CHAPTER 1
Veterinary Parasitology: basic concepts

1.1 Introduction

The primary aim of this book is to provide a 'student-friendly' introduction to Veterinary Parasitology for those aspiring to become veterinarians, veterinary nurses or veterinary scientists. It also offers an accessible resource for those already qualified and wishing to refresh or expand their general knowledge of the topic. Others engaged in the many and varied facets of animal health and veterinary public health will also find information relevant to their interests.

This first chapter explores the nature of parasitism while Chapters 2–7 examine clinically relevant relationships and interactions between the parasite, its host and the environment. Finally, Chapters 8 and 9 recognise that, in the real world, veterinarians and animal health workers are not usually presented with a parasite as such, but with a problem concerning some bodily dysfunction affecting a flock, herd or individual.

To fulfil the aims of this book, the emphasis throughout has a clinical bias. Academic information is restricted to that necessary to gain a broad understanding of the pathogenesis, epidemiology, diagnosis and control of the commonest parasitic diseases. Key words are defined in the text or, if printed in a blue typeface, explained in a nearby 'Help box'. A glossary is provided on the website that accompanies this book.

Wherever possible, concepts are described in straightforward language, and unnecessary jargon or detail is avoided. Further aids to learning are provided in 'Help boxes', while

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Companion website: www.wiley.com/go/jacobs/principles-veterinary-parasitology

'Extra Information Boxes' offer additional insights for more advanced readers. Cross-references within the book are given in the format (see Section 9.2.3), (see Table 9.10) etc. These are to assist readers who may wish to follow up on particular points, but they can otherwise be ignored.

The emphasis with regard to parasite identification and the diagnosis of associated disease is on 'how it's done' rather than 'how to do it'. Latin names and taxonomic relationships are introduced only where these provide a useful foundation for comprehension, learning or further reading. The number of parasites that might be encountered in veterinary practice is so great that to mention them all would transform this 'guide to learning' into an encyclopaedia, which would defeat the purpose of the book. Selected examples are therefore given to provide an understanding of underlying principles and to illustrate the range and diversity that exists within the wonderful world of Veterinary Parasitology.

Help box 1.1

Definition of some key technical terms

Aetiology/ aetiological agent: the cause or origin of a disease.
Biotic potential: an expression of the rate at which a parasitic species can multiply. It depends on the number of offspring produced ('fecundity') and the number of generations each year ('generation time').
Endemic: a term used to describe a population or area within which a pathogen is established, replicating and being transmitted between hosts.
Epidemiology: the science that describes and explains patterns of disease in the host population (i.e. the distribution and determinants of disease).
Eukaryote: an organism with a cytoskeleton and complex subcellular structures enclosed within membranes (including a nucleus containing chromosomes). Examples: protozoa and metazoa.
Incidence: the number of new cases of infection per unit time.
Pathogen/pathogenicity/pathogenesis: an organism that causes disease / the severity of the damage caused / the mechanism of the disease process.
Prevalence: proportion of host population infected at a point in time.
Prokaryote: an organism without a nucleus or other membrane-bound subcellular structures; DNA in circular plasmid. Example: bacteria.
Species: the basic unit of biodiversity. Although everyone knows what a species is, there is no exact definition as boundaries are often blurred. Two commonly cited definitions are: 'a group of organisms capable of interbreeding and producing fertile off-spring' and 'a separately evolving lineage that forms a single gene-pool'.
Taxonomic: relating to the laws and science of describing, identifying, naming and classifying organisms.

1.1.1 What is Veterinary Parasitology?

Animal disease can have noninfectious or infectious origins. Noninfectious diseases result from genetic defect, physiological abnormality, structural dysfunction or external factors such as injury, radiation or poisoning. In contrast, infectious diseases are associated with invasive self-replicating agents that have evolved to occupy an animal body as their ecological niche in just the same way as a koala bear has become adapted for life in a particular species of *Eucalyptus* tree.

By convention, the study of infectious agents is divided into Microbiology, which embraces noncellular and prokaryotic organisms, like viruses and bacteria, and Parasitology, which is concerned with eukaryotic life-forms. Fungi are an anomaly in this scheme as, although they are eukaryotes, they are traditionally taught as part of Microbiology in most veterinary schools and so have been omitted from this book.

Veterinary Parasitology is a composite of three distinct disciplines, each with its own set of host–parasite interactions, clinical considerations and vocabulary. The three topics that make up the bulk of Veterinary Parasitology are:

a – Veterinary entomology: the study of parasitic arthropods, including insects, ticks and mites (see Chapters 2 and 3);

b – Veterinary protozoology: a subject that embraces the wide range of single-celled eukaryotic organisms that comprise the parasitic protozoa (see Chapter 4);

c – Veterinary helminthology: which covers three main groups of parasitic worms – trematodes (flukes), cestodes (tapeworms) and nematodes (roundworms), as well as some minor groups such as the thorny-headed worms (see Chapters 5–7).

1.2 Parasitism and parasites

1.2.1 Parasitism

Parasitism is part of a spectrum of intimate zoological relationships between unrelated organisms which includes:

a – Commensalism: two species living together for the benefit of one or both, but without detriment to either

party, and without any metabolic dependence (e.g. cattle egrets and cattle).

b – Symbiosis: two species living together, each dependent on the other for their mutual well-being and survival (e.g. cellulose-digesting organisms in the caecum of a horse).

c – Parasitism: two species living together, where one of the pair (the parasite) is living at the expense of the other (the host).

d – Parasitoidism: two species living together as in parasitism except that the host invariably dies (or is at least rendered incapable of functioning) once the parasitoid has extracted the sustenance it needs for that stage of its development. Familiar examples include parasitoidal wasps used in horticulture that lay their eggs on or in other insects to provide a food-source for their larvae.

Parasitism implies nutritional dependence on the host for at least part of the life-cycle. It also involves a high degree of specialised adaptation as the animal body is not a passive ecological niche (like a rotten tree-trunk harbouring beetles, for example) but is responsive and hostile to foreign invasion. A parasite must be able to overcome host defences and evade immunological attack. Mechanisms must also be in place to ensure transfer of infection, both geographically from host to host ('horizontal transmission') and temporally from generation to generation ('vertical transmission'). This often entails an intricate integration of the life-cycle of the parasite with that of its host.

Parasites can themselves be victims or beneficiaries of invading organisms. Fleas, for example, are exploited by larval stages of both tapeworms and nematodes, while the canine heartworm, *Dirofilaria*, is metabolically dependent on a symbiotic bacterium, *Wohlbachia*.

1.2.2 Classification

The unwise student could approach every parasitic infection as a separate entity, but this would be an enormous task and a very inefficient approach to learning. It would soon become apparent that similarities exist between some diseases and this would prompt the question: 'what are the common factors?' So, classification is an inherent attribute of human curiosity. It has been noted already that Veterinary Parasitology embraces at least three types of arthropod, several types of protozoa and at least three types of parasitic worm, and so the value of classifying aetiological agents of disease is already becoming apparent.

Taxonomy is a powerful and essential component of biological understanding, although, from a clinician's viewpoint, it is a tool rather than an end in itself. Knowledge of the relationship between parasites often allows similarities in life-cycle, epidemiology, pathogenesis and drug susceptibility to be predicted. Thus, if used intelligently, classification provides a valuable framework for learning and reduces considerably the amount that has to be committed to memory. The classification in this book is kept at the simplest level compatible with this objective.

Help box 1.2

Classification

The animal kingdom is divided into some 35 phyla (singular 'phylum'), which in turn are subdivided successively into Class, Order, Family, Genus and Species, with a species being the basic replicating entity. Subclass, Suborder and Superfamily groupings are also useful in some contexts. Relationships are deduced from morphological, biological and, more recently, molecular evidence and so taxonomic charts (and, confusingly, parasite names on occasion) have to be revised as knowledge accumulates. This can lead to discrepancies between different information sources.

Nomenclature

The identity of every organism is defined by using a combination of its genus and species names. Thus, the protozoan parasite that causes redwater fever in northern European cattle is *Babesia divergens*, while the related species *Babesia bovis* and *Babesia bigemina* cause similar diseases in warmer regions. By international agreement, the ending -osis is placed on a parasite name to indicate the disease caused by that parasite, e.g. babesiosis. By tradition, the ending -iasis is sometimes preferred in human medicine and may occasionally be found in veterinary publications.

It is sometimes useful in Veterinary Parasitology to refer to the common characteristics of a larger grouping of parasites such as a family, which always has a technical name ending in -idae (e.g. the Ixodidae, which is anglicised as 'ixodid ticks'), or even a superfamily with the suffix -oidea (e.g. the Trichostrongyloidea, which becomes 'trichostrongyloid worms').

Help box 1.3

Writing parasite names

When writing parasite names, the genus name always starts with a capital letter while the species name is lower case throughout. The convention in parasitology as in all biological disciplines is to italicise these. The first time a parasite is mentioned in a text, the full name is used, but thereafter the genus name is abbreviated, e.g. *Babesia divergens* becomes *B. divergens*. The word 'species' can be abbreviated to sp. (singular) or spp. (plural), so '*Babesia* sp.' means an unnamed *Babesia* species, while '*Babesia* spp.' refers to more than one species in that genus.

Why use Latin names?

Latin names are universal, whatever language is being used for communication. Local names can be parochial (for example, babesiosis is known as 'Red-water fever' in the UK but as 'Texas fever' in the USA) or ambiguous ('sand-fly' for example refers to phlebotomine sand-flies in most countries, but is the colloquial term for biting midges in some others).

Pronouncing Latin names

There is no right or wrong way to pronounce a Latin scientific name. Some are tongue-twisters and with these it helps to know how the word can be broken down into syllables. Some of the most troublesome Latin names are listed in the Pronunciation Guide on the website that accompanies this book.

1.2.3 Host–parasite relationships

Parasites and their hosts have evolved together over many millions of years. Every host is vulnerable to infection by several, if not many, parasitic species. Thus, there are many more parasitic species on this planet than host species! It is not surprising, therefore, that a great diversity of host–parasite relationships exists. These are often amazingly intricate and are part of the fascination of parasitology, as will become apparent when the life-cycles of individual parasites are described in later chapters.

Parasites

Parasites can be broadly categorised according to their location on or in the body of their host:

a – Ectoparasites: live or feed on the surface of the host, or embed themselves into superficial or adjacent underlying tissues. Ectoparasites engage in host–parasite associations ranging from flies that land fleetingly to feed on secretions from the eyes, nose or other orifices to mites that spend nearly their whole lives in skin tunnels.

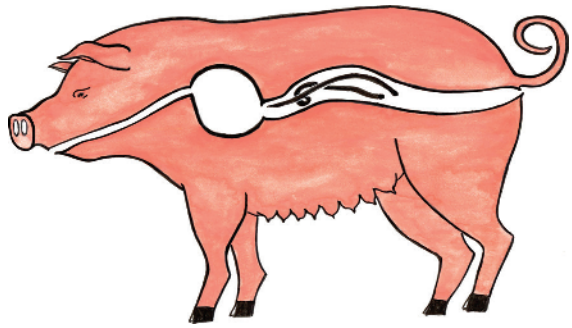

Figure 1.1 Gastrointestinal parasites such as the worms depicted here in black are technically 'outside' of any body tissue.

b – Endoparasites: live within the body of the host. Parasites may be found in every body tissue except, perhaps, bone and keratin. Those free in the lumen of the gastrointestinal tract are, technically speaking, lying outside of any host tissue (see Figure 1.1), but they are nevertheless included in this category.

A fundamental distinction that influences both the pathogenesis of infection and options for control is the relationship of the parasite to the tissue it inhabits:

a – Extracellular parasites: these live on or within host tissues but do not penetrate into host cells. Examples include almost all metazoan and also many protozoan parasites.

b – Intracellular parasites: these live inside a host cell modifying its genomic expression to cater for their needs, e.g. many protozoan parasites and at least one nematode genus (*Trichinella*).

Parasites can also be differentiated on the basis of their reproductive behaviour in the final host (see Figure 1.2). This distinction is useful as it points towards fundamental biological differences that influence pathogenesis, epidemiology, control and treatment:

a – Microparasites: these multiply within their host. Consequently, each organism that enters the body is capable of initiating a massive infection if not checked by host defences or by chemotherapy. This category includes the parasitic protozoa (as well as microorganisms such as bacteria).

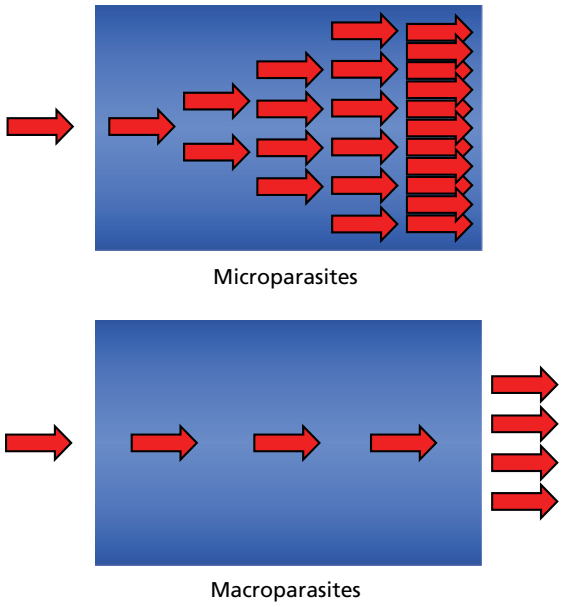

Figure 1.2 Microparasites (above) multiply their numbers within the host; whereas the number of mature macroparasites (below) never exceeds the number that invaded the host (with a few exceptions).

b – Macroparasites: these do not generally increase in number while they are on or within the final host. They may produce eggs or larvae but these are dispersed into the environment. Thus, the number of mature parasites on or in the final host never exceeds the number of infective units that originally invaded the body. This category includes arthropods and helminths, although there are a few species that break the general rule by multiplying on or in the host (for example: lice, mites and a few nematodes, e.g. some *Strongyloides* species).

c – Microcarnivores: these visit the host transiently to feed but leave again before undergoing any development or producing offspring. Many parasitic arthropods, such as mosquitoes, can be included in this designation.

With such a diverse spectrum of host–parasite associations, there are inevitably some organisms that do not fit conveniently into these broad groupings.

Hosts

Some parasites require just one host to complete their developmental cycle and produce progeny. Others utilise two or more animals. Hosts can be exploited in different ways and the following terminology is used to differentiate between these:

a – Final (or definitive) host: a term used to identify the host in which sexual reproduction of the parasite takes place.

b – Intermediate host: this is a host in which only immature stages grow and develop. Asexual replication may occur (but not sexual reproduction).

c – Transport and paratenic hosts: no parasitic development of any kind takes place in these and they are not a necessary part of the life-cycle. The parasite takes advantage of another animal by using it as a vehicle to increase its chances of reaching its next essential host. The word 'paratenic' implies an intimate relationship in which the parasite becomes embedded within the tissues of its host. The corresponding association with a transport host is more casual and often passive in nature. The two terms are sometimes used interchangeably with less precision.

d – Reservoir host: as the name suggests, this depicts a host population that acts as a source of infection for other animals.

e – Vector: this is a vague term for an insect, tick or other creature that carries (transmits) a disease-causing organism from one host to another.

Life-cycles are described as being:

a – Indirect (or heteroxenous): if an intermediate host is involved; or

b – Direct (or homoxenous): if there is no intermediate host.

Zoonoses

Parasitic zoonoses are diseases of mankind associated with animal parasites (see Section 9.3). They can be classified according to the various biological pathways that lead to human infection (see Figure 1.3):

a – Direct zoonoses: direct transfer from animal to human, e.g. *Cheyletiella* mites from an infested lap-dog.

b – Cyclozoonoses: where humans infect animals and vice versa in strict rotation, e.g. the beef tapeworm.

c – Metazoonoses: these involve a vector as intermediary, e.g. phlebotomine sandflies carrying *Leishmania* from dogs to humans.

d – Saprozoonoses: indirect transfer via the environment, e.g. children playing on ground contaminated with *Toxocara* eggs from a dog or fox.

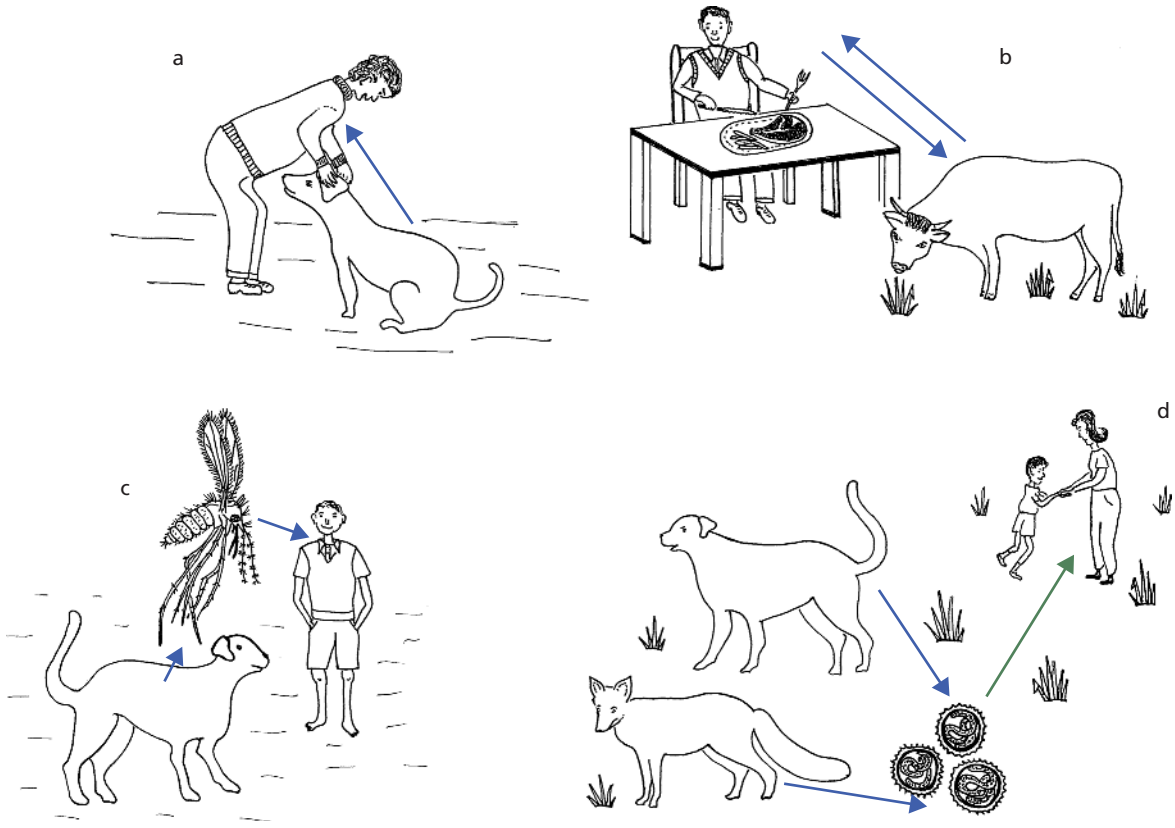

Figure 1.3 Ecological relationships that expose humans to zoonotic parasites: a – direct zoonoses; b – cyclozoonoses; c – metazoonoses; d – saprozoonoses (further explanation in text which uses same lettering as shown above). Sandfly redrawn after Mönnig from Lapage, 1962 with permission of Wolters Kluwer Health - Lippincott, Williams & Wilkins.

1.3 Host–parasite interactions

Hosts rarely gain any benefit from the presence of parasites and are often harmed by them. Defence mechanisms have therefore evolved which, if totally effective, would have extinguished parasitism as a lifestyle. But the continued existence of an abundance of parasites indicates that successful counter-strategies have arisen through natural selection. These in turn have driven the development of further protective measures and so the cycle known as the 'parasitic arms-race' continues. Coevolution has resulted in host–parasite interactions of such complexity that they can be reviewed only at a superficial level in an introductory text such as this.

1.3.1 Host defences

Hosts have evolved many behavioural and other strategies to reduce the risk of succumbing to parasitism. Herbivores, for example, will not eat the lush grass close to a faecal deposit where the greatest concentration of infective worm larvae occurs (the 'zone of repugnance'). The most powerful form of defence, however, is the immune system. This comprises a battery of chemical and cellular weaponry used to combat invasive organisms. Immune reactions may completely or partially disable the attacker or they may alleviate the clinical consequences of infection.

Ideally, immunity should protect against reinfection after the invading parasites have been eliminated. This is called 'sterile immunity'. It can last for a lifetime but often wanes with time. Sometimes, however, such protection persists

only as long as a few parasites survive to continually boost the immune processes. This is known as 'premunity'.

In some cases, parasite evasion has gained an evolutionary advantage that renders host immunity relatively ineffective, so the host remains vulnerable despite being repeatedly exposed to infection (e.g. sheep with liver fluke). Some immune reactions directed at a parasite can produce collateral damage to host tissues. Hypersensitivity and allergy are well-known examples.

Innate and acquired immunity

Vertebrates have evolved two separate but closely linked systems to provide protection against invasive pathogens. These are known as innate and acquired immune responses.

Innate immunity

The innate (or nonspecific) immune response is the body's first line of defence. It functions similarly whatever the nature of the invader and whether or not the host has experienced similar attack before. It comprises a series of natural physical, chemical and cellular barriers that are either permanent features (such as the integrity of skin and mucosae or the acidity of the stomach) or that can be quickly mobilised. The latter include a variety of cell-types with different modes of attack as well as humoral factors such as complement. A spectrum of communication molecules (cytokines and chemokines) released by white blood cells (leukocytes) enables the innate immune system to interact with the acquired immune system.

Help box 1.4

Some key immunological and pathological terms

Antibodies: macromolecules (immunoglobulins) produced by the host adaptive immune system to recognise specific receptor sites on alien molecules (antigens) and to initiate or assist in their neutralisation or destruction. There are different classes of antibody that are labelled IgM, IgG, IgE etc.
Antigen: molecule presented to a host that invokes an adaptive immune response.
Apoptosis: controlled and purposeful cell death (as opposed to necrosis, which is cell death due to an acute insult or injury, and autophagy, which is related to recycling cell components).
Chemokines: a specific class of cytokines that attract cells towards each other (chemotaxis), e.g. immune cells to the site of infection.
Complement: a biochemical cascade of small plasma and membrane-bound protein molecules that assist in the destruction of some invading organisms. One such cascade is a nonspecific innate response (the 'alternative pathway') while another is antibody-dependent (the 'classical pathway').
Cytokines: signalling molecules that cells use to communicate with each other. The term includes the interleukins (with names such as IL-2 and IFN-γ) that serve to modulate immune responses.
Eosinophilia: an increase in the number of eosinophils (white blood cells with red-staining granules) in the blood.
Humoral: a word used to describe aspects of immunity mediated by macromolecules in the blood or other body fluids (as opposed to cell-mediated immunity).
Hyperplasia: greater than normal proliferation of a particular cell type or tissue.
Lymphocytes: mononuclear white blood cells. There are several types: NK (natural killer) cells involved in innate immunity; B cells that produce antibodies; T cells involved in cell-mediated immunity, including Th (helper cells) that produce cytokines and cytotoxic cells that can kill parasitized host cells. There are also memory cells which enable pathogens to be quickly recognised on reinfection.
MHC: Major Histocompatibilty Complex. Molecules that carry parasite antigen to the surface of the host cell so that it can be recognised by antigen-processing cells.
Phagocyte: A cell that engulfs and ingests foreign particles.

Acquired immunity

Acquired (also called 'adaptive' or 'specific') immune responses come into action more slowly than innate reactions as they are tailor-made to combat the particular nature of each new challenge. A quicker response occurs when an animal is subsequently re-exposed to the same pathogen as the system is already primed for that specific reaction. Acquired immunity starts with the detection of foreign molecules (antigens) and the processing of these by antigen-presenting cells. This process generates two forms of adaptive response which are strongly linked to each other:

i) a cellular response characterised by T-lymphocyte participation, and
ii) humoral immune reactions mediated by B-lymphocytes and antibody-producing plasma cells.

Extra information box 1.1

The Th1/Th2 dichotomy

Different Th-lymphocyte subpopulations have different cytokine profiles and therefore play different roles. As either the Th1 or the Th2 subpopulation tends to predominate in a particular parasitic infection, the 'Th1/Th2 dichotomy' is an important determinant in the pathogenesis of infection and in the design of vaccination strategies. Th1-mediated responses are concerned mainly with cellular immunity and lead to the activation of effector cells, such as macrophages and dendritic cells. Th2-mediated responses are primarily associated with humoral immunity, with cytokines that result in anti-inflammatory reactions accompanied by an increase of specific antibody production, in particular IgE. Mast cells and eosinophils are also activated. These contain granules which, when released onto the surface of larger organisms, are capable of initiating enzymatic digestion.

In general, antigen-presenting cells processing bacterial and protozoan antigens tend to produce IL-12 which leads to an expansion of the Th1 population, whereas antigens derived from helminths and arthropods trigger mainly IL-4 and IL-6 which stimulate Th2-cell proliferation.

Immunity to arthropods

Most parasitic arthropods are ectoparasites. The degree of contact they have with body tissues and the time they spend on the host vary greatly – from a mosquito's fleeting visit to mites that burrow into the superficial epidermis. A few, like warble fly larvae, are true endoparasites, penetrating much more deeply into the body. Thus, opportunity for host detection of arthropod antigens varies accordingly, influencing both the nature and effectiveness of the subsequent immune responses.

In cases where contact is intimate and prolonged, as with some mange mites, a cell-mediated and partially protective immunity often develops. But where the antigens presented to the host are confined to those in the saliva injected during transient feeding behaviour (e.g. biting insects), immune responses may be limited to a local hypersensitivity. Such reactions do little to discourage further flies from biting and can become very itchy (pruritic). This may be of benefit if it encourages animals to move away from infested land or to adopt a more effective grooming behaviour (e.g. in flea or louse infestations), but pruritus can also provoke excessive scratching, rubbing and biting.

Ixodid ticks are rather different as, although they are temporary parasites, they remain attached to their host for several days while taking a blood meal. This provides greater opportunity for immune attack and, over time, parasitized hosts can develop a partially effective species-specific immunity. This acts by interrupting blood-sucking processes, thereby reducing the well-being and reproductive capability of the tick.

Immunity to protozoa

Parasitic protozoa that establish in extracellular positions within the body are exposed to humoral immune responses and are thereby susceptible to destruction by membrane disruption or ingestion by phagocytes. Those that have adopted an intracellular lifestyle will be shielded from such attack (except when moving between host cells) and cellular immune mechanisms are then more likely to be effective.

Extra information box 1.2

Some immune effector mechanisms

Lysis: A complement-dependent process in which the alternative pathway is activated by parasite surface antigens leading to destruction of the parasite by membrane disruption.

Opsonisation: A process whereby a pathogen is 'labelled' with a molecule (e.g. complement factors or a specific antibody) that attracts destructive cells such as phagocytes.

Phagocytosis: Phagocytes such as neutrophils, macrophages, monocytes and dendritic cells will ingest opsonised protozoa or parasitized host cells and attempt to kill them with oxidants, nitrous oxide, etc. and to digest them with enzymes.

Immunity to helminths

In contrast to protozoa, helminths are multicellular, relatively large and have a less intimate relationship with host tissues. Generally, they are extracellular and do not multiply within the host. Consequently, it is more difficult for the host to respond effectively. This is especially true for the many helminths that live in the lumen of the gastrointestinal tract as they are not in direct contact with any body tissue (see Figure 1.1). Immune attack has to be multifaceted and is often aimed at securing the parasite's demise by long-term attrition rather than swift execution.

Expulsion of nematodes from the gastrointestinal tract is a complex two-stage process. Firstly, the mucosal lining has to become permeable to macromolecules so

that specific antibodies (e.g. IgA) can 'leak' into the lumen at the site of parasitism. During this process goblet cell hyperplasia results in excess mucus formation. This helps to dislodge some helminths while others exploit it as their primary food-source, which illustrates the complexity and fascination of host–parasite relationships.

Extra information box 1.3

Immune effector mechanisms against helminths in the gastrointestinal tract

Immune protection against gastrointestinal helminths is largely orchestrated by Th2-cells situated in the Peyer's Patches (prominent thickenings of the gut wall). When activated by excretory/secretory (ES) helminth antigens, these cells produce a range of cytokines and chemokines which stimulate IgE production and eosinophilia, together with hyperplasia of mast and goblet cells. The IgE triggers mast cells to release granules containing vasoactive amines and histamine. These substances not only damage helminths directly but also increase gut permeability (permitting an outflow of specific antibodies). They also increase smooth muscle contractions in the gut wall (which helps to dislodge weakened parasites from their predilection sites).

Many gastrointestinal helminths migrate through body tissues en route to their predilection site and may consequently elicit different sets of immune responses during their parasitic life-cycle. They are likely to have reached the gut before acquired immunity to the tissue-stage becomes functional, but the activation of these adaptive responses will help protect the host against future invasion by the same species. Thus, there is an important difference between immunity that protects against reinfection and immunity that eliminates or ameliorates an existing infection.

Extra information box 1.4

Immune effector mechanisms against helminths within host tissues

Protection against tissue-dwelling helminths is predominantly of a cellular nature, reflecting their more intimate contact with their host. They are particularly prone to destruction by an antibody-dependent cell-mediated mechanism. IgE antibodies formed against surface antigens enable host cells such as eosinophils, neutrophils, macrophages and platelets to attach to the parasite and flatten out to ensure tight adhesion. The cells then secrete cationic proteins that are highly toxic to the helminth.

1.3.2 Parasite evasion of immunity

The survival of parasitic species is dependent on being able to escape the immune responses of its host. Such evasion strategies are multifaceted and can be divided into several main groups:

a – Sequestration: making it as difficult as possible for immune processes to reach the parasite. There are two main ways of doing this:

- **i)** by adopting a relatively inaccessible predilection site, e.g. within particular cell types or organs (such as the CNS or within the lumen of the gastrointestinal tract);
- **ii)** by generating a protective capsule, membrane or cyst wall.

b – Masking or changing surface antigens – examples include:

- **i)** incorporation of host molecules onto the surface of the parasite;
- **ii)** synthesis of parasite antigens which mimic host molecules;
- **iii)** antigen variance – periodic changes of surface antigens, thereby rendering previous host adaptive responses ineffective. Some parasites have stage specific antigens that serve the same purpose.

c – Disturbance of immunological effector mechanisms – examples include:

- **i)** surface shedding to remove adhering immune cells or specific antibodies bound to parasite antigen;
- **ii)** enzymatic digestion of antibodies;
- **iii)** inhibition of oxidative products synthesised by leukocytes;
- **iv)** reducing MHC-expression on the surface of infected cells, thereby inhibiting antigen presentation to the immune system.

d – Modulation of the host immune response – this can be achieved in various ways, for example:

- **i)** induction of multiple clones of T- and B-cells that produce nonspecific antibodies (polyclonal activation), thereby disabling the host's ability to manufacture in sufficient quantity the specific antibodies needed to combat the invading parasite;
- **ii)** induction of immune complexes in the blood and cleavage of antibody/ complement factors, both of which result in severe immune suppression.

e – Influencing apoptosis:

- **i)** release of pro-apoptotic factors that shorten the life of leukocytes that might threaten the parasite;
- **ii)** synthesis of anti-apoptotic factors by an intracellular protozoan parasite to prolong the life-span of its host cell.

f – Arrested development and hypobiosis: Some parasites are able to pause their development at a strategic point in their parasitic life-cycle. This waiting phase (termed 'arrested development') is used to synchronise parasitic development with host or environmental events (e.g. parturition or the onset of a favourable season of the year). There are various biological advantages to be gained from this (see for example Section 6.3.1). During this process, parasites often 'hide' from targeted host immune responses by slowing or shutting down vulnerable metabolic processes ('hypobiosis').

1.4 Parasitic disease

1.4.1 The host–parasite balance

In nature, the coevolution of host defence mechanisms and parasite evasion strategies has resulted in an uneasy equilibrium whereby there is no undue threat to the continued existence of either at a population level, although the well-being or survival of individuals (host or parasite) may be compromised. The parasite needs to feed and reproduce, yet it faces extinction should infection jeopardise the survival of the host population. In a stable ecosystem, a well-adapted parasitic species is one that survives in the host long enough to replicate but provokes no more than tolerable damage to the host population.

Disease generally indicates a disturbance of this ecological balance. This may be caused by naturally occurring factors, such as unusual weather conditions, but is often due to human intervention. Compare, for example, zebra roaming the African savannah carrying large worm burdens seemingly without ill-effect, with the vulnerability of horses confined to small paddocks.

The host–parasite relationship can be perturbed in two ways:

a – Increased host susceptibility – for example, if animals are:

- **i)** stressed, debilitated or immunocompromised;
- **ii)** exposed to parasites with which they have not coevolved (e.g. European cattle placed in a tropical environment);
- **iii)** not allowed to express natural behaviour (e.g. restrained so they cannot groom to remove ectoparasites);
- **iv)** selectively bred for production traits at the expense of natural ability to resist infection (innate or acquired);
- **v)** inbred (e.g. some canine blood lines are particularly vulnerable to demodectic mange).

b – Increased parasite numbers – exposure to host-seeking (infective) life-cycle stages may increase, for example, if:

- **i)** host stocking density is increased, thereby increasing the output of parasite eggs / larvae etc. per unit area (or per kg forage);
- **ii)** parasitized animals are introduced into a previously clean area (e.g. through livestock movements, global trade etc.), thereby infecting susceptible local livestock, potential wild-life reservoirs or vectors;
- **iii)** short-term weather patterns or longer-term trends such as global warming produce conditions more favourable for the development of preparasitic life-cycle stages;
- **iv)** there is a surge in the population of intermediate hosts or vectors, or an increase in the number infected or their accessibility;
- **v)** the parasite population becomes resistant to anti-parasitic medication.

As host defences and parasite immune evasion are both contributory elements to a stable host–parasite relationship, the total elimination of a parasite from the host population can have unintended consequences. For example, without the immuno-modulatory effect of parasites, the human immune system can go into 'overdrive' in some individuals. This may, at least in part, account for the recent increase in allergies and immune-mediated diseases recorded in affluent societies (see Section 7.1.6).

1.4.2 Why parasites are important

Many microbial diseases sweep through populations as dramatic and sometimes devastating epidemics. While parasites can also kill or provoke acute disease, their greatest effect is in the form of chronic, low-grade and debilitating damage. Frequently, the deleterious consequences of parasitism are not readily apparent on clinical examination and so the term 'subclinical disease' is often employed. The various ways in which parasites impact veterinary medicine can be summarised as follows:

a – Animal welfare: many parasitic infections cause pain, discomfort or are otherwise distressing to the host.

b – Agriculture: as well as obvious losses due to death and disease, subclinical disease is of significance as it prevents farm animals from attaining their full genetic potential. The constant drain on bodily resources, imposed by the need to maintain the immunological battle against parasites and to repair the physiological and structural damage they cause, can lead to reduced weight-gain or an increased food conversion ratio, or to a reduction in meat, milk or fibre (e.g. wool) yield and quality. This obviously affects agricultural production and economics. In impoverished rural communities, it deprives the human population of much needed sustenance and diminishes the animal power available to work the land and carry produce to market.

c – Veterinary public health: many parasites of animals are transmissible to humans and capable of causing disease. Parasite vectors can also transfer microbial diseases from animals to humans, e.g. ticks carrying the Lyme disease bacterium. Veterinary input is important in food hygiene to ensure that zoonotic parasites, such as the nematode *Trichinella*, are excluded from the food chain (see Section 9.3.1).

d – Aesthetic considerations: animal owners and consumers often find the sight or thought of parasites repugnant, even though there may be no immediate danger to themselves or their pets, e.g. a cat passing a tapeworm segment, or foodstuffs harbouring an innocuous parasite. Affected meat may be condemned at the abattoir, even though the parasite concerned is neither capable of infecting humans nor of causing overt disease in animals, e.g. *Taenia ovis*.

1.4.3 Pathogenic mechanisms

There are many ways in which parasites can damage tissues or adversely influence bodily functions. These include traumatic outcomes and mechanical defects, parasite-induced cellular and pathophysiological changes, together with detrimental cellular and immunological 'own-goals'. Intracellular parasites not only use their host cell as a food source but may also reprogram its genomic expression to meet their physiological requirements. A selection of the most commonly encountered pathologies is listed in Table 1.1. These and other mechanisms are described in later chapters.

Table 1.1 Some examples of how parasites damage their hosts

Type of damage	An example	More information in Section:
Space occupying lesions	Hydatid disease	5.3.4
Intestinal obstruction/perforation	Ascarid infections	7.1.3
Mechanical damage	Blowfly myiasis	2.2.6
Cell damage/necrosis by intracellular parasites	Coccidiosis	4.6.2
Fibrosis	Liver fluke disease	5.6.2
Epithelial hyperplasia: protein-losing enteropathies	Parasitic gastroenteritis	6.3.2
Malabsorption: villous atrophy	Coccidiosis	4.6.2
Plug feeding	*Strongylus vulgaris*	6.3.3
Anaemia: blood sucking	Hookworms	6.3.4
Anaemia: haemolysis	Babesiosis	4.8.1
Thrombosis	*Strongylus vulgaris*	6.3.3
Lung damage	Bovine lungworm	6.3.5
Heart malfunction	Canine heartworm	7.1.5
Immunological damage	Leishmaniosis	4.5.1
Inflammatory damage	Sheep scab	3.3.3
Neurological damage	*Sarcocystis neurona*	4.7.1
Secretion of pharmacologically active substances	Canine heartworm	7.1.5
Secretion of toxins	Some ticks	3.2.1
Abortion	Toxoplasmosis	4.7.3
Dermatitis	Flea infestation	2.2.2
Tumour formation	*Spirocerca*	7.1.5
Transmission of other pathogens	Many dipteran flies	2.2.5

1.5 Diagnostic techniques

Accurate diagnosis is an essential prerequisite for effective treatment and control. Sometimes the cause of disease may be obvious from clinical signs and history. On many occasions, however, the root of the problem may be obscure or confirmation may be required in order to rule out other possibilities. Diagnosis involves demonstrating parasitic involvement, determining the identity of the organism and, if necessary, quantifying the intensity of infection. Detection of a causal agent can be by direct observation of life-cycle stages in faeces, blood etc. or by gathering indirect evidence, such as the occurrence of specific antigens, antibodies or DNA-sequences. Sometimes, particular biochemical changes are associated with a parasitic infection (e.g. elevated serum pepsinogen concentrations in bovine ostertagiosis). Similarly, quantification can be direct (e.g. worm counts at autopsy) or it may provide an indirect indication (e.g. eggs per gram of faeces or an antibody titre).

1.5.1 Direct detection methods

Some ectoparasites, such as blowfly maggots, are easily accessible and large enough to be collected manually for identification. Others, such as parasitic mites, are too small or too deeply embedded in the skin and so brushing or scraping techniques are needed, with collected material subsequently prepared for microscopic examination.

Haematogenous parasites (i.e. carried in the blood) can be demonstrated in blood samples, which can be prepared as wet or dry smears, centrifuged or filtered as appropriate. Other endoparasites may present a greater challenge, but biopsy may be an option in specific cases. If deaths have occurred, autopsy of representative animals provides an opportunity for investigating the whole body for parasites or parasitic damage.

With living animals, however, faecal examination ('coproscopy') is probably the commonest laboratory diagnostic procedure for demonstrating the presence of endoparasites. Many parasites living in the respiratory system, liver or gastrointestinal tract have life-cycle stages that leave the animal with digestive waste. Sometimes microscopic examination of a fresh faecal smear may suffice, particularly if motile forms are present in large numbers. More often, there are only a few parasitic structures in a large faecal volume. Concentration techniques are therefore needed to assist detection.

Flotation

An appropriate amount of faeces is mixed with a larger volume of an aqueous solution (such as saturated sodium chloride, sodium nitrate or sugar) with a specific gravity that allows lighter parasitic structures, such as eggs, cysts or oocysts, to float while heavier faecal debris sinks. If known weights and volumes are used, a quantitative estimate can be made, e.g. eggs or oocysts per gram of faeces (abbreviated to e.p.g. and o.p.g., respectively). A McMaster counting chamber is often used for this purpose (see Figure 1.4). A subsample (aliquot) of the faecal suspension is pipetted into each of the two chambers on the slide. Eggs that rise and come to rest within the boundaries of the marked grids are identified microscopically and counted. As the volume of fluid beneath each square is known (0.15 ml), the e.p.g. value can easily be calculated.

Sedimentation

Some parasitic structures (e.g. trematode eggs) are too heavy to rise reliably in commonly used flotation fluids and so, in these cases, the faecal sample is mixed with a large volume of water, sieved to remove larger particles, and allowed to stand in a tall vessel. The sediment is examined after an appropriate period.

There are also centrifugation techniques that increase the speed and sensitivity of flotation and sedimentation. Some parasitic structures are more delicate than others and a technique must be selected that does not distort or destroy the object being sought.

It is sometimes necessary to 'culture' faecal samples to encourage development to life-cycle stages that are easier to identify, e.g. by hatching strongyle eggs and

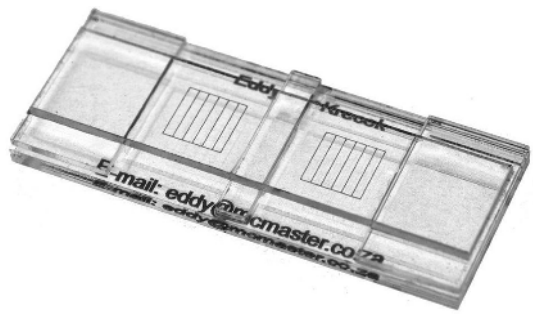

Figure 1.4 McMaster chamber (used for counting helminth eggs and/or coccidian oocysts in faecal samples). Reproduced with permission of T.E. Krecek.

allowing the emerging larvae to develop to the infective stage (see Figure 6.17) or by sporulating coccidian oocysts (see Figure 4.6). This is done by incubating the sample at an appropriate temperature in the presence of adequate air and humidity.

Practical tip box 1.1

Faecal samples

Faecal samples for parasitological examination should be freshly passed by the animal or, preferably, taken directly from the rectum. Faeces on the ground are quickly invaded by free-living nematodes and other organisms that can complicate interpretation. Samples should be examined as quickly as possible and kept refrigerated in the meantime. Even then there may be nonparasitic structures present that could confuse the unwary, e.g. pollen grains or fungal spores that have passed through the animal, or even small air bubbles introduced during preparation. Plant hairs can be easily mistaken for larvae. Such objects are sometimes called 'pseudoparasites'. It should also be remembered that herbivores harbour symbiotic protozoa in their digestive systems.

Nevertheless, most parasitic eggs, cysts and oocysts are easy to recognise with care and practice. Many of these are illustrated or briefly described in this book, as are some other parasitic life-cycle stages. However, reference to identification keys or other detailed publications will be needed for definitive identification.

Figure 1.5 Baermann apparatus (used for recovering larvae from faecal samples).

Migration

Some motile parasitic life-cycle stages, e.g. nematode larvae, will migrate out of the faecal mass into water. This phenomenon is exploited in methods such as the Baermann technique in which a faecal sample is placed on a sieve or in a gauze bag in contact with water (see Figure 1.5). Any larvae that emerge are collected in a funnel. Such approaches are also useful for recovering nematode larvae from other materials, e.g. grass washings if the infectivity of a pasture is being investigated.

1.5.2 Indirect detection methods

Often, more information can be gained by searching for indirect evidence of infection than by looking for the parasite itself. Serological methods for detecting antibodies or antigens, and PCR techniques indicating the occurrence of unique genetic sequences are becoming more frequently used as diagnostic tools in Veterinary Parasitology.

Until recently, indirect diagnostic procedures were mostly the domain of laboratories with expensive equipment and highly trained personnel. But technology is advancing rapidly and easy-to-use diagnostic kits are increasingly becoming available for field use.

Immunological assays

Immunological diagnostic tests are designed to detect either antibodies generated in response to infection, or parasite antigen present in blood, tissue fluids or faeces. They are often known by acronyms such as IFAT, ELISA etc. This section outlines the principles that lay behind the commonest of the tests currently in use.

Indirect fluorescence antibody test (IFAT)

The IFAT (see Figure 1.6) is a means of making visible the occurrence of specific antigen on the surface

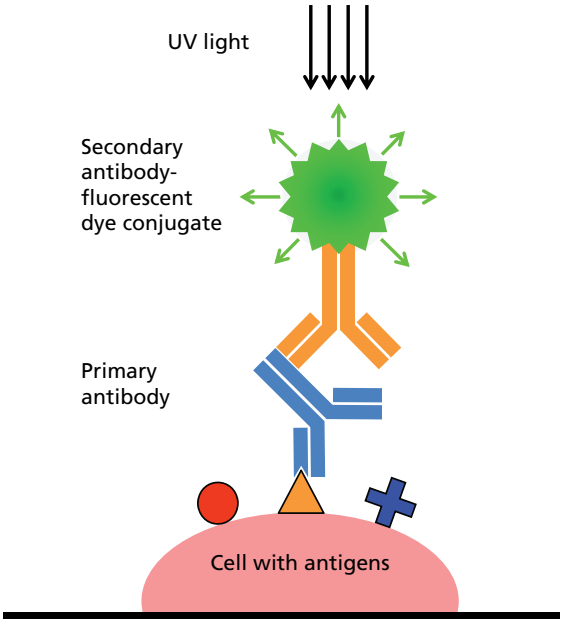

Figure 1.6 Diagram illustrating the principle of the IFAT. Redrawn after https://wiki.cites.illinois.edu/wiki/display/BIOE414/Background with permission of University of Illinois at Urbana-Champagne.

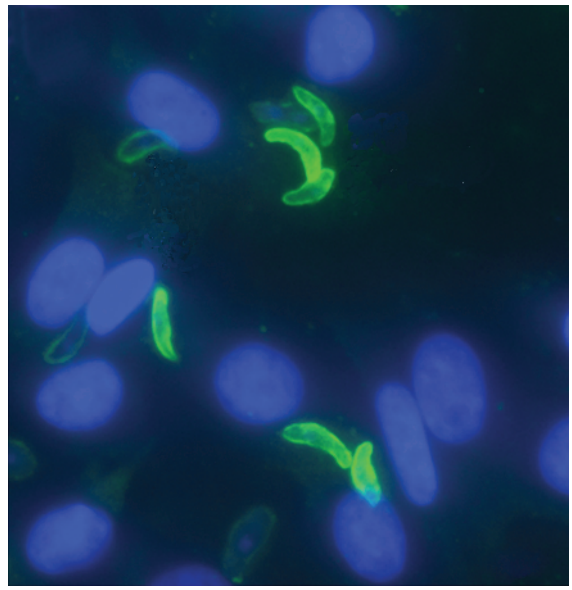

Figure 1.7 IFAT: protozoan parasites (*Eimeria* merozoites) fluorescing green under UV light (with host cells stained blue). Reproduced with permission of D.J. Ferguson.

of a parasite (or within a cut section of the parasite). It is particularly useful for detecting and identifying some protozoan parasites, but it also has applications in other parasitic groups. The principle of the test is as follows:

a – The specimen is fixed on a microscope slide.

b – It is exposed to a reagent containing antibody specific for a particular parasite antigen (the primary antibody).

c – If the antibody meets matching antigen on the surface of the specimen, they will bind together; if there is no match, the antibody is removed when the slide is washed.

d – The next reagent is a secondary antibody designed to attach to any member of the antibody class (e.g. IgG or IgM) used in the first reagent.

e – The secondary antibody has previously been tagged ('conjugated') with a molecule that can be excited by UV-light.

f – Thus, the parasite antigen, if present, is indicated by luminescence when the slide is examined by fluorescent microscopy (see Figure 1.7).

Enzyme-linked immunosorbent assay (ELISA)

The ELISA is used to detect specific antibodies (e.g. in serum samples) or soluble antigen (e.g. in faecal samples). It is a highly sensitive method as antibody-antigen binding triggers a chain reaction which gives a measurable colour change. The tests are performed in a series of wells on a plastic microtitre plate (see Figure 1.8). This is often done manually but the process can be automated.

ELISAs can be performed in a number of ways for different purposes, one of which (the 'indirect' ELISA for detecting specific antibodies) is illustrated in Figure 1.9:

a – The wells of a microtitre plate are coated with purified antigen which binds to the plastic.

b – A series of dilutions of the test serum is prepared and pipetted into the wells. If specific antibodies are present they will bind to the antigen and remain when the plate is washed.

c – The next reagent contains a secondary antibody (as in the IFAT above) that is conjugated with an enzyme (such as alkaline phosphatase or horse radish peroxidise).

d – After further incubation and washing, a substrate is added that changes colour when digested. This will

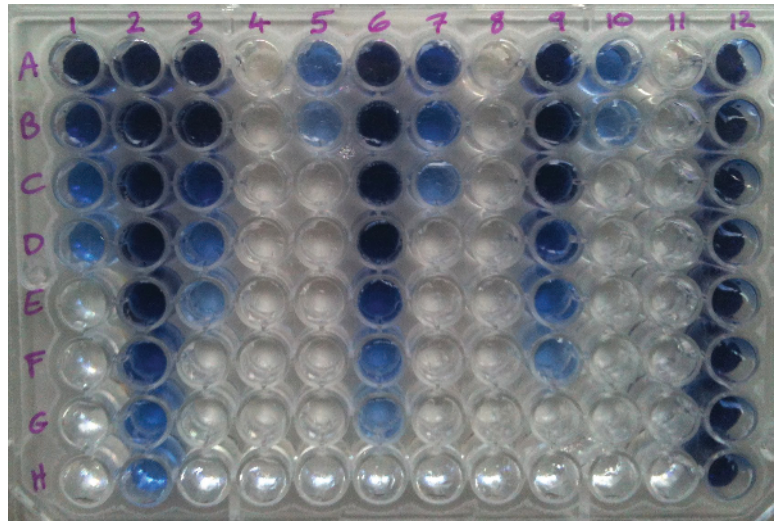

Figure 1.8 Microtitre plate displaying results of an ELISA assay: lanes 11 and 12 – negative and positive control sera, respectively; lanes 1 to 10 – test samples (two-fold dilutions from 1:20 from row A downwards). Reproduced with permission of B. Catchpole.

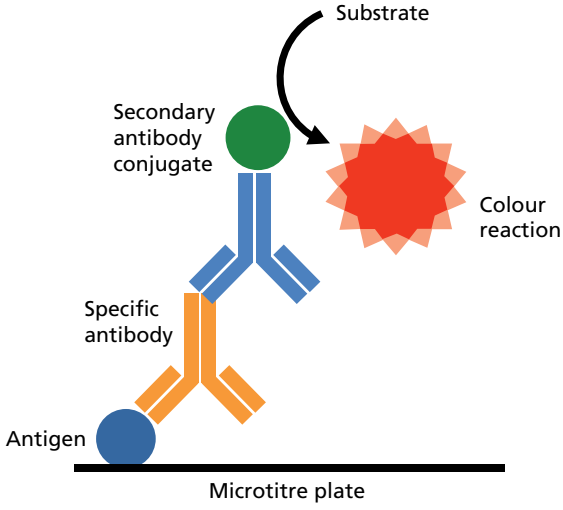

Figure 1.9 Diagram illustrating the principle of the indirect ELISA. Redrawn after http://www.abnova.com/support/resources/ELISA.asp with permission of Abnova Corporation.

happen only if the enzyme that triggers the reaction has been bound, via the two antibodies, to the antigen.

Complement fixation test (CFT)

The principle of this diagnostic test relies on the capacity of antibody-antigen-complexes to bind complement factors. In the CFT assay a specific parasite antigen is incubated with the test serum in the presence of complement. An indicator system is used, based on sensitised sheep erythrocytes. If the sample is positive, the antibody-antigen-complexes that form will bind to the complement and the erythrocytes will remain intact. On the other hand, if no specific antibodies are present in the test serum, haemolysis will occur since unbound complement factors will destroy the erythrocytes. The CFT is used routinely for detecting equine piroplasmosis, an important protozoan infection.

Western blotting

Antigens carry an electrical charge and so they can be spatially separated from other molecules, and from each other, by electrophoresis. They can then be transferred to a carrier membrane and incubated with a test serum. An enzyme-conjugated antibody (as in the ELISA above) is then used to indicate each site on the membrane where specific binding has taken place. A positive result will be depicted by a characteristic pattern.

The specificity of Western blotting is often higher than can be obtained with other techniques as electrophoresis generally separates out all the antigenic components in a sample, thereby isolating any cross-reacting molecules that might complicate interpretation in the other tests.

DNA techniques

As in all other branches of the life-sciences, DNA technology has opened up new possibilities for diagnosis

and investigation. Specific primers are used in the polymerase chain reaction (PCR) to amplify unique segments of the parasite genome either for confirmation of identity or to provide fragments of DNA in sufficient quantity for sequencing. Such techniques have proved to be valuable tools for research, giving precise results from minute amounts of material, and have given many new insights into taxonomy and epidemiology. In particular, subtle but important variations can be detected between organisms that are morphologically identical. An important example is the recent differentiation of the protozoan species, *Giardia duodenalis*, into epidemiologically distinct 'genotypes' (see Section 4.5.2). To date, few of these tests are available for routine diagnosis as it can be difficult to extract standardised DNA-samples from field samples and the reagents tend to be expensive. These obstacles are being overcome and this technology will surely play an ever increasing role in the future, not only for identifying the cause of disease but for identifying other significant traits such as drug resistance.

1.5.3 Limitations

Diagnostic tests do not always give a reliable or definitive answer – results have to be interpreted with care. The limitations of each test have to be understood. This applies particularly with regard to sensitivity and specificity, which are rarely 100% even for the most sophisticated tests (except, perhaps, some DNA procedures):

a – Sensitivity: a measure of the proportion of infected animals in a population that will show up as positive using the test in question (e.g. if 100 animals in a flock or herd are infected and the test shows a positive result for 98 of them, then the sensitivity of the test is 98%).

b – Specificity: a measure of the proportion of uninfected animals that will actually register as 'negative' (the remainder being 'false positives'). Thus, if 1 of 100 uninfected animals gives a positive reaction, then the specificity of the test is 99%. False positives are usually caused by cross-reactivity with similar antigens expressed by other parasites or microbial organisms.

Finally, it has to be stressed that the demonstration of a parasitic infection does not necessarily mean that a diagnosis has been made, as the disease may be due to another concurrent aetiological factor.

1.6 Treatment and control

Veterinary Parasitology may be a fascinating topic for academic study but the primary purpose for its inclusion in the veterinary curriculum is to empower the practitioner with the knowledge and understanding needed to combat parasitic disease in an effective and sustainable way.

1.6.1 Key concepts

Although interrelated and often combined, treatment and control are distinct and separate concepts:

a – Treatment: Treatment is a short-term measure aimed at producing an immediate impact on the parasite population. The intended benefit could be alleviation of suffering, enhancement of productivity or prevention of further parasite replication. Additional supportive therapy is often given to help repair damage and restore health.

b – Control: Control has a longer-term perspective and is aimed at preventing future infection and minimising disease risk. It implies the development and implementation of a plan.

The aim of most control programmes is to prevent the parasitic population building up to pathogenic proportions, or to enhance the host's ability to withstand infection (e.g. by vaccination), or both. Such schemes often include strategically timed treatments, but nonchemical approaches can augment, reduce or even replace drug usage. For example, the coccidiosis risk in a poultry house can be lessened considerably by keeping the floor-litter dry so oocysts cannot develop to the infective stage.

Experience has shown that treatments rarely yield longer-term benefits if given in a haphazard or arbitrary way. On the other hand, over-reliance on routine treatments can give diminishing returns over time as parasite strains become resistant to the chemicals used (see Section 1.6.3). This is why control programmes require planned interventions based on epidemiological knowledge.

Very often the concepts of treatment and control are combined. When presented with an outbreak of parasitic disease, a veterinarian will first treat the animals to restore health and then give advice on how to prevent a recurrence of the problem.

1.6.2 Chemotherapy

Selective toxicity

Chemotherapeutic agents are medicinal compounds ('toxicants') that come as close as possible to the ideal of killing or disabling a target parasite without also poisoning the host. This differential effect is termed 'selective toxicity'. In some cases, it is achieved by exploiting differences in biochemical pathways between parasite and host. More often, a good margin of safety is assured because the drug molecule binds more readily or more enduringly to parasite receptors than it does to the host equivalent.

It has already been noted that the life-cycles of 'microparasites', such as protozoa and bacteria, are fundamentally different from those of 'macroparasites' such as helminths and arthropods (see Figure 1.2). Consequently, a different approach to chemotherapy is required in each case. The primary objective for antiprotozoal therapy is to curtail parasite replication within the host, ensuring that population growth does not reach a level that will overwhelm host immunity. Macroparasites (with some exceptions) do not multiply within the final host and the emphasis therefore is on disrupting the transmission of nervous impulses at synaptic and neuromuscular junctions. This action paralyses the parasite, thereby removing its ability to feed or maintain its favoured position ('predilection site') in or on the body. Some parasiticides achieve a similar end-result by impeding energy metabolism.

Formulation

Parasiticides can be administered in different ways depending on purpose and convenience. This may be by mouth, by injection or by skin exposure (dips, sprays, etc.). The term 'topical' is used to describe preparations applied externally with activity confined to the skin. Compounds that are carried to their site of action via the blood-stream are said to act 'systemically'.

In commercial products, the active ingredient is blended with inert materials to produce the required physical presentation, e.g. as a solution, suspension, shampoo, capsule, powder, granule, paste, pellet, etc. This process is known as 'formulation' and can also be used to modify biological and pharmacological properties (increasing potency or reducing toxicity, for example). Slow-release formulations emit their active compound continuously over a period of time (e.g. insecticidal collars, ear-tags, tail-bands etc.) thereby extending the duration of action. A similar effect can be obtained if the active compound is eliminated only slowly from the body, e.g. if a particular tissue such as fat acts as a reservoir. The dynamics of drug storage, metabolism and excretion are termed 'pharmacokinetics' and influence the way in which many parasiticidal drugs are used.

Help box 1.5

Some commonly used terms for antiparasitic drugs

Parasitic group	Collective term for drugs	Notes
Parasites	Parasiticides	
Ectoparasites	Ectoparasiticides	
Insects	Insecticides	This term often used to include ticks and mites as well
	Insect growth regulators	Do not kill adults but stop larval development
Acarina (ticks and mites)	Acaricides	
Protozoa	Antiprotozoals	
Coccidia	Coccidiocides/coccidiostats	These have slightly different meanings (Section 4.10.2)
Helminth	Anthelmintics	Note the correct spelling (left); 'antihelminthics' is sometimes seen but is wrong
Cestodes (tapeworms)	Cestocides	
Trematodes (flukes)	Flukicides	
Nematodes	Anthelmintics	This generic term is used as 'nematocide' is restricted by convention to chemicals used against plant-parasitic nematodes

Help box 1.6

Some pharmaceutical terms

Selective toxicity: the ability of a parasiticide to kill the target organism without harming the host.
Synergism: occurs when the biological activity resulting from the simultaneous application of two substances is greater than their additive effect.
Safety margin: The ratio between the recommended therapeutic and maximum safe doses of a drug formulation.

While prolonged activity may be of advantage in parasite control, residues of biologically active molecules when above defined safety levels render meat and milk products unfit for human consumption. Label instructions for farm animal medicinal products therefore stipulate a statutory interval between treatment and slaughter for meat, or release of milk for human consumption (the 'product withdrawal period').

Formulations providing prolonged delivery should be designed so that drug concentrations decline rapidly at the end of the therapeutic period. This is because a slow decline may expose some parasites to suboptimum doses which could encourage the onset of resistance (as explained in the next section).

1.6.3 Resistance to parasiticides

Many parasitic arthropod, protozoan and helminth species are capable of developing strains resistant to the effects of chemotherapeutic agents. This phenomenon is becoming an ever greater problem worldwide. Resistance not only restricts options for effective treatment but has already imposed severe constraints on agricultural practice in some parts of the world. For example, failing tick control in parts of the wet tropics prohibits the local use of susceptible European cattle (see Section 8.2.4), while sheep farming has been abandoned in parts of South Africa and Australia due to multiresistant stomach worms (see Section 8.2.1).

Most major classes of parasiticide have been in widespread use for several decades. Few chemical entities with a novel mode of action have been brought to the market in recent years. It is therefore important that control programmes do not encourage new resistant strains to develop.

Selection

'Resistance' is defined as the ability of a strain of an organism to tolerate doses of a chemotherapeutic agent that would prove lethal to a majority of individuals in a normal population.

Resistance has a genetic basis. If a treatment is, for example, 98% effective, it is likely that the proportion of individuals expressing genes for resistance will be higher in the 2% that survive than was the case in the whole population prior to exposure. Survivors will continue to produce offspring and the next generation will therefore have an increased prevalence of resistance genes. Thus, selection makes more common the resistance genes (either innate or the result of recent mutation) found originally in only a few individuals. Exposure of subsequent generations to the same or similar treatment will magnify this effect until a breakdown in control becomes clinically evident.

There is always a risk that newly acquired animals may be harbouring parasites carrying resistance genes. To avoid introducing these into the local gene-pool, such animals should be treated with an effective compound and quarantined until shown to be parasite-free.

Selection pressure

'Selection pressure' describes the intensity of the selection process and will be greatest when a high proportion of the parasite population (on a farm, for example) is exposed to the toxicant. The majority of the next generation will then be offspring of treatment survivors and the prevalence of resistance genes will be correspondingly higher. If only part of the parasite population is exposed, the untreated sector (which is said to be '*in refugia*') provides a reservoir of susceptibility genes that will dilute the prevalence of resistance genes in the general gene pool and thereby slow the development of resistance.

Rate of development

When a parasite population is regularly exposed to a particular chemical group, the onset of resistance can vary from a few months to several decades depending on:

a – Parasite factors: such as the nature of the resistance mechanism and its genetic basis; the initial prevalence of the resistance gene(s) in the population; and the biology of the parasite, particularly its biotic potential;

b – Management factors: such as husbandry, treatment strategies (e.g. timing and frequency of dosing), accuracy of dosing, opportunities for parasites to remain *in refugia* etc.;

c – Extraneous factors: such as climate – resistance is likely to occur sooner in warm humid regions where more intensive control measures are needed and parasite generation times are shorter.

Multiple resistance

A parasite population can become resistant to two or more parasiticides, including compounds to which it has never been exposed. This can happen in two ways:

a – Side-resistance: when resistance develops following exposure to a particular compound, it is likely that the parasite strain will also be resistant to other members of that same chemical class (since they will have a similar mode of action).

b – Cross-resistance: a strain resistant to one chemical group may also be resistant to a structurally unrelated class if both act on the same parasite target site (for example, descendents of a fly population resistant to DDT may be resistant to synthetic pyrethroids).

Reversion

Reversion to susceptibility can sometimes occur over time if a parasite population is no longer exposed to the chemical group that induced resistance, but unfortunately this does not always happen. Whether or not reversion occurs depends on:

a – Gene-flow dynamics: e.g. the rate of influx of susceptibility genes into the general gene-pool from parasites *in refugia* or in newly introduced hosts, or (in the case of mobile parasites such as insects) from neighbouring parasite populations;

b – Genetic mechanism of resistance: the genetic change responsible for resistance is sometimes irreversible (e.g. if a susceptibility gene has been deleted).

Treatment failures

It is easy to blame treatment failures onto resistance, but experience has shown that the majority of such events are due to other factors, such as:

i) an inappropriate choice of parasiticide;
ii) an inadequate dose-rate;
iii) incompetent administration or faulty dosing equipment;
iv) posttreatment exposure to overwhelming parasitic challenge;
v) hepatic damage (e.g. liver fluke infection) – some compounds need to be modified in the body (metabolised) before becoming active.

Evidence in the form of results from *in vivo* (animal) or *in vitro* (laboratory) tests is necessary before a suspicion of resistance can be confirmed.

1.6.4 Integrated parasite management

Integrated parasite management (IPM) is a concept that is increasingly being adopted in animal parasite control. It is based on the integrated pest management philosophy already well-established in agricultural and horticultural crop protection. IPM builds on acceptance of the view that total dependence on chemical control is no longer a sustainable option because:

a – Resistance: is limiting the use/ usefulness of many existing pesticides/ parasiticides;

b – Few replacement products: the rate of introduction of products active against resistant strains is restricted by the high cost of discovery, development, safety-testing, registration and marketing of novel chemical entities;

c – Meat and milk residues: are potential problems associated with chemotherapy if products are used inappropriately;

d – Ecological concerns: arise if significant quantities of biologically active materials are released into the environment.

The IPM model aims to manage pest or parasite populations, rather than eliminate them, by using chemicals intelligently as one component of a wider integrated control strategy based on epidemiological principles. This encompasses husbandry and hygiene measures, together with vaccines and other technological aids, if available. The use of parasiticides, when needed, is limited to sustainable strategic interventions.

1.6.5 Vaccination

Vaccination primes the host immune system so that it can react more quickly and effectively on encountering antigens associated with specific pathogens. Protozoan and metazoan parasites are structurally complex

and have a broad range of strategies for avoiding or subverting host immune responses (see Section 1.3.2). Developing vaccines to protect against parasitic disease therefore presents formidable technological challenges and, consequently, relatively few such products are yet available for veterinary or medical use. Furthermore, their employment has to be economically justifiable within the constraints of veterinary and agricultural practice. Several innovative and technically effective vaccines have proved to be commercial failures in recent years. Nevertheless, vaccine technology is advancing and there is expectation that this situation will improve.

Vaccines tilt the host–parasite balance in favour of the host and this can be exploited in different ways. Most often, they are used to prevent parasite numbers building up in the host to pathogenic proportions, thereby protecting the vaccinated individual against disease. Not all vaccines block parasite establishment completely, however, and so vaccinated animals can still act as a source of infection for others (e.g. calves vaccinated against lungworm, see Section 8.2.2).

With some vaccines (e.g. the hidden antigen tick vaccine described below), parasites establishing on vaccinated animals may be weakened, but not necessarily killed. Their reproductive potential is greatly reduced with the result that fewer infective stages will accumulate on the pasture over the coming weeks and months. With this type of vaccine, therefore, the main beneficiary is not necessarily the vaccinated individual itself but the animals that subsequently graze the now-safer pasture (see Section 8.2.4). In such cases, vaccination is used for the future benefit of the herd or flock while not necessarily protecting the immediate health of the recipient of the vaccine dose – a concept that can be difficult for some farmers to accept.

Natural antigen vaccines

Vaccines can be prepared in a number of ways. The most obvious approach is to utilise antigens that stimulate immunity in natural infections. Sometimes a single antigen extracted from the parasite or its excretory/secretory products ('ES antigens') can induce a strong protective response. Molecular cloning techniques are used for large-scale manufacture of such molecules and the product is known as a 'recombinant vaccine'. One promising vaccine, currently being evaluated in the tropics, prevents pigs from becoming infected with a tapeworm cyst that is transmissible to humans (*Taenia solium*; see Section 9.3.1).

Hidden antigen vaccines

Many attempts at producing recombinant vaccines have yielded disappointing results as parasites generally only succumb when exposed to an array of antibody and cell-mediated attacks. This is partly because of their complexity but also due to their ability to evade or neutralise many specific host responses. An ingenious way has been found to circumvent this latter problem by exploiting molecules that are vital to the parasite's survival but are not detected ('seen') by the host immune system. Such molecules are called 'hidden' or 'concealed' antigens.

In nature, hidden antigens do not induce an immune response, and it follows that the parasite cannot have evolved any corresponding evasive strategy. The parasite is, therefore, highly vulnerable if a way can be found to promote host recognition of this antigen.

This concept is best illustrated with an example (see Figure 1.10). Troublesome ticks, such as *Boophilus*, have potentially antigenic molecules incorporated into their mid-gut wall but these are fixed and so 'hidden' from the host's immune system. After a tick attaches to the skin of its host to feed, it sucks a large quantity of blood into its mid-gut. If the host has been bitten previously by that tick species, the blood will contain antibodies to substances in tick saliva, but these do little harm to the tick and confer little protection to the host. If, however, the host has been vaccinated with a preparation made by cloning 'hidden antigen' isolated from the *Boophilus* mid-gut in the laboratory, then the feeding tick will ingest specific antibodies capable of attacking its gut lining with destructive effect.

One disadvantage of the hidden antigen approach is that the immunity of the vaccinated animal is not reinforced on exposure to natural reinfection (as no gut-surface antigens are presented to the host). This means that booster vaccine doses are needed at regular intervals to prevent immunity from waning.

Attenuated vaccines

One way of ensuring that the broadest spectrum of antigens is presented during the vaccination process is to expose the host to the living parasite. Of course, this has to be done without invoking the disease that we are trying to prevent. This can be achieved by using parasite strains of low virulence. These can be naturally occurring, or their pathogenicity can be diminished artificially, in which case the organism is said to

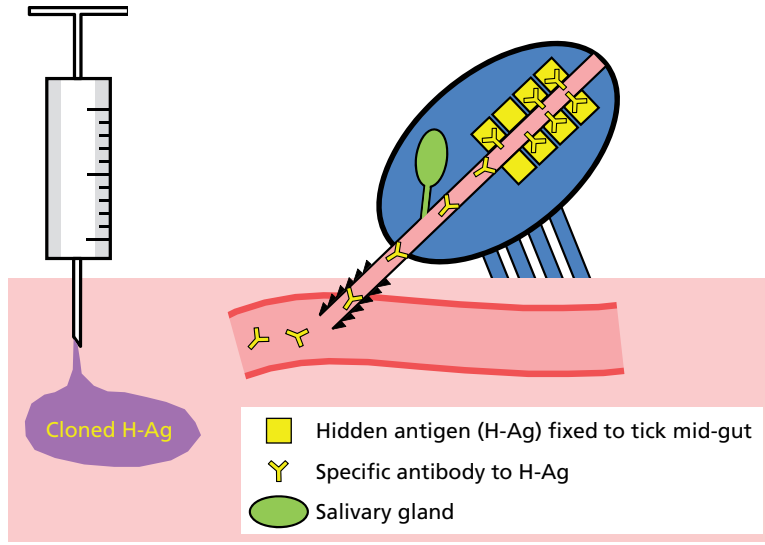

Figure 1.10 Diagram illustrating the principle of the hidden antigen tick-vaccine.

be 'attenuated'. For example, the blood parasite *Babesia* can be attenuated by infecting a splenectomised calf and using blood from this animal to infect another, and so on, until the desired effect is achieved. This process is known as 'passaging'. Such vaccines have been used with success in some tropical regions where babesiosis is a severe constraint on cattle production. With this approach, however, there is the theoretical possibility that, as the parasite can complete its life-cycle, it might revert to virulence and be transmitted to other animals.

Other attenuated vaccines have addressed this problem by using parasites that have been modified to such an extent that there is no possibility of onward-transmission. For example, the protozoan parasite *Toxoplasma gondii* normally enjoys a short period of uninhibited multiplication in a new host before encysting to hide from immune attack (see Section 4.7.3). The attenuated strain, which is the product of numerous rapid passages through laboratory mice, has lost the ability to encyst. When used as a vaccine, therefore, the resulting infection is self-limiting as the parasites are all killed by the protective immune responses that they themselves have engendered.

Another example is the cattle lungworm vaccine. This comprises living infective larvae that have been exposed to γ-radiation. When administered orally to calves, irradiated larvae migrate to the lungs in the usual way but die before they are able to do significant damage. This vaccine has been successfully employed for over 40 years.

More recently, an effective vaccine for coccidiosis in poultry was created by passaging several species of the causal protozoan parasite, *Eimeria*, through chick embryos. The attenuated organism has a shortened ('precocious') parasitic cycle in which the most pathogenic of the several developmental stages has been lost and is skipped over. Once again, the host is exposed to the full array of antigens needed to induce a solid protective immunity but without risk of the live vaccine provoking disease.

Attenuated vaccines do have some limitations. As they consist of living organisms, they tend to have short shelf-lives and they require exacting conditions for transport and storage.

1.6.6 Alternative technologies

Concerns about the continued sustainability of some control methods, the current limitations of vaccines and the affordability of high-tech solutions for farmers in poorer countries, have lead to a great deal of research on alternative and low-input approaches to parasite control. The main aims are to maintain the health and productivity of livestock while reducing dependency on chemicals, and to maintain the susceptibility of parasite populations to parasiticides for as long as possible. In other words, to enhance host resistance to parasites while delaying the development of parasite resistance to parasiticides.

Enhancing host resistance

Genetic diversity within host populations influences the ability of individual animals to withstand parasitism. Typically, a few animals within a flock or herd will harbour large numbers of parasites while a greater number will have few or none. The usual spread of parasites through a host population is said to be 'overdispersed', i.e. it does not follow a normal distribution curve (see Figure 1.11).

This difference in vulnerability of individual animals to parasitism is often referred to as 'host resistance', although the phenomenon actually has two distinct, although interlinked, components:

a – Resistance: this is the ability of an animal to defend itself against parasitic attack by means of innate and acquired immune responses (see Section 1.3.1). The main methods of enhancing host resistance are by ensuring adequate nutrition and by vaccination.

b – Resilience: this is the ability of an animal to tolerate the presence of parasites. For example, two animals may harbour identical parasitic burdens yet only one may show signs of clinical disease. The healthier animal is better able to limit, or to compensate for, the damage caused by the parasites.

Breeding for resistance/resilience

Resilience is, to a greater or lesser degree, a heritable trait. In the case of gastrointestinal nematodes in some breeds of sheep, heritability is of the same magnitude as that for milk yield in dairy cows. Breeding programmes can therefore improve this trait if a suitable marker can be found for identifying the more resilient rams.

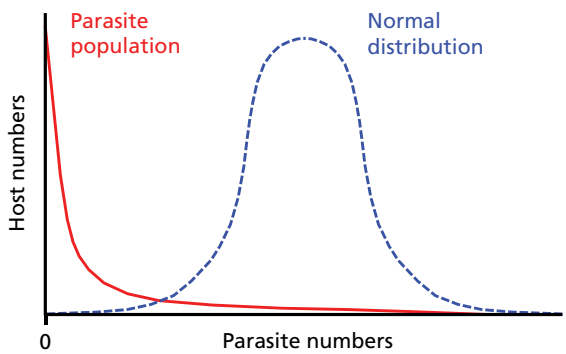

Figure 1.11 The typically skewed distribution of parasites within a host population compared with a 'normal' distribution curve (notional diagram).

Stock hybridisation is sometimes employed to achieve resistance or resilience. For example, *Bos indicus*-type cattle (humped tropical breeds) are much more tolerant of tick infestations than are European (*Bos taurus*) breeds. Cross-breeding can enable European cattle to survive in heavily tick-infested areas with fewer acaricidal treatments, albeit at a cost of reduced productivity.

Nutrition

It is well-recognised that parasitism is more pronounced in malnourished animals and that an adequate and well-balanced diet will boost both resistance and resilience. Some food-stuffs ('bioactive crops') can be particularly beneficial in this respect. For example, tannin-rich fodders such as lucerne (alfalfa) and clover reduce the establishment of infective nematode larvae. They also protect protein from microbial degradation in the rumen, thereby increasing the supply of plant protein to the abomasum. Many other plants, e.g. chicory, have antiparasitic properties that could theoretically be exploited, either as forages in a grazing programme or as feed supplements.

Delaying parasite resistance

It was noted in Section 1.6.3 that resistance to parasiticides is a selection process in which resistance genes become more prevalent in successive generations. Strategies to delay the onset of resistance must therefore block, dilute or reverse this trend. One approach is to combine or alternate the use of drugs with different modes of action, so that parasites surviving one treatment are killed by the next. There is, however, a risk that ill-considered dosing schedules could potentially lead to multiresistance.

Low-input approaches aim to keep a sufficient proportion of the parasite population *in refugia* to maintain the diversity of the gene pool (Section 1.6.3). To achieve this aim, interventions are designed to maintain the overall level of parasitism within tolerable limits, while allowing sufficient numbers of parasites not exposed to the drug to reproduce. This can be done in at least two ways:

a – Selective treatments: treatments are restricted to those animals in greatest clinical need, e.g. those exhibiting diarrhoea or anaemia;

b – Targeted treatments: these are given only to those individuals within the group that are contributing most to the infectivity of the environment.

Targeted treatments can be used, for example, in horse stables. Faecal egg-counts identify the few animals in a group that excrete large numbers of worm eggs and these are treated. This reduces pasture contamination substantially while the genetic diversity of the parasite population is maintained by the untreated members of the herd. The risk of future disease is not completely averted, however, and so good clinical judgement, careful management and regular monitoring are required.

Biological control

Biological control measures are widely used in horticulture, especially in greenhouses (e.g. parasitoid wasps to control aphids or nematodes for slugs). Similar approaches are being investigated for animal health application. For example, nematodes that kill flea larvae are commercially available for outdoor use in some countries.

A promising innovation that may have veterinary application in the future is the use of nematophagous fungi. A number of nematode-trapping species have been used successfully in field trials for reducing the numbers of infective gastrointestinal nematode larvae appearing on pasture. Fungal spores fed to ruminants pass through the animal and produce networks of hyphae throughout faecal deposits. These release compounds that attract nematodes, which are captured, killed and digested by a variety of means including adhesive nets (see Figure 1.12), nonadhesive constrictive rings or by deposition of droplets of paralytic toxin.

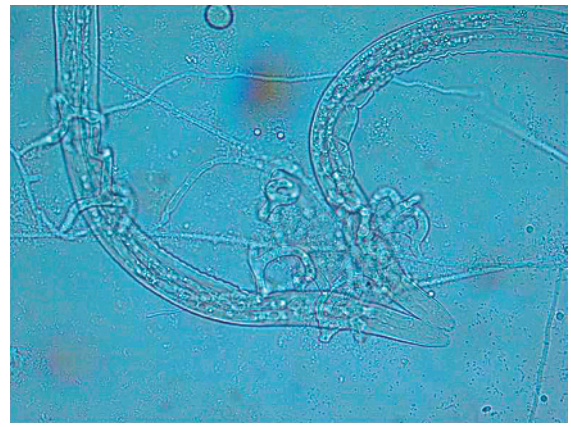

Figure 1.12 Nematode trapping fungi: two nematode larvae entrapped in an adhesive 3D network of hyphae. Reproduced with permission of N. Soto-Barrientos.

1.6.7 Concluding remarks

It will have become apparent during the course of this chapter that parasite control is not always straightforward but often requires a range of skills. It demands knowledge of the biology and epidemiology of each type of parasite, the ability to interpret laboratory findings and make an accurate diagnosis, an understanding of the rationale underlying different control methods, and an appreciation of the strengths and limitations of available parasiticides and vaccines. This is why the expertise of the animal health professional is so valuable to the animal owner.