures, the trophozoite divides, resulting in up to 8 merozoites per infected red cell. These merozoites do not always completely separate after division, which can lead to some interesting formations, including the four-ringed 'Maltese cross'. It is possible to identify species of *Babesia* accurately from blood slides, given time and

skill. However, it can be difficult as there are fewer distinguishing features than between the various species of *Plasmodium* and parasitaemia in peripheral blood can be rather low. The species of *Babesia* are divided into 'small' and 'large' piroplasms according to their morphology, which can give an indication of the species. For example, in dogs, *Babesia canis* is described as a 'large' species while *Babesia gibsoni* is a 'small' one. Inside the red cells, *Babesia canis* parasites have a pear-shaped appearance and often appear in pairs; they are about twice the size of *Babesia gibsoni* merozoites, which tend towards the four-pronged, Maltese cross formation.

9.5.3 Morphological identification of *Taenia* tapeworms

The Taeniidae family of tapeworms contains the genera *Taenia* and *Echinococcus* and there are important human and animal pathogens within both. Humans are the definitive host for some species in this family and accidental intermediate (sometimes called 'aberrant') hosts for others. Despite morphological similarities between the species, the pathology that arises within the host as a result of infection can be different, so it can be very important to identify to species level.

A faecal sample from a person or animal infected with adult tapeworms can sometimes contain whole worms which have been eliminated from the host, or portions of the strobila which have broken off naturally. The species of the host can give some indication of the possible identity of the cestode. For example, humans are the definitive host for *Taenia saginata* and *Taenia solium* whereas dogs are the definitive hosts of *Taenia pisiformis*. So you might find an adult *Taenia pisiformis* in the faeces of a dog, but not in the faeces of the dog's owner. The length and shape of an intact worm are a useful taxonomic indicator and if a scolex (head) is found within the host's faeces, then this can be examined microscopically for anatomical structures which indicate species. It is important to search for the scolex in the patient's faeces following treatment with an anthelmintic to confirm that they have been cured. If the scolex remains attached to the gut lumen, then it will grow a new strobila and continue producing eggs.

Where sections of the adult strobila are found but no scolex, there is another way of distinguishing between some species, which is to look for gravid proglottids (i.e. segments containing fertilised eggs). The proglottid contains a uterus, which has branches containing the eggs. By carefully injecting the proglottid with a dye such as Indian ink, it is possible to count the number of main uterine branches, which is characteristic of the species (see Colour Plate 31). For example, *Taenia solium* has fewer than 13 branches, while *Taenia saginata* has more than 13.

In the absence of the scolex or gravid proglottids, one can search for tapeworm eggs in the faeces. First, the eggs are concentrated using a suitable technique and a saline wet preparation should be made and examined for the presence of helminth eggs at $\times 10$ and $\times 40$ magnification. *Taenia* spp. eggs are indistinguishable but in terms of making a diagnosis of 'tapeworm infection', this can be helpful. The eggs are orangey brown in colour with a characteristic round (bordering on oval) shape, about 40μm in diameter and with a thick striated wall. By focusing the microscope up and down it is possible to see the hooks inside the embryonic worm. Although only *Taenia solium* has hooks as an adult, all species have this feature at the embryonic stage.

9.5.4 Morphological identification of filarial nematode infections

Observations of the patient can provide useful information in filarial infections. In lymphatic filariasis, adult helminths can be observed in the lymphatic vessels in the pelvis and legs on an

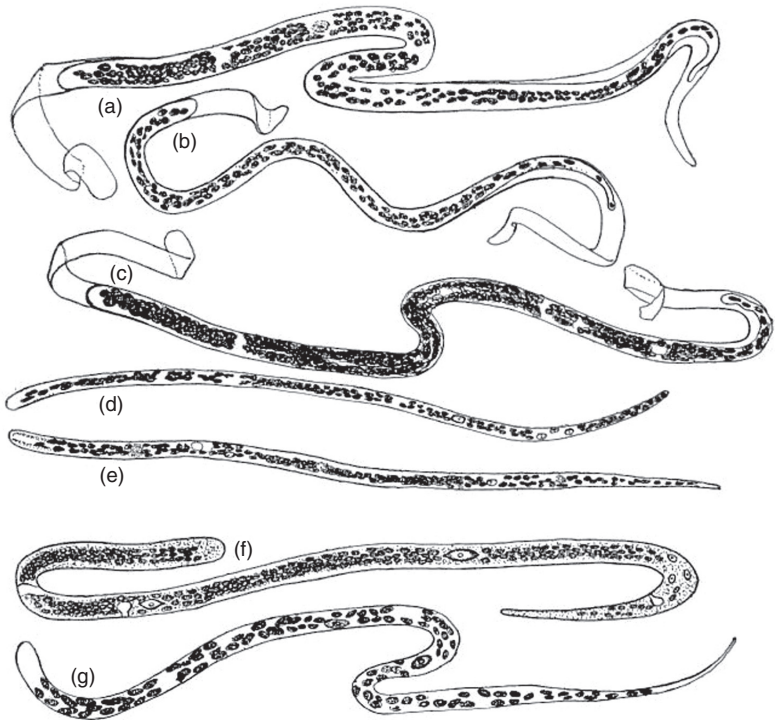

Figure 9.3 Different species of microfilariae drawn to scale. (a) = *Wuchereria bancrofti* (sheathed, no nuclei in tip of tail); (b) = *Brugia malayi* (sheathed, two nuclei in tail); (c) = *Loa loa* (sheathed, nuclei to tip of tail); (d) = *Dipetalonema perstans* (no sheath, tail blunt with nuclei to tip); (e) = *Mansonella ozzardi* (no sheath, pointed tail without nuclei at tip); (f) = *Onchocerca volvulus* (no sheath, no nuclei in end of tail); (g) = *Dirofilaria immitis* (no sheath, sharp tail without nuclei in end). Source: Chandler and Read, 1961

ultrasound scan. *Loa loa* worms are sometimes visible in the eye. Microfilariae can be observed and identified in blood films (Figure 9.3), but an understanding of the timing of the parasitaemia is important when collecting samples. The concentration of some microfilariae in peripheral blood exhibits 'periodicity' which means that it varies throughout a 24-hour period and this variation depends on the nematode species. Some species are usually present in higher numbers during the night, i.e. 'nocturnal periodicity' (e.g. *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* in humans). This means that to detect and identify these species, the person collecting the blood sample must wake the patient up at midnight! (Interestingly, this periodicity seems to be a response by the parasite to physiological conditions within the host over a 24-hour period of being active, eating and sleeping, such that the peak parasitaemia timing is found to be reversed in people who regularly work night shifts.) Other microfilariarae are at peak concentration in the middle of the day (e.g. *Loa loa* in humans) which means that the patient's lunch might be interrupted for blood collection) or mid-afternoon (e.g. *Dirofilaria* spp. in canids). However, some species do not show marked periodicity such as *Mansonella* spp. in humans.

Microfilariae can be identified in thick blood films made from blood collected from finger pricks. However, whenever possible, it is better to take an anti-coagulated whole blood sample which should be concentrated by centrifugation or filtration before processing, to enhance

sensitivity (Cheesbrough, 2006). The microfilariae of all species look like 'small worms' in blood films, but it is possible to distinguish between species by their sheath and the arrangement of their nuclei, which is why staining is so useful (Figure 9.3).

The best place to find *Onchocerca* and *Mansonella* microfilariae is usually skin snip biopsies around nodules which might contain adult worms. If the adult worms are located in the pelvic region, then the microfilariae may be found in the urine. Again, a slide is prepared and stained with Giemsa or similar stain, allowing the morphology of the worm, sheath and organisation of the nuclei to be observed.

9.6 Biochemical techniques

The biochemical variation in enzymes in different strains or closely related species of organisms can be exploited to aid accurate laboratory identification. Enzyme electrophoresis involves separating out specific enzymes from cultured parasites or homogenates by electrophoresis. Isoenzymes have different separation properties and, by observing the patterns, isolates with similar patterns of iosenzymes (called 'zymodemes') can be grouped together. Research into isoenzymes of isolates which were assumed to be different strains of *Entamoeba histolytica* spp. in humans led to the conclusion that there were actually two distinct species of *Entamoeba*: the pathogenic *Entamoeba histolytica* and the non-pathogenic *Entamoeba dispar* (Diamond and Clark, 1993). While it is a valuable technique, it does require specialist expertise and equipment. In the case of organisms such as *Entamoeba*, the amoebae have to be grown in a culture before the biochemical analysis can take place. Nowadays, isozyme analysis has largely been superseded by molecular methods of identification.

Differences in isoenzymes have also been exploited in malaria detection and species identification. The observation that *Plasmodium* has a specific parasite lactate dehydrogenase enzyme which is different from that of the host cell and that *Plasmodium falciparum* produces a version which is biochemically distinct from other *Plasmodium* spp. led to the development of the OptiMAL test for human malaria (Moody *et al*., 2000). However, this test is an 'antigen capture' assay rather than an enzyme activity assay. It uses specific antibodies embedded in a cellulose strip to detect the presence of parasite isoenzymes using the immunochromatographic lateral flow format (see section 9.9).

9.7 Immunological techniques

The use of immunologically based assays to detect both antibodies against specific pathogens and the antigens themselves in clinical specimens has proved to be very successful in diagnostic microbiology. Whatever the format, the assay is always based on providing the right conditions for particular antigen–antibody reactions to occur and a suitable system to visualise and measure the reaction. Tests to detect parasite antigens are particularly useful when direct observation of the organism in a specimen by microscopy is difficult. One of the most commonly used methods is enzyme-linked immunosorbent assay (ELISA), also known as enzyme immunoassay (EIA), to detect parasite cell surface antigens. Antibodies are raised against specific parasite antigens and these may be linked (conjugated) to an indicator molecule such as a dye or an enzyme that initiates a chromogenic (colour-forming) reaction. This enables the presence of the antibody to be

determined. Alternatively, the primary specific antibody is not conjugated and it is detected through a secondary (conjugated) antibody that is raised against it. The sample containing the presumed antigens or the antibodies is immobilised onto a solid support (i.e. solid phase) such as the wells of a microtitre plate or latex beads. Then, depending upon which component is the solid phase, either antibodies or the sample antigens are added. If the parasite antigens are present, then the antibodies will bind to them. The wells are then washed to remove unbound reagents, and the presence of the antigen–antibody complex is demonstrated through the indicator molecule or through adding a secondary conjugated antibody. If the parasite antigen is not present, there will be no reaction. Immunofluorescence antigen testing (IFAT) uses the same principle with fluorescent dyes acting as the indicator molecules and is widely used to test for the presence of parasites in faeces, tissues, and body fluid samples.

ELISA is a commonly used laboratory technique to detect the presence of *Entamoeba histolytica* in faecal samples. The solid phase is a monoclonal antibody raised against an *Entamoeba histolytica*- specific cell surface antigen, such as the Gal/GalNAc lectin. This has been widely evaluated in studies in areas of low and high prevalence throughout the world and is reported to have generally good sensitivity and specificity. However, the formalin used in the concentration of faecal samples (and sometimes to preserve samples during transport to the laboratory) can have a detrimental effect on the lectin and, if this occurs, it necessitates a new aliquot of the faeces to be collected (Fotedar, *et al*., 2007). Although the more severe and life-threatening clinical conditions such as dysentery and amoebic liver abscess are associated with *Entamoeba histolytica*, abdominal symptoms sometimes occur as a consequence of *Entamoeba moshkovskii* and *Entamoeba dispar* (Fotedar *et al*., 2007). There is some evidence for cross-reactivity between these three *Entamoeba* species in commercially available ELISA tests, so some samples might give a false positive result for *Entamoeba histolytica* (Furrows *et al*., 2004). Also, the epidemiology and relative distribution of the three species are not really known. Thus, while the ELISA tests allow the identification of *Entamoeba histolytica*, it might still be important to determine whether *Entamoeba dispar* or *Entamoeba moshkovskii* is present in a sample and, if so, which one.

Although the detection of ova in faeces is a relatively quick and simple way to diagnose tapeworm infection, it is not always very sensitive, as an infected person or animal does not excrete eggs continuously. As a consequence, an immunoassay technique to detect *Taenia* antigens in human faeces and *Echinococcus* antigens in canine faeces was developed in the 1990s (Allan and Craig, 2006). This 'coproantigen' ELISA is a capture assay, designed to detect antigens from eggs or proglottids in a faecal sample. The solid phase is an antibody raised in rabbits, against antigens extracted from adult worms. The assay has better sensitivity than microscopy, but it cannot distinguish between the species (Allan and Craig, 2006), due to antibody cross-reactivity to genus-specific antigens contained in the worm extract. More recently, a *Taenia solium*-specific assay using a different antigen preparation method to raise antibodies has been reported (Guezala *et al*., 2009). This is clinically useful because it enables identification of patients at risk of developing cysticercosis.

ELISA and IFAT methods can also be used to detect serum antibodies, which are widely used in diagnostic microbiology as markers of infection. Protozoa and helminths are more complex organisms than viruses and bacteria and they present a variety of antigenic components to the host immune system during their life cycles. This means that unfortunately, the immune response to most parasites is poorly understood, which has made the development of assays for clinically useful antibodies difficult. While in assays to detect antibodies to virus or bacterial infections, the

solid phase is purified, usually a monoclonal antibody, in the case of parasitic infections, it is often not possible to identify the key antigen which the host responds to. Also, as the parasite changes morphologically during its life cycle stages, it also changes biochemically and the host response to a particular antigen might be quite transient. For these reasons, there are a limited number of assays to detect antibody response to protozoa and helminths, and those which are available usually detect total antibody or IgG, rather than IgM, which would indicate current or recent infection. Nevertheless, the detection of parasite-specific antibodies is particularly useful in the diagnosis of neurocysticercosis. Neurocysticercosis can be acquired by ingestion of *Taenia solium* eggs, so the patient may not be harbouring an adult worm themselves, meaning faecal analysis would not be helpful. For human *Taenia solium* cysticercosis, the enzyme-linked immuno-electrotransfer blot (EITB) is considered the best method of antibody detection (Garcia *et al*., 2003). It incorporates purified preparations from seven different cysticercus proteins and the antibody–antigen reaction is detected by chromatography (Dorny *et al*., 2003). There are also some sandwich ELISA assays available which are simpler and cheaper to run (Dorny *et al*., 2003) but they are considered to be less sensitive than EITB (Garcia *et al*., 2003). All these serological assays measure total or IgG antibody, which is the main class of IgG produced in response to *Taenia* infections. This makes serology limited to use for screening or epidemiology, because it cannot distinguish between active and resolved, past infection. However, it is very helpful for the initial diagnosis of cysticercosis in conjunction with clinical symptoms and a CT or an MRI scan (Garcia *et al*., 2003). Also these assays can be quantitative, meaning that they can be used to monitor the progress of patients on treatment. Similarly, an ELISA to detect *Taenia saginata* cysticerci in cattle has been developed (Ogunremi and Benjamin, 2010).

9.8 Molecular techniques

An obvious means of identifying organisms is through their unique DNA profile. However, sequencing the whole genome would be far too costly and time-consuming to undertake on a routine basis. Consequently, scientists look for regions of the genome that exhibit variability. There is no single locus that is suitable for all organisms but among the eukaryotes, the mitochondrial genes for cytochrome oxidase I (COI) and cytochrome oxidase II (COII) have proved effective for differentiating between many animal species. Some parasitic protozoa lack functional mitochondria, therefore they tend to be differentiated on the basis of a variety of different markers, though the 18S ribosomal RNA (18S rRNA) gene has proved particularly useful. There are standard protocols for the extraction and analysis of DNA and this facilitates standardisation between laboratories. The amount of parasite DNA within a sample can be extremely small but the region of the genome of interest can be amplified using the polymerase chain reaction (PCR). The primers used to identify the beginning and end of the sequence of interest can be labelled with differently coloured fluorescent dyes. Consequently, after separation (e.g. electrophoresis), the PCR products can be detected by exposure to a laser beam that induces fluorescence at specific emission wavelengths that are then detected in a recording CCD camera.

Quantitative (real-time) PCR is a PCR-based technique that can be used to both amplify and quantify the targeted DNA. Two of the most common means of quantification are the inclusion of a fluorescent dye (e.g. SYBR® Green) in the PCR reaction that intercalates with the DNA as it is produced and the TaqMan® assay. The TaqMan® assay uses custom-designed sequence-specific

primers and is therefore better at detecting individual species or variants within a species than the fluorescent dye intercalation method. It is also possible to include several different primers within an assay to detect multiple targets – this is known as a 'multiplex assay'. Unfortunately, at present, it is still more expensive and more complicated to perform TaqMan® assays than the intercalation method. The latter method, however, is relatively non-specific because SYBR® Green will bind to any double-stranded DNA molecule. Consequently, it will bind to primer-dimers and other PCR artefacts and thereby give rise to false positives. In both techniques, with each cycle of the PCR process, more DNA is produced and this is measured as an increase in fluorescence. The DNA product is therefore 'quantified' as it increases in 'real time', hence the terms 'real time' and 'quantitative' PCR. Once sufficient DNA has been produced, it can be subject to further analysis such as sequencing or Southern Blotting. A good quality check is to subject the PCR products to DNA dissociation (melting) curve analysis to confirm that the correct product was formed: artefacts would yield a different-shaped dissociation curve. A new type of PCR analysis called digital PCR could replace quantitative PCR in the future (Heyries *et al*., 2011). This procedure amplifies DNA exclusively from a single template and converts the signals to a digital output (cf. the linear output generated by conventional PCR). Consequently, digital PCR is more effective than real-time PCR at detecting and amplifying low copy number templates, which would be the situation where there was low parasite density. In addition, it is easier for the operator to quantify the amount of DNA present and undertake statistical analysis of the PCR product. Further details on molecular diagnostic techniques can be found in Buckingham and Flaws (2007).

In addition to identifying suitable regions of the parasite genome, it is important to be able to extract the DNA for analysis. Faecal samples present particular difficulties because they contain bile salts and plant polysaccharides that interfere with standard DNA extraction and PCR techniques. The parasite DNA also has to be differentiated from the enormous amount of bacterial and viral DNA that is naturally present in faeces. However, there is now a commercially available kit for the extraction of DNA from faeces called the QIAGEN QIAmp® DNA Stool Mini Kit. This will also extract DNA from other samples containing high levels of PCR inhibitors.

Molecular techniques are available for the identification of an increasing number of parasites, though a number of these are still at the research stage and require standardisation and optimisation. At present, these tend to be used mainly for research and in reference laboratories, but they will probably achieve more widespread use in the near future. Molecular techniques are particularly valuable for the detection of parasitic protozoa which are difficult to identify using conventional light microscope techniques. Parasitic protozoa are especially hard to detect at low densities and there are often several species that are morphologically very similar or identical in appearance. Molecular techniques can also be performed quickly and once they have been optimised, they can provide a highly specific diagnosis. For example, PCR assays are now available that can distinguish between *Entamoeba histolytica*, *Entamoeba dispar*, and *Entamoeba moshkovski* (Stark *et al*., 2008b). Similar PCR assays have been developed for identifying a range of apicomplexan, trypanosome and *Leishmania* parasites (e.g. Deborggraeve and Büscher, 2010; Reithinger and Dujardin, 2007; Steenkeste *et al*., 2009). A common criticism of molecular techniques is that they can only detect the parasite being tested for but this can be overcome to some extent through multiplex assays. For example, Muhanguzi *et al*. (2010) have devised a system to detect a wide selection of species of *Babesia* and *Theilera* in cattle, which they reported to be both sensitive and specific. Similarly, it is might be possible to design multiplexes to simultaneously detect *Leishmania* and HIV where co-infection is a possibility.

Box 9.2 How diagnostic techniques can influence epidemiological studies

Molecular diagnostic techniques sometimes make us reassess our understanding of the epidemiology of parasitic infections. For example, in *Giardia duodenalis*, phylogenetic analysis of sequences of loci within the small subunit 18S ribosomal RNA, particularly the regions designated *gdh* and *tpi*, suggests that the 'species' comprises distinct 'strains'. These strains fall into one of eight genetic groups (also called 'Assemblages', i.e. A-H), based on genotype and host mammal(s). The genotypes associated with human infections fall into two broad groupings called variously in the literature 'Polish group' and 'Belgian group', 'Group 1 /2' and 'Group 3', 'Genotype A' and 'Genotype B' or 'Assemblage A' and 'Assemblage B', with most authors tending to favour the latter. Isolates with sequences which place them in Assemblage A have been recovered from humans, livestock, dogs, cats, beavers, guinea pigs, slow loris, and for Assemblage B humans, dogs, beavers, slow loris, chinchillas and siamang (Thompson, 2004). All human isolates fall into Assemblages A or B. In Assemblage A, there are two distinct 'subgroups' I and II. Group AI contains isolates from humans and some other mammals, but AII comprises genotypes found in humans only. Assemblage B is a more diverse grouping. The other groups have more restricted host ranges – for example, Assemblage C genotypes have only been found in dogs – and there is no evidence of human infection.

The ability to identify strains of *Giardia duodenalis* more precisely can provide the opportunity for clearer insights into the organism's epidemiology. For example, it is now possible to trace sources of outbreaks accurately and to ascertain whether zoonotic transmission has occurred (Thompson *et al*., 2008). The evidence from the limited number of such studies that have so far occurred is intriguing. All *Giardia* isolates from humans which have been sequenced so far fall into Assemblages A or B; if humans could be infected zoonotically from domestic or farm animals, a mixture of genotypes would be expected, including some from Assemblage C (dog-specific-types), Assemblage D (cat-specific) and Assemblage E (livestock-specific). However, in three distinct studies, sequencing of isolates from domestic dogs in Australia and India and gorillas in Uganda revealed that some of the animals were infected with 'human' strains from Assemblages A and B. Another study of *Giardia* in domestic cats in Australia found genotypes previously thought to have been restricted to dogs. Thus, human-to-animal transmission does seem to occur! One does, however, need to be careful with studies based on recovering organisms from faeces because there is always the possibility that they might have got there by accident. For example, dogs are coprophagic and therefore it is possible that they could act as transport hosts – the *Giardia* cysts consumed with faeces travelling through the gut and not hatching. Similarly, dogs, like cats and other carnivores will consume the guts of prey and may therefore pass the parasites acquired with this meal with their own faeces.

Molecular techniques could also prove useful for the diagnosis of a number of helminth infections. For example, it is impossible to distinguish between the eggs of *Taenia* tapeworms on the basis of their morphology, nor is the coproantigen ELISA very effective at distinguishing between species. A multiplex PCR assay has been developed for the diagnosis of human taeniasis in faecal samples (Yamasaki *et al*., 2004) which exploits sequence variation in the mitochondrial cytochrome oxidase I gene (COI). It uses primers which are designed to produce different-sized PCR

products which can be identified by comparison with a size marker when run out onto an agarose gel. Another method of DNA detection, the loop-mediated isothermal amplification (LAMP), has also been adapted to identification of *Taenia* species, based on detection of differences in gene sequences coding for cytochrome oxidase I and cathepsin L-like cysteine peptidase (clp) (Nkouawa *et al*., 2010). As an amplification system, LAMP is reported to produce results faster and to be technically simpler than PCR (Nkouawa *et al*., 2010). PCR assays have been developed using species-specific primers that are effective in the determination of the prevalence of the filarial nematodes *Brugia* spp. and *Wuchereria bancrofti*, both in mammalian hosts and in mosquito vectors (Mishra *et al*., 2007; Nuchprayoon, 2009). However, at the time of writing, the Rapid Diagnostic Test for circulating filarial antigens is still the method of choice for detection of microfilariae in humans due to the technical simplicity of the test.

9.9 Rapid diagnostic tests (RDTs)

There is increasing interest in the development of rapid diagnostic tests (RDT) for a range of medical conditions. These tests do not require skilled operatives and can be performed by a nurse, doctor, or vet while they are examining the patient or animal. They are therefore sometimes referred to as 'point of care tests'. They are particularly useful in developing countries that lack the resources to support dedicated laboratory diagnostic services. They can also be useful when a quick decision is required about patient treatment such as in an emergency situation where cerebral malaria needs to be confirmed or excluded as soon as possible.

Most RDTs work by attaching an antigen or antibody to a solid substrate and when the corresponding antibody or antigen binds to it, a chemical reaction is initiated that results in a coloured product being formed that can be seen with the naked eye. Lateral flow or immunochromatographic (ICS) tests are one of the most commonly used forms of rapid diagnostic test. Commercial ICS tests are currently available for several parasitic diseases including malaria and lymphatic filariasis and there is a possibility that one will soon be available for cysticercosis (Handali *et al*., 2010). ICS tests can be designed to detect the presence of specific antigens, antibodies or the products of PCR reactions. A single test strip can even be used to detect the presence of several substances at the same time. The ICS test typically consists of a long rectangular strip of nitrocellulose onto which the sample (which may be blood, serum, urine, faeces, or saliva) is placed. Addition of a buffer causes the contents of the sample to migrate up the test strip by capillary action as in chromatography. A common ICS design is to have the sample molecules first come into contact with a 'sample pad' within which are adsorbed detection molecules such as a colloidal metal or dye bound to the antigen/antibody designed to detect the sample molecule of interest – this is known as the 'detection conjugate'. The antibody/antigen in the sample interacts with the antigen/antibody in the sample pad and the resultant complex continues up the ICS strip. The complex is then captured by immobilised antibodies/antigens at the test and control reagent lines. The test line captures the antibody–antigen–conjugate complex while the control line captures the 'detection conjugate' and thereby confirms that it is present and has migrated up the strip. If the antibody/antigen of interest is present in the sample, there will be two lines but if only the control line is present, then the antibody/antigen is absent. If there is no control line it indicates that the test has not worked and must be repeated. Most ICS strips can be read with the naked eye within 2–15 minutes. There is a move towards developing tests that are read by a machine which would enhance sensitivity and allow the possibility of quantifying the result.

For the routine diagnosis of faecal parasites, the initial clinical question is often focused on whether the patient has one of a range of possible pathogens. So, for example, an RDT which can detect *Entamoeba histolytica*, *Giardia lamblia* and *Cryptosporidium parvum* has been developed. The test requires a small amount of fresh (or frozen, but not fixed) faeces and the antigens are extracted using a buffer before addition of the sample to the test strip. This is coated with antibodies against specific antigens for all three species. This assay appears to have good specificity, which is not surprising, but reports about its sensitivity are less conclusive (Fotedar *et al*., 2007).

9.9.1 Rapid diagnostic tests for malaria

A substantial amount of work has been undertaken to develop rapid diagnostic tests for malaria over the past 20 years. The first test to detect malaria parasites in patients' peripheral blood which came onto the market was designed to detect a protein produced uniquely by *Plasmodium falciparum,* called histidine rich protein-2 (HRP-2) (Uguen *et al*., 1995). This had the major disadvantage of not being able to detect the other species of *Plasmodium,* so anyone who had malaria but was not infected with *Plasmodium falciparum* would be given a negative result. The test has been refined to include another marker, an aldolase which is produced by all *Plasmodium* spp. – usually referred to as 'pan-malarial antigen' (PMA). Although it cannot distinguish between species, a negative HRP-2 but positive PMA result would indicate a diagnosis of non-*falciparum* malaria (Wongrichanalai *et al*., 2007). The second type of assay relies on the fact that there is a specific *Plasmodium* isoform of lactate dehydrogenase, usually called parasite LDH (p-LDH) (Moody *et al*., 2000). The test is designed to detect both *Plasmodium falciparum*-specific p-LDH and a p-LDH produced by all species of *Plasmodium*, which means that it should be able to pick up any species, but also specifically identify *Plasmodium falciparum*.

Both the HRP-2 based and the p-LDH-based assays use the ICS antigen capture principle with monoclonal antibodies raised against the particular antigen. A small amount of the patient's blood is lysed and added to the test strip, allowing the liquid to move along by capillary action. When antigen in the sample meets antibody on the strip, it becomes bound. An in-built negative control shows whether the test has been performed correctly and all reagents are reacting as expected (Figure 9.4).

HRP-2 is produced by the parasite in the peripheral blood stages and is present at highest concentrations as the schizonts burst (Uguen *et al*., 1995). Thus it is considered to be a marker of active infection. However, HRP-2 is reported to be detectable in patients' blood for several weeks after successful treatment of the asexual (trophozoite and schizont) stages, suggesting that it is also present in gametocytes (Wongrichanalai *et al*., 2007). The addition of the pan-malarial antigen to this test did not resolve this problem – in fact, the aldolase was found to persist longer and be more strongly associated with gametocytaemia than HRP-2 (Tjitra *et al*., 2001). In contrast, p-LDH levels appear to reflect parasitaemia with the asexual stages (Moody *et al*., 2000), so is a better test to use when monitoring a patient's response to treatment (Wongrichanalai *et al*., 2007).

The p-LDH-based RDT performs well in developed countries (Moody *et al*., 2000), although early formats of the kit were affected by temperature and humidity during transport into the field in endemic areas (Moody and Chiodini, 2002). Individual packaging of strips has addressed this problem, but there is another issue which is related to the pan-malarial p-LDH antigen in the assay. This has been found to be detectable in *Plasmodium vivax* and *Plasmodium malariae*, but the test is reported to perform badly against *Plasmodium ovale* infection (Moody and Chiodini, 2002).

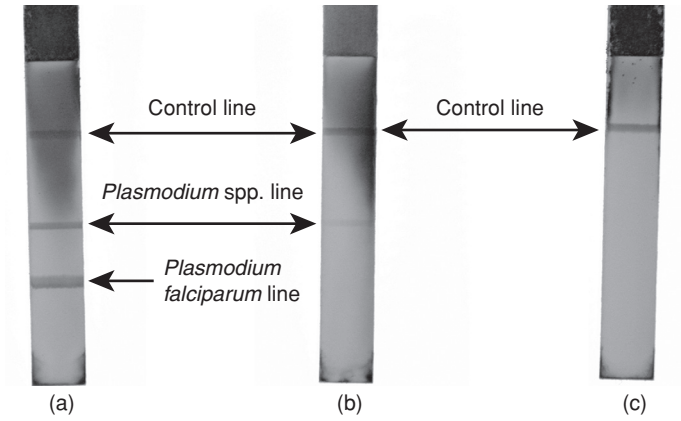

Figure 9.4 OptiMAL RDT for malaria. (a) = *Plasmodium falciparum* positive; (b) = *Plasmodium* spp. positive; (c) = Control positive

RDTs for malaria are reported to have acceptable sensitivities when compared with the 'gold standard' of microscopy, although the limits of detection are about 500 parasites per μL of blood (Moody and Chiodini, 2002; Wongrichanalai *et al*., 2007). The nature of the markers used in both RDTs means that they vary in their effectiveness at detecting different species of *Plasmodium*. Both the HRP-2 and the p-LDH assays are designed to detect *Plasmodium falciparum*-specific antigens, so tend to perform well for this species. The addition of the pan-malarial antigen to the HRP-2 marker allows detection of non- *falciparum* malaria, but cannot confirm the species. In an area where *Plasmodium vivax* is prevalent, species identification could be important, because a separate anti-hypnozoite treatment is required. The pan- malarial p-LDH strip in the other form of the assay is very useful for detecting *Plasmodium vivax*, but performs poorly against *Plasmodium ovale,* possibly because the latter produces a different isoform of the enzyme (Moody and Chiodini, 2002). Problems with false positive test results in patients who have rheumatoid factor and false negatives during pregnancy have also been reported (Wongrichanalai *et al*., 2007).

Box 9.3 Future prospects of malaria diagnosis

At present, the choice of a test for malaria used by a particular diagnostic laboratory will depend on a number of factors. First, whether to use microscopy which is highly sensitive and very specific when performed by trained operators. In situations where an RDT is chosen, then it is necessary to think about how the assay will be used. If monitoring patients during a course of treatment will be an important part of the laboratory's role, then the p-LDH version will be more suitable. However, in a *Plasmodium ovale* endemic area, the HRP-2/PMA assay would be preferable.

Issues with sensitivity and specificity in RDTs have led researchers to explore other areas, which might be appropriate in areas of high malaria transmission, to complement rather than compete with existing tests. An interesting possibility which is in the early stages of development is the use of computerised recognition systems to screen blood films for parasites (Tek *et al*., 2010). This could potentially save staff time, by screening large numbers of slides and flagging

up those which are positive and need further investigation. Another issue is whether molecular techniques such as polymerase chain reaction (PCR) could be adapted to 'field' conditions in endemic areas such as Sub-Saharan Africa. The prospect of improved sensitivity and better ability to distinguish species than RDTs is outweighed at the moment by the requirement for costly equipment and a reliable electricity supply. However, low cost and robust PCR technologies are becoming available and ecologists are now using PCR in field situations to support their work. It is therefore likely that PCR-based diagnostic techniques that are suitable for developing countries will soon become a possibility. For example, Mens *et al*. (2008) report a method of PCR which uses specific primers to allow distinction between the four human species of *Plasmodium* and detection of the PCR products based on biotinylated primers as an alternative to gel electrophoresis.

9.9.2 Rapid diagnostic test for filariasis

An RDT lateral flow immunoassay test for filariasis is currently available to test specifically for *Wuchereria bancrofti*. It comes as a test card comprising two layers and is a straightforward antibody–antigen–antibody sandwich format. The bottom section encloses a strip impregnated with antibody raised against *Wuchereria bancrofti* labelled with colloidal gold. A drop of the patient's blood sample (either a finger prick or anticoagulated venous blood) is added to this. The top section, which includes a membrane containing a second aliquot of anti-*Wuchereria bancrofti* antibody, is laid on top and the card is left for 10 minutes before reading the results (www.binax.com). The sensitivity of this test is reported to be very good and compares favourably with the traditional microscopic methods. For example, a study in an endemic area of Sri Lanka found it to be more sensitive and specific than thick blood films even after concentration of the blood sample by filtration (Chandrasena *et al*., 2002). It can detect microfilaria in blood at very low counts and blood specimens for testing can be taken at any time of the day (Melrose *et al*., 2004). This test is currently advocated for use in filarial control programmes to assess prevalence in a particular area and to evaluate the effectiveness of interventions (Molyneux, 2009).

9.10 MALDI-TOF MS

MALDI-TOF MS is a relatively new diagnostic technique that is promising to revolutionise the identification of microorganisms. MALDI-TOF MS is an abbreviation of matrix-assisted laser desorption/ionisation time of flight mass spectrometry. MALDI-TOF MS is commonly used to analyse large organic molecules that are too fragile to analyse using more conventional mass spectrometry. It has the advantages of requiring minimal sample preparation and is both highly accurate and quick to perform (Seng *et al*., 2009). However, the initial cost of the equipment is high and it can only be used in well-equipped laboratories. It is very popular in proteomics for determining the mass of peptides formed during digestion with specific protease enzymes. For example, Hewitson *et al*. (2008) used the technique to determine the proteomic profile of excretory-secretory products released by the filarial nematode *Brugia malayi*. The possibility of using proteomic fingerprinting for the identification of parasitic pathogens has been attempted but not yet developed beyond the research laboratory. For example, Agranoff *et al*. (2005) have

suggested it might be used in the diagnosis of Human African Trypanosomiasis. MALDI-TOF MS has a great future for the diagnosis of a range of medically important bacteria (e.g. Hsieh *et al*., 2008; Veloo *et al*., 2011), but its potential as a diagnostic tool for parasites remains to be ascertained. Nevertheless, Von Bergen *et al*. (2009) have demonstrated that it is possible to differentiate between all species and strains of the pathogenic alga *Prototheca*. This is useful because, although the algae can be observed in histological sections, species identification of *Prototheca* is difficult. This is because it currently depends upon cultivation of the algae and this is time-consuming – especially as they are rather slow-growing.

Questions

1. Name two differences in the way that thick and thin blood films are prepared and one advantage of each method of preparation.
2. Why is it important to examine a stool sample macroscopically before proceeding with laboratory investigations?
3. Describe (or draw) an ELISA to detect the antigen of a particular parasite in a blood or faecal sample.
4. Name one parasite which can be detected using a commercially available immunochromatographic, lateral flow rapid test.
5. List two advantages and two disadvantages of using light microscopy to detect faecal parasites.
6. If you found a *Taenia* spp. ovum in a faecal sample, indicating that the patient was infected with this helminth, how could you determine the species of tapeworm?
7. Choose one of the available immunochromographic lateral flow RDT assays for malaria and describe how it distinguishes between *Plasmodium* spp.
8. What is meant by the term ‘nocturnal periodicity’ and why is it important to understand this when attempting to detect microfilariae?
9. Comparing light microscopy to PCR for the detection of *Entamoeba* spp. in faecal samples, name one advantage and one disadvantage of each method.
10. Briefly describe how the EnteroTest works and state one species of parasite it can be used to detect.

10

Parasite treatment and control

10.1 Introduction

There is a wide variety of parasites and an equally wide range of substances and practices used in their treatment and control. We have therefore not attempted to list and compare individual classes of drugs and their modes of action or to go into great depth on the different approaches to control programmes. Good reviews of these topics are provided by Melhorn (2001) and Molyneaux (2006). Detailed information about how infections with particular human parasite species can be treated and prevented is covered by Garcia (2007) for human pathogens and Taylor *et al*. (2007) for parasites of domestic animals. We will focus upon the general concepts and also introduce some of the most recent advances that may become the means of treating and preventing parasitic infections in the future. In many cases these also exemplify how developments in fields such as cancer research and the economy can influence parasitology.

10.2 Importance of understanding parasite life cycles for effective treatment and control

Attempting to understand parasite life cycles is like learning German grammar: it is complicated and full of obscure exceptions to the rule. However, without the grammar, one cannot speak the language correctly, and without knowledge of a parasite's life cycle, one cannot begin to understand how it is transmitted and how it causes disease. Consequently, if one knows the life cycle, one can begin to work out many aspects relevant to treatment and control (Table 10.1). For example, in the UK, rainfall and temperature are the key factors determining the transmission efficiency of *Fasciola hepatica* – principally through their effect upon the snail intermediate host. This has enabled the development of a liver fluke forecasting scheme that is operated by the National Animal Disease Information Service (NADIS) (http://www.nadis.org.uk). Farmers can use this to determine when their flock is most at risk of infection and should therefore be treated with anthelmintics or, if possible, moved to less risky pasture. Similar liver fluke forecasting schemes have been developed in other countries (Fox *et al*., 2011; Malone and Yilma, 1999; Yilma and Malone, 1998).

Parasites with simple direct life cycles involve only a single host species (or group of related host species) and there is usually no reproduction outside the definitive host. For example, *Ascaris lumbricoides* lives in the human intestine and each egg gives rise to one adult worm. One might

Parasitology: An Integrated Approach, First Edition. Alan Gunn and Sarah J. Pitt.

Table 10.1 How a parasite's life cycle can influence its treatment and control

Life cycle factor	Importance in treatment/control	Application of life cycle knowledge
Direct life cycle	Provision of sanitation and basic hygiene practices can prevent many gastrointestinal parasitic diseases	Washing fruit and vegetables in clean water can remove protozoan cysts and helminth eggs
Life cycle involves one or more species of vector	Disease transmission can be controlled by targeting the vectors	Bed-nets can prevent mosquitoes transmitting malaria
Life cycle involves one or more intermediate hosts	Disease transmission can be controlled by targeting the intermediate hosts	Drainage to remove the habitat of snail intermediate hosts of *Fasciola hepatica*
Parasite has a variety of definitive hosts	Reservoir hosts are a potential source of reinfection	*Schistosoma japonicum* has numerous reservoir hosts which can contaminate paddy fields, etc. with eggs
Parasite has life cycle stages that are exposed to the environment	Environmental conditions can promote or limit infections	Composting can kill the infective stages of many gastro-intestinal parasites
Sequence and timing of life cycle stages within a host	Optimal time for diagnosis	Microfilariae of *Wuchereria bancrofti* exhibit periodicity
Location within host	Optimal time for treatment	Cattle should be treated for warble fly infections before the larvae reach their winter resting sites

therefore expect that these parasites would be easier to control than those with intermediate hosts or vectors, but this is not always the case. The eggs of this helminth are transmitted through faeces, so prevention of faecal contamination of the food and water supply should limit the transmission of *Ascaris lumbricoides* and many other gastrointestinal parasites. This, however, is easier said than done, since provision of basic sanitation demands cooperation on the part of the public and constant maintenance. Anyone who has attended a large open air event such as a pop concert or rally will be well aware of how there are seldom sufficient toilets and these soon become so noisome that people take to urinating and defecating wherever they can find a sufficiently secluded spot. For some residents of remote rural areas in developing countries and displaced people in refugee camps, such poor toilet facilities are a fact of everyday life, which means that faecal-oral transmission of disease can be rife. Furthermore, once a person in the family has become infected, it can be difficult to stop the parasite infecting others. Although simple and cost effective measures can help control gastrointestinal parasite infections, the provision of sanitation is not photogenic and therefore lacks appeal as a media or research topic: vaccines are far more glamorous and enable photographs of cute babies in the arms of concerned-looking young doctors.

By contrast to *Ascaris lumbricoides*, *Onchocerca volvulus* (river blindness) has a complex life cycle which involves development within and transmission by *Simulium* (blackfly) vectors. Nevertheless, in parts of West Africa, this parasite has been successfully controlled solely through the actions of professional teams treating rivers with insecticides to kill off the blackfly larvae. In this case, control did not rely heavily upon the cooperation of the local people and they do not need to change the way they lived.

10.3 Treatment of parasitic diseases

10.3.1 The ideal antiparasitic drug

The properties required in an antiparasitic drug are in many respects no different from those looked for in most other pharmaceutical products (Table 10.2) (Pink *et al*., 2005). Obviously, the drug needs to be effective against the intended pathogen and it needs to kill all of the parasites found in (or on) the body of the host. It should be less harmful to the host cells than to the parasite. This selectively toxicity is harder to achieve for antiparasitic drugs than for treatments against some other microorganisms, since both the host and parasite are eukaryotes. Anti-bacterial agents exploit the differences in cell biology and metabolism between the eukaryotic host and the prokaryotic bacteria, which is why it has been easier to develop antibiotics. It is not unusual for a host to harbour two or more life cycle stages of the same species of parasite, but drugs can vary in their activity against these. For example, praziquantel is effective against adult schistosomes but not very good at killing the developing schistosomulae. If any parasites remain alive after drug treatment, they may be able to multiply and cause disease again or become a source of infection for other animals or humans. In addition, the exposure of parasites to sub-lethal levels of drugs increases the risk of resistance developing. Drugs that have a broad spectrum of action are beneficial since they can be used to treat a variety of parasites. For example, the avermectin drugs such as ivermectin and doramectin are excellent in this regard as they are active against a range of gastrointestinal helminths as well as ectoparasites, such as lice, fleas, and ticks. It is not unusual for a selection of these parasites to be found simultaneously infecting an individual human or animal host. Drugs that kill the parasite rapidly reduce the chances of resistance developing, since the less time the parasite has to 'interact' with the drug, the less chance there is of it evolving a physiological means of counteracting it. Where the host is gravely ill, it is important to remove the parasite from the host as quickly as possible. However, the sudden death of large numbers of parasites within the body's tissues may release massive amounts of parasite antigens that trigger a harmful immune reaction or even a potentially fatal anaphylactic shock. For example, diethylcarbamzine is no longer recommended for the treatment of onchocerciasis because it inactivates all the microfilariae in the host at once and this can be quite large numbers. The consequences of this for the host include unbearable itching, fever, tachycardia, and hypotension. These symptoms are caused by the immune

Table 10.2 Properties of an ideal antiparasitic drug or treatment regime

Kills 100% of the parasites (including all life cycle stages in the host)
Broad spectrum
Rapid action
Provides long-lasting protection
Simple to administer (e.g. does not require invasive procedures or medical supervision)
Requires only one or two treatments to achieve a cure
Safe (does not cause harmful side-effects)
Does not have contra-indications (i.e. does not interfere with other medical/veterinary treatments or cause problems if the host is pregnant or is suffering from an underlying medical/veterinary condition)
Affordable to the individual/population
Chemically stable with a long shelf life
Does not cause harm to the environment

response to the symbiotic *Wolbachia* bacteria released by the dead and dying microfilariae, which then trigger an acute inflammatory reaction.

Drugs are seldom administered as compounds on their own. Instead, they are 'formulated' with a cocktail of chemicals, the composition of which varies with the intended means of delivery (e.g. liquid, tablet, or injection), and alters the effectiveness of the drug. The 'formulation' can influence a drug's stability, toxicity to both host and target parasite, rates of absorption and excretion, bioavailability, and pharmacokinetics. For example, formulations of insect growth regulators, natural pyrethrin and synthetic pyrethroid insecticides often include piperonyl butoxide (PBO) because it acts as a synergist and enhances their activity. A relatively new area of research is to investigate the possibility of extending the usefulness of drugs to which resistance has evolved by employing so-called 'resistance reversers' within drug formulations. Resistance reversers interfere with the physiological processes that confer a parasite's resistance to a particular drug or group of related compounds (Rosenthal, 2003).

Ease of administration is important in order to ensure compliance and so that people and animals can be treated quickly and with the minimum of fuss. For humans, drugs that can be delivered as tablets or liquids are preferred, since the patient can take these without supervision and the chances of them taking the recommended dosage and completing the course of treatment are high. Injections, especially intravenous or intra-peritoneal, usually have to be given by trained medical personnel – and this limits the situations in which the drugs can be dispensed. Also most people are more willing to take a pill than receive an injection. By contrast, oral dosing of animals is not always easy – as anyone who has ever tried to get a pill inside a recalcitrant cat will attest. It is sometimes possible to deliver the drug via the animal's food but it is then difficult to control the dose the animal consumes. For domestic livestock such as sheep and cattle, special 'dosing guns' are produced that deliver a known quantity of 'drench' (i.e. drug) to the back of the animal's throat so that it has to swallow it. In this way the farmer can dose large numbers of animals relatively quickly. Injections, especially intramuscular injections, are often preferred where there are large numbers of animals to be treated. A trained person can rapidly inject many animals with minimal handling – and therefore reducing stress to both. An even simpler means of drug delivery is through 'pour on' or 'spot on' formulations in which the drug is simply poured or spotted onto the back or neck of the animal and it is then absorbed across the skin. For example, eprinomectin (an avermectin type drug) can be poured onto the back of sheep to control both gastrointestinal nematode infections and the sheep nostril fly *Oestrus ovis* (Hoste *et al*., 2004). Ivermectin and some of the other anthelmintics used to treat ruminants can be given in a specially designed canister called a 'slow-release bolus' that is placed into the rumen using a special device. The bolus releases a set amount of drug every day over a prolonged period of time. This provides long-lasting protection where there is a constant risk of reinfection but is unsuitable for lactating cows because the drug would appear in the milk. There is a compulsory withdrawal period after any drug treatment in food-producing animals to avoid the risk of drug residues being found in the meat or milk. The withdrawal period varies between drugs and can vary from several weeks to a few days; milk from cows treated with eprinomectin can be used straight away. Similarly, some antiparasitic drugs are considered safe to use in adult humans but are not suitable for treating women who are pregnant or are likely to become pregnant. This is due to the potential risk they pose to the developing foetus. For example, metronidazole and miltefosine are considered potentially teratogenic and therefore not suitable for treating infections during pregnancy.

The fewer the number of treatments required to remove the parasite, the better the chance of patient compliance – especially if the treatment has to be delivered at a medical centre or

veterinary surgery. If repetitive treatments are required, then there is a high possibility that when the patient starts to feel better or the animal seems to be improving the patient or owner will cease or forget to complete the treatment regime. This not only increases the possibility that the parasite will persist but also increases the chances of any resistance developing.

Antiparasitic drugs are intended to kill living organisms and therefore there is always a risk that they will also harm the host. The chance of this is reduced if the drug selectively acts upon a physiological process that does not occur in the host. For example, drugs such as cyromazine (Vetrazin®) and diflubenzuron that target the moulting process in arthropods are safe for use in mammals because there is no comparable metabolic pathway. Cyromazine interferes with the deposition of chitin in the cuticle while diflubenzuron inhibits chitin synthesis. However, all chemicals are toxic if taken in sufficient concentration and it is very rare for a drug to be so specific that it only interacts with a single physiological process. Patients are unlikely to complete a treatment regime if the drug induces unpleasant side effects such as nausea and vomiting. If the drug is so toxic that it has to be given under medical supervision – such as antimonials for the treatment of leishmaniasis – then it further reduces the situations in which the drug can be employed and adds to the cost of treatment. The host's health and genetic constitution can influence the way it responds to the drug. For example, ivermectin is considered safe for use in most mammals but in certain breeds of domestic dogs very low concentrations (sub-therapeutic dose) induce neurological symptoms that include hypersalivation, ataxia, blindness, respiratory distress, and which can prove fatal. The reaction is most pronounced in sub-populations of Border Collies: not all Border Collies are susceptible and the condition has also been described in other breeds such as Collies, Australian Shepherds, white German shepherd dogs and Shetland sheepdogs. Veterinarians used to adopt the adage 'white feet – don't treat' although this is now recognised to be too sweeping a generalisation and the adage is now 'white feet – test before you treat'. The condition is caused by a deletion mutation in the MDR1 gene that encodes a protein called P-glycoprotein. P-glycoprotein is a transporter protein that is found on the apical border of a variety of cells including intestinal cells, biliary canalicular cells, renal tubular cells, the placenta, and testes. However, in the case of ivermectin sensitivity, it is the role of P-glycoprotein in the blood–brain barrier that is most important (Mealy *et al*., 2001). P-glycoprotein is a key constituent of brain capillary endothelial cells and transports a wide variety of structurally unrelated compounds that include ivermectin. It therefore regulates the movement of many drugs across the blood–brain barrier. Dogs that are homozygous for the MDR1 deletion are unable to control the movement of ivermectin into and out of the brain and the levels can rise to a point at which it exerts toxic effects (Merola *et al*., 2009). These breeds of dog can also suffer from adverse sensitivity to other drugs that are substrates for P-glycoprotein. Dogs that are homozygous for the deletion mutation can also have problems excreting ivermectin and other P-glycoprotein substrate drugs via the bile or urine and this could then result in the concentration of the drug within the circulation remaining higher than in other breeds of dog.

Cost is, of course, a major consideration for any treatment regime but especially so for poor people living in developing countries. The definition of 'affordable' varies between individuals, countries, and situations. Even if a drug is ideal in every way, if it is too expensive, then it becomes irrelevant to all but those who are rich. Of 17 antiparasitic drugs that were developed between 1974 and 2004, only four of them were judged suitable for use by poor countries (Moran *et al*., 2007). Drugs that are still in patent are usually expensive because the manufacturer needs to make sufficient profit to recoup the costs of development and fund the development of new drugs. In addition, pharmaceutical companies are not charities and must make a profit for their

investors. However, sometimes a company makes specific drugs available at reduced price as part of a control programme. Once a drug is out of patent, it can be manufactured by any commercial concern and its cost usually declines, which can radically alter the approach to the treatment and control of parasitic diseases. This is exemplified in the cases of albendazole and praziquantel. Albendazole is a broad spectrum benzimidazole anthelmintic that is used to treat gastrointestinal helminth infections and has the added advantage of being highly effective against *Giardia*. Praziquantel is also a broad spectrum anthelmintic but is most commonly used to treat schistosome infections. In the early 2000s, tablets of albendazole typically sold for about US $0.20 per tablet while those of praziquantel cost about US $3.00 per tablet. By the end of the decade the drugs were out of patent, and costs had fallen to about US $0.02 per tablet for albendazole and US $0.07 per tablet for praziquantel. As a consequence, it became more expensive to identify infected individuals through mass diagnostic screening than it was to simply treat a whole population. The emphasis therefore changed from how to identify the individual in need of treatment to treating everyone regardless of whether they needed it. This approach is possible with drugs such as albendazole and praziquantel because they have been used for many years and are known to be safe with relatively few instances of harmful side effects.

The cost of a drug is partly linked to its chemical stability since a drug that is stable, has a long shelf life, and does not need to be stored in a fridge is usually going to be cheaper than one that lacks some or all of these attributes. This is because it can be bought in bulk and can then be stored and transported at low cost and will be instantly available when needed. This can be a major consideration in developing countries where the temperatures are high, the distances long, and the electricity supply unreliable.

Finally, one must also consider the environmental impact of the drug. The tendency is always to concentrate on the immediate need but all the drugs that go into us and our animals are ultimately passed out of us in one way or another and they then enter the environment. Sometimes the drugs are metabolised completely but very often breakdown products or unmetabolised drug are passed in the urine or faeces and in the case of lactating animals, it can be found in the milk. In animals reared for slaughter, drug residues may be present in the meat. The potential environmental impact of avermectin drugs is a controversial topic, with some workers considering them to be serious pollutants while others believe them to be a minor problem that affects only certain ecosystems at certain times of year. Although avermectins such as ivermectin have proved very safe in human and veterinary medicine (except in animals homozygous for the MDR1 deletion), the majority of the drug is rapidly excreted unmetabolised with the animal's faeces. In addition, residues can persist in the environment for weeks under suitable conditions and therefore affect susceptible invertebrates both in the soil (Figure 10.1) and in surrounding water systems (Fernández *et al*., 2011). This may be considered beneficial since they can affect the development of larvae of parasitic nematodes and of pest insects such as stable flies (*Stomoxys calcitrans*) and horn flies (*Haematobia irritans)*. However, the residues can also harm susceptible non-target invertebrates such as dung beetles and other insects that breed in dung (Kolar *et al*., 2008). This may lead to dung decaying more slowly than it would normally and also the reduction in the insect population means that there is less food for insectivores such as certain bats and birds. If dung decays slowly, it results in excessive fouling of the pastureland. Sheep and cattle avoid plants that are contaminated with faeces and therefore the pasture becomes covered in rank grass and the quality of the pasture declines. This problem is particularly acute in dry climates such as parts of Australia in which dung beetles are vitally important for the breakdown of dung (Halley *et al*., 1989; Herd, 1995; McKeller, 1997).

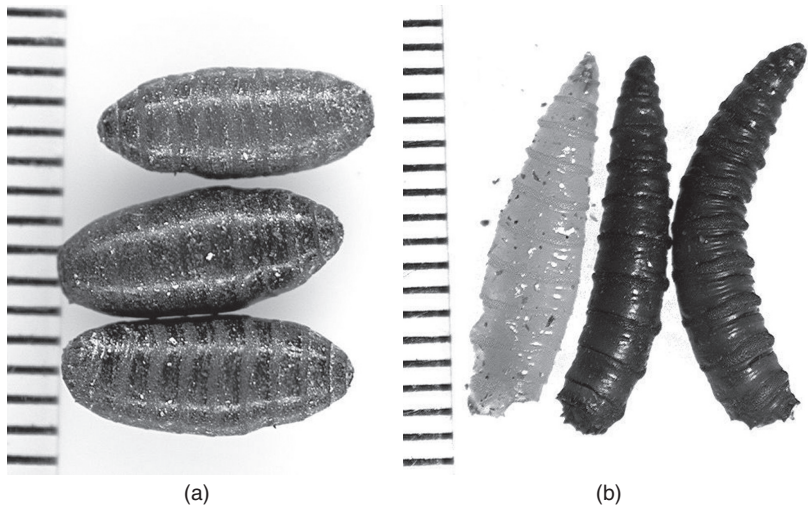

Figure 10.1 Influence of ivermectin residues on the pupariation of *Calliphora vicina*: (a) healthy, barrel-shaped, puparia that develop following contraction of the third instar larva; (b) elongate, malformed, puparia that develop from larvae exposed to ivermectin residues. Adult flies never emerge from malformed puparia

10.3.2 Pharmaceutical drugs

Most pharmaceutical antiparasitic drugs are manufactured from basic chemicals although some are derived from microbes. For example, the avermectins and milbemycins are derived from fermentation products of the actinomycete bacteria *Streptomyces avermiltilis* and *Streptomyces cyanogriseus* respectively. Most of the current antiparasitic drugs used on humans were initially developed for the treatment of other medical and veterinary conditions or for the control of crop pests: it was only subsequently that their antiparasitic properties were identified. Although this may appear to place an undue reliance upon serendipity, it must be borne in mind that the market for new antiparasitic drugs for treating people is relatively small. In 2006, it was estimated that the global market for antiparasitic drugs and chemicals for use in human medicine was US $0.5 billion, while for veterinary applications it was US $10 billion (Brown *et al*., 2006). Pet owners are willing to spend considerable sums of money on their animals and therefore it makes more commercial sense to develop drugs for this market than it does for humans: in 2008, the market for antiparasitic drugs for dogs and cats was put at US $3.4 billion (Woods and Knauer, 2010). These may sound like large sums of money but they must be set against the much bigger markets for other pharmaceutical products. For example, the market for heart disease drugs in the USA was estimated to be US $273 billion year^{-1} in 2010 and could rise to US $818 billion year^{-1} by 2030 (Heidenreich *et al*., 2011). Furthermore, the enormous cost of developing a drug from initial synthesis to final marketing means that there is little commercial incentive for introducing new antiparasitic drugs. The cost of bringing a new antimalarial drug to the market has been put at US $300 million and the chances of a candidate drug failing during phase II clinical trials are as high as 50% (Pink *et al*., 2005). Consequently, there are economies of scale if novel compounds are first tested for their effectiveness in a range of uses for which there are larger markets. Subsequently,

the knowledge gained from a chemical's use in one particular application can be transferred to its use against parasites. Nevertheless, some of the existing antiparasitic drugs are highly toxic (e.g. antimonials) and resistance is becoming an increasing problem (e.g. chloroquine). There is therefore a serious need for new drugs to treat a wide range of parasites. Unfortunately, new drugs are not reaching the market fast enough and many scientists are advocating novel approaches to speed up the process of drug identification. One way of speeding up the identification of novel drugs and vaccines is through the establishment of public–private initiatives such as COST Action B22 (http://www.icp.ucl.ac.be/cost/costB22/b22_front_cover.htm) in which scientists in pharmaceutical companies, universities, and institutes cooperate to form 'virtual companies' to identify potential new targets and undertake the initial screening novel compounds.

The traditional means of screening compounds for antiparasitic activity is a slow and costly process. This is because most parasites cannot be cultured *in vitro* and therefore activity is assessed by giving the drug to a laboratory animal infected with the parasite or one of its close relatives. For example, antimalarial drug activity can be assessed by treating mice infected with *Plasmodium berghei*. The use of laboratory animals is always a controversial topic and apart from the ethical considerations, it can be criticised on the basis that laboratory models do not always provide a reliable basis for understanding how the intended parasite target would respond to a particular drug in its normal host. Nevertheless, up until recently this was virtually the only way in which potential antiparasitic drugs could be screened. The approach to identifying candidate drugs has now become more selective using a combination of bioinformatics and chemoinformatics (Krasky *et al*., 2007). This approach ensures that only those chemicals with a high chance of success are tested in laboratory animals. The process is based on identifying potential target molecules from computational analysis of genome and protein sequences and then identifying chemical structures that should interact with the target molecule without causing pathology in the host (Müller and Hemphill, 2011). The full or partial sequences of several parasite species are now known, so it is becoming easier to assign a function to many genes. This makes it possible to identify unique features that could be targeted by either novel drugs or those already known to express particular structure-activity relationships (Crowther *et al*., 2011; Duncan *et al*., 2011). Clearly, it is not enough to simply identify a unique parasite protein, it must also be shown that the protein performs an essential function within the parasite. This is relatively easy for those protozoan parasites that can be maintained in culture or in laboratory animals by selectively activating or inhibiting the target protein using knockout/knockdown mutants or RNAi (RNA interference) experiments. For example, Tiwari *et al*. (2010) undertook an analysis of the genome of *Plasmodium berghei* and discovered that 23 out of 66 genes coding for protein kinases were functionally redundant for asexual development within red blood cells and therefore not suitable targets. Protein kinases are essential for many cellular processes and therefore logical drug targets. By identifying the specific protein kinases that are essential at particular life cycle stages, it is possible to narrow down the list of genes to be targeted, and this helps to prioritise further research. Similarly, there are two isoprenoid biosynthesis pathways: the mevalonate pathway that is found in vertebrates, fungi and some bacteria and the MEP (2-*C*-methyl-D-erythritol 4 phosphate) pathway that occurs in many bacteria, algae, higher plants and those organisms with a photosynthetic lineage. The latter includes parasites such as *Plasmodium* and *Prototheca*. Consequently, by selectively targeting enzymes involved in the MEP pathway, it may be possible to design safe and selective drugs that will simultaneously kill parasites and some of the accompanying secondary bacterial infections (Kuntz *et al*., 2005). Unfortunately, similar phenotypic experiments are more difficult to perform in platyhelminth and nematode parasites because of their complex life cycles and protein function has to

be inferred from model organisms such as *Caenorhabditis elegans* (Brown *et al*., 2006). This is less than ideal because the model organisms often lack the metabolic pathways the parasites utilise to achieve such functions as break down haemoglobin and avoid the host immune response.

10.3.3 DNA/RNA technology

With every passing year it is becoming easier and cheaper to sequence whole genomes. As a consequence, it is often said that we are living in the 'post-genomic era' during which we will revolutionise medicine, agriculture, and many other aspects of our lives. In particular, we are promised personalised medicine in which we will receive treatment that is tailored to our own particular physiological and genomic idiosyncrasies. For example, our genetic constitution affects the way in which we metabolise and are affected by drugs and other chemicals. Individual genomic sequencing would prevent people being given drugs which would not work for them or would induce harmful side effects. However, the molecular mechanisms controlling our bodies are enormously complicated and transforming laboratory experiments into commercial products will not be easy. It was once believed that a gene coded for a single protein but we now know that a single gene can code for tens or even thousands of different proteins. Consequently, achieving a desired physiological effect is not necessarily a question of switching on or off a few genes. Furthermore, the new technologies are not cheap and at least in the near future there is little likelihood of personalised medicine becoming an important factor for the treatment of parasitic diseases of poor people living in developing countries.

One of the most fundamental discoveries in recent years is that epigenetic mechanisms are responsible for many aspects of cell regulation (Goldberg *et al*., 2007). Epigenetic factors are those mechanisms that regulate genetic expression without changing the DNA sequence. Epigenetic regulation is important in all organisms and has particular relevance for host parasite relationships because it governs the host's immune response and the parasite's life cycle transformations, virulence, ability to overcome the host's immune system, and adapt to drugs (e.g. Cosseau *et al*., 2010; Hakimi and Deitsch, 2007; Lopez-Rubio *et al*., 2007; Poulin and Thomas, 2008). It is also considered to be a potential target for antiparasite therapies because it may prove possible to selectively target unique epigenetic pathways in parasites without harming the host (Zucca and Savoia, 2011).

Epigenetic factors include DNA methylation, histone modification, and regulatory RNA molecules. DNA methylation occurs through the addition of methyl groups to cytosine to produce 5-methylcytosine. This normally takes place at CpG sites (cytosine-phosphate-guanine) and extensive methylation of CpG sites within a gene sequence results in the gene being silenced. Chromatin consists of DNA wrapped around the large structural protein histone. If the sequence of amino acids that comprise histone is modified, it alters the three-dimensional shape of the molecule and this affects the expression of gene activity of the associated DNA. Histone modification can occur in several different ways (e.g. acetylation or methylation) and these have different effects on gene expression. It was once thought that that RNA was merely a 'messenger molecule' in cells but we now know that there are a variety of single- and double-stranded RNA molecules and that small non-coding micro RNA molecules are involved in the regulation of gene expression at the level of translation through mechanisms such as RNA interference.

RNA interference regulates gene activity and is also part of a cell's natural means of protection against viruses (Ambros, 2004; Hannon, 2002; Mello and Conte, 2004). It is brought

about by short sequences of double-stranded RNA and in many but not all organisms the RNAi pathway is as follows. Specific double-/ stranded RNA is cleaved by a ribonuclease enzyme called 'dicer' to form small (short) interfering RNA (siRNA) consisting of about 20–25 nucleotides. The small interfering RNA is then assembled to form an 'RNA-induced silencing complex' (RISC) that includes the antisense strand of the targeted mRNA and an endonuclease enzyme. The silencing complex binds to the mRNA and then the endonuclease enzyme ('Argonaute') brings about its degradation (Militello *et al*., 2008). Consequently, the mRNA is not translated and the protein it codes for is not formed. This natural process can be exploited by exposing cells to synthetic double-stranded RNA or siRNA and thereby influencing their gene activity. However, the effectiveness of this varies between organisms and some, such as *Babesia bovis*, *Leishmania major*, *Theileria* spp., *Trypanosoma cruzi* and several other parasitic protozoa, lack an active RNA interference pathway (Militello *et al*., 2008). There is also some doubt about whether synthetic siRNA exerts its effects directly through the RNAi pathway or through some other mechanism. Kleinman *et al*. (2008) found that in a mouse model of macular degeneration, siRNA bound to Toll-like receptor 3 (TLR3) on the surface of cells and induced the cytokines gamma interferon and interleukin-12. Perhaps the mode of delivery is important since Davis *et al*. (2010) subsequently found that siRNA delivered via nanoparticles were effective at inducing the RNAi pathway in human melanoma patients.

Box 10.1 Antisense DNA and RNA

Within a cell, the first step in the production of a protein is when the gene coding for it in the cell's DNA is transcribed into a sequence of messenger RNA (mRNA) oligonucleotides. The single-stranded mRNA molecule then moves to the ribosomes where it is translated into a sequence of amino acids. The mRNA is referred to as a 'sense' strand while its non-coding complementary strand is the 'antisense strand'. For example, if the sense strand had the sequence 5′-AACGAAUUAC-3′, its antisense strand would be 3′-UUGCUUAAUG-5′. If sense and antisense strands came into contact, they would bind together to form a non-functional duplex molecule. Consequently, the sense strand would not be translated and the protein molecule it coded for would not be formed. Natural antisense RNA transcripts can be found in both prokaryotic and eukaryotic organisms (including protozoan and helminth parasites) in which they presumably function in the regulation of RNA although their purpose is not always apparent. Engineered antisense RNA inhibition is accomplished by inserting a gene which codes for the antisense sequence of mRNA into an organism's DNA. This blocks the expression of a specific protein and is a basis for the production of transgenic organisms and many studies on gene function. It could also be a potential area of development for therapies.

The DNA helix consists of two complementary strands of DNA: these strands are known as the 'sense' and antisense' strands. The antisense strand is transcribed to produce the sense strand of mRNA. Short strands (oligodeoxynucleotides) of synthetic antisense DNA will bind to mRNA to form an RNA/DNA heteroduplex which is then a substrate for endogenous cellular RNAases. Consequently, the protein coded for by the mRNA is not formed. This can be used as means of studying gene function and also to selectively kill target cells/organisms by preventing them

from producing essential molecules. Oligonucleotides are rapidly broken down within cells and therefore modified phosphorothioate oligodeoxynucleotides that are more resistant to nuclease attack are usually employed. For example, antisense phosphorothioate deoxynucleotides inhibit the growth of *Leishmania amazonensis* and *Plasmodium falciparum* (Mishra *et al*., 2001; Barker *et al*., 1996) and have been considered as potential chemotherapeutic drugs.

While manipulating epigenetic processes offers the prospect of killing pathogens and enhancing the host immune response, the technology to accomplish this is still at the experimental stage. Nevertheless RNAi therapy for cancer and viral diseases is advancing rapidly and will undoubtedly inform the future treatment of parasitic diseases. It is, however, necessary to proceed with care. Although RNAi therapy is theoretically more specific in its mode of action, it suffers from many of the concerns that affect conventional pharmaceutical drugs (Castanotto and Rossi, 2009). In particular, the siRNA sequences have to be delivered to the target cells and remain there long enough to exert an effect. There also remain safety concerns such as the risks of siRNA effects on non-target gene sequences and what the consequences of overstimulation of the RNAi pathways would be. The latter may not be a problem if synthetic siRNA does not act directly on RNAi pathways. However, if they act non-specifically through Toll-like receptor 3 pathways, then this could lead to a more widespread reaction that affected non-target tissues (Kleinman *et al*., 2008).

10.3.4 Molecular chaperones (heat shock proteins)

Nuttall's Standard Dictionary (1921 edition) quaintly describes a chaperone as 'a matron who attends a young lady in public places as a protector'. Molecular chaperones do not ward off predatory suitors but they do ensure that their charges are able to engage in their duties in a seemly fashion! They are protein molecules that facilitate the non-covalent folding/unfolding and assembly/disassembly of other macromolecules. They do not, however, form part of the functioning macromolecule after it is assembled or correctly folded. Molecular chaperones are found in both prokaryotic and eukaryotic organisms and play an essential role in many basic functions such as responding to environmental cues, signal transduction, differentiation, and development. The best known of the molecular chaperones are the group known as the 'heat shock proteins'. They were given this name following the discovery that they are expressed in response to heat stress. The term is a bit of a misnomer since it was subsequently found that 'heat shock proteins' are also expressed in response to numerous stresses, including infection, and as part of normal physiological processes (i.e. they are 'constitutively expressed'). Heat shock proteins (Hsp) are classed into families according to their molecular weight (e.g. Hsp20, Hsp40, Hsp60, Hsp70, Hsp90, and Hsp100) and members of each family tend to show structural and functional similarity. An organism may have several heat shock proteins within each family and some of them work by interacting with co-chaperones. Heat shock proteins are present in parasitic protozoa, helminths, and arthropods and their importance in development would suggest that they might be suitable vaccine or drug targets (Acharya *et al*., 2007). However, until recently it has proved difficult to target specific Hsp molecules. This is because families of Hsps in different organisms are structurally very similar and within an organism an Hsp can be involved with many different macromolecules.

Heat shock protein 90 (Hsp90) is currently the most promising drug target for a number of pathogens and also for the treatment of cancer. Clinical trials of experimental drugs targeting Hsp90 for the treatment of breast cancer and other neoplasms are ongoing (Calderwood, 2010) and this research is informing the development of antiparasitic drugs. Hsp90 is expressed by all parasites investigated to date. Among the parasitic protozoa *Plasmodium falciparum*, *Leishmania donovani*, *Toxoplasma gondii* and *Trypansoma cruzi*, Hsp90 functions include regulation of life cycle stage transformations (Neckers and Tatu, 2008). Hsp90 is found in free-living, plant parasitic, and animal parasitic nematodes and at least some of its roles vary with the nematode's lifestyle (Him *et al*., 2009). Hsp90 is also expressed by trematodes, cestodes, and arthropods.

Plasmodium falciparum has three Hsp90 proteins and one of these has been found to be sufficiently different from human Hsp90 to be targeted specifically. The drug 'geldanamycin' and its analogues are effective inhibitors of Hsp90 in *Plasmodium falciparum* and also *Trypanosoma evansi*, *Schistosoma japonicum*, the filarial nematodes *Brugia pahangi* and *Brugia malayi* and schistosomes (Pallavi *et al*., 2010; Taldone *et al*., 2010; Wenkert *et al*., 2010). Geldanamycin is a benzoquinone-containing ansamycin that was originally identified from the actinomycete bacterium *Streptomyces hygroscopicus*. Interestingly, although the Hsp90 found in the nematodes *Brugia* spp. and *Caenorhabditis elegans* share considerable structural similarity, those worms of the free-living species are not affected by geldanamycin. Indeed, among nematodes, geldanamycin only binds to Hsp90 in those species that lack free-living stages or where the transmission stage is enclosed within an egg (Him *et al*., 2009). The reason for this is probably because the activity of Hsp90 is regulated through a number of co-chaperones and post-translational modifications that vary with the task it is undertaking (Taldone *et al*., 2010). Consequently, in the case of nematodes, the ability of geldanamycin to bind to Hsp90 is related to the nematode's life cycle strategy. This also emphasises the problems associated with using free-living organisms as models for parasitic species when attempting to identify potential drugs and drug targets.

Other heat shock proteins are also involved in the life cycle of parasites. Examples include Hsp100, which is important for the infectivity and survival of *Leishmania* within macrophages, while Hsp70 enables *Toxoplasma gondii* to avoid the host's inflammatory response. Genomic analysis of *Plasmodium falciparum* indicates the presence of six Hsp70s and these could be targeted by direct inhibition or through preventing them from interacting with their co-chaperones (Pesce *et al*., 2010).

10.3.5 Nanotechnology

Nanomaterials are solid colloidal particles 1–100 nm in diameter with applications in computing, clothing, engineering, and pharmaceuticals. Nanomaterials can be manufactured from elements such as gold, silver, and carbon, from compounds such as iron oxide, as well as from organic polymers such as chitosan. Perhaps surprisingly there is currently no accepted legal definition of what constitutes an engineered nanomaterial and Maynard (2011) has argued that this should remain the case. For parasitologists, their main interest is as drug/antigen/gene carriers, vaccine adjuvents, and diagnostic tools (Date *et al*., 2007; Hauck *et al*., 2010). For example, antisense oligodeoxynucleotides delivered using chitosan nanoparticles are effective at preventing the growth of *Plasmodium falciparum* (Föger *et al*., 2006) while polypeptide nanoparticles have been used to deliver antigens in an experimental anti-malarial vaccine (Kaba *et al*., 2009). Borate glass nanofibres are under investigation for the treatment of venous stasis and other medical problems. In addition to preventing bacterial infections, they also stimulate bone regeneration and

wound healing without associated scarring (Wray, 2011). They might therefore prove useful for the treatment of the slowly healing ulcers and wounds caused by parasites.

Nanoparticles come in a wide variety of shapes and sizes. Nanocapsules have a polymer membrane and the chemical to be delivered is encapsulated within it or adsorbed onto its outer wall. Nanospheres are solid and the associated chemical is either dispersed within its matrix or adsorbed onto its outer surface. As their name suggests, nanotubes have a tubular profile but may be short, long, solid, hollow, single-walled or multi-walled. Particles that are less than 100 nm in size have an enormous surface area to volume ratio and exhibit very different chemical and physical properties to larger particles. This can prove useful for delivering drugs that are either bound to or enclosed within nanoparticles. By altering the size and shape of the nanoparticle, one can radically alter a drug's characteristics. In particular, it is possible to improve a drug's solubility in water. Solubility is an important attribute if a drug is to be absorbed and dispersed around the body, and lack of solubility is the reason that many candidate drugs fail during screening. In addition, by delivering drugs via nanoparticles, it is possible to extend the period of time they remain in the circulation, adjust the rate at which they are released, target them to a specific organ, and combine two or more drugs where combination therapy is required (Zhang *et al*., 2008). For example, andrographolide is a natural product derived from the leaves of *Andrographis paniculata* that combines anti-*Leishmania* activity with low toxicity. However, its therapeutic use has not been exploited because it has a short plasma half life and does not reach its target in sufficient concentration to exert a strong antiparasitic effect. By delivering the drug using nanoparticles, Roy *et al*. (2010) were able to target the drug to macrophages and increase its effect on *Leishmania donovani*. However, this was an *in vitro* assay and it remains to be determined whether a curative effect could be obtained *in vivo*.

Nanoparticles have particular promise for the treatment of intracellular parasites, since the drug-bearing particles can be targeted to specific cell types which would then internalise them through endocytosis. Nanoparticle delivery could also extend the usefulness of older drugs for which the patent has expired so that they can be reintroduced to the market at comparatively low cost. In the process of reformulating older drugs using nanoparticles, they can become more effective and have fewer side effects than in their original guise. For example, nanoencapsulation of quinine has been found to increase its efficacy against *Plasmodium berghei* in rats (Haas *et al*., 2009).

Box 10.2 Gold nanoparticles for the diagnosis and treatment of parasitic infections

Gold is chemically stable and does not corrode. However, although gold is inert, gold nanoparticles will adsorb protein and other organic molecules onto their surface and therefore they make good delivery vehicles. Gold nanoparticles can be manufactured in a variety of shapes and sizes depending upon the physical and chemical properties required. They have great potential for the treatment of cancer and they could prove equally useful for combating parasitic diseases (Pissuwan *et al*., 2011). For example, gold nanoparticles have been used as carriers of hydrophobic drugs and by conjugating an antibody to the surface of the particles, they can be used to target specific cells. Gold nanoparticles are also potential vehicles for non-viral gene delivery.

Raman reporters or 'Raman tags' are molecules that are excited when stimulated by specific wavelengths (Gong *et al*., 2006). When Raman reporters are attached to gold nanoparticles, they can be visualised after administration using a technique called Surface Enhanced Raman

Spectroscopy. Consequently, the location of a tumour or parasite can be determined using gold nanoparticles bearing the appropriate antibodies and Raman reporters.

Certain types of gold nanoparticles convert absorbed light into near infra-red radiation (heat) and have potential for laser photoablation (photodynamic therapy). The basis of this approach is that the nanoparticles are targeted to specific cell types, then a laser beam is directed onto them. This results in localised heating that kills the cells to which the gold nanoparticles have bound. Pissuwan *et al*. (2009) used this approach to kill the tachyzoites of *Toxoplasma gondii* and it could presumably also be used for other parasites. Alternatively, a laser can deliver a specific wavelength that stimulates gold nanoparticles that have reached their target to release bound molecules, such as drugs. This ensures that the target cells experience a sudden therapeutic dose of the drug.

Agglutination-based immunoassays are based on the detection of aggregates that develop when an antigen binds to an antibody. Gold nanoparticles can be used as the solid phase in agglutination assays. Aggregation of gold nanoparticles within a fluid shifts the absorption peak to a longer wavelength and this can be detected colorimetrically. Alternatively, the binding of antigens or antibodies with nanoparticles can be measured using piezoelectric biosensors that respond to the mass change that follows binding. Wang *et al*. (2004) describe how people or animals infected with *Toxoplasma gondii* can be identified by detecting anti-*Toxoplasma gondii* immunoglobulins in their serum using gold nanoparticles coated with *Toxoplasma gondii* antigen.

Although nanoparticles have great promise in human and veterinary medicine, most potential applications are still at the experimental stage. Nanoparticles are not without their problems and risks, although these are difficult to predict. For example, nanoparticles are prone to aggregation and this can make them difficult to handle in both liquid and dry formulations. Substances that are safe in one particular size range may become poisonous or carcinogenic at another size. In addition, the chemistry of the nanomaterials, their shape, and their porosity are all factors that influence their toxicity. Silver nanoparticles have numerous domestic and industrial applications, including incorporation into socks to kill bacteria and reduce smells, and they are already finding their way into soil ecosystems. Unfortunately, silver is a toxic metal, and silver nanoparticles could potentially affect microbial and invertebrate communities (Hänsch and Emmerling, 2010; Roh *et al*., 2009). There is also evidence that nanoparticles might accumulate through food chains since gold nanoparticles biomagnify in caterpillars that consume plants grown in a hydroponic medium containing the nanoparticles (Judy *et al*., 2011). More worrying is the observation that cobalt-chromium nanoparticles can induce DNA damage in human fibroblast cells without ever crossing the cell membrane (Bhabra *et al*., 2009). A review of the health and environmental concerns about nanoparticles is provided by Lam *et al*. (2006).

10.3.6 Quantum dots

Quantum dots are nanocrystal semiconductors (e.g. CdSe/ZnS) that are of great interest for their electronic and optical characteristics. In biology, they have many potential uses for bioimaging because they can be attached to molecules or cells and thereby enable their movements to be

tracked in real time. For example, quantum dots have been used to monitor the invasion of erythrocytes by *Plasmodium falciparum* and as tools to identify *Plasmodium*-infected erythrocytes using flow cytometry (Ku *et al*., 2011; Tokumasu *et al*., 2005). Not only can quantum dots be used to identify particular cells,they can also deliver gene silencing RNA (siRNA) (Chen *et al*., 2005). Consequently, quantum dots may also prove useful in the treatment of parasitic diseases. However, there are various quantum dots and they differ in their metabolism and excretion and some of them could prove harmful to both the recipient and the environment (Hardman, 2006). Consequently, before the full potential of quantum dots in human and animal medicine can be realised, more information is required on their toxicological properties.

10.3.7 Natural remedies

Until the development of the pharmaceutical industry in the late 1800s, the only drugs available for treating parasitic diseases were based on naturally occurring substances derived from plants, animals and mineral substances. For example, gastrointestinal nematode infections in both humans and domestic animals were commonly treated with turpentine, which is a volatile oil distilled from pine resin, male fern extract (*Dryopteris felix-mas*), or areca nut powder (*Areca catechu*). These remedies were less effective than modern pharmaceuticals and often deeply unpleasant to the patient. However, some natural remedies were undoubtedly 'life savers'. For example, bark from South American Cinchona trees contains quinine and for hundreds of years was the basis of the only available effective treatment for malaria (Sherman, 2011).

Natural products are often inconsistent in their effectiveness because of varying levels of the active ingredients. For example, there are many species of Cinchona tree and their barks vary in their quinine content. Consequently, once chemists became skilled in manufacturing substances that were both more effective and could be delivered in precise dosages, many people lost interest in 'natural remedies'. However, over time, parasites have developed resistance against many widely-used drugs and therefore pharmaceutical companies and researchers are now interested in identifying novel substances in plants and animals. These chemicals could then be either manufactured *de novo* or through biologically engineered bacteria or if the structure is too complicated, they could be extracted from cultured plants or animals. For example, the cysteine proteases contained within the latex of fig trees (*Ficus* spp.) and the papaya (*Carica papaya*) break down the cuticle of nematodes but have a low oral toxicity in mammals. They therefore show considerable promise for the treatment of gastrointestinal nematode infections (Stepek *et al*., 2004). Sponges (Porifera) contain a group of chemicals called kalihinol*s* that are toxic to several species of human parasitic protozoa, including *Plasmodium* (Encarnacion *et al*., 2000; Kayser *et al*., 2003; Miyaoka *et al*., 1998). However, major problems remain in developing artificial culturing conditions for sponges (Mendola, 2003), and in formulating the chemicals so that they can reach the sites where they will need to have an effect in sufficient concentration and without losing their biological activity.

There is a resurgence of interest in traditional remedies in both developing and industrialised countries. In the developing countries, the interest is being caused (at least in part) by the high cost of pharmaceutical drugs (Fajimi and Taiwo, 2005), while in the industrialised countries some people distrust modern Western-style medicine and are wary of (or prejudiced against) major drug companies. Many people are also scared by reports of medicines that cause harmful side effects and they are attracted to cures that are purported to be more 'natural'. In addition, many diseases

are difficult or impossible to cure and therefore those who suffer from them feel let down by the medical profession and are looking for alternatives.

Box 10.3 Artemisinin and the treatment of malaria

References to the antimalarial properties of extracts of sweet wormwood ('Qinghao', *Artemisia annua*) can be found in Chinese traditional medicine texts that date back several hundred years. However, it was not until the 1970s that any serious attempt was made to determine the active ingredient and exploit it on a widespread basis. As is so often the case with parasitic diseases, the initial impetus for the research came not so much from humanitarian concerns, but to support troops engaged in warfare. In this case, the Chinese government was seeking novel antimalarial drugs that could be used to treat Vietnamese Communist soldiers who were engaged in a prolonged jungle war with the Americans (Sherman, 2011).

The active ingredient of the wormwood extract is a sesquiterpene trioxane lactone called 'qinghaosu' or 'artemisinin' which has the empirical formula $C_{15}H_{22}O_5$. The complicated structure makes it difficult to synthesise artificially, though strains of yeast have been biologically engineered to produce artemisinic acid that can then be converted to artemisinin (Ro *et al*., 2006). The amount of artemisinin contained within sweet wormwood varies between strains of the plant and is also affected by the soil and climate in which it is grown. There are hopes that high-yield hybrid plants currently being grown in Madagascar, South Africa and certain other African countries may boost the production of artemisinin (Ellman, 2010).

Artemisinin is highly effective for the treatment of malaria and in particular *Plasmodium falciparum*. The discovery of artemisinin coincided with the development of widespread resistance to chloroquine and sulfadoxine-pyrimethamine and it was soon the treatment of choice. Sweet wormwood therefore became an important cash crop for many farmers in China, Vietnam and elsewhere in Asia. However, by 2007, the supply outstripped demand and the value of sweet wormwood fell from a high of US$ 1,100 kg^{-1} to a low of US$ 200 kg^{-1}. Farmers therefore turned their attention to more valuable crops and this has been further exacerbated by a coincidental increase in world food prices. No sooner had this happened than there was an increase in the demand for sweet wormwood as a consequence of the Global Fund to Fight AIDS, Tuberculosis and Malaria releasing funds to subsidise cheap drugs under its Affordable Medicines Facility – Malaria. Thus the availability and price of the drug have become closely tied to the vagaries of marketplace and sudden injections of cash from large aid organisations (Van Noorden, 2010).

Artemisinin has poor bioavailability and is usually modified to produce semi-synthetic derivatives such as artesunate, artemotil, and artelinic acid. The WHO demands that artemisinin and its derivates are used in combination with other drugs (artimisinin-based combination therapies – ACTSs) in order to ensure that all the parasites are killed and therefore reduce the chances of resistance developing. For example, in many parts of the world, artesunate is used in combination with mefloquine to treat *Plasmodium falciparum* infections. Unfortunately, many manufacturers are failing to heed the WHO and resistance to artemisinin has already developed in the area between the borders of Thailand and Cambodia (Dondorp *et al*., 2010). In addition, there is a widespread problem of counterfeit drugs that contain either very low levels of artemisinin derivatives or none at all (Newton *et al*., 2006, 2007). The low concentrations of artemisinin

derivatives increase the risk of drug resistance developing. The counterfeit drugs lacking artemisinin derivatives are equally harmful because not only will they not cure the patient's malaria but they sometimes contain carcinogens such as benzene and safrole.

The mode of action of artemisinin and its derivatives is still uncertain and there are several competing theories (O'Neil *et al*., 2010). There is strong evidence that the drug is first activated by interacting with iron, though whether this is free iron, that present in haem, or both is not known. For example, one suggestion is that the drug interacts with the haem moiety in haemozoin, which is a waste product of *Plasmodium* metabolism, to produce free radicals that then kill the parasite by damaging DNA and cell membranes and/or interfering with the physiological processes necessary for further production of haemozoin. Artemisinin may also harm parasites without interacting with iron, since Hartwig *et al*. (2009) have shown that derivatives accumulate within the membranes of *Plasmodium falciparum* where they directly induce oxidative damage.

In addition to *Plasmodium*, artemisinin derivatives are also effective against other parasites including *Toxoplasma gondii* (D'Angelo *et al*., 2009), and schistosomes and other flukes (Utzinger *et al*., 2007). However, there are contradictory reports on the effectiveness of artemisinin and its derivatives on parasitic nematodes (Squires *et al*., 2011). Artemisinin derivatives also show promise in cancer chemotherapy and they can prevent the proliferation of a number of cancer cell lines (O'Neil *et al*., 2010). Artemisinin-based drug therapies therefore show promise for the treatment of malaria co-infections and also carcinogenic trematode parasites.

Although traditional remedies are often considered by the public to be safe, they have seldom undergone the stringent efficacy and safety testing required of pharmaceutical products. This is not surprising because those who prepare natural remedies seldom have the resources to comply with the requisite legislation and the products often contain a mixture of chemicals which vary between batches. Pharmaceutical companies and their proponents complain about this, since they spend enormous sums of money in developing drugs while companies that market natural products seldom conduct clinical trials and there is limited information on the health risks associated with taking the remedies (De Smet, 2004; Tilburt and Kaptchuk, 2008). In addition, Asian herbal remedies sometimes contain high levels of heavy metals and undeclared prescription drugs (Ernst, 2002). Sometimes natural remedies may not only make matters worse, they can be positively lethal. For example, Kriel and Joubert (1996) report that in South Africa, native healers called '*baloi*' have been known to incorporate the ground-up segments of *Taenia solium* into medicines to treat tapeworm infections. This is presumably on a similar basis to homeopathy in that small amounts of a poison can be used to treat a greater poison – but in this case the consequences would be dire.

10.3.8 Homeopathy

Few therapies are more guaranteed to induce fury among scientists and medical professionals than homeopathy. However, it would be perverse to ignore totally 'remedies' that are routinely used by hundreds of thousands of people around the world. Homeopathy is based upon the concept that a disease can be cured by consuming dilute concentrations of substances that would provoke similar signs and symptoms in a healthy person. The idea was first proposed in the 1790s by the German

physician Dr Samuel Hahnemann. He observed that drugs obtained from the bark of Cinchona that were used at the time to treat malaria induced malaria-like symptoms in those who were uninfected by the disease. Homeopathy has since grown enormously and supports registered practitioners in human and veterinary homeopathic medicine. The global market for homeopathic remedies is worth millions of pounds. Although there are experimental studies reporting beneficial effects of homeopathic treatments for parasitic diseases, these tend to be published in journals of homeopathy and on specialist websites rather than in the mainstream scientific press (e.g. Rodrigues de Almeida *et al*., 2008; Zacharias *et al*., 2008). Many scientists argue that the concentration of the purported 'active ingredient' in homeopathic remedies is so low that one might as well drink distilled water, and that there is no rational scientific basis for homeopathy. In human medicine, the reported beneficial results of homeopathic remedies have usually been ascribed to the 'placebo effect' (i.e. basically, if one believes that something is doing you good, then you often feel better), and published evidence suggests that the effect of homeopathic medicine is no better than a placebo (Ernst, 2002b). One could argue that as long as a person feels better and the treatment is not harmful, then it doesn't matter how the effect is achieved. However, to knowingly prescribe someone a placebo is not consistent with informed patient choice and some doctors would consider it unethical. Of more concern, especially with parasitic infections, is the possibility that an infected person who initially resorts to homeopathy might put off consulting a medical doctor until the disease has caused serious pathology.

10.4 Vaccines against parasitic diseases

Drugs may provide a complete cure for an infection but unless the host and parasite are subsequently prevented from coming into contact, reinfection is often an almost certainty. Wherever parasite exposure is a regular occurrence, long-lasting protection can only come from the development of a protective immune response. In the past, the only way in which immunity to a disease could be acquired was through surviving the infection. Indeed, in the UK and the USA some parents still organise ill-advised 'chicken pox parties' so that their offspring have the opportunity to become infected with the disease. Once a child has recovered from chicken pox, they usually have a long-lasting protective immunity to the condition. However, natural infections can have serious consequences. Although chicken pox is usually a mild disease in children, the blisters it causes can become infected with bacteria and some children become seriously ill. The purpose of vaccination is to stimulate a protective immune response without the risks associated with a natural infection. However, no vaccine can be expected to give 100% protection and even with preparations which closely mimic the natural infection, lifelong immunity is not guaranteed. This is because the immune system is not infallible, and as animals age, their physiological and regulatory processes tend to slow down and become subject to errors.

However, vaccines have been very effective in controlling viral and bacterial diseases (and in the case of smallpox, it has been eradicated altogether thanks to an effective vaccination programme). Due to these successes, many people see antiparasite vaccines as a sort of 'holy grail' that will solve the problems of diseases such as malaria, trypanosomiasis, and amoebic dysentery. The great hope is that with a few injections, it will be possible to reduce the expensive disease burden that afflicts so many developing countries. To this end, since the end of the Second World War, hundreds of millions of pounds have been invested in the development of antiparasitic vaccines. However, very few have progressed beyond the trial stage and entered widespread use. This has

Table 10.3 Types of vaccines

Vaccine description	Vaccine component
Attenuated	Live non-virulent organism
Killed	Dead pathogen
Sub-unit	Antigenic component
Toxoid	Inactivated toxin
DNA	Specific gene(s)

led some to question whether the money has been well spent (Desowitz, 1991) or even, in some cases whether a vaccine is desirable in the first place. For example, Gryseels (2000) has argued that if an effective vaccine for human schistosomiasis was developed, it would reduce the impetus for providing safe drinking water, sanitation, and health care and thereby compromise the control of other important but less high profile pathogens. He also points out that human schistosomiasis is a serious disease for only a minority of people, who can already be diagnosed and treated at the primary health care level. Indeed, in parts of Pakistan, current vaccination campaigns against diseases such as polio, measles and bacterial meningitis are running into problems because the public perceives that these have not been accompanied by any improvement in health services (Anon, 2011b).

There are a variety of approaches to vaccine design (Table 10.3) but to be effective within a control programme, one must first understand the biology and life cycle of the parasite and also how the immune response is mounted against it (Tarleton, 2005). For example, an anti-sporozoite vaccine would block new infections with malaria and be particularly useful for people who have never been exposed to the disease and are visiting an endemic region. It would also reduce the chances of those already infected from acquiring a more serious infection through repeated challenges. However, an anti-sporozoite vaccine would not help cure an existing infection and a person already infected with *Plasmodium vivax* or *Plasmodium ovale* may continue to suffer relapses without appropriate drug treatment since these species have a hypnozoite stage that gives rise to future generations of merozoites. The different species of *Plasmodium* overlap in their geographical distribution but they are taxonomically very distinct so any anti-sporozoite vaccine would probably have to contain components specific for each species. Nevertheless, phase III field trials with the RTS,S/AS01 malaria vaccine that targets the circumsporozoite protein are encouraging. This vaccine was tested on babies who were 6–12 weeks old and slightly older children aged 5–17 months. Both groups were given either three staggered doses of the vaccine or a control, and the level of protection conferred was assessed over 14 months from the date of the first vaccination. A complete analysis of the data was not available at the time of writing, but the results for the first 6000 children aged 5–17 months indicated that the vaccine provided 50.4% efficacy at preventing clinical malaria and 45.1% efficacy at preventing severe malaria (RTS,S Clinical Trials Partnership *et al*., 2011). These are the best results to date of any large-scale field trial for an anti-malaria vaccine. However, it should be noted that the overall efficacy of the vaccine at preventing severe malaria in both age groups was only 31.3%.

An anti-gametocyte vaccine would potentially block transmission of the disease within a locality. This approach to control would be particularly effective for parasite species such as *Plasmodium falciparum* in which there are no wild or domestic animal reservoirs of infection. However, an anti-gametocyte vaccine would not directly benefit anyone already suffering from malaria and it would not prevent someone from becoming infected should they be bitten by an infectious

mosquito. In addition, as a means of control it might run into problems if infected people regularly visited the control programme region (e.g. refugees, border regions) and thereby maintained a level of infection among the local mosquitoes. A good review of the progress to date and future prospects in malaria vaccine development is provided by Girard *et al*. (2007).

The nature of the host immune response to parasite challenge is a crucial factor in the development of an effective vaccine. Since eukaryotes are more complex organisms than bacteria and viruses, it has proved much more difficult to determine suitable targets for vaccine preparations. Although most parasites generate an immune response in their host, in many cases this is not protective. This is often because the parasite is able to avoid the immune response by hiding within host cells (e.g. *Leishmania*) or because it has evolved sophisticated mechanisms for avoiding, depressing, and confusing the host immune system such as variable surface coat antigens generated by trypanosomes. Consequently, the vaccine must be able to do more than stimulate the normal host immune response to the parasite, which is essentially all that is required of an antiviral vaccine. Instead it must induce an immune response that is able to reach the parasite, overcome the parasite defence mechanisms, and induce a much stronger immune response or exploit targets that are usually protected.

Vaccination can be expected to exert strong selection pressure upon a pathogen and some workers feel that we should be careful about the type of vaccine we try to develop. Sylvain Gandon and co-workers (Gandon *et al*., 2001; Gandon and Day 2008) have suggested that vaccines that reduce the growth rate of pathogens or neutralise the harmful effects of toxins could alter the natural selection within parasite populations in favour of more highly virulent strains. Consequently, those who are not vaccinated may encounter more dangerous varieties of the pathogen. By contrast, they argue that vaccines that block infection may result in selection for less virulent pathogens.

10.4.1 Attenuated vaccines

Attenuated vaccines utilise live organisms that are biologically the same as the ‘wild type’ pathogen but do not induce disease or only cause mild symptoms. The attenuated organisms might be obtained through the selection of non-pathogenic strains or through treatment of the wild type with mutagens or passage through laboratory animals or cell cultures. Because the vaccine is ‘live’, the injected organism will grow and multiply within the host thus exposing it to a variety of different life cycle stages. Consequently, live vaccines elicit an immune response similar to that of the pathogen but without its associated pathology. They are therefore more likely to induce a T cell-mediated immune response than a killed vaccine and this is important for combating intracellular parasites. However, there are always concerns over whether attenuated pathogen might revert to the ‘wild type’ or induce disease. For example, a vaccine against *Ancylostoma caninum* that utilised X-ray attenuated third-stage larvae was developed in the 1960s (Miller, 1965) and subsequently entered commercial production in the 1970s. The vaccine gave up to 90% protection in dogs but was ultimately withdrawn because it sometimes gave rise to patent infections, and there were also the common problems associated with live vaccines of expensive production costs and a short shelf life (Fujiwara *et al*., 2006). Another problem with developing attenuated anti-parasite vaccines is that many organisms cannot be grown in culture, and those that can be cultured change their phenotype over successive generations so that they become less like the ‘wild type’ and genetically more homogeneous. Nevertheless, there is continued interest in developing an anti-*Leishmania* attenuated vaccine (Handman, 2001) and an anti-*Theileria*

annulata vaccine has been used successfully to treat cattle for many years (Hall *et al*., 1999; Innes *et al*., 2011). There is also interest in using live attenuated organisms as vehicles for recombinant antigens. For example, the yellow fever virus vaccine YF17D is extremely safe and effective and can be genetically engineered to express particular antigens to which an immune response is subsequently generated. By inserting circumsporozoite antigen into the YF17D vaccine, Stoyanov *et al*. (2010) were able to immunise mice against *Plasmodium yoelli* and thereby protect them from subsequent infection. Similarly, a live attenuated *Salmonella enterica* var *typhimurium* mutant has been used as the vehicle to deliver recombinant *Echinococcus granulosus* antigens to dogs. The resultant oral vaccine was found to confer 74–79% protection in field trials (Petavy *et al*., 2008).

10.4.2 Killed vaccines

Killed vaccines involve growing the pathogen in culture and then killing it before using it as a vaccine. This obviously overcomes some of the safety worries associated with live vaccines, although quality control is essential because if even a small number of live pathogens were injected, it might result in disease. In addition, if the pathogen produces a toxin, this must be removed during vaccine preparation. This approach is limited to those parasites that can be grown under culture conditions but experimental vaccines with variable degrees of efficacy have been developed against a range of protozoa including *Plasmodium*, *Leishmania*, *Cryptosporidium parvum*, *Neospora caninum*, and *Toxoplasma gondii* (Good, 2011; Innes *et al*., 2011; Noazin *et al*., 2009).

Many parasite antigens induce an immune response but in only a few instances is this protective. Sub-unit vaccines are those that incorporate only those antigens that are known to induce a protective immune response. This approach therefore depends upon biochemically characterising the pathogen and then testing different components for their ability to induce an immune response. Once a likely candidate has been identified, it can be purified from cultured parasites. Alternatively, a recombinant vaccine can be manufactured if the gene coding for the antigen is identified and cloned into bacteria or yeast cells. There is increasing interest in producing vaccines in plants, since it is cheaper to set up greenhouse facilities than it is a bioreactor. Plant-based H5N1 avian flu virus vaccines are already in clinical trials (e.g. Landry *et al*., 2010) but plant-based antiparasite vaccines are still at the early experimental stages of development (Daniell *et al*., 2009).

10.4.3 Recombinant vaccines

Just as parasite genome sequencing has revolutionised our approach to drug design, it is also helping to identify new vaccine candidates (Tarun *et al*., 2008). Recombinant vaccines enable large amounts of specific antigens to be produced without the problems of parasite culture: it is therefore a particularly useful approach for protozoan life cycle stages that can normally only be obtained in very small numbers and for helminth parasites. Furthermore, in sub-unit vaccines the antigens are isolated from the rest of the pathogen and therefore they are potentially much safer than ‘live’ or ‘killed’ vaccines.

Recombinant sub-unit vaccines that provide a high level of protection have been developed against a range of cestode parasites but for various reasons they have not yet entered commercial production (Lightowlers, 2006). For example, there is an effective recombinant antigen vaccine

that prevents the development of the cysticerci of *Taenia ovis* in sheep. After a great deal of research, the vaccine was registered and 20 million doses prepared – but it was never released as commercial product. The reasons for this were a combination of commercial, scientific, and political factors that are admirably covered by Rickard *et al*. (1995). The final vaccine was developed to reduce the prevalence of cysticercosis in older lambs before they were sent to slaughter. However, if the vaccine was to be useful in a parasite eradication campaign, it would need to stimulate an immune response that could be transferred via a ewe's colostrum to her lambs so that these would be protected while their own immune system developed. More scientific work was needed to accomplish this and therefore rather than spend yet more money, the decision was made to put into production a product that was not entirely suitable for a control programme. There was then the question of who should pay for the vaccine, since the cost of the infection was predominantly borne by the slaughterhouse through condemned meat rather than the farmer. On top of this was a complex political situation revolving around who was responsible for what, legislation, and how the use of the vaccine would fit into an existing control programme based on treating dogs – which are the definitive hosts of *Taenia ovis*. It can be argued it is cheaper to target a comparatively small number of dogs rather than have to vaccinate hundreds of sheep (although that depends upon having the means to compel all owners to treat or vaccinate their dogs). Anyway, this is a classic example of how even if an effective vaccine, drug, or control measure can be developed, it may not make commercial sense to put it on the market.

10.4.4 Toxoid vaccines

Toxoid or anti-toxin vaccines are used where the toxins produced a pathogen are the main virulence factor. This toxin production is a feature of a range of bacterial diseases such as anthrax and diphtheria. The vaccine is prepared by isolating the toxin and then inactivating it, for example, using treatment with formaldehyde. Because the chemical mimics the toxin biochemically, but is not actually active, it is called a 'toxoid', for example, the diphtheria and tetanus toxoids in the DPT vaccine. Much of the pathology associated with malaria is a consequence of glycophosphatidyl inositol and some workers consider it to have the characteristics of a toxin. Synthetic glycophosphatidyl inositol has been used as the basis for an anti-toxin vaccine that provides protection against severe malaria in mice challenged with *Plasmodium berghei* (Schofield *et al*., 2002). This vaccine could therefore protect against the most severe consequences of disease but it would not protect against infection and it would not necessarily seriously affect transmission. Parasites do not, on the whole, produce toxins but they do release antigenic excretory/secretory products that have been explored as potential vaccine candidates. These are complex mixtures that often contain cysteine proteases which play an important part both in the nutrition of the parasites and in the pathology they cause. Effective experimental vaccines using cysteine proteases have been designed against protozoa (e.g. *Trypanosoma cruzi* [Duschak and Couto, 2009]), nematodes (e.g. *Haemonchus contortus* [Bakker *et al*., 2004] and *Ostertagia ostertagi* [Geldhof *et al*., 2002]), trematodes (e.g. *Fasciola hepatica* [Jayaraj *et al*., 2010; Wijffels *et al*., 1994]) and cysteine proteases are also considered to be potential vaccine targets for a variety of mite and tick parasites (Nisbet and Huntley, 2006). In addition to cysteine proteases, many other enzymes, proteins and components of the excretory/secretory products have shown promise as vaccine candidates against a range of schistosome and nematode species (e.g. El Ridi and Tallima, 2009; Hewitson *et al*., 2008; Nagaraj *et al*., 2008).

Protective immunity to most parasitic diseases depends to a greater or lesser extent upon the development of a cellular immune response. This is particularly true of those parasites in which there are intracellular life cycle stages. Live attenuated vaccines will stimulate both a cellular and a humoral response, but there are often concerns about their safety and practicality. Killed and subunit vaccines offer greater safety, but they stimulate a predominantly humoral immune response. Consequently, although they provide exceptionally good protection against a variety of bacterial and viral diseases, they have proved less efficacious against protozoan and metazoan parasites. DNA vaccines offer considerable promise against these parasites because they stimulate both a humoral and a cellular immune response (Carvalho *et al*., 2010; Laddy and Weiner, 2006).

10.4.5 DNA vaccines

DNA vaccines evolved rapidly from experiments performed in the late 1980s and early 1990s demonstrating that animals injected with plasmid DNA encoding specific genes developed both an antigen-specific humoral response and major histocompatibility complex (MHC) class I-restricted CD8+ T cell responses (Gurunathan *et al*., 2000). DNA vaccines are prepared by cloning a gene that codes for a specific antigen into a bacterial plasmid or recombinant viral vector that is then injected into the subject. Promotor sequences are also incorporated into the plasmid to boost the production of the antigen. The immune response to DNA vaccination has been investigated almost exclusively using mice but it is not clear that similar responses are generated in other animals. The reason for the uncertainty is because vaccines that work effectively in mice seldom provide similar levels of protection when tested using larger mammals. Furthermore, the manner in which encoded antigen is processed and presented to the immune system is influenced by whether the vaccine is delivered as an intramuscular injection or using a 'gene gun' that injects tiny particles of gold or other heavy metal coated with the plasmid (Smooker *et al*., 2004). Depending upon the mode of vaccine delivery, the plasmid directly transfects skeletal muscle cells (myocytes) or antigen-presenting cells (e.g. dendritic cells) and initiates gene transcription followed by antigen production. The antigens are processed by the host cells in a similar manner to how they would be if they had originated from the parasite, after which they are detected by both major histocompatibility complex (MHC) classes (I and II) and an immune response is generated. Further details are provided by Kutzler and Weiner (2008). In addition, the plasmid DNA can stimulate an immune response directly through recognition by dendritic cells of specific immunostimulatory sequences composed of unmethylated CpG (cytosine-phosphate-guanine) motifs (Higgens *et al*., 2007).

As well as stimulating both a cellular and a humoral immune response, DNA vaccines are cheaper to develop and manufacture than live attenuated and subunit vaccines. They are also relatively stable and can be stored at room temperature. DNA vaccines are also considered to be safe (Le *et al*., 2000), though concerns have been raised over the possibility that they might affect the genes controlling cell growth or cause an autoimmune reaction against host DNA (Glenting and Wessels, 2005). Not surprisingly, the potential of DNA vaccines for the treatment of various disease conditions has been researched intensively. However, it has proved difficult to transfer candidate vaccines from a laboratory situation to the field. This is at least partly a consequence of the candidate DNA vaccines not generating as strong a response in humans and domestic animals as they do in mice. Nevertheless, at the time of writing, progress was being made in the development of DNA vaccines against protozoa (e.g. *Plasmodium* [Shuaibu *et al*., 2010] and *Leishmania* [Doroud *et al*., 2011]), trematodes (e.g. *Schistosoma japonicum* [Da'Dara *et al*., 2008]),

nematodes (e.g. *Haemonchus contortus* [Zhao *et al*., 2011]) and arthropods (e.g. *Boophilus microplus* [Ruiz *et al*., 2007]).

As with drugs, vaccines are formulated with a variety of substances that help to preserve the active ingredient and have immunostimulatory properties. In particular, vaccines usually contain an 'adjuvant' that accelerates, prolongs, or enhances the immune response, but is not itself antigenic. Numerous adjuvants have been described including aluminium salts, squalene, and oil-based substances. DNA vaccines may incorporate conventional adjuvants or specific immunostimulatory sequences may be inserted into the plasmid genome (Higgens *et al*., 2007). Genes coding for cytokines such as interleukin-12 have also been placed into the plasmid genome to enhance a specific immune response (Sin *et al*., 1999).

10.4.6 Vaccine administration

Vaccines are normally given as an injection that may be intravenous, intramuscular, or intradermal. A few vaccines, such as the oral polio vaccine, can be swallowed and there is an increasing focus on the possibility of delivering vaccines as a nasal spray. Injections are seldom popular with humans, especially when they cause painful or systemic flu-like reactions and if a series of injections are required, it can seriously limit compliance. Ideally, therefore, a vaccination should only require a single injection. In addition, although the health risks associated with re-using needles are now well known, the practice still occurs in some countries. Oral vaccines and nasal spray vaccinations are much more 'patient friendly' and are therefore likely to be accepted by the overwhelming majority of the population, which is required if 'herd immunity' is to be obtained. Carcaboso *et al*. (2004) demonstrated that it was possible to induce a prolonged rise in antibody levels in mice using an anti-malarial vaccine containing the synthetic peptide SPf66 delivered as a nasal spray. Similarly, Yu *et al*. (2010) were able to induce immunity in mice to *Cryptosporidium parvum* using a DNA vaccine delivered as a nasal spray.

Needle-free injection devices and drug delivery systems are being developed that can be used for intradermal, subcutaneous and intramuscular delivery using accelerated liquids or powder grains. The actual injection takes as little as 40msec using a high pressure jet and is delivered into a much smaller area of skin than a conventional injection. The injection causes very little damage to the underlying tissues, reduces the risk of needle-borne contamination and is said to be virtually pain-free. Needle-free injections often provide a greater antibody response than conventional injections (e.g. Williams *et al*., 2000) and this has also been found to be the case with anti-malaria vaccines (Arguiar *et al*., 2001). Gene guns are needle-free delivery systems used to deliver DNA or RNA attached to gold nanoparticles. The gold nanoparticles are accelerated to supersonic speed in a stream of helium gas and forced into the subcutaneous skin. In field trials, Moorthy *et al*. (2003) found that an anti-malaria vaccine delivered using a gene gun gave an equivalent response to intramuscular injections.

10.5 Control of parasitic diseases

10.5.1 Eradication, elimination and control of parasitic diseases

There is a world of difference between eradication and control (Molyneaux, 2006). Eradication indicates that the parasite no longer exists anywhere in the world and therefore there is no need to

Table 10.4 Factors contributing to the success of the smallpox eradication campaign

Live attenuated vaccine confers long-lasting protective immunity
Single dose of vaccine required, easily delivered
Natural exposure confers long-lasting protective immunity
Absence of animal reservoir hosts
Fear of disease ensures public acceptance of vaccine
Infected individuals always symptomatic and thus identified
Effective co-ordinated vaccine delivery and education campaign
Government cooperation
Funding

maintain treatment regimes or control programmes. By contrast, if a parasite is merely controlled, then it continues to exist, though its incidence and prevalence, morbidity and mortality in particular areas will be reduced as a consequence of the ongoing control measures. In between these two extremes is localised elimination of the parasite from a specific geographical location although this condition can usually only be sustained by control programmes to prevent the parasite from reinvading from surrounding regions.

Although there is good evidence that small parasite burdens are useful for the maintenance of a well-regulated immune system (Chapter 8), parasites are usually viewed with such loathing that most people would favour their complete eradication. This, however, is seldom a realistic goal owing to a variety of biological, sociological, economic, and geographical reasons. Indeed, very few pathogens have been intentionally eradicated from the world and these are all special cases. For example, the smallpox virus no longer exists in the wild although two stocks continue to be held in laboratories in the USA and Russia. If these stocks are destroyed (which currently seems unlikely), then the virus can finally be declared extinct. Many factors have contributed to the success of the smallpox eradication campaign, but fundamental to this was the availability of a vaccine that provided a high level of protective immunity (Table 10.4). Since the eradication of smallpox in1980, the rinderpest virus was eradicated in 2011 and there are hopes that the polio virus will be eradicated in the next few years.

There are currently no examples of successful parasite eradication campaigns, though it is possible that the Guinea worm *Dracunculus medinensis* may be eradicated in the near future. Islands and geographically isolated regions (e.g. cut off by mountain ranges) have a good chance of achieving success in local elimination campaigns provided they are suitably policed and the parasite is not able to re-enter from the nearest endemic region. For example, the warble flies *Hypoderma bovis* and *Hypoderma lineatum* have been virtually eradicated from the UK as a consequence of the availability of effective drugs and the stringent imposition of regulations on the treatment and movement of cattle. Although the warble flies have wild hosts and also infect horses (that are not covered in the legislation), their numbers are not sufficient to maintain more than a very small localised reservoir population. In developed countries many human parasites have been eradicated as a consequence of the rise in living standards, effective waste disposal, and improved hygiene. Similarly, in some developed countries, improved meat hygiene inspection and concern over zoonotic diseases have led to the virtual elimination of helminths such as *Echinococcus granulosus* and *Taenia saginata*. Nevertheless, these parasites remain common in many other countries and would undoubtedly return to those in which they are currently eliminated should the opportunity arise. This is one of the reasons why many countries have stringent regulations concerning the

movement of live animals within and across their borders and also for the import and sale of meat and meat products.

Even local elimination of parasitic diseases is seldom feasible and therefore the authorities are usually considering the best means of controlling them. This means that the parasite will persist and the community, farmer or individual must consider both the cost of the control measures and the level of infection that is considered acceptable. For crop pests it is relatively easy to calculate the economic costs of control by determining the 'gain threshold': this is derived by dividing the management costs by the market value of the crop. Therefore, if the management costs for the application of a pesticide are €100 hectare^{-1} and the market value of the crop is €20 tonne^{-1}, the gain threshold would be 5 tonnes hectare^{-1}. That is, the farmer would need to save at least 5 tonnes hectare^{-1} for it to be worth his while applying the pesticide. Although a similar procedure could be applied to domestic animals (e.g. by reference to live weight gain, milk production, etc.), the farmer would also have to consider the welfare of his animals. For example, although treatment may not make economic sense, by not treating his animals he may cause them suffering that he would consider unacceptable (although farming is a business, most farmers like their animals). In addition, it might lead him to being prosecuted under animal welfare legislation. Also there may be local legislation compelling farmers to treat their animals should they become infected with a specific disease (e.g. sheep scab *Psoroptes ovis*). Furthermore, the farmer must bear in mind that parasitic diseases are generally chronic infections and that an infected animal is a potential source of infection for other animals both in the present and the future (the transmission stages of some parasites can persist for months or even years). The determination of an 'acceptable level of infection' for domestic livestock is therefore not always a simple procedure. For human parasitic diseases any attempt at calculating an acceptable level of infection is fraught with ethical issues. However, even in the developed nations there is never sufficient money to treat all those who require medical attention and some means of prioritising needs has to be developed, such as the DALY calculations referred to in Chapter 1.

10.5.2 Education

Parasitic infections tend to have their most severe impact upon those who are poorly educated (e.g. Quihui *et al*., 2006) but the risk of infection can often be reduced by simple changes in behaviour. It therefore follows that education is usually a key feature of parasite control programmes. For example, if people understand that the large cysts that grow inside them result from eating and drinking substances that are contaminated with dog faeces, then, theoretically, it should be relatively easy to reduce the incidence of hydatid disease among a population by making them reassess their relationship with dogs. Educational material is usually disseminated via leaflets, posters, radio and television programmes. However, as the world becomes increasingly commercialised, the worthy but often dull and simplistic health information can battle to gain the intended recipient's attention. The production of written health information is (or should be) a skilled job since the target audience is seldom highly literate. The information has to be presented clearly and concisely and in a language that the target audience can understand. For example, in India, and parts of Africa and Asia, there are numerous local languages and it cannot be assumed that everyone will be conversant with the country's main official language. Avoiding scientific terminology and employing illustrations and photographs can help. In addition, a large number of people in both developed and developing countries are illiterate and therefore cannot understand even simple written guidance. For these people, radio and television can be important sources of information.

China has achieved good success with so-called 'barefoot doctors', who dispense simple treatment and verbal guidance to isolated communities. Health messages are often delivered via teachers in schools since the young are usually more impressionable and are likely to pass on their knowledge to their parents or guardians. Health clinics usually provide an opportunity to talk to the women in the community who may otherwise be very difficult to reach (especially in patriarchal societies), but who can often have a strong influence on the behaviour of all members of the family.

Unfortunately, although education is important, just because someone has information, does not mean that they are willing or able to act on it. If life were this simple, there would not be such immense problems with alcoholism, drug abuse and unwanted pregnancies. For example, insecticide-treated bed-nets are effective at preventing malaria, provided that they used correctly (Hill *et al*., 2006). As a consequence, aid agencies in parts of Africa distribute free bed-nets and instruct people on how to use them. However, despite this, the level of infant mortality from malaria sometimes remains high. This is partly because the nets are not always appropriate for the way people actually live. For a start, the bed-nets that are distributed often have to be hung from the ceiling and unless there is sufficient space they need to be erected and disassembled every day. This can quickly become a chore and can be the responsibility of a single person, and if they are not present, the bed-nets are not used. In many countries, people like to sleep outside at night because it is too hot inside, and there is therefore often nowhere to hang the bed-net. Even if the people are sleeping indoors, the bed-net traps heat, which can then make it difficult to sleep. If the only source of light in the room is a naked flame, it would be considered too risky to use a bed-net in close proximity. Some people also consider bed-nets to be a potential danger to young unsupervised children and think that they could become trapped within or strangle themselves trying to get in and out. In many African cultures, the young children sleep with their mother until they are weaned and two bodies under a single bed-net can make it uncomfortably hot (Galvin *et al*., 2011). In short, people might be well aware of the benefits of a particular behaviour (in this case, sleeping under a bed-net), but for a whole variety of reasons or excuses they choose to ignore it. Similarly, although people might know that freezing or thorough cooking can kill the infective stages of most parasites, they may be unable to do either of these things. Poor people seldom have access to freezers, and firewood or other fuel may be expensive or unavailable. In addition, local practices handed down over generations are a major determinant of how food is prepared and whether it is cooked at all.

Nevertheless, in some countries, education has proved enormously successful in the control of certain parasitic diseases. For example, during the 1800s in Iceland, hydatid disease was effectively controlled for many years through the provision of educational pamphlets. It probably helped that although the population was literate the only competing reading material in Icelandic at the time was the Bible and some Viking sagas. Subsequently, legislation and more effective treatments became available and by the 1960s the disease had been eliminated from the country (Craig and Larrieu, 2006). Similarly, a large part of the success of the Guinea worm eradication programme is a consequence of the effectiveness of the education programme that encourages people living in regions where *Dracunculus medinensis* is endemic to only drink water that is passed through a filter to remove infected copepods.

10.5.3 Environmental modification and cultural control

Until the development of effective drugs and pesticides, environmental modification and cultural control were the main means of combating pests, parasites, and diseases. It was seldom understood

why certain practices were effective but experience taught us to do or not do certain things at certain times of the year. For example, for many years people thought that malaria was caused by breathing in the air that surrounds marshy land. People therefore avoided living close to wetlands or they drained the land. This also improved the health of domestic animals by reducing their risk of exposure to *Fasciola hepatica* as well as reducing the survival of eggs and infectious stages of many parasitic nematodes. However, it is a difficult and expensive business to drain land and we now realise that it can have harmful ecological consequences.

Box 10.4 Organic livestock farming and parasitic diseases

There is an increasing market for organic produce in many parts of the world and this fetches a premium in the marketplace. However, officially certificated organic farming poses a problem for livestock farmers because they are unable to employ many of the drugs that are used to control parasitic infections. Consequently, organically-farmed animals often contain higher parasite burdens than animals that are farmed conventionally. Not only can parasites reduce productivity to the point at which the losses make farming uneconomic but they also raise welfare issues.

Organic farmers can utilise certain approved natural products to control parasites. For example, diatomaceous earths can be used as supplements to treat *Eimeria* and gut nematodes in hens or as a dust to remove ectoparasitic mites (Bennett *et al*., 2011). Diatomaceous earths are naturally occurring soils that are comprised largely of the fossilised remains of diatoms. They have a wide variety of applications from a component of cat litter to DNA purification. They have also been trialled for use against helminth infections in ruminants but more research is required to confirm their effectiveness (McLean *et al*., 2005). Many organic farmers provide their animals with nutritional supplements to offset the losses they suffer from larger worm burdens compared to conventionally-reared stock.

Natural products are seldom as effective as pharmaceutical drugs at controlling parasites, so organic farmers have to make greater use of cultural control techniques. Lower animal stocking rates can reduce the risk of transfer of infections and contamination of the land with parasitic protozoan cysts or helminth eggs. Nevertheless, over time the number of infective stages on a piece of land will steadily increase. Farmers with plenty of land may be able to rest pasture for prolonged periods to allow time for the transmission stages to starve to death – how long would depend upon the potential pathogen species and environmental conditions – but this is seldom feasible. The number of transmission stages can also be reduced by alternating grazing with other livestock species and/or combining grazing with using the land for a hay crop (Waller, 2006). The influence of plant species composition on parasite transmission remains poorly understood. Work in New Zealand has indicated that grass species composition has a major impact on parasite burdens and productivity in lambs. Furthermore, forage crops containing high levels of condensed tannins (e.g. *Hedysarum coronarium*) could have natural anthelminthic properties (Niezen *et al*., 1996). The difficulty of determining the protective effect of forage composition is due to the composition and concentration of plant secondary compounds being affected by a host of genetic and environmental variables. Some grasses, such as molasses grass *Melinis multiflora*, have a deterrent effect on ticks and reduce their survival (Thompson *et al*., 1978) but they have not been utilised to any great extent. A more robust anti-tick response is generated by the legume *Stylosanthes* that captures and kills ticks on sticky secretions (Stutherst *et al*., 1982).

Parasites are susceptible to pathogens just like other organisms and biological control could be exploited in organic farming. However, while fungi will kill helminth eggs and some fungi do specialise in capturing and killing nematodes (Waller and Larsen, 1993), it has proved difficult to utilise them as part of a routine control programme. Currently, most interest is focused upon the nematophagous fungus *Duddingtonia flagrans*. The spores of this fungus can survive passage through the gut of a ruminant and will then germinate and grow rapidly once passed with the faeces. Consequently, the spores can be incorporated into feed blocks or controlled release devices and the fungi will kill parasitic nematode larvae that are passed with the faeces or subsequently hatch from eggs while in the faecal pat. The fungi are not specific predators of nematode parasites and will also attack free-living nematodes but there are no reports of it having an adverse effect on the environment (Waller, 2006).

In countries with seasonal climates, the risk of infection is often most pronounced at certain times of year and it is sometimes possible to predict when or whether a parasite will be a particular problem and take appropriate action. For example, the fluke-forecasting scheme can provide warning of when it is appropriate to move sheep to safer pastures. This, of course, assumes that a farmer has alternative less risky pasture on which to move his animals, and poor people seldom have the opportunity to move to a less disease-prone environment.

Relatively simple environmental modifications can reduce the risk of exposure to many parasitic diseases. For example, increasing the water flow rate in irrigation channels and removing plant growth can make them unsuitable for the snails that act as the intermediate hosts of schistosomes. Similarly, concrete farmyards and animal pens are easier to keep clean of faeces and solid hut or house walls and ceilings do not provide hiding places for the hemipteran bug vectors of *Trypanosoma cruzi*. Although simple environmental modifications are usually relatively cheap, they may still be too expensive for the poorest people who would benefit most. Their implementation therefore often requires funding by government or aid organisations and they usually require constant upkeep to remain effective.

The safe disposal of human and animal faeces is central to the control of many parasitic diseases. However, human and animal waste is used as a fertiliser for the growth of crops and in aquaculture in many parts of the world and this can inadvertently contribute to the spread of pathogens. This is because alternative industrial nitrogen fertiliser is either not available or too expensive. Furthermore, if waste were not used as a fertiliser, it would have to be disposed of in some other manner that would undoubtedly be expensive or pose environmental problems. The infective stages of many parasites are protected by thick cyst walls (e.g. *Entamoeba histolytica*) or egg shells (e.g. *Ascaris lumbricoides*) and can survive for weeks or even years under ideal conditions (WHO, 2006b). However, if the faeces are composted in an appropriate manner for a period of time before it is applied to crops, then all the parasite infective stages will be killed. There are two basic composting processes: aerobic and anaerobic. Aerobic composting is where microorganisms that utilise oxygen break down the organic matter and respire carbon dioxide. The oxidation of organic compounds releases a lot of heat but as long as the temperature remains below 45°C, the process of decomposition is slow. The arrival of thermophilic species enables the temperature to rise to above 60°C and the speed of decomposition increases dramatically. Anaerobic decomposition takes place in the absence of oxygen and the breakdown products are mostly ammonia, hydrogen sulphide, organic acids and methane although some carbon dioxide is formed.

Anaerobic decomposition does not yield much heat and is slower than aerobic decomposition – it also yields a lot more unpleasant smells. Although anaerobic decomposition will kill parasite transmission stages eventually, the low temperature means that it is less effective than aerobic decomposition. Consequently, aerobic composting is generally recommended for the composting of farm animal waste and in villages that lack mains waste disposal facilities. Where anaerobic composting is undertaken, it is often preceded by a period of aerobic composting to destroy the pathogens. There are many ways of undertaking aerobic composting but when it is done effectively the inner temperature can rise to over 60°C while the outer temperature can reach 50°C. Exposure to temperatures above 45°C for 5 or so days can be sufficient to inactivate even parasite transmission stages as robust as *Ascaris* eggs (Koné *et al*., 2007).

10.5.4 Remote Sensing (RS) and GIS technology

Remote Sensing (RS) satellite data and Geographic Information Systems (GIS) technology are proving increasingly useful for monitoring the epidemiology of parasites and forecasting the risk of disease outbreaks. Remote sensing is a means of monitoring the environment without actually making physical contact with it. This is most commonly achieved through satellite technology using a combination of passive and active monitoring devices. Passive detectors monitor different wavelengths that are emitted or reflected from the land beneath. Active detectors emit particular wavelengths and measure the time taken for them to return. Remote sensing can be used to monitor temperature, ground cover, forestation, and many other environmental variables on a regular basis in regions in which it would be impossible to gain ground-based measurements owing to the cost and/or risks involved. A variety of RS satellite datasets are available including LANDSAT, MODIS, NDVI, and SRTM DEM. Geographic Information Systems are means of capturing, storing, updating, retrieving, analysing, and displaying any form of geographically-referenced digital information. It is not a single entity but a collection of computer hardware, software, and geographical data: a GIS that is suitable for one situation is not necessarily suitable for another application.

GIS is particularly useful for parasite surveillance and simulating the consequences of particular intervention strategies or changes in the environment (e.g. global warming). Through GIS it is possible to map simultaneously one or more of the following on either a regional, national, or global scale: the occurrence of the parasite, the disease it causes, its host, vector/intermediate host, co-infections, and environmental variables (Brooker and Clements, 2009). For example, disease maps are a quick and simple means of visualising spatial and temporal 'hot spots' of where disease is clustering, the linkages between parasite distribution and environmental variables and the effectiveness of control measures. Similarly, remote sensing can identify those environmental variables that promote the breeding of vectors and intermediate hosts and therefore where problems are likely to arise.

Although GIS has enormous potential, it has remained a specialist topic rather than a day-to-day tool for workers in human and veterinary science. This is because many of the GIS tools such as ArcGIS are complicated and take months of dedicated practice to master. In addition, the hardware and software can be expensive and do not always integrate well with modelling software that uses spatial statistics. For example, Agent-Based Modelling is popular as a means of simulating pathogen transmission but it has proved difficult to use it in conjunction with GIS (Kennedy *et al*., 2009). Nevertheless, GIS does not have to be expensive. Freeware GIS software

such as DIVAGIS can be downloaded from the internet and is suitable for mapping the vectors and intermediate hosts of parasitic diseases. For example, Cuervo *et al*. (2010) used DIVAGIS software to identify areas suitable for colonisation by the snail intermediate hosts of *Fasciola hepatica*. Furthermore, spatial datasets of parasites, intermediate hosts and vectors are available for download through biodiversity databases such as GBIF (http://www.gbif.org/). However, it worth noting that for those working with human diseases, there are confidentiality concerns, since location and patient data are often linked together and consequently, it would possible to reverse engineer maps to identify the home address of individual patients (Brownstein *et al*., 2006).

10.5.5 Treating the individual or the population

The traditional basis of disease control programmes has always been to reduce the prevalence of infection – ideally to zero. However, for many parasitic diseases, and in particular human gastrointestinal helminth infections in developing countries, this is now considered to be an expensive and unrealistic goal. This is because there is little prospect of breaking the transmission cycle of parasites such as *Ascaris lumbricoides* until there is adequate sanitation. In the absence of sanitation, people who are cured of their infection are usually reinfected within a short period of time. Therefore, the prevalence of infection among a community remains the same or soon returns to its original level after a brief decline.

Low burdens of gastrointestinal helminths seldom cause serious pathology unless the patient is malnourished or suffering from another health problem. Consequently, the new philosophy is to aim to reduce the worm burden within individuals rather than the number of people infected with worms. In particular, it is aimed at reducing the number of people harbouring high worm burdens. In humans, as in other animals, the majority of parasites are usually found within a minority of the population. These individuals suffer the most harmful effects from parasitism and are also a source of infection for the rest of the population. It may be too costly to identify these highly susceptible individuals, but if they are treated on a regular basis along with everyone else, then it can prevent them from developing a serious infection. The fact that many people remain infected with worms can be used as an argument for halting control programmes on the basis that it is a waste of time and money. However, reducing the worm burden to sub-clinical levels can result in marked increases in health and productivity.

Treating the whole population works well for gastrointestinal parasites of humans and domestic animals for which there are cheap safe drugs. Low parasite burdens are seldom pathogenic. Nevertheless, as is the case with any mass medication scheme, there is always going to be a small minority of people who react badly to a drug or vaccine. And the more people who are involved means that there will be more reports of harmful reactions and this can result in bad publicity, compromise the control programme and lead to claims for compensation. Where the only available drugs are expensive or have serious side effects, it is important that those who are infected are identified and treated on an individual basis.

In other situations, identifying exactly which individuals have the disease and targeting the treatment appropriately is not only important for the individual's welfare, but can be the key to effective control. Recent developments in laboratory tests have proved helpful in this regard. For example in areas where malaria is endemic, it still relatively common to treat everyone with malaria-like symptoms with anti-malarial drugs. Even when laboratory tests are available, there can be a perception among the doctors and nurses treating patients that a 'negative' blood slide

result might not be correct and so the person should be treated anyway. Although this 'presumptive treatment' should of course mean that those who are infected with *Plasmodium* spp. are treated, thus relieving their symptoms and reducing the likelihood of transmission to others, it does have some risks. One is that the patient may not have malaria at all, but another serious infection and without the appropriate treatment, their condition could become worse or even fatal. Another is that the indiscriminate use of anti-malarials, particularly chloroquine, has already encouraged the development of drug-resistant strains of the parasite in many parts of the world and these are therefore harder to control. Drakeley and Reyburn (2009) argue that the immunochromatogenic rapid diagnostic tests (RDTs) for malaria are making it possible to abandon or at least reconsider the strategy of treating everyone with anti-malarials, particularly in areas where transmission is at a low level. The tests have been shown in evaluation studies to be reliable and sensitive and provide a quick result. As a consequence of education and experience, healthcare workers increasingly perceive the results from RDTs to be as trustworthy as those from blood slides. In regions where malaria transmission is at low levels, this targeted approach means that the anti-malarials would be available only for the patients who actually need them. This is a better use of limited resources and should enhance the control efforts.

10.5.6 Piggy-backing control programmes

Although centrally administered control programmes or those delivered by aid organisations can provide excellent results, they are seldom sustainable. A change in government priorities or the withdrawal of aid can result in a control programme collapsing and the prevalence of the target parasite (or other disease) increases. The return of the disease can be severe since the local population may have lost some of its immunity during the period of reduced parasite exposure. The WHO is therefore advocating a new approach to disease control in which the control measures are 'piggy-backed' onto existing networks. This approach is primarily being used for the control of gastrointestinal parasites in children but could be adapted for other parasites and infectious diseases. By using existing networks there is a reduced need for trained personnel and many of the people involved are already employed doing other jobs. Consequently, the long-term delivery of the control programme is inherently more stable and less susceptible to the vagaries of external funding. It is also less expensive and reduces the burden on the existing local health system that is usually lacking in staff and resources. Young children are particularly vulnerable to the harmful effects of gastrointestinal parasites and schools are ideal venues for contacting them. Many children within a given locality will attend school and the school itself is a 'non-threatening' focal point of the community: the same might not be the case with other 'official buildings'. In poor communities, some children do not go to school or attend only sporadically but they are almost certainly going to be familiar with the school and can be encouraged to attend for a 'special free event'. In addition, in poor communities teachers are often respected members of society whose views and opinions are valued. Therefore, if the teachers explain why it is important for all children to receive a pill, it is more likely that the community will participate than if an outside organisation suddenly turned up 'out of the blue'. Because they are educated, teachers can be quickly trained to organise 'de-worming days' during which they administer tablets of albendazole or some other safe anthelmintic for which there is no need for complicated treatment regimes, injections, or worries about potentially serious reactions. Although it could be argued that the good will of the teachers is being exploited, there are clear advantages for them since children who are healthy are

more likely to attend regularly and perform better in their lessons than those who are chronically ill. In addition, high-risk children would benefit the most from this approach to parasite control – that is, the children from the poorest families and girls (who are often discriminated against in patriarchal societies). 'De-worming days' can be organised on a regular basis to ensure that those who are most vulnerable continue to receive adequate protection. The piggy-backing approach can, however, also be used in conjunction with 'one-off' mass health campaigns such as vitamin A supplementation programmes, feeding programmes, and water and sanitation initiatives, during which there is a unique opportunity to reach thousands of people.

10.5.7 Disruptions to control programmes

However well planned and supported a control programme is, it can be disrupted by unforeseen circumstances such as famine, flood or war. These events often lead to mass migration of people from one geographical area to another and in some cases involves them crossing national borders. Hastily erected refugee camps are usually a good source of faeco-orally transmitted disease, but also people carry their infections with them. So when migrants move from an area where a particular parasite is endemic to one where it is not, it can create public health problems for the indigenous population. For example, in regions where malaria is endemic, people infected with *Plasmodium* could still be active enough to travel. This might lead to the reintroduction of malaria into an area which had finally eliminated the disease, or refugees could carry drug-resistant strains or new species to areas where these had not previously been found. Provided that a suitable vector mosquito is available in the new area, then transmission can resume. This occurred in Tajikistan during the civil war in the 1990s. As a result of the fighting, some people sought refuge in neighbouring Afghanistan, where a chloroquine-resistant strain of *Plasmodium falciparum* was circulating. Once Tajikistan became a safer place to live, people started returning home and some brought malaria with them. Although *Plasmodium vivax* infection was quite common, *Plasmodium falciparum* had not been reported in Tajikistan for many years; however, a species of *Anopheles* which was capable of acting as a vector was present and so transmission was observed (Pitt *et al*., 1998). This case also highlights another important point in effective control. Efforts to identify this problem were initially hampered by lack of accurate diagnostic facilities, since laboratory staff had only been trained to detect *Plasmodium vivax* in blood slides and were reporting *Plasmodium falciparum* infections as 'negative'.

10.5.8 Role of governments, foundations, and aid organisations

The control of many parasitic diseases depends upon a co-ordinated national or even international response and this can only be achieved through the active cooperation of governments. This requires stability within the country and a suitable infrastructure for distribution of funds, communication and travel for expert personnel. A control programme can be organised through national government, local government, or some combination of the two depending upon the way the country operates. Governments accumulate revenue from taxes and determine funding priorities. They therefore determine the extent to which human and animal health services are funded and allocate the priority given to particular diseases. Even within the richest countries there is never enough money to meet all the health needs of the population and decisions as to which diseases

receive the most funding can be heavily influenced by political considerations. Only governments have the power to pass and enforce legislation. They can also ensure that different authorities and departments work together to mount a strategic response. Control programmes work best where there is a strong, stable government that is honest and appreciates the importance of the control programme. Governments also give permission for aid organisations or other charitable bodies to undertake work in their country or region: no matter how serious a disease problem or natural disaster might be, aid organisations cannot undertake operations without the agreement of a country's national or regional government. However, all governments tend to think of their own survival first: fighting a rival for power can be far more important to a government than fighting a disease.

Aid organisations and health authorities must work in the real world and therefore politics, advertising, and media relations play a large part in determining whether a control programme can be undertaken, how it is undertaken, and its ultimate success. Unless they are carefully managed at both a national and local level, mass vaccination and other health campaigns can be perceived as vehicles for outside agencies, which are almost invariably seen as having an ulterior and nefarious purpose. For example, the polio eradication campaign ran into problems in Nigeria because a rumour spread that the vaccine contained substances that made Muslims infertile. Similarly, a rumour spread in Pakistan that the CIA was mounting a fake vaccination campaign in their attempts to find Osama bin Laden (head of the terrorist organisation Al-Qaeda). The rumour was that the CIA knew that bin Laden had young children travelling with him and if they were taken to be vaccinated, his locality might be identified (Anon, 2011b). Such rumours are not confined to developing countries and similar health scares accompany most mass vaccination campaigns. Consequently, the discovery of an effective drug or vaccine to treat a parasitic disease is just one small part of a control programme.

Legislation can be a highly effective tool in the control of parasitic diseases. For example, legislation can force local authorities to provide safe water and sanitation, make it an offence to import pets or domestic animals without a certificate indicating that they are free of disease, and require farmers to dispose of dead stock appropriately. However, legislation serves little purpose if it is not enforced or where the penalties are so low that people are not put off committing the offence (see Colour Plate 32). Similarly, the target of the legislation must have a realistic chance of obeying the rules. For example, the owners of livestock will continue to slaughter and butcher animals at home if there is no official slaughterhouse within easy reach or it charges too much for its services. Effective legislation needs to be backed up by education because laws that the public does not understand or deem to be irrelevant or an unjust imposition are seldom enforceable except in a police state.

Governments in developed countries are a major source of aid and they also influence how the money is spent within the recipient country. This can cause tensions and conflicts of interest since donor governments often favour the purchase of products and services from their own country. It can be argued that the recipient of aid has a better appreciation of the best ways in which the donated money could be spent but this must be balanced by the disappearance of huge amounts of aid money through corruption and government mismanagement.

In addition to governments, there are a wide variety of charities and aid organisations that fund or deliver parasite control programmes. The Bill and Melinda Gates Foundation has put enormous sums of money into the treatment and control of malaria while several public–private partnerships have been established to fund the sequencing of parasite genomes and the development of antiparasitic drugs and vaccines (e.g. Drugs for Neglected Diseases Initiative, Institute for One World

Health, Medicines for Malaria Venture) (Nwaka and Ridley 2003; Nishtar, 2004). Large donors will inevitably influence policy decisions. Although this should not necessarily be considered a bad thing, it can be a source of concern for those who hold different views. For example, in 2009, following pressure from the Bill and Melinda Gates Foundation, the PATH Malaria Vaccine Initiative (MVI) changed the way in which it identified and funded candidate malaria vaccines. In particular, a much greater emphasis was placed upon identifying transmission-blocking vaccines because these are seen as having greater potential in contributing to the long-term eradication of the disease.

Regardless of the source of funding for a control programme, its delivery is often influenced by the prevailing management ethos. Many industrialised nations have a short-term target-based culture but this does not always transfer well to disease control in the developing world. For example, when considering how best to control malaria, the distribution of insecticide-impregnated bed-nets is easily measured and can therefore become an end in itself. As a result, little consideration is given to whether the type of bed-nets is appropriate for the lifestyle of the recipients and whether bed-nets are being used effectively. In addition, the distribution of free bed-nets does not contribute to sustainable malaria control since it can put the local manufacturers out of business. Consequently, after a couple of years when the donated nets need to be repaired or replaced, there is no one able to do the job (Moyo, 2009). In addition, some scientists are worried that the exposure of mosquitoes to sub-lethal levels of insecticides within the bed-nets may encourage the development of insecticide resistance (Butler, 2011).

Questions

1. State four properties of an ideal antiparasitic drug. Briefly discuss the reasons for each of these choices.
2. Using named examples, state four ways in which an understanding of parasite life cycles can inform control.
3. Why is it difficult to obtain reproducible results on the effectiveness of natural plant-based remedies for the treatment of parasitic diseases?
4. Briefly discuss why it has proved difficult to develop commercial vaccines against human parasitic infections.
5. Why is RNAi therapy considered to offer so much potential for the treatment of parasitic diseases?
6. With the aid of named examples, explain the difference between eradication, elimination and control of parasitic diseases.
7. Give two examples of how nanoparticles could prove useful in the treatment of parasitic diseases.
8. Briefly explain why human and animal faeces should be composted before being used in agriculture. Include in your answer an explanation of whether aerobic or anaerobic composting is the better option.
9. With the aid of an example, briefly discuss what is meant by ‘piggy-backing’ parasite control measures.
10. What are the advantages and limitations of legislation as a means of enforcing the control of parasitic diseases?

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Parasitology: An Integrated Approach, First Edition. Alan Gunn and Sarah J. Pitt.

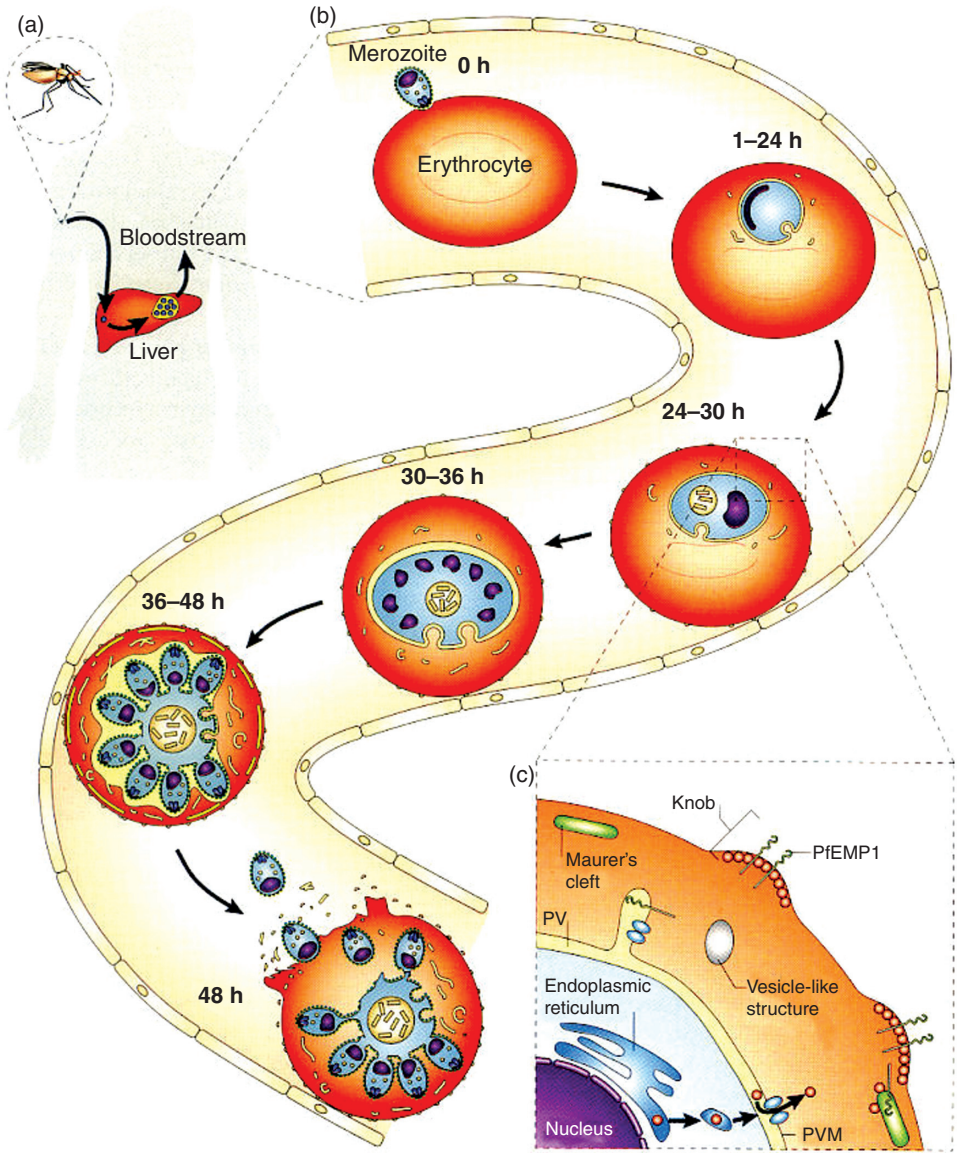


Plate 1 Schizogony in *Plasmodium falciparum*. (a) = Mosquito injects sporozoites into the bloodstream and these then travel to the liver. The sporozoites invade the hepatocytes and undergo exoerythrocytic schizogony during which they produce merozoites. (b) = Merozoites leave the liver cells, enter the circulation and infect red blood cells. Within the red blood cells the merozoites undergo erythrocytic schizogony over a period of 48 hours. During the first 24 hours the ring stage starts to grow; during 24–36 hours the parasite enters the trophozoite stage in which it grows and DNA replication takes place; between 36 and 48 hours the schizont is formed that culminates in the formation of merozoites and the death of the red blood cell. (c) = The parasites export proteins through the parasitophorous membrane (PVM) that re-model the cell membrane of infected red blood cells so that 'knobs' are formed. Exported proteins include *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1). Source: Goldberg and Cowman, 2010

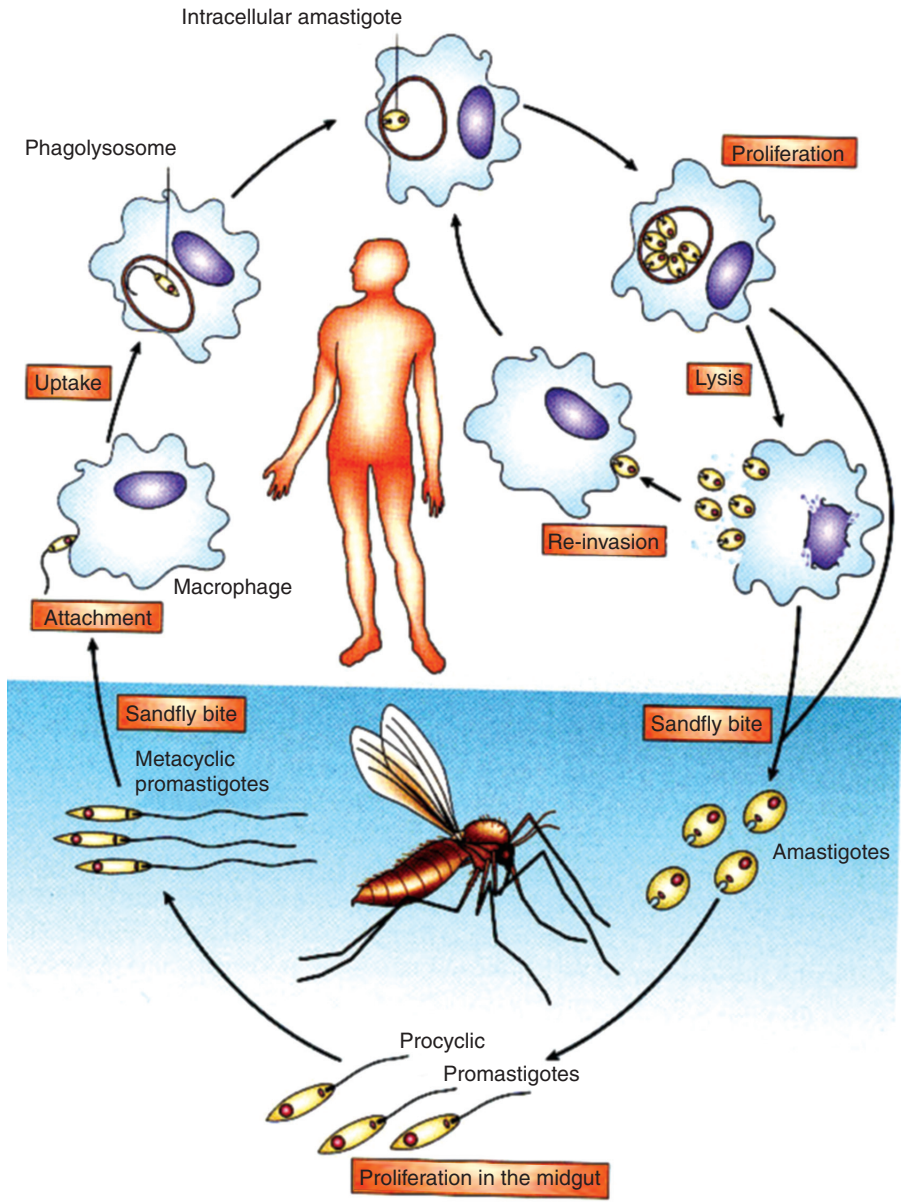


Plate 2 Diagrammatic representation of the life cycle of *Leishmania donovani*. Source: Chappuis *et al*., 2007. Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? *Nature Reviews Microbiology* **5**, 873–882.

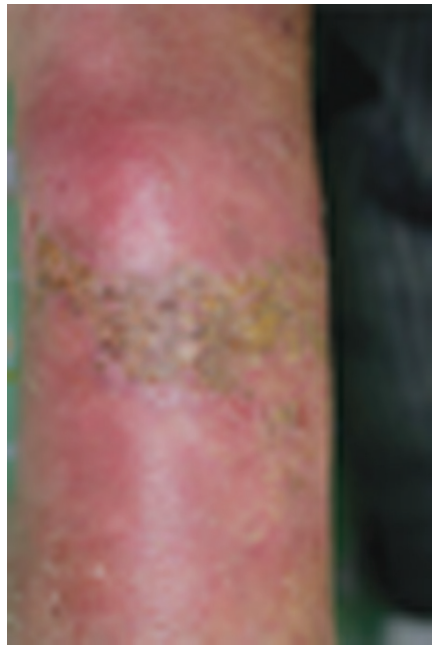


Plate 3 Forearm of patient suffering from a cutaneous infection of *Prototheca cutis*. Note the cellulitis-like inflammation and ulcer. Source: Satoh *et al*., 2010. (Prototheca cutis sp. Nov., a newly discovered pathogen of protothecosis isolated from inflamed human skin. International Journal of Systematic and Evolutionary Microbiology 60, 1236–1240. Reproduced by permission of Society for General Microbiology)

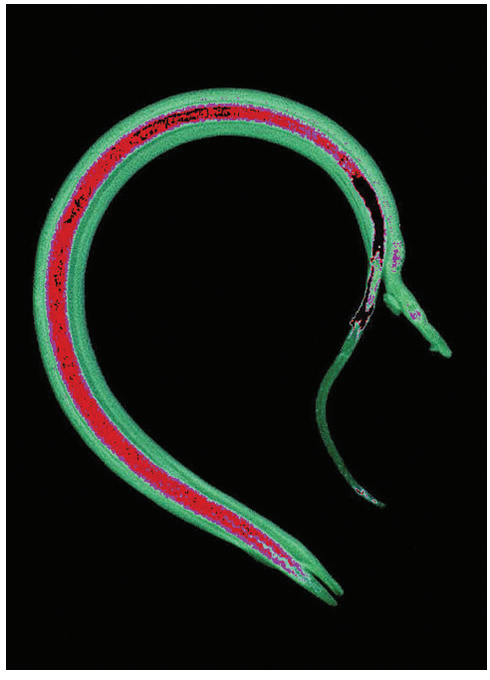


Plate 4 Adult schistosomes. The long slender female is carried in the gynaecophoric canal of the shorter, more stumpy-shaped male

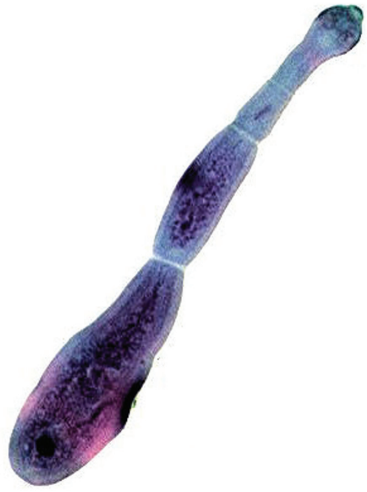


Plate 5 *Echinococcus granulosus*: Adult worm. Adults of this species are small (this specimen is 5 mm in length) and usually consist of 3–4 segments (proglottids). Note the presence of a rostellum at the tip of the scolex. The rostellum is armed with two rows of hooks.

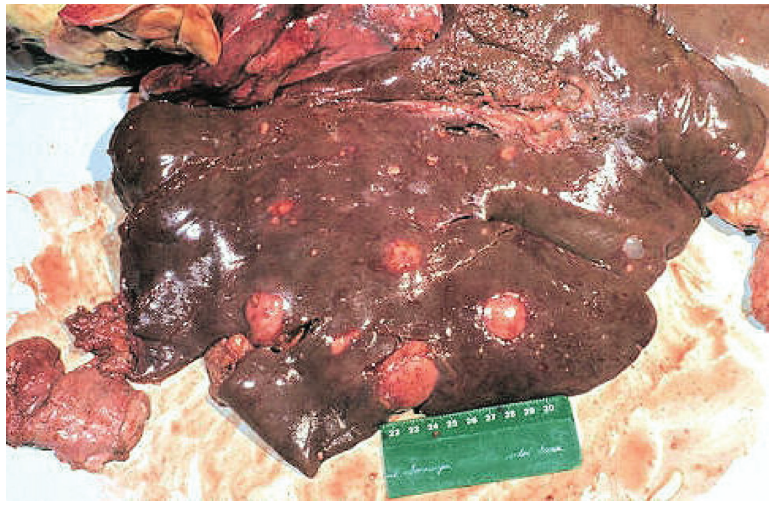


Plate 6 *Echinococcus granulosus*: hydatid cysts in the liver of a donkey

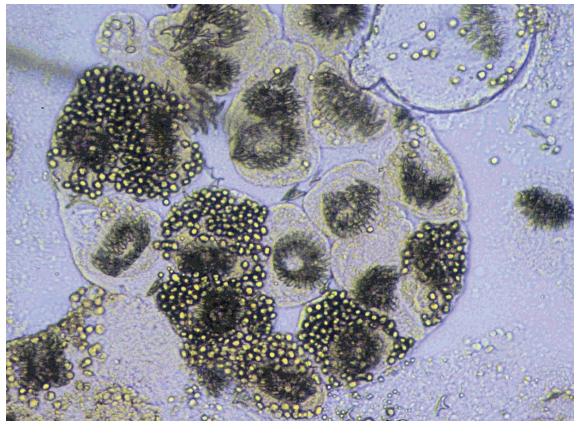


Plate 7 Hydatid ‘sand’ from inside a hydatid cyst of *Echinococcus granulosus* showing developing protoscolices

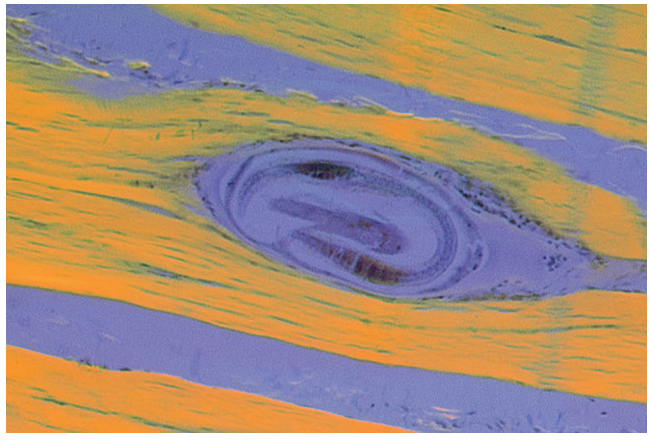


Plate 8 Encysted larva of *Trichinella spiralis*

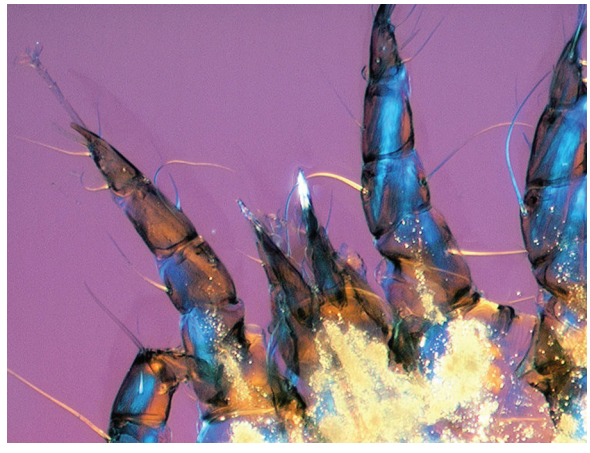


Plate 9 Adult sheep scab mite *Psoroptes ovis* showing the mouthparts.

Plate 10 Engorged hard tick (family Ixodidae). Note how the mouthparts can be seen from above.

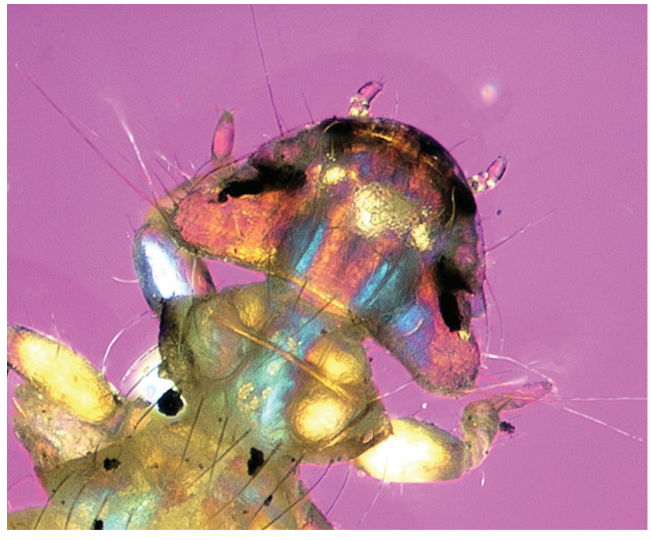


Plate 11 Mallophaga, biting louse. Note how the triangular-shaped head is wider than the thorax and the unmodified tarsi ('feet')

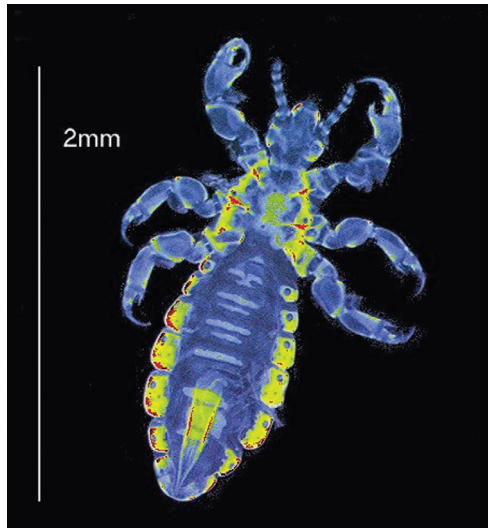


Plate 12 Anoplura, sucking louse, *Pediculus humanus capitatis*. Note how the head is narrower than the thorax. The tibia and tarsi have evolved to become claw-like and adapted for clinging onto hairs. This specimen is a male as evidenced by the sword-like aedeagus at the posterior of the abdomen.

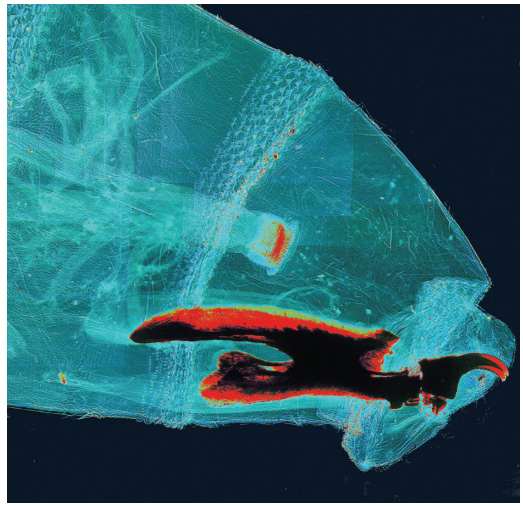


Plate 13 Cephalopharyngeal skeleton of the third instar larva of the blowfly *Calliphora vomitoria*. Note the curved mouth-hooks

Plate 14 Sheep blowfly strike caused by *Wohlfahrtia magnifica*. The sheep is a fat-tailed Awassi breed and the larvae have almost completely destroyed the tail. Note how deeply the larvae have burrowed into the flesh

Plate 15 Third instar larva *Oestrus ovis* within the turbinate bones of a sheep. Note how the shape of the larva ensures it is tightly wedged within the cavities

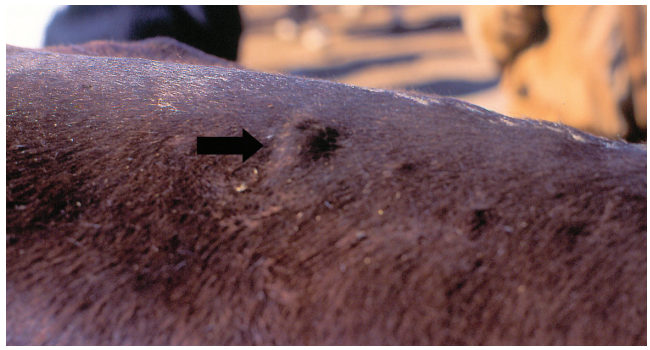


Plate 16 *Hypoderma lineatum*: warble (arrow) on the back of a cow

Plate 17 *Hypoderma lineatum*: mature larva removed from the warble illustrated in Plate 16. Scale in mm

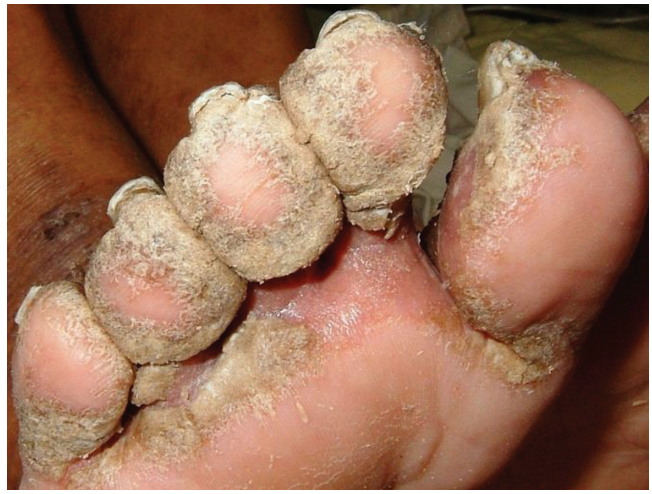


Plate 18 Crusted scabies is highly debilitating and infectious. This photograph illustrates the foot of a 51-year-old woman admitted to hospital suffering from pulmonary tuberculosis and acute respiratory failure. Scabies can become severe in patients whose immune system is compromised by illness. From http://dermatlas.med.jhmi.edu/derm/indexDisplay.cfm?ImageID=-1428734691. (Copyright Vincent C.B. Lin. MD, Dermatlas; http://www.dermatlas.org)

Plate 19 Coenurus of the tapeworm *Taenia multiceps* removed from the brain of a sheep. Note the large number of protoscolices budding from the germinal membrane

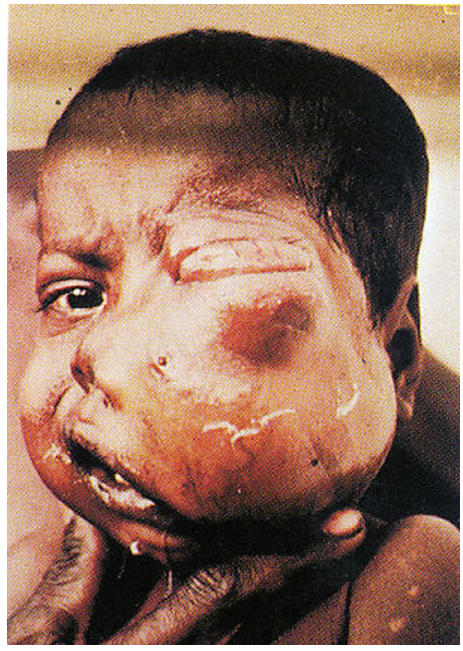


Plate 20 Burkitt's tumour. This severely disfiguring and fatal tumour is associated with co-infection with EBV (Epstein-Barr virus) and malaria. Source: Peters and Gilles, 1989

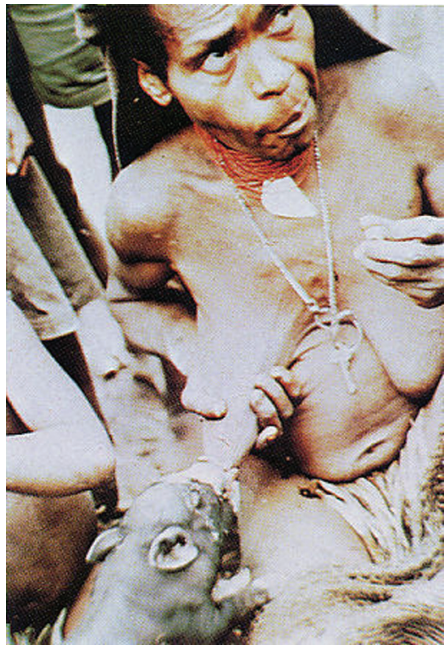


Plate 21 Woman feeding her piglet. In parts of the New Guinea highlands pigs play such an important role in culture that piglets may be fed in preference to children. Source: Peters and Gilles (1977)

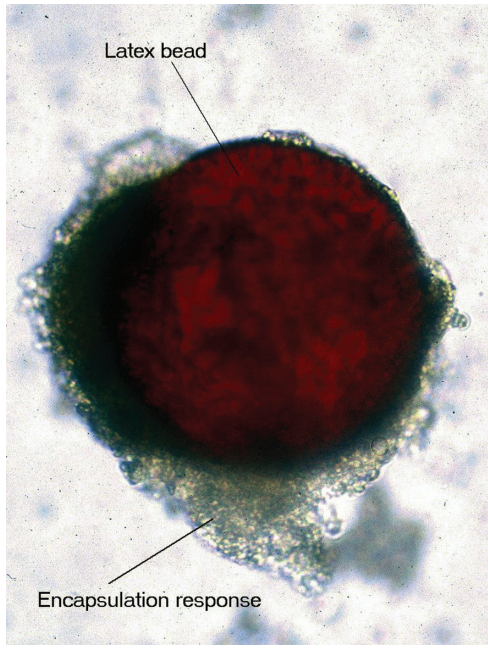


Plate 22 Encapsulation response to a latex bead injected into the haemolymph of the final instar larva of the moth *Spodoptera exempta*

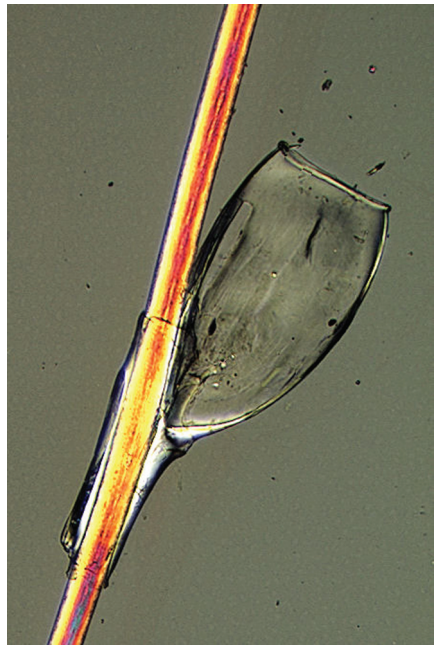


Plate 23 Hatched egg ('nit') of the head louse *Pediculus humanus capititis*

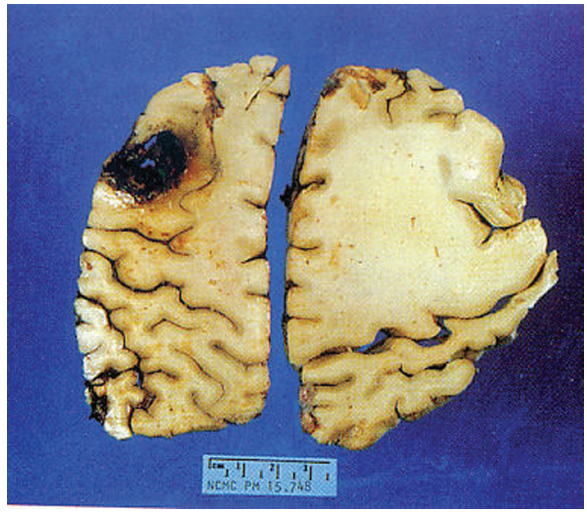


Plate 24 Fatal toxoplasmic brain abscess from a patient who had AIDS. Source: Kean *et al*., 1991

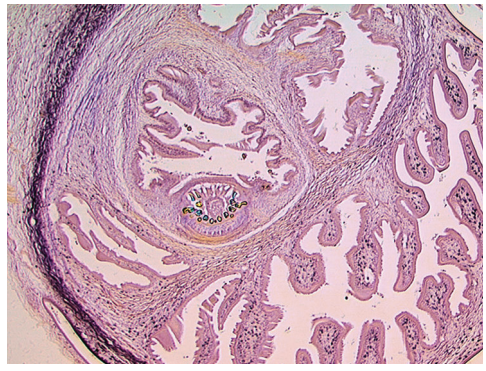


Plate 25 Light microscope section through a cysticercus of *Taenia solium*. Note that there is only one protoscolex and budding does not occur

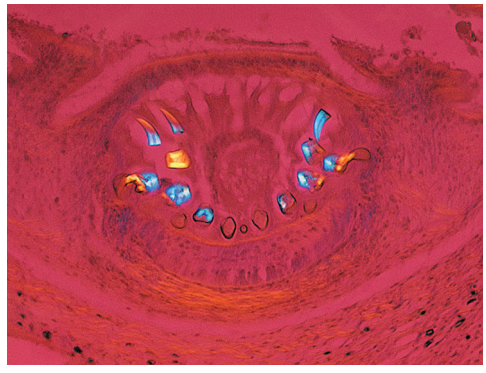


Plate 26 Light microscope section through a cysticercus of *Taenia solium*. Note that the protoscolex is armed with hooks

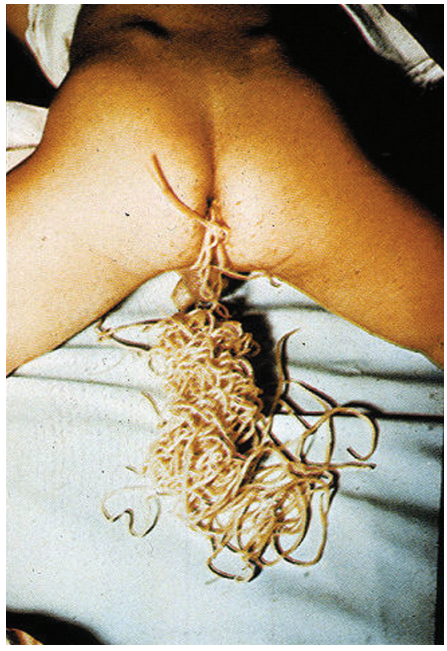


Plate 27 Huge numbers of *Ascaris lumbricoides* passed by a young child following anthelmintic treatment. When such large numbers are present, they can block the gastrointestinal tract. Source: Peters and Gilles, 1989

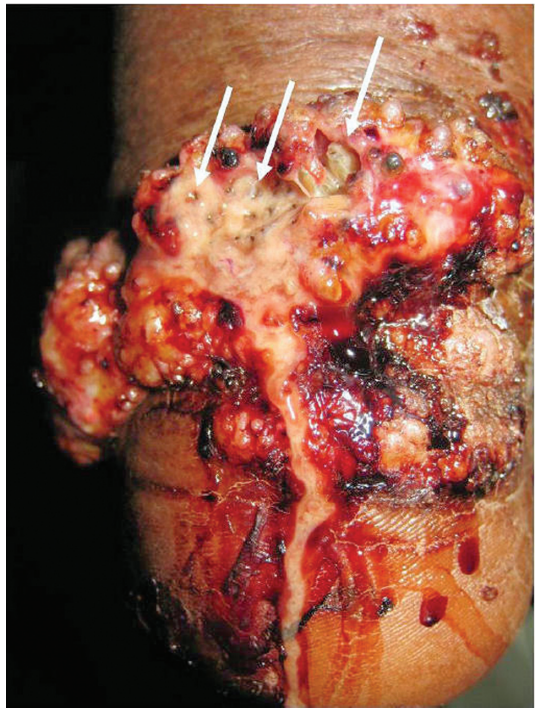
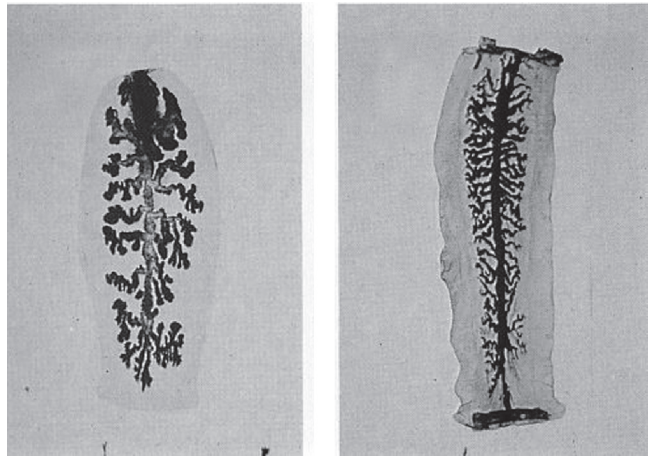


Plate 28 Heel of a man suffering from a co-infection of chromoblastomycosis and cutaneous myiasis. The rear ends of some of the larvae of *Chrysomya bezziana* are arrowed. Source: Slesak *et al*., 2011

Plate 29 After this man's tongue was re-attached, leeches were applied to relieve congestion until the venous blood supply was re-established. Source: Kim, J.S. *et al*., 2007. (Reprinted from Journal of Plastic, Reconstructive and Aesthetic Surgery, 60, Kim, J.S. *et al*., 1152–1155, 2007, with permission from Elsevier)

Plate 30 Cerebrospinal fluid is required for some tests to determine whether trypanosomes have invaded the central nervous system. This is an unpleasant procedure for the patient and carries risks of causing nerve damage. Source: Peters and Gilles, 1989



(a) (b)

Plate 31 Gravid proglottids of *Taenia saginata* (a) and *Taenia solium* (b) injected with ink to show the difference in the number of uterine branches. Source: Kean *et al*., 1991

Plate 32 The Middle Eastern country where this photograph was taken had regulations requiring sheep to be slaughtered at official slaughterhouses where the meat could be inspected and waste disposed of safely. However, numerous small unlicensed butchers could be found throughout the country