1 Nematode parasites

For pastorally reared livestock, internal parasite infections represent the greatest threat to health and productivity. Parasites generally achieve maximal importance in young (non-immune) animals kept at pasture and returning regularly to areas they have grazed before, and when the climate is generally warm and wet, and winters are mild. Nevertheless, some studies have shown that even where access to pasture is considerably reduced, parasitism can still cause considerable problems and some parasites can adapt to husbandry conditions that would normally be considered incompatible with parasite biology. Of all the helminth parasites of livestock, the gastrointestinal nematodes (GIN) have arguably the greatest overall impact.

The nematodes

In the past, nematodes have been ascribed to various phyla, namely, the Aschelminthes or Nemathelminthes, but are now generally recognised as belonging to their own unique phylum, the Nematoda (Hodda, 2007). The nematodes are biologically quite distinct from other 'helminths' such as the Platyhelminthes (cestodes and trematodes) and Acanthocephala (the thorny-headed worms). Indeed, nematode moulting behaviour has encouraged some to consider them as being more closely related to other moulting organisms (forming the superphylum, the Ecdysozoa) than they are to the other helminth taxa (Aguinaldo *et al.*, 1997). Acceptance for this new arrangement is by no means universal and there are strong arguments against it (reviewed by Hodda, 2007), but taken at face value such a relationship could explain why a drug like ivermectin can have excellent activity against nematodes and arthropods, but none against flukes or tapeworms.

Traditionally, two major classes of nematodes have been recognised (Anderson, 2000; Chitwood, 1950), the Secernentea and the Adenophorea – the

vast majority of 'adenophorean' nematodes being aquatic with this class contributing only a handful of vertebrate parasites (e.g. *Trichuris* spp., *Trichinella* spp.). The major parasites of terrestrial vertebrates were placed within the Secernentea and were split amongst the following orders: the Ascaridida, the Oxyurida, the Rhabditida, the Spirurida and finally the Strongylida (the strongylids). This latter order includes the vast majority of nematode species causing gastrointestinal disease in ruminants.

The strongylid order contains several superfamilies, the bulk of ruminant nematode parasites being found in one, the Trichostrongyloidea. The trichostrongyloid nematodes are all rather slender and tend to be small, the majority being around 2 cm or less. With simple, small mouths they are described as mucosal browsers, i.e. feed on small particulate matter, mucus and dissolved molecules on the mucosal surface. A small number of important genera can be found in other superfamilies such as the Ancylostomatoidea and Strongyloidea. Nematodes in both of these superfamilies tend to be stouter than the trichostrongyloids. The Ancylostomatoidea contains the blood-feeding hookworms, whereas the Strongyloidea contains a number of plug-feeding nematodes – nematodes that ingest a plug of host tissue which is liquefied in the buccal capsule by enzymatic action and the liquid material is swallowed.

The important nematode genera and species parasitising ruminant livestock

Climate and geographical location, management factors, the classes of stock present on a farm and the presence or absence of other stock or wildlife that may act as reservoirs of parasites can all have a major impact on the exact balance of species present. It is generally accepted that the major parasites of sheep do not establish that well in cattle and *vice versa*, thus few parasites are common to both when sheep and cattle share the same grazing, whereas many of the major parasites of sheep will establish readily in goats. *Bos taurus* and *Ovis aries* are farmed on all inhabited continents, having arrived there largely as the result of human activity and their parasites have gone with them. Thus a parasite such as *Ostertagia ostertagi* is ubiquitous in cattle in temperate climates, whether this is in North or South America, in Europe and Asia or in Australia and New Zealand.

In the following discussion of the major genera and species of livestock, the parasites will be grouped according to the organ they infest rather than on any taxonomic basis. Unless stated otherwise, it can be assumed that the parasites mentioned are strongylids, from the superfamily Trichostrongyloidea. The list has concentrated on the more economically important parasites and thus has a bias towards GIN of temperate climates. Many nematodes are not discussed, but it should be born in mind that parasites such as *Mecistocirrus digitatus*, though prevalent and important in some parts of the world, have similar biology and behaviour to some of those listed in the following sections – *Haemonchus* spp. in the case of *M. digitatus* (Aken *et al.*, 1997). Likewise, the camelid strongylid, *Camelostrongylus mentulatus*, when it occurs in sheep, is effectively a straight swap for *Teladorsagia* spp. (Hilton *et al.*, 1978).

Abomasal genera

Haemonchus

Main species: *H. contortus, H. placei*
Being the largest of the common abomasal parasites, *Haemonchus* spp. are also the most pathogenic being unusual for trichostrongyloids in feeding on blood (see later). Also regarded as one of the most prolific parasites, females produce up to 10,000 eggs per day. *H. contortus* is primarily a parasite of sheep and goats, but can be found in cattle and some species of deer, whereas *H. placei* is primarily a parasite of cattle. For some time there was a debate as to whether *H. contortus* and *H. placei* were separate species, but there is genetic evidence that this is indeed so (Blouin *et al.*, 1997), and hybrids of the two species may be infertile (Le Jambre, 1995). In New Zealand where *H. placei* has not been recorded, small numbers of *H. contortus* may be found in cattle, but clinical disease is exceptional. A rarer third species, *H. similis*, has been recorded in cattle and deer in North America and Europe. *Haemonchus* spp. generally prefer warm, moist conditions and hence are more of a problem in tropical and subtropical conditions, the free-living stages of the parasites struggle to overwinter in cooler climates. In temperate countries such as the United Kingdom and New Zealand, the prevalence and the risk of disease have traditionally been greater in the warmer southern and northern parts of the respective countries. There are however reports of increasing numbers of cases of ovine haemonchosis occurring in Scotland and Sweden (Sargison *et al.*, 2007; Waller *et al.*, 2005).

Common names of *Haemonchus* spp. include twisted stomach worm, wire worm and Barber's pole worm and one can see why when viewing freshly collected females in which the pale uterus entwines around the red, blood-filled intestine. Adults of all three species are 2–3 cm in length and are therefore easily seen on the mucosal surface during post-mortem examination.

Ostertagia

Main species: *O. ostertagi (O. lyrata)*
O. ostertagi is a cosmopolitan parasite of cattle and is considered the most important parasite of cattle in temperate regions. It is also important in subtropical climates with adequate winter rainfall. Well adapted to cooler climates, *O. ostertagi* survives reasonably well over winter as L3 on pasture or in soil, or as arrested larvae inside the animal. Originally described as a separate species, based on the presence within populations of small numbers of male nematodes with different morphology, *O. lyrata* is now considered as a morphological variant of *O. ostertagi*. A number of studies have examined this, including studies of ribosomal and mitochondrial DNA (Zarlenga *et al.*, 1998), and so far none have refuted the hypothesis that *O. ostertagi* and *O. lyrata* are the same species. Insofar as *O. lyrata* males are usually present

in smaller numbers than *O. ostertagi* males, *O. lyrata* is considered the minor morphotype, *O. ostertagi*, the major morphotype.

The adults are slender, brownish-red worms reaching approximately 1 cm in length and can be observed on the mucosal surface, from where they can be difficult to remove, being embedded within the mucus layer. *Ostertagia* spp. females are considered of low fecundity, laying as few as 50 eggs per day.

O. leptospicularis is primarily considered a parasite of cervids, but has occasionally been recovered from cattle and sheep.

Teladorsagia

Main species: *T. circumcincta (T. (Ostertagia) trifurcata, T. davtiani)*

Teladorsagia spp. essentially occupy the niche in sheep and goats that *O. ostertagi* does in cattle, and their behaviour and effects are very similar. For a long time, these organisms were in the genus *Ostertagia* (*O. circumcincta*). As with *O. ostertagi*/*O. lyrata*, *T. circumcincta*/*T. trifurcata*/*T. davtiani* are considered one species with *T. trifurcata* and *T. davtiani*, minor morphotypes. Recent genetic analyses found more variation between different strains of *T. circumcincta*, including a comparison of goat vs. sheep *T. circumcincta*, than existed between the major and minor morphotypes (Grillo *et al.*, 2007, 2008). *T. circumcincta* may thus include a cryptic species, but *T. trifurcata*/*T. davtiani* are not implicated.

Both *Ostertagia* spp. and *Teladorsagia* spp. are sometimes referred to as the brown stomach worms.

Trichostrongylus

Main species: *T. axei*

The only species of this genus found consistently in the gastric compartment, *T. axei* has been found in cattle, sheep, goats, deer, pigs and horses. It is the smallest of the common abomasal nematodes and is relatively easily overlooked when alongside the others (*Haemonchus*, *Ostertagia*/*Teladorsagia*). *T. axei* burrows between the epithelial cells and thus occupies a slightly different niche than the other abomasal GIN species.

Small intestinal genera

Trichostrongylus

Main species: *T. colubriformis, T. vitrinus, T. longispicularis, T. rugatus*

These are relatively small worms, less than 1 cm in length. *T. longispicularis* is primarily a parasite (minor pathogen) of cattle, whereas *T. colubriformis* and *T. vitrinus* are important parasites of sheep and goats. In the warmer

parts of temperate regions moving into subtropical areas, small intestinal *Trichostrongylus* spp. make a greater contribution to ill health in small ruminants, and the infections can exhibit very high egg counts. In a study of South Australian sheep, *T. rugatus* dominated in low rainfall areas, *T. vitrinus* in wetter districts whereas *T. colubriformis* was common throughout the state, but was rarely dominant (Beveridge & Ford, 1982).

Worms in this genus are sometimes referred to as the black scour worms.

Nematodirus

Main species: *N. battus, N. filicollis, N. spathiger, N. helvetianus*

These are sometimes called the thin-necked or thread-necked worms as the anterior part of the female is noticeably narrower than the posterior. This is primarily the result of the very large eggs produced by this parasite. *Nematodirus* spp. are fairly cosmopolitan in distribution. The distribution of *N. battus* is the most restricted being confined to the northern United Kingdom, parts of Northern Europe and Canada, possibly reflecting only a recent cross-over from deer – disease due to *N. battus* was not recorded in sheep before 1951 (Winter, 2002). *N. Helvetianus* is primarily a parasite of cattle, whereas the others are more prevalent in small ruminants. *N. battus* does however infect cattle, particularly young calves. The biology of *Nematodirus* spp. is atypical for the trichostrongyloids. Larvae develop to the ensheathed L3 stage within the egg, hence the larger egg size; development is generally slow, and the eggs of *N. battus* and *N. filicollis* appear to enter a period of developmental arrest and require a period of chilling to trigger hatching (Chapter 3). Thus, for *N. battus* and *N. filicollis*, there may be only one generation of the parasites each year. Infection is passed from one year's lambs or calves to those born the next year, with a negligible role for adult stock – transmission that can be encouraged by the use of specific paddocks for calving/lambing from one year to the next. Disease is seen in the spring and early summer and follows the (synchronous) hatching of a large number of eggs (Sargison, 2004). Deaths of young stock are not uncommon. Immunity to these worms develops rapidly, thus curtailing the period of risk, and a definite age resistance has been observed in sheep, with parasite-naive 8-month old lambs refractory to infection (Winter *et al.*, 1997).

Cooperia

Main species: *C. oncophora, C. punctata, C. pectinata, C. surnabada (C. mcmasteri), C. curticei*

Cooperia spp. are cosmopolitan parasites of cattle and sheep, with *C. curticei* most often recovered from sheep. These species are generally considered to be of only mild pathogenicity, although *C. punctata* and *C. pectinata* may be more damaging, which may relate to a more invasive behaviour of their larvae (Taylor *et al.*, 2007).

Large intestinal genera

Oesophagostomum

Main species: *Oe. radiatum, Oe. columbianum, Oe. venulosum*
Up to 2 cm in length, these strongyloid nematodes are sometimes called nodule worms because of the (inflammatory) nodules that develop around larvae that have burrowed into the submucosa of the host intestine. *Oe. radiatum* is found in cattle worldwide, whereas *Oe. venulosum* and *Oe. columbianum* occur in sheep. *Oe. venulosum* is the more common species of the two sheep species and is the least pathogenic. *Oesophagostomum* spp. are strongyloid, plug-feeding nematodes, but the shallow buccal capsule in the adult renders this stage relatively non-pathogenic. Pathogenicity relates principally to the encystment of larvae in the submucosa of the distal small intestine and the large intestine. These nodules may comprise a significant inflammatory component and may eventually fill with green, eosinophilic pus. Nodules may attain 2–3 cm in diameter and tend to be more pronounced following repeated exposure. Clinical signs, including diarrhoea, often accompany the emergence of the parasites from confinement within the nodules. These parasites prefer warmer conditions, struggling in areas with colder winters.

Chabertia ovina

Ch. ovina is another strongyloid nematode, but in contrast to *Oesophagostomum* spp., *Ch. ovina* has a very large, bell-shaped buccal capsule and is capable of taking a significant bite of the intestinal mucosa. It is therefore considered to be quite pathogenic, but seldom occurs in large numbers. As few as 300 adults may however be enough to cause clinical signs. Disease specifically due to this nematode has been recorded in the winter rainfall areas of Australia and South Africa (Taylor *et al.*, 2007).

Nematode evolution

The oldest known fossil of a nematode is of an insect parasite preserved in 135 million year old amber (Poinar, 2003), but otherwise, as with most small, soft-bodied invertebrates, nematode history cannot be traced through fossil evidence. Nevertheless, researchers have used various tools to hypothesise that the nematodes may date back as long as 1000 million years ago (Hedges, 2002; Meldal *et al.*, 2007; Vanfleteren *et al.*, 1994) and most likely originated in the sulphide-rich sediments present, then and now, at the bottom of all major bodies of water (Bryant, 1994). These benthic deposits are still rich in nematode species today and interestingly share many of the characteristics of the contents of the gastrointestinal tracts of vertebrate animals, being a slurry of organic (and inorganic) material with low oxygen levels. Despite this aquatic ancestry, parasitic nematodes are far more abundant in terrestrial animal hosts than in

their marine and freshwater counterparts and the major reason for this is that nematodes, lacking adaptations for swimming, enjoy little contact with the vast majority of pelagic animal species. Nematodes eventually spread to the land, where they have become one of the most abundant organisms in soil. From here, nematodes switched from a free-living existence to a parasitic one, a process thought to have occurred on at least six occasions (Dorris *et al.*, 1999).

Recent reviews of nematode evolution have challenged much of the older classification of nematode taxonomy (Blaxter, 2003; De Ley & Blaxter, 2002, 2004; Hodda, 2007), with a major change affecting the strongylid order, which may now be reduced to the level of superfamily (becoming Strongyloidea) within the order Rhabditida, and the various superfamilies (Trichostrongyloidea, Strongyloidea, etc.) reduced to family level (Trichostrongylidae, Strongylidae). However, since the older nomenclature is commonly used throughout the veterinary literature, this book will continue to reflect the older systematics. The Rhabditida order contains many free-living and microbivorous nematodes and includes one of the most well-studied organisms in biology, *Caenorhabditis elegans* (Dorris *et al.*, 1999; Mitreva *et al.*, 2005), and there are in fact many similarities in morphology and behaviour of the free-living rhabditids and the earlier larval stages of many parasites.

The history of the strongylids as parasites may date as far back as the late Devonian or early Carboniferous (approximately 350 million years), to sometime after the first appearance of amphibians on land (Durette-Desset *et al.*, 1994). The ancestral strongylid parasite was undoubtedly a close relative of a free-living ancestor of contemporary free-livers such as *C. elegans* (Chilton *et al.*, 2006). Pivotal to this origin of strongylid parasitism may have been the relative ease with which the larvae of these newly parasitic forms could penetrate the softer amphibian skin – oral routes of infection evolving only later.

The subsequent history of strongylid evolution is undoubtedly complex, but involved all of the terrestrial vertebrate phyla and rather than following a close path of co-evolution of host and parasite, there have been numerous instances of host-switching, combined with explosive radiations of parasite species taking advantage of radically different new host forms. The evolutionary histories of the nematode fauna of herbivores such as the horses, macropodid marsupials and ruminants offer such examples. The predominant nematode parasites of kangaroos and horses belong to the same superfamily of strongylid nematodes, the Strongyloidea, whereas the dominant parasites of ruminants are trichostrongyloids. Strongyloid parasites of ruminants are nevertheless present in genera such as *Chabertia* and *Oesophagostomum*. The equids, in common with many earlier herbivores (including ruminant ancestors), developed an enlarged caecum as the site for bacterial fermentation of plant material. The development of such a vast digestive organ may have considerably lessened competition between nematode species allowing the great diversification seen in the equine strongyloids (Durette-Desset *et al.*, 1994), and in extant adult equids, the majority of nematode parasites are large intestinal species with much of the rest of the gastrointestinal tract, the small intestine in particular, rather bereft of parasites. In contrast, both the large kangaroo species and the ruminants developed as foregut fermenters with

associated changes in anatomy (and an inferred anterior shift in the availability of nutrients) that led to an extensive radiation of nematode species in the stomach and small intestine (Durette-Desset *et al.*, 1994). In the case of the kangaroos, the strongyloid nematodes of the cloacine family were the principal beneficiaries of these profound changes whereas in the ruminants it was the trichostrongyloids which took advantage of the modified niches.

A major contributor to the success of strongylid nematode species was the evolution and spread of the grasses which became widespread in the mid to high planetary latitudes from about 15 million years ago. This period is also associated with a gradual replacement of browsing herbivores by grazing species (Janis *et al.*, 2000). By ingesting herbage closer to the ground and therefore facing more faecal contamination, and because grasses generally support the presence of the free-living stages of nematode parasites to a greater extent than do broadleaf species, grazing animals are typically more exposed to nematode parasitism than are browsers.

The domestication and subsequent movement of livestock has undoubtedly had a major impact on the distribution and prevalence of parasites in these species. Parasites such as *H. contortus*, *H. placei*, *O. ostertagia* and *T. circumcincta* have been transported far beyond the regions where they would have originally evolved. Sheep may first have been domesticated in Mesopotamia, and parasites such as *T. circumcincta* were likely present in their wild ancestors. In contrast, sheep may have become exposed to *H. contortus* only once humans imported them to Africa and into contact with other ruminant species such as antelope (Hoberg *et al.*, 2004).

The transition to parasitism

Of the more than 25,000 recognised species of nematodes, approximately 60% are parasites of plants or animals (De Meeus & Renaud, 2002). The nematodes have thus proven extremely successful in adopting both free-living and parasitic lifestyles. As stated previously, before shifting to parasitic lifestyles, free-living nematodes first successfully adapted to niches in the terrestrial environment. As such they had to develop the ability to cope with highly variable levels of moisture, something that is a major limiter of nematode activity on land. Immediately, one of the benefits, perhaps the most important, of parasitism becomes apparent. In environments prone to drying out, the ability of the host animal to locate water and then conserve it within its own tissues can far exceed those of a small invertebrate. Likewise, the host can be relied upon to be more adept at locating nutrients, presenting nourishment to its resident parasites in the form of either digesta or nutrients already assimilated into host tissues and secretions or held within commensal organisms of the gut microflora. Within the host, nematodes are also (presumably) spared predation by nematophagous organisms, including other nematode species, various metazoans and fungi.

Within the host, parasites also benefit from more stable temperatures. Even within so-called cold-blooded hosts, temperatures will vary less than in the

external environment, whilst in avian and mammalian hosts, temperature will vary little. Overall, a parasitic nematode inhabits an environment that is far more stable than do their free-living relatives. The relative protection afforded by the host environment has even allowed parasitic nematodes to become considerably larger than their free-living counterparts (Yeates & Boag, 2006).

Most free-living nematodes are quite small, reaching maximum lengths of only a few millimetres. The small size and simplicity of free-living nematodes is largely a function of the constraints placed on them by the external environment. In moving through the liquid phase surrounding the particulate matter of soil and sediments, both small size and a lack of appendages are favourable physical characteristics. However, removed from these restrictions, some parasites have achieved massive proportions, with the largest nematode ever described (*Placentonema gigantissima*) reaching over 8 m in length in the placentas of whales. A counter-argument to larger size in nematodes being primarily a benefit of parasitism is that it is rather a necessity. Larger females can lay more eggs and this may be an absolute requirement for some nematode species to ensure that at least some of their progeny will encounter and infect a suitable host. *Ostertagia* spp. and *Teladorsagia* spp. in their bovine and ovine hosts, respectively, are arguably amongst the most successful of the world's nematode parasites, despite their smaller size and reputations for being of relatively poor fecundity. This suggests that other factors than egg output *per se* may be far more important in determining the success of a species.

Nematode biology

Morphology

Nematodes are morphologically very similar, sharing a relatively simple body plan, viz. an essentially cylindrical, unsegmented tube. The mouth is terminally situated at the anterior end of the body, whilst the anus emerges on the ventral surface behind the tip of the tale.

The nematode surface

The external surface consists of a collagen-rich cuticle that is secreted by the underlying epidermis (hypodermis). The cuticle is tough and protective, but is known to be permeable to a limited range of molecules such as water and ammonia. The relative inability of the cuticle to stretch and enlarge is thought to be the major reason for moulting during growth, nevertheless some of the larger parasites, such as the ascarids, achieve remarkable rates of elongation even after the final moult. In addition to collagens, the cuticle may contain various other components. The outer surface is usually covered by a glycoprotein-rich surface coat. In addition, the cuticle may also contain water, lipids, various enzymes and haemoglobin-like molecules. As such, the cuticle is very much a 'living tissue'.

Cuticle structure has been shown to vary markedly between different nematode groups. Innervated, finger-like projections (papillae), transverse annulations or longitudinal ridges, wing-like alae and circumferential inflations (dilations) are important features that can be used in the identification of nematode species. For many of the trichostrongyloid nematodes, longitudinal ridges are arranged in a specific pattern known as the synlophe. This is thought to play an important role in allowing nematodes to remain in intimate contact with the mucosal surface.

The composition of the cuticle may also vary according to the life-cycle stage. In adult nematodes, 80% or more of the cuticle may be composed of collagens (Fetterer & Rhoads, 1993), and in a study of expressed sequence tags (ESTs) of (growing) L4 *T. circumcincta*, a large proportion of overall gene expression was for collagens (Nisbet *et al.*, 2008). In free-living stages, the non-collagen protein, cuticlin, is important and probably plays a role in resisting the more noxious environmental conditions (DeGiorgi *et al.*, 1997; Fetterer & Rhoads, 1993). Other studies have identified a number of stage-specific substances. One example is a 35 kDa epicuticular glycan (CarLa) found initially in *T. colubriformis* (Harrison *et al.*, 2003) and subsequently in all of the major GIN species investigated (Shaw, personal communication). CarLa is present in the L3, but not in the L4 or adult stages; neither is the molecule found in *C. elegans*. It is also a major antigen – one of the three surface-associated larval antigens shown to dominate the natural mucosal antibody response directed against the L3 (Maass *et al.*, 2007). In summary, therefore, each parasitic stage – L3, L4, adult – may present a vastly different set of antigenically distinct molecules to the host.

The nematode body

Below the cuticle, the epidermis forms a continuous tube and extends into the interior in four longitudinal cords – two lateral, one dorsal and one ventral. The dorsal and ventral cords contain nerves whereas the lateral cords contain the canals of the excretory system. Beneath the epidermis are longitudinal muscle fibres, arranged in ventral and dorsal blocks.

The internal body cavity of nematodes is described as a pseudocoelom. In many nematodes, but by no means all, the pseudocoelom is fluid-filled and this fluid is under pressure thus providing antagonism to the muscles of the body wall, and contraction of either the dorsal or ventral muscle blocks causes flexion. When the muscles relax, the pressure of the pseudocoelomic fluid allows the worm to then straighten. Contraction/relaxation of the muscles thus produces alternating dorso-ventral flexion allowing nematodes to move in an undulating manner. This basic sinusoidal movement is easily seen in cultured larvae suspended in water, but in more appropriate conditions, e.g. *C. elegans* on the surface of agar, the worms are capable of very fine movements, particularly of the head during feeding, and they can move backwards as well as forwards (Burr & Robinson, 2004).

Maintenance of the pressure of the pseudocoelomic fluid is of great importance to the worms and is thought to be the major role of the nematode excretory

system. In the secernentean nematodes, this system comprises the two lateral longitudinal ducts running the length of the body, a transverse duct connecting the two and a further duct connecting the transverse duct to the excretory pore on the ventral surface of the body. This external pore is usually situated anteriorly. In some worm genera, e.g. *Trichostrongylus* spp., the excretory pore is situated in a small depression (notch). Secretory cells may be associated with the excretory system, extending its function beyond just osmoregulation. For example, secreted enzymes may be involved in the extracorporeal digestion of substrates, whilst other substances may be absorbed onto the cuticular surface, contributing to the surface coat.

The gut and feeding

The nematode gut is a straight tube extending from the mouth to the anus. The structure of the mouth is one of the most variable parts of nematode anatomy and is highly adapted to the mode of feeding of individual species/stages. The mouth may be a simple invagination of the cuticle or it may be surrounded by two, three or six lips, or a series of fused sensory papillae (such as the external leaf crown of strongyloid nematodes such as *Oesophagostomum* spp.). The diameter of the oral opening varies considerably; nematodes feeding on fluids, fine particulate matter or bacteria have narrow openings, whereas those feeding on tissues (the plug feeders) have wider mouths. In the case of the latter, this usually gives the anterior end of the worm a blunter appearance rather than a smooth taper, whereas in an organism like *Ch. ovina*, the anterior end is visibly flared. Behind the mouth lies the buccal cavity. In the simple-mouthed trichostrongylids, such as *Ostertagia* spp., *Teladorsagia* spp. and *Trichostrongylus* spp., the buccal cavity is much reduced and the oesophagus (pharynx) begins almost immediately. In *H. contortus*, a 13-μm long and 3-μm wide tooth or lancet arises from the dorsal lining of the buccal cavity. This buccal lancet is used to pierce the vasculature of the host's mucosa to allow blood feeding. In the plug-feeding strongyloids and blood-feeding hookworms, a much broader cavity (capsule) is present that may possess additional adornments such as teeth, gutters and, in the hookworms, cutting plates.

Oral structure can vary between the different stages of the nematode life cycle. Thus the preparasitic, early larval stages of strongylid nematodes have cylindrical buccal cavities adapted for bacterial feeding that are very similar to those of their close relatives, the rhabditids (Figure 1.1).

This rhabditiform arrangement is lost by the (non-feeding) L3 stage. In *H. contortus*, the buccal lancet develops first in the L4 stage, while the relatively shallow, cylindrical buccal capsule found in adult *Oesophagostomum* spp. is different from the more globular (more primitive) version found in the L4.

Behind the mouth is the oesophagus; this muscular structure pumps food into the intestine and as such has to resist the pressure of the pseudocoelomic fluid, which tends to compress the intestine thus resisting filling. A valve is therefore present between the oesophagus and the intestine to prevent regurgitation. The structure and appearance of the oesophagus can vary markedly

Figure 1.1 The anterior end of a newly hatched L1 *T. circumcincta*. The cylindrical buccal capsule (arrow) and the 'grinder' (arrow head), structures found only in the L1 and L2, are visible. Similar structures can be found in other microbivorous nematodes such as the free-living rhabditid *C. elegans*. Image courtesy of Laura Green and Kevin Pedley.

between different taxonomic groups, but also between different life-cycle stages. For example, adult strongylid nematodes typically have a filariform (from the Latin for thread, *filum*) oesophagus that is relatively straight with only a slight posterior thickening. In others, the posterior swelling is more pronounced and bulb-shaped. In the microbivorous rhabditids, and in the first and second larval stages of strongylid nematodes, the oesophagus is described as rhabditiform and the prominent posterior bulb contains a specialised area – the grinder (Figure 1.1) – in which the cuticle is thickened to allow ingested bacteria to be physically disrupted (Bird & Bird, 1991; Munn & Munn, 2002). In addition to muscle cells, the oesophagus may also contain cells responsible for secreting digestive enzymes.

The intestine consists of a simple epithelial tube, and its cells may be individually discernible or may be arranged in a syncitium. The luminal surface of the cells is covered by prominent microvilli, whilst the outer basal surface bears only a thin basement membrane. The intestinal cells may contain large stores of lipids and proteins: the lipid deposits are very obvious in the L3 of the strongylid nematodes and act as their sole energy supply until they are ingested by a host. Up to 30% of the dry weight of an infective larva may be stored lipid (Bird & Bird, 1991).

Posteriorly, the intestine terminates in the rectum and anus (cloaca in males), where a muscular sphincter is situated to control defecation. Defecation in nematodes can occur surprisingly frequently – every 3–5 min – and can be

quite an energetic process with some of the larger ascarids capable of generating (in air) a 60-cm jet of faeces (Bird & Bird, 1991).

The genital tracts of male and female nematodes

Male nematodes are usually smaller than the females, sometimes remarkably so. Most males (all strongylids) have one testis, which is essentially a blind-ending tube terminating at the cloaca. Spermatogenesis is usually confined to the blunt tip of the testis, and mature spermatozoa are stored distally in a more dilated section, the seminal vesicle. The seminal vesicle then merges into the vas deferens, the most terminal part of which is muscular, forming the ejaculatory duct that controls the release of the sperm during copulation. Males typically possess one or two chitinous spicules associated with the terminal portion of the reproductive tract. The spicules are pushed into the vulva of the female during copulation to direct the flow of ejaculate. Usually, but not always, symmetrical, the shape of the spicules varies uniquely according to nematode species and is an important tool in identification. The exception occurs in a small number of species in which different morphological variants of the same species (morphotypes) possess different shaped spicules. A third, usually smaller, chitinous structure, the gubernaculum, may also be present.

A variety of other structures may be associated with reproduction in the male. The tale is usually adorned with sensory papillae and some, e.g. some of the ascarid nematodes, have suckers for attaching to the body of the female. In the strongylid nematodes this function is performed by a very prominent structure, the copulatory bursa – hence the strongylids are sometimes referred to as the 'bursate' nematodes. The copulatory bursa is essentially an expanded area of cuticle forming two lateroventral membranes or lobes with a further dorsal lobe. These structures are strengthened by several integral bursal rays. The dorsal lobe may fuse with the lateroventral ones or be distinct. In the trichostrongyloids, the dorsal lobe is distinct, but is much reduced. The structure of the various lobes and the spacing and patterning of the bursal rays in particular are also important tools used in identification. A homologous structure can be found in male *C. elegans*, and given the relative simplicity of the rest of the body, it is perhaps no surprise that 40% of the cells of a male *C. elegans* are sexually differentiated (Bird & Bird, 1991).

Female nematodes may possess one or two genital tracts, i.e. are monodelphic or didelphic. In didelphic nematodes such as the trichostrongyloids, the two tracts share a common vulva. The position of the vulva may vary considerably. For many trichostrongyloid nematodes the vulva is situated within the mid-portion of the body, but still towards the tail. As a result, one of the two tracts heads anteriorly, whereas the other extends towards the tail – a condition known as amphidelphy. As with the males, meiosis occurs generally at the blind tip of the tract (ovary) adjacent to which is the spermatheca in which spermatozoa are stored after mating. The spermatheca leads to the uterus, which in mature females contains eggs in various stages of development. The number of eggs is another feature that varies between species, larger females producing

and storing more eggs. The release of eggs out of the uterus may be controlled by muscular sphincters (the ovejector apparatus). In the strongylid nematodes, these (paired) sphincters are conspicuous and the area between the two sphincters is termed the vagina. The vulva is protected by a cuticular flap in some species – an additional feature used in identification. In some nematodes, the genital tract is entwined around the intestine; this is particularly obvious in *H. contortus*.

The nematode nervous system

The nervous system of *C. elegans* has been extensively studied and is thought to be fairly typical of secernentean worms. Due mostly to its large size, the nervous system of *Ascaris suum* has also been fairly well studied and is broadly similar to that of *C. elegans*. *C. elegans* hermaphrodites have just over 300 neurons in total; males have more (around 380) the bulk of the difference representing the innervation of the copulatory bursa and the 40 or so muscles controlling the tail, spicules and bursa of the male. Despite its much greater size, *A. suum* actually has slightly fewer neurons (about 250).

The nervous system is organised into a number of different ganglia situated mostly in the anterior and posterior ends, and into the dorsal and ventral nerve cords, which run for most of the body length alongside ridges of the epidermis. The larger ventral nerve cord arises from a ring of nerves that surrounds the oesophagus. The somatic musculature is innervated either by the nerve ring (anteriorly) or by motor neurons in the ventral nerve cord. Both the ventral and dorsal muscle blocks are innervated by motor neurons whose cell bodies are wholly within the ventral cord. The dorsal and ventral cords are linked via regular circumferential connections or commissures.

Nematodes possess various sensory structures and are capable of responding to a range of stimuli – chemical, thermal and mechanical, while only a very few nematodes are capable of responding to light. Sense organs are predominantly confined to the cuticle of the anterior and posterior ends (especially in the male), but there are also a number of internal sensory organs as well and the gut is richly endowed with sensory capability.

A large range of chemicals are utilised as neurotransmitters. Acetylcholine, gamma-aminobutyric acid (GABA), glutamate, serotonin (5-HT), and various peptides, especially the FMRFamide-like peptides, have all been demonstrated in nematodes. Other putative neurotransmitters include histamine, nitric oxide (NO) and catecholamines such as dopamine, adrenaline/noradrenaline. The nematode nervous system is the target of several classes of anthelmintic drugs (Chapter 4).

Nematode genetics

One of the fundamental aspects of nematode biology is that despite their relative morphological simplicity, they are in fact genetically and biochemically

complex. The completion of the genome project for *C. elegans* revealed a genome size of approximately 100 Mb, representing approximately 20,000 genes, at least half of which may be unique to nematodes (Blaxter, 2003). Comparisons can be made to the estimated 15,000 genes of the fruit fly *Drosophila melanogaster* and the 30,000–40,000 genes of mammals. Work is currently under way to sequence the *H. contortus* genome, which based on flow cytometry results has been estimated at 53 Mb (Leroy *et al.*, 2003). The same technology has been used to estimate the genome of *T. circumcincta* to be slightly larger at 59 Mb. It remains to be seen as to whether the smaller genome sizes of these economically important strongylid parasites are reflected in a smaller number of genes. A smaller genome may simply reflect a reduction in non-coding material. Interestingly, estimates for other nematode parasites reveal much bigger genomes, e.g. 230 Mb for *Ascaris suum* and 350 Mb for the canine hookworm *Ancylostoma caninum* (Abubucker *et al.*, 2008).

Chromosome number in nematodes has been shown to vary quite considerably, from $n = 1$ to 25 (Blaxter, 2000), however, most strongylid organisms examined so far have $n = 6$, as does *C. elegans*, and sex determination is by an XX–XO mechanism (seldom XX–XY), with females having 12 chromosomes in total ($2n$), males 11.

Recent studies show a high level of genetic diversity within populations of various economically important parasite species, likely due to very large effective population sizes (Anderson *et al.*, 1998; Grillo *et al.*, 2007; Prichard, 2001).

Nematode physiology

The mechanisms that nematodes use to generate energy have been shown to vary not just between species, but also between different life-cycle stages. Nematodes utilise aerobic and anaerobic pathways and may switch from one to the other during their development. Aerobic pathways generate more energy (38 moles of ATP for the complete oxidation of 1 mole of glucose, compared to 2 moles of ATP when glucose is fermented to lactate) and allow for the more complete catabolism of carbon skeletons. Lacking a circulatory system and residing in a low oxygen tension environment, nematodes such as the ascarids are too big for oxygen to penetrate interior tissues by diffusion, and thus they respire anaerobically as adults despite being largely aerobic as eggs and earlier larval stages (L1 and L2) (Kita, 1992; Kita *et al.*, 1997). Availability of oxygen is less of a problem for nematodes such as the trichostrongyloids, which are of much smaller diameter and reside much closer to the mucosal surface in an environment with a higher oxygen tensions than that found in the luminal fluid. As a result, nematodes such as *T. circumcincta* and *Nippostrongylus brasiliensis* are likely to be largely aerobic (Fry *et al.*, 1983; Kita *et al.*, 1997; Simcock *et al.*, 2006). Interestingly, *H. contortus* may respire anaerobically, despite its diet of oxyhaemoglobin, fermenting glucose to products such as acetate, succinate and propionate (Roos & Tielens, 1994).

One indication of which pathway is utilised is the main metabolite stored. Anaerobic nematodes/stages tend to store carbohydrate, either as glycogen or as trehalose, whereas aerobic nematodes/stages tend to store lipid.

A number of nematodes have been shown to produce haemoglobin-like molecules – nemoglobins (Blaxter, 1993; Weber & Vinogradov, 2001). Nemoglobins exist in three forms: (1) a monomeric globin domain containing a single haem group found intracellularly in the body wall and oesophageal muscles, (2) a tetramer found extracellularly in the cuticle and (3) an octamer of two-domain subunits also found extracellularly, but in the pseudocoelomic fluid. The intracellular, monomeric nemoglobins may function in a manner similar to mammalian myoglobin, facilitating oxygen capture and usage in the relatively low oxygen tension of the gut. This form may be present in virtually all nematodes including free-livers such as *C. elegans* and its presence has been identified in several strongylid parasites of livestock such as *T. colubriformis* (Frenkel *et al.*, 1992), *O. ostertagi* (DeGraaf *et al.*, 1996), *T. circumcincta* (Nisbet *et al.*, 2008) and, despite its purported anaerobiosis, *H. contortus* (Fetterer *et al.*, 1999). *N. brasiliensis* has the intracellular form and the cuticular form, suggesting that other strongylids may have the cuticular form as well (Blaxter *et al.*, 1994), whereas the larger, pseudocoelomic form has only been detected in the ascarids.

In most parasitic nematodes, expression of the nemoglobin genes increases in the later parasitic stages. Nemoglobins may, however, perform functions other than oxygen utilisation, for example NO detoxification (Barrett & Brophy, 2000), and certainly, although the pseudocoelomic form in *A. suum* binds exceptionally well to oxygen, it does not readily release it, even in a vacuum, and thus likely serves to maintain anaerobic conditions within the nematode rather than facilitate aerobic activity. Possession of nemoglobins is the reason why many nematodes appear reddish in colour e.g. *Ostertagia* and *Teladorsagia* spp., which are sometimes therefore described as the brown stomach worms.

A further reminder of the relative complexity of nematodes is the demonstration of oxidative and reductive pathways that enable nematodes to detoxify harmful chemicals encountered in the host and also in the external environment. Reductive pathways may be more important in parasitic stages given the lower oxygen tensions involved. Indeed, initial attempts to identify oxidative catalysts such as cytochrome P450 were unsuccessful (Barrett, 1997); however, recent experiments suggest that nematodes do possess cytochrome P450 (Kotze *et al.*, 2006). Reductive and hydrolytic pathways are probably more important in the first steps of nematode metabolism (Barrett, 1997), followed by conjugation reactions with glutathione, a reaction catalysed by glutathione transferase. This latter enzyme also plays a role in nematode defence against host-derived reactive oxygen species, alongside superoxide dismutase, catalase and glutathione peroxidase (Callahan *et al.*, 1988).

Interestingly, recent work has suggested that nematode detoxification pathways play some role in allowing the worms to metabolise anthelmintic molecules. Solana and co-workers (Solana *et al.*, 2001) showed that cytosolic and microsomal fractions of *A. suum* were able to sulphoxidate albendazole to the

less active sulphoxide; however, only fractions from the trematode *Fasciola hepatica* could oxidise the sulphoxide to the fully inactive sulphone. An additional mechanism that equips nematodes to deal with xenobiotics is the possession of the ATP-binding cassette (ABC) transporters. These molecules actively pump foreign substances out of cells and are well represented in nematodes, including *C. elegans*. *C. elegans* has three families of ABC transporters (Lespine *et al.*, 2008), the P-glycoproteins for which there are at least 14 separate genes, and the HAF and MRP families. Homologues of various *C. elegans* transporters have been found in parasitic nematodes including *H. contortus* and *O. ostertagi*. Recent work has demonstrated that treatment of *H. contortus* with macrocyclic lactone anthelmintics selects the constitutive or inducible overexpression of at least five P-glycoproteins (Prichard & Roulet, 2007).

The dauer larva

In response to adverse environmental factors – declining food availability, increased competition, or desiccation – many free-living nematodes are able to generate an alternate life-cycle stage that is better suited to outlast the period of adversity and resume normal development once conditions improve. This typically involves the development of a third larval stage that is structurally, behaviourally and biochemically distinct from normal L3 (Burnell *et al.*, 2005; Elling *et al.*, 2007). The cuticle of these more 'enduring' (dauer) larvae is modified (is more protective and better able to resist desiccation); the gut is non-functional and movement is usually considerably curtailed. Further development does not proceed whilst conditions remain adverse. Dauer larvae of free-living nematodes also show a pronounced phoretic association with other invertebrates such as dung beetles and use these larger, more mobile organisms as a means of reaching new food supplies. The pathways and processes controlling entry and exit to and from the dauer stage are undoubtedly complex, but include sensory information received as far back as the L1 stage. Such input includes nematode pheromones, which obviously increase in concentration in times of overcrowding.

Many view the infective L3 of GIN as a dauer equivalent and consider the ability to form the dauer stage as an important pre-existing adaptation of free-living nematodes for their eventual transition to parasitism – having a dauer stage facilitating the 'sit and wait' approach necessary for infective stages to persist in the environment prior to encountering and infecting a suitable host. There are however many differences between the true dauer larvae and the infective stages of nematode parasites (Elling *et al.*, 2007), suggesting that the two may have developed independently or that there has at least been considerable divergence in biology since free-livers and parasites last shared a common ancestor.

Anhydrobiosis

Terrestrial nematodes have adapted to cope with highly varying levels of environmental moisture. Obviously, relative humidity is an important factor as it predicts

the rate at which soils, faecal material etc. will dry out, but the main variable is the presence or absence of liquid water. As water disappears, nematode activity reduces and they become quiescent; all movement and activity ceases and the worm's tissues begin to dehydrate. For many nematodes, marked water loss proves fatal, although some have developed the ability to survive almost the complete loss of body water (anhydrobiosis). The ability of individual nematodes to undergo anhydrobiosis can be expected to vary widely, with those species that inhabit desiccation-prone environments more likely to evolve this trait than others. Likewise, species vary in their ability to tolerate fast or slow rates of water loss, with the probable majority requiring a slower rate. A slow rate of desiccation is thought to allow nematodes the time to manufacture chemicals such as trehalose and glycerol, which protect membrane integrity in the absence of water. Coiling behaviour helps slow the rate of water loss by reducing surface area, some nematodes aggregating in clumps to achieve the same effect. Likewise, some rely on additional features to slow the rate of desiccation – the presence of the sheath for infective L3 or, for unhatched stages, the eggshell. Several workers have shown that as the cuticles and sheaths of nematodes dry they become less permeable, thus further slowing rates of desiccation (Bird & Bird, 1991).

While anhydrobiotic nematodes are metabolically inactive, recovery from anhydrobiosis can be exceptionally rapid, locomotion returning in as little as 2 hours once liquid water returns.

Lettini and Sukhdeo (2006) examined the ability of four nematode species to undergo anhydrobiosis. Whilst *H. contortus* and *T. colubriformis* L3 could undergo several cycles of desiccation/rehydration, L3 of two rodent strongylids, *Heligmosomoides polygyrus* and *N. brasiliensis*, were killed by a single desiccating event.

The nematode life cycle

Across most nematode groups the life cycle is largely identical, with sexually dioecious adults (occasionally parthenogenic females or self-fertilising hermaphrodites) producing eggs out of which a larval stage emerges that grows via a series of four moults until the adult form is again achieved. Moulting allows the identification of four distinct larval stages (L1–L4). Some texts refer to a fifth larval stage – the L5 – essentially the immature adult, but since no further moult is involved in its further maturation, this term is not used here. Some (plant nematodologists especially) prefer the use of the term juvenile instead of larva, but as pointed out by Bird and Bird (1991) this largely anthropomorphic term has no strict parallel in biology, and by definition, the term juvenile does not implicitly rule out reproductive capability.

Pre-parasitic development

Few nematode parasites can complete their life cycles entirely within the host. Instead, a defined stage of the life cycle will leave the definitive host and pursue

further development in the environment, sometimes in one or more additional host species. Development proceeds to another defined life-cycle stage capable of infecting the definitive host. For most strongylid nematodes parasitising livestock, it is the egg, at various stages of development, that exits the host; occasionally, as in lungworms such as *Dictyocaulus viviparus*, it is a newly hatched larva (L1). For most strongylid nematodes, eggs hatch whilst still in the faeces and larval development proceeds as far as the L3. It is this stage that exits the faeces and is infective for the definitive host. For some parasites, the L3 develops within the egg. For this to occur, all of the required nutrients for larval development must have been invested in the egg *in utero*. The L3 may then remain within the egg until the egg is ingested by a suitable host, as occurs with the ascarids, or the L3 may emerge into the environment (e.g. *Nematodirus* spp.). Development of the embryo is fuelled principally by its lipid stores and thus requires oxygen, and levels of dissolved O_2 in gut fluid are probably too low to allow much development before the egg exits the host animal in its faeces. Strongylid eggs appear in freshly voided faeces with the embryo still in the morula stage.

The fully formed L1 may develop in as little as 24 hours and then hatching takes place, presumably once a defined level of development has been achieved. Hatching involves an increase in the permeability of the eggshell, which allows an intake of water into the egg, increased hydration of the L1 and the exit of trehalose out of the egg (Perry, 2002). Emergence of the L1 out of the egg requires the muscular activity that only a fully hydrated larva can generate and is probably assisted by various enzymes including lipases, chitinases and metalloproteinases. In many instances, the eggshell softens prior to hatching and the action of the anterior, or posterior, end of the L1 forces an opening.

At approximately 300-μm long, emerged L1 quickly commence moving and feeding within the faecal material. As stated earlier, the L1 and L2 are both microbivorous, feeding on bacteria present in the faeces.

There are two moults in the pre-parasitic phase of development – the L1 to the L2 and then the L2 to the L3. Moulting is essentially a multi-stage process. After a period of feeding, the larva typically enters a period of inactivity (lethargus). Next, the old cuticle separates from the epidermis (apolysis), which then proceeds to secrete a new cuticle. The next stage (ecdysis) involves rupture of the old cuticle and is followed by the emergence of the subsequent life-cycle stage. Characteristically for many parasitic nematodes, apolysis occurs during the second moult, but ecdysis is delayed, and the larva retains the cuticle of the L2. The L3 is therefore smaller than the L2. For most strongylids the L2 (and hence L3) is between 600 and 1000 μm long.

With its retained sheath, the ensheathed L3 cannot feed and represents a point of arrest in the nematode life cycle – there will be no further development until a host is encountered. The L3 is however still active and begins to migrate away from the faeces into the external environment. The gut of an L3 is non-patent, but the intestinal cells are packed with stored metabolites and are usually clearly visible. The number of intestinal cells can be used to identify the L3 of different species, as can the length of the gap between the tip of the tail of the L3 and the actual termination of the sheath.

With a significant part of the life cycle spent outside the host animal, environmental factors have a major impact on developmental success. Predictably, moisture levels, temperature and the availability of oxygen are the key drivers, affecting not only how quickly eggs hatch and larvae develop, but also how long larvae (and eggs) survive (Chapter 3). In changing from a feeding stage (L2) to the infective stage (ensheathed L3) nematodes change their behaviour dramatically, from actively feeding to migration away from the food source. The factors that trigger this behavioural change are poorly understood, but those that affect its success are better known.

As stated earlier, the presence of liquid water is vital for nematode activity. Free-living larvae move through the fluid phase of the faecal material and once the third larval stage has been achieved, a film of moisture on the surface of the adjacent vegetation is an absolute requirement for migration away from the faeces and onto herbage. The depth of the moisture film, in comparison to the diameter of the nematode, is critical in determining how rapidly nematodes can make progress, too little or too much, and nematode movement slows and eventually stops (Burr & Robinson, 2004). Solid masses of faeces retain moisture even when the adjacent pasture is relatively dry. This plays an important role in facilitating the survival of larvae over shorter dry periods. The hard crust that develops on the dung exterior reduces evaporation, but also prevents larvae from escaping, and mechanical disruption of the faeces may eventually be required to allow larvae to escape. In contrast to normal faeces, watery, diarrhoeic faeces offer little protection against desiccation. Experimentally, nematodes have been shown to be capable of surviving dehydration to a remarkable degree, yet in the field, prolonged desiccation is widely recognised as one of the few phenomena to seriously deplete free-living stages.

Oxygen is required for hatching and larval development. In the solid dung mass, oxygen levels, particularly towards the centre, may in fact be limiting, which slows development. Disruption of the pat increases the availability of oxygen, and in moist and warm conditions, accelerates development. Once the ensheathed L3 has exited the dung, the availability of oxygen increases and is presumably no longer limiting.

At colder temperatures (10°C and below) nematode biology slows dramatically and at some point all development and activity ceases. Within a particular species, genetic variation allows some individuals to be more cold-tolerant – or, conversely, more heat-tolerant – than others, and this allows climate-adapted strains to eventually develop. Experimentally, eggs and larvae remain viable for extended periods at sub-zero temperatures, but survival will eventually be curtailed. Freezing represents the greatest threat, with the formation of ice crystals causing considerable damage to membranes and structures. Nematodes may either be freeze-resistant or freeze-tolerant. Unhatched eggs and some ensheathed L3s may supercool at freezing temperatures, i.e. ice crystals do not form. This is particularly true in the absence of liquid water, which prevents inoculative freezing – the extension of ice crystals by direct contact. The eggshell, and to a lesser extent the extra sheath of infective L3s, can be a barrier to inoculative freezing. Free-living larval stages may achieve a degree of freeze-tolerance by utilising molecules such as trehalose and glycerol

as cryoprotectants (Wharton, 2002). Ultimately, however, the more freeze–thaw cycles nematodes undergo, the more likely they are to die.

The optimum temperature for development is generally quoted as being around 25°C. At higher temperatures, metabolic activity increases further, but mortality markedly increases. Thermotolerance has been shown for a number of nematode species (Wharton, 2002) and may be partly mediated by classic heat-shock proteins (HSPs), which can both protect proteins against denaturation and repair damaged proteins. At sub-lethal, warm temperatures, increased activity depletes the stored nutrient reserves of larvae and thus ultimately reduces longevity; the optimum temperature for survival of ensheathed larvae is thus back at around 10°C. The epidemiological significance of the development and survival of eggs to L3 of the major GIN species is discussed in Chapter 3.

The parasitic phase of the life cycle

The parasitic phase of the life cycle can only commence when the L3 encounters the host. For many species, this is a largely passive process – the grazing animal inadvertently ingesting larvae as it feeds. Once in the host, the first step in the transition to the parasitic phase is the completion (ecdysis) of the second moult, i.e. loss of the retained sheath – exsheathment. Exsheathment is triggered by the chemical conditions present in the proximal gastrointestinal tract of the host.

It is generally assumed that exsheathment typically occurs in the part of the gut immediately anterior to the actual site occupied by the adult parasite; thus abomasal parasites exsheath in the rumen, whereas small intestinal species exsheath in the abomasum. However, a considerable proportion of the L3s of small intestinal species may still exsheath in the rumen (Hertzberg *et al.*, 2002). In the rumen, the equilibrium between bicarbonate (HCO_3^-) and carbonic acid (H_2CO_3) at the near neutral pH of rumen fluid, and the relatively high levels of CO_2 are thought to be pivotal to exsheathment. In contrast, in the abomasum, the presence of pepsin/HCl is considered important. As with other moults in the life cycle, exsheathment is an active process involving the activity of enzymes on the cuticle and the physical activity of the larva to achieve egress. A number of enzymes may be contained in the so-called exsheathing fluid secreted by the larva (Lee, 2002). For example, a zinc metalloproteinase has been identified, which attacks a specific annular portion of the sheath of *H. contortus* L3 (Gamble *et al.*, 1989). Its action causes the anterior end of the sheath to break off and the L3 then emerges – other secreted substances may act as lubricants. Amongst the parasite-derived factors influencing exsheathment are eicosanoid metabolites such as leukotrienes (LT). Administration of the lipoxygenase inhibitor diethylcarbamazine to ensheathed L3 of *Oesophagostomum dentatum* caused the complete inhibition of exsheathment (Joachim *et al.*, 2005). This inhibition was reversible by washing or the administration of LT. Exsheathment can be rapidly triggered *in vitro* using solutions of hypochlorite.

Exsheathment generally occurs rapidly and the exsheathed larvae may start to appear in more distal parts of the gut within 24 hours of infection. There is some evidence that persistence of larvae in the rumen for beyond 12–24 hours increases their mortality (Hertzberg *et al.*, 2002).

Upon entering the abomasum, the larvae of species such as *Haemonchus* spp., *Ostertagia* spp. and *Teladorsagia* spp. immediately penetrate into the pits and glands of the mucosa. This may preferentially occur in either the fundic or pyloric areas although in general the fundic mucosa is more frequently targeted. Once confined within the pits/glands the L3 presumably commences feeding, but on what precisely is not known. All undergo at least the next moult to the L4 within this mucosal niche. The L4 of *Haemonchus* is thought to then emerge onto the mucosal surface and completes its development here (Charleston, 1965), whilst *Ostertagia* and *Teladorsagia* spp. remain in the pits/glands to undergo the final moult. Similarly, larvae of small and large intestinal species penetrate into the glands and crypts of the intestines. Some, e.g. *Oesophagostomum* spp., may penetrate through the epithelium to the lamina propria of the submucosa (McCracken & Ross, 1970). This has important immunological considerations since these more invasive larvae arguably expose more of themselves to the host's immune system, whilst the majority of other larvae remain on the other side of the epithelium.

The mucosal phase of development is sometimes referred to as the histotropic or histotrophic phase. These terms suggest a much closer association with the cells of the host than typically occurs and are probably more appropriately applied to the life cycles of parasites such as *Trichuris* spp. and *Trichinella* spp., which do actually penetrate and feed on individual cells.

The reasons GIN enter this mucosal phase are poorly understood. By encysting in the mucosa, larvae may access higher oxygen levels or alternate substrates. Some see mucosal invasion as a relic of the evolutionary past of nematodes, reflecting the ancestral behaviour of nematodes as necessarily migratory following invasion across the skin. An alternative reason may be that by encysting, larvae are able to undergo the lethargus associated with moulting without risking losing their place in the gut; this implies that emerged nematode stages must actively maintain their position and that any reduction in activity, including the paralysis caused by anthelmintics, threatens this. However, the observation that *Haemonchus* is able to undergo the final moult on the mucosal surface must make this open to conjecture.

Hypobiosis

In some circumstances, parasitic development may become arrested. Arrested development (sometimes referred to as inhibited development or hypobiosis) has been defined as *the temporary cessation of development of nematodes at a precise point in early parasitic development when such an interruption contains a facultative element, occurring in certain hosts, certain circumstances or certain times of the year and often affecting only a proportion of the worms* (Michel, 1978). True hypobiosis needs to be carefully distinguished from the

apparent delay/interference in development that can manifest as a slower transition between stages or the stunting of eventual adults that can occur when larger numbers of worms are competing for limited resources (Hong *et al.*, 1986) or due to immune interference in development. For many arrest-prone species, but not all, a cessation of growth occurs early in the fourth larval stage, and hypobiotic larvae have little, but variable, metabolic activity. The underlying genetic and physiological mechanisms responsible for parasites either entering or leaving the hypobiotic state are poorly understood. Hypobiosis is discussed more fully in Chapter 3.

Pre-patent periods

The pre-patent period is defined as the time taken from ingestion of larvae/eggs to when the infection becomes patent, i.e. the first appearance of eggs/larvae in the host animal's faeces. Given that the conditions parasites encounter in the host are far more stable than those in the environment, development within the host, assuming that the nematodes do not undergo hypobiosis, generally proceeds at a fairly predictable rate and for many of the important GIN, eggs first appear in the faeces of infected animals 2–4 weeks after infection, although for some species, e.g. some *Cooperia* spp., this may occur slightly sooner (Anderson, 2000).

Niches occupied by parasitic nematodes within the vertebrate host

The gut of the vertebrate host consists of numerous niches, which GIN can occupy. In addition to the different organs themselves, there are several available niches within each organ – mucosal tissue, a layer of secreted mucus, and the digesta present within the lumen.

Larger parasites such as the ascarids generally occupy the lumen of the gut. They are better able to resist peristaltic contractions and thus maintain their position in the small intestine. Smaller parasites such as the trichostrongyloids would struggle to do this and if present in the luminal contents would undoubtedly be swept along with the bulk flow of digesta and hence out of their preferred organ and even out of the animal. Instead, the trichostrongyloids are found in much more intimate association with the mucosal surface. Some species such as the abomasal parasites *Teladorsagia* spp., *Ostertagia* spp. and *Haemonchus* spp. can be found within the mucus layer covering the mucosal surface (Figure 1.2).

In contrast, *T. axei* pushes between the epithelial cells of the superficial mucosa and persists in the tunnels it creates.

Interestingly, whilst *Teladorsagia* spp., *Ostertagia* spp. and *Haemonchus* spp. all have obvious synlophes, *T. axei* does not. It may be that the synlophe assists in allowing those nematodes that have it to better embed themselves within the mucus. The mucus layer of the abomasum is, however, of finite

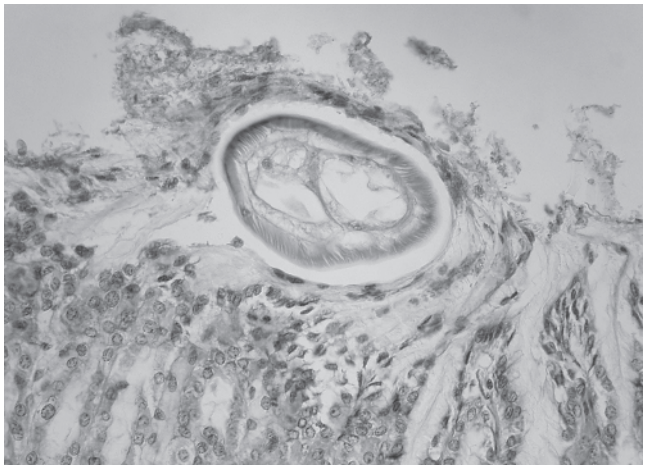

Figure 1.2 Section of abomasal fundic mucosa from a sheep infected with *T. circumcincta*. A cross section of an adult nematode can be seen on the mucosal surface and appears embedded in a tunnel of mucus and cellular debris.

dimensions. Since it is seldom preserved with routine histological methods, there is little information on the depth of the mucus layer within the abomasa of ruminants, but studies in humans have estimated a depth of approximately 100 μm for the basal, firmly adherent mucus layer in the gastric fundus, with a similar depth of loosely adherent mucus above this, and the total mucus depth increases to closer to 300 μm in the human pylorus (Atuma *et al.*, 2001). It is likely that similar dimensions are involved in the ovine and bovine stomach, and thus the depth of the mucus layer is broadly similar to the diameter of the trichostrongyloid nematodes that infest it – effectively the worms are tunnelling within the mucus layer itself. This raises the question of whether larger nematode species could be accommodated at all.

In the small intestine, the other species of *Trichostrongylus*, e.g. *T. colubriformis*, which also lack a synlophe, reside for at least part of their length in tunnels within the epithelium (Figure 1.3), whereas synlophe-bearing genera such as *Nematodirus* and *Cooperia* adopt an entirely different strategy. These nematodes appear to entwine themselves around the villi (Durette-Desset, 1985) using the ridges of the synlophe to grip the villous epithelium, and it is of note that specimens of both *Nematodirus* spp. and *Cooperia* spp. are often visibly coiled when freshly recovered from small intestinal washes.

The different intestinal species also vary in their precise location along the gut with *Trichostrongylus* spp. considered to prefer a more anterior niche in comparison to *Cooperia* spp. or *Nematodirus* spp. (Davey, 1938; Sommerville, 1963; Tetley, 1937). Interestingly, the luminal pH of the anterior small intestine continues to be acidic for some distance away from the pylorus. Eventually, the pH is brought to near normal by the secretion of bicarbonate. Davey (1938) cited pH and the presence of bile salts as two factors influencing predilection sites.

When 30,000 *T. vitrinus* and *T. colubriformis* L3 were co-administered to sheep, *T. vitrinus* preferentially established in the most anterior part of the gut,

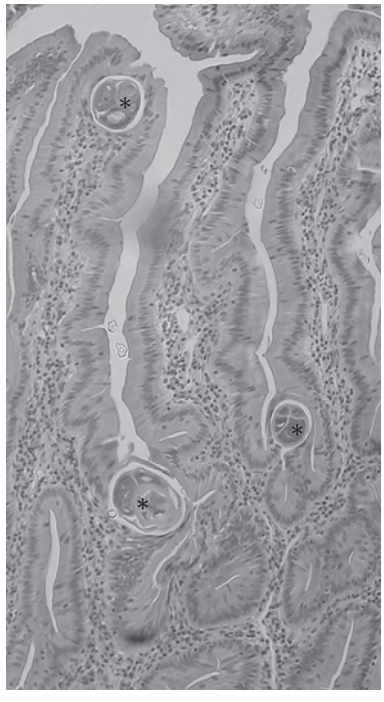

Figure 1.3 Section of proximal small intestine of a goat infected with *T. colubriformis*. Cross sections of adult nematodes (*) can be seen between the epithelial cells of the villi.

displacing *T. colubriformis* posteriorly (Roy *et al.*, 2004). In addition, fewer *T. colubriformis* established, however, at lower larval doses, this competition was not evident. In contrast, when *T. vitrinus*, *T. colubriformis* and *T. rugatus* were co-administered, *T. colubriformis* and *T. vitrinus* did not compete and established in equal numbers in the anterior small intestine, but *T. rugatus* was displaced (Beveridge *et al.*, 1989). In calves infected with *C. oncophora* (Armour *et al.*, 1987), the majority of worms were present in the duodenum, but in at least one animal there was a posterior shift of the population with the bulk of the nematodes in the more distal small intestine, with approximately equal numbers of worms in the jejunum and ileum.

A posterior shift in worm population has also been reported in association with anthelmintic use in instances of anthelmintic resistance. In one study (Bogan *et al.*, 1988) worm counts were performed on sheep infected with *C. curticei*, some of which had been treated with ivermectin. Efficacy of treatment was calculated 7 and 14 days post-treatment at 61 and 90%, respectively. In performing the worm counts, the small intestine had been divided into four equal quarters, and whilst most worms were present in the first half of the gut of the untreated control animals, post-ivermectin, more worms were recovered from the third and fourth quarters. The hypothesis advanced was that the ivermectin successfully paralysed the resistant parasites, which therefore lost their grip of the mucosa and were swept down the gut, but once they had recovered motility, they were able to re-establish contact with the mucosa.

The particular niche occupied by the various species of GIN may ultimately influence which immune responses will be more effective in expelling individual GIN species. Nawa *et al.* (1994) compared the immune expulsion by rats of *Strongyloides* spp., which are burrowing nematodes, and *N. brasiliensis*, a nematode that wraps itself around villi utilising a very well-developed synlophe. Whilst mast cell function was seen as pivotal for expulsion of *Strongyloides* spp., hyperplasia of goblet cells was required for the elimination of *N. brasiliensis*. If similar mechanisms operate in the ruminant small intestine, the immune mechanisms required to expel *Trichostrongylus* spp. may be quite different from those required to deal with *Cooperia* spp.

The lifespan of parasitic nematodes

The lifespan of free-living nematodes such as *C. elegans* is relatively short, most dying within 1–2 months. Temperature has a marked effect on survival with increased lifespan at cooler temperatures (32 days at 16°C vs. 11 days at 25.5°C) (Klass, 1977). Some free-living species, such as the vinegar eels, *Turbatrix aceti*, achieve much longer lifespans. Vogel (1974) maintained *T. aceti* at 15°C for over 200 days, whereas at 30°C this reduced to 70 days. Interestingly, dietary restriction prolongs lifespan in nematodes (Sutphin & Kaeberlein, 2008), whilst reducing fecundity. Thus it might be expected that well-fed parasitic species, kept at the high temperatures of their mammalian hosts, would also be relatively short-lived, yet the longest nematode lifespans recorded are for parasitic species. Human hookworms have been recorded as living for as long as 15 years (Gems, 2002), whereas the filarial parasite, *Dirofilaria immitis*, the canine heartworm, may live as long as 7 years. In contrast, some parasites continue to have fairly short life expectancies. Many of the important GIN species of livestock probably live only for a few months. An important determinant of how long nematodes can persist in the host is, of course, host immunity. Many of the adults of the ascarid parasites are rejected once the host has reached approximately 6–12 months of age even though the host developed the ability to reject or limit the development of newly acquired larvae much earlier. For young grazing ruminants, immunity may not limit the lifespan of some of the first nematodes to establish, but it will certainly eliminate others that are acquired subsequently.

Knowing the lifespan of parasites can be important for several reasons. For example, in the context of the development of anthelmintic resistance, long-lived survivors of a drench may be capable of releasing resistant progeny for a prolonged period. Or, in the case of immune status, an ability to prevent the establishment of the L3 of short-lived parasite species may be relatively more important than removing the mature adults. It remains unclear as to what extent the relative pathogenicity of the individual GIN species determines the length of survival, or indeed how quickly protective immunity may develop. These concepts are discussed in more detail in the subsequent chapters.

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