Andrei Daniel MIHALCA

# Textbook of Veterinary Parasitology

## Introduction to parasitology. Protozoology.

AcademicPres

**Andrei D. MIHALCA**

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Introduction to parasitology

Protozoology

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# 1 INTRODUCTION TO PARASITOLOGY

## 1.1 Defining parasitology. Diversity of parasitism in nature.

The amount of information in the field of parasitology is huge, and more than 20 international peer-reviewed scientific journals are currently being published in this biomedical field. Parasitology is, arguably, one of the most intriguing and fascinating biological sciences and certainly one of the most complex ones, involving a broad interdisciplinary approach. Knowledge from fields like taxonomy, phylogeny, ecology, biochemistry, genetics, molecular biology, immunology, epidemiology, pharmacology, pathology, etc. is required for this complex approach. We will try to limit this chapter to the essentials.

When attempting to define ***parasitology*** you get a snowball effect. To make it simple, parasitology represents the study of ***parasitism***. Parasitism is a very complex interaction between two species, one called parasite and the other called host. However, to give a more complex definition is trickier than expected, as suggestively emphasized by Bush et al. (2001): "*if you assemble 10 scientists and ask them to define parasitism, you would obtain 10 different answers*".

Etymologically, the word parasite derives from the association of two Greek words: *para* (beside) and *sitos* (grain, food). If we summarize different definitions given to parasites, there are many similarities but also notable differences (**table 1.1**).

**Table 1.1** Different definitions given to the parasite

| |
|---|
| An animal that lives completely at the expense of plants, animals, or humans[1] |
| A plant or animal that lives upon or within another living organism at whose expense it obtains some advantage[2] |
| An organism which lives in or on another organism and benefits at the other's expense[3] |
| An organism that lives in or on and takes its nourishment from another organism[4] |

1 - Mehlhorn (2008); 2 - Gosling (2005); 3 - Soanes (2008); 4 - Webster's New World Medical Dictionary (2008)

Based on most of these definitions, many organisms could be considered parasitic. Even if all viruses, many bacteria, many fungi, but even some plants or vertebrates are ecologically parasitic organisms, customarily, parasitology comprises the study of parasitic protists and invertebrate animals (mostly helminthes and arthropods, but also some other small groups of metazoans). Parasitism is a relatively common way of life, and practically every major phylum of Kingdom Animalia includes parasitic species, some of them exclusively, at least in one stage of their life cycle (**table 1.2**).

**Table 1.2** Animal phyla with parasitic representatives*

| Phylum[1] | Percent of parasitic species[2] |
|---|---|
| Porifera | <1 |
| Placozoa | 0 |
| Myxozoa | 100 |
| Cnidaria | <1 |
| Ctenophora | ~1 |
| Priapula | 0 |
| Nematomorpha | 100 |
| Nematoda | ~50 |
| Loricifera | 0 |
| Kinorhyncha | 0 |
| Onychophora | 0 |
| Arthropoda[3] | ~3 |
| Tardigrada[4] | 0 |
| Platyhelminthes | ~80 |
| Mollusca | ~1 |
| Annelida | ~6 |
| Sipuncula | 0 |
| Rotifera | ~1 |
| Acanthocephala[5] | 100 |
| Nemertea | <1 |
| Phoronida | 0 |
| Bryozoa | 0 |
| Brachiopoda | 0 |
| Gastrotricha | 0 |
| Entoprocta | 0 |
| Chaetognatha | 0 |
| Gnathostomulida | 0 |
| Echinodermata | <1 |
| Hemichordata | 0 |
| Chordata | <1 |
| Cycliophora | ? |
| Rhombozoa | 100 |
| Orthonectida | 100 |

* Adapted from Bush et al. (2001)

1 Several classifications are available in the literature. The phyla name used in this book are those listed on Animal Diversity Web (ADW): http://animaldiversity.ummz.umich.edu
2 At least in one stage of their life-cycle
3 Herbivorous insects are not included
4 Including pentastomes (according to ADW)
5 According to ADW, Acanthocephala is part of phylum Rotifera

Some estimates state that about half of the known species on Earth are parasitic at least in one of their life stage.

## 1.2 Parasitism as an interspecific interaction

Any interaction in which a species of organism spends a part of or its entire life in association with another species is called symbiosis. The word ***symbiosis*** (Greek: *syn* = along with, together; *bios* = life), as originally used by Heinrich Anton de Bary (1831-1888) refers to organisms living together. Some suggestions to use this term only for the bilateral positive interspecific relationships (equal to mutualism) are confusing and will not be considered herein.

Partner species that are involved in a symbiosis may benefit from, be harmed by, or not be affected by the association. Despite some expected overlaps, there are five main types of symbiotic relations: phoresis, inquilinism, mutualism, commensalism and parasitism. Phoresis and inquilinism do not imply trophic interactions.

***Phoresis*** (also called phoresy) (Greek: *pherein* = to bear) refers to interactions where one partner (the host) mechanically carries one or more individuals from another species (the phoretic organism) (**figure 1.1**). Nevertheless, some phoretic species can eventually cause harm to their host mainly because of overburdens. In the pictured case, the interaction can arguably be called parasitism, as the damage is purely mechanical and no physiological or trophic mechanisms are involved.

***Inquilinism*** (also called inquilism) is another type of pure mechanical interaction where two or more organisms

of different species share a dwelling place.

In a ***mutualistic*** symbiosis, both partners benefit from the relationship (**figure 1.2**). The extent to which each partner benefits, is difficult to assess, but it is generally accepted that for any benefit there is a certain biological cost.

**Figure 1.1** Phoretic mites on insects are extremely frequent in nature. *(Photo Andrei D. Mihalca)*

The term ***commensal*** (literally meaning "together at the same table") was first used by the Belgian parasitologist P.J. van Beneden (1809-1894) to explain the associations in which one animal shares food obtained by another animal. There is some controversy on how broad this meaning should be. Some authors consider that commensalism do not involve any physiological interaction nor dependency between the partners, and only the spatial proximity allows the commensal to feed on nutrients captured or ingested by the host. Yet, the meaning used in this work refers to the broader concept, where commensalism includes all types of associations when one partner (the commensal) benefits and the other (the host) is not harmed. Moreover, some others consider phoresis and inquilinism particular types of commensalism.

**Figure 1.2** One of the most common mutualistic interactions, the lichen. *(Photo Andrei D. Mihalca)*

With the view of all above, ***parasitism*** can be defined as a symbiosis (certainly the most common one) in which one of the symbionts (the parasite) benefits at the expense of the other (the host). All the main aspects of parasite-host interactions as well as the types of hosts and parasites will be detailed in the following chapters. An interesting type of parasitic-like interaction is known to occur in echiurans, a small group of vermiform, bottom-dwelling marine organisms. In this case, males (1-3 mm in length) are parasitic in the kidneys of

females (80 mm in length). It is highly arguable if this particular association could be considered parasitism, as it is an intraspecific interaction not an interspecific one.

To include a certain symbiotic interaction (**figure 1.3**) in one of the types defined above is more or less conventional. Many relationships are dynamic, and there may be frequent transitions from one type to another. Symbiotic associations may change because of external factors (environmental or host-dependent) or due to internal influences (symbionts-dependent).

The evolutionary approach to symbiotic interactions is the most interesting one. For instance, a parasitic association can evolve into mutualism or commensalism. With high probability, most mutualistic symbioses probably began as parasitic ones, with one organism attempting to exploit another one. As brilliantly perceived by Paracer and Ahmadjian (2000) in their monograph on symbiosis, if one considers parasitism as an antagonistic relationship then mutualism can be regarded as a standoff or a draw between the two antagonists. The widely accepted theories of the origins of some cell organelles like mitochondria or chloroplast consider that these are transformed bacteria that may have begun as parasitic symbionts in larger prokaryotic cells. On the contrary, a mutualistic or commensalistic association may degenerate into a parasitic one if the defense mechanisms of the host are decreased.

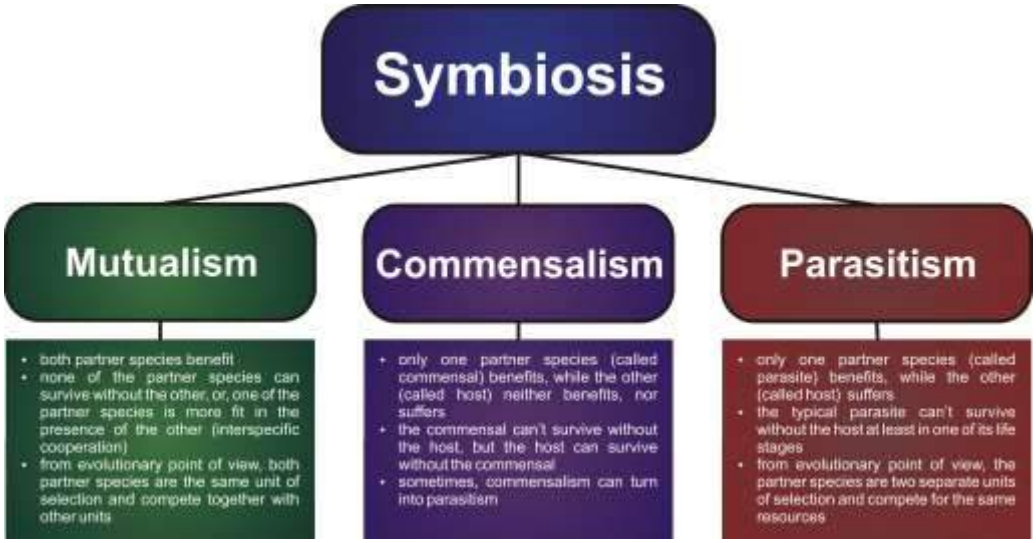



**Figure 1.3** Main features of symbiotic associations which involve trophic interactions (phoresis and inquilinism are excluded).

The first exhaustive attempt to classify symbiotic interactions was made by M.P. Star in 1975. He used several criteria for his classification like location of symbiont within the host, persistence, dependence and specificity of symbiosis. Parasitism basically fits to this system with some peculiarities detailed in Chapter 1.5.

## 1.3 An ecological approach to parasitology

We can regard parasites from many points of view, but certainly the medical aspects of parasitism, especially in humans and domestic animals are the most considered worldwide. However, especially in wild animals, parasites do not cause apparent disease, and the relative balance between parasites and hosts is the result of a complex and long coevolution.

If we consider parasitology a branch of ecology, the habitat and environment of the parasite is provided by another organism, the host. Many ecological principles which apply to free-living organisms can be applied to parasites. The only particular situation is that the environment of the parasite is alive and actively fights against it. Therefore, the main issues for such an approach to parasitology include adaptations of parasites to their environment and life style, adaptations of hosts to antagonize parasites and mainly the nature of host-parasite relationship. To put it simply, ecological parasitology studies the complex relationships between parasites, hosts and environment. The environment of the parasite however, differs. During the parasitic phase, as shown above, the environment of the parasite is the host's body. However, the vast majority of parasites have at least one stage in the external environment during their life cycle, also very important in the understanding of this complex interaction.

Ecology of the host itself greatly influences the adaptations of the parasite in order that it could easily infect the host. The best examples with this view refer to parasites with heteroxenous life-cycles (see Chapter 1.7).

An important question of an ecological approach to parasitology is what the role of parasites within the ecosystem is. There are well-defined theories stating that parasitism plays a major role in the evolution of species diversity and regulation of host populations.

Another question is if and how parasites are able to regulate biodiversity in general and host populations in particular. Some consider parasites as a threat to biodiversity, mainly in the case of endangered hosts. Nevertheless, parasites are part of our biodiversity as are their hosts. Parasites of endangered species can sometimes play a significant role in the conservation efforts. At least one documented case of parasite-mediated extinction was described in the snail *Partula turgida*, where the last-known individual was killed in captivity by a microsporidian parasite. However, in the case of highly host-specific parasites, the extinction of the host equals with the extinction of the parasite itself (host-parasite co-extinction). It might be the

case of the rhino ticks (i.e. *Dermacentor rhinocerontis*) from Africa.

One thing is for sure. A world without parasites would look completely different than the world we know today, not necessarily in a good way.

## 1.4 A brief history of parasitology

History of medical sciences is a fascinating topic and basic knowledge is required for a proper understanding of progress dynamics in certain fields. There are several reviews on history of parasitology, most of them focusing on human parasites. Some of these works focus also on general parasitology, and a great resource is the review of Cook GC (in Gillespie SH, Pearson RD. 2001. Principles and Practice of Clinical Parasitology. Wiley. 752 pp.).

**Early records.** Since humans became aware of their social and ecological identity, they were also probably aware of some macroparasites like larger helminthes or cutaneous arthropods living associated with *Homo sapiens* or animals nearby (domesticated or hunted). However, the first written documentation on a parasitic organism is found in the Papyrus Ebers (~1550 BC). In ancient Egypt, several other writers were aware of some major helminthic infections of humans, like schistosomiasis, dracunculiasis or ascariasis. Dead female *Dracunculus* worms have been found in Egyptian mummies older than 3000 years. In ancient Greece, Aristotle (384-322 BC) mentioned in his writings parasitic helminthes of dogs, pigs and fish. Galen of Pergamum (131-199), the Roman physician and philosopher of Greek origin (**figure 1.4**), recognized three macroparasites of humans: *Ascaris*, *Taenia* and *Enterobius*. Human hydatidosis was already known by Aretaeus of Cappadocia (81-138).

**Figure 1.4** Galen of Pergamum. *(reproduced from a lithograph by Pierre Roche Vigneron)*

**Spontaneous generation.** All these early records and many other observations did not have a real biological background. The general belief until the mid-nineteenth century was that parasites, like all other living organisms, appear through spontaneous generation. This theory, synthesized by Aristotle, was firstly doubted on by the Dutch biologist Jan Swammerdam (1637-1680) and by the Italian physician Franceso Redi (1626-1697). The later did not agree that flies arouse spontaneously from rotting meat. Despite his morphological proof on

the sexual reproduction of *A. lumbricoides*, the British anatomist Edward Tyson (1650-1708) was also an adept of spontaneous generation. A particular application of this theory was extrapolated to parasitology by Marcus Bloch (1723-1799) and Johan Göze (1731-1793). The two famous European parasitologists embraced the opinion that parasites were "inborn in their host". The concept of spontaneous generation was finally abandoned after strong experimental proofs brought by Luis Pasteur (1822-1895).

**Emergence of parasitology as a science (17th and 18th centuries).** Although most historical records of parasites are related to humans, the birth of parasitology as a science is linked to veterinary medicine.

**Figure 1.5** Francesco Redi. *(reproduced from an engraving by Lodovico Pelli)*

Francesco Redi (**figure 1.5**) is considered the father of parasitology, after he published in 1694 the first scientific monograph on parasitic organisms "*Osservazioni intorno agli animali viventi che si trovano negli animali viventi*" [*Notes on living animals found in living animals*] (**figure 1.6**). Five years later, Nicolas Andry (1658-1742) was the first to illustrate the scolex of a human tapeworm, *Taenia saginata* in his "*De la génération des vers dans le corps de l'homme*" [*On the generations of worms inside the human body*] (**figure 1.7**).

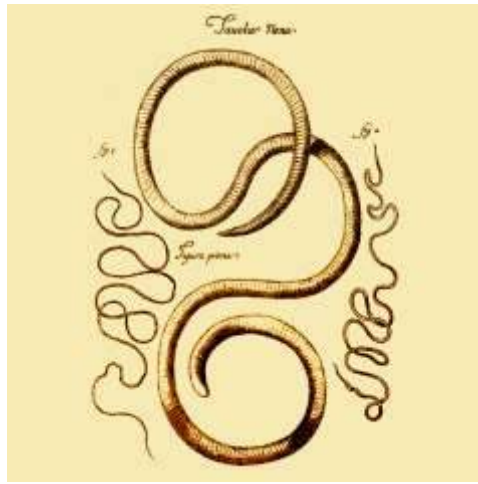


**Figure 1.6** Original drawing from Redi's "*Osservazioni intorno agli animali viventi che si trovano negli animali viventi*" depicting: "*a big worm found in the kidney of a marten*" (in the middle); "*a worm found under the skin of a lion*" (right); "*a worm very frequently found under the skin of martens and skunks*". (left)

Eighteenth century brought three major contributions in parasitology. In 1760, Pierre Pallas (1741-1811) wrote a dissertation called "*De infestis viventibus itraviventia*". Göze, who discovered the scolex of *Echinococcus* in hydatid cysts,

published in 1787 his "*Versuch einer Naturgeschichte der Eingeweidewürmer tiericher Körper*" [*Natural history of intestinal worms from the body of animals*]. Last but not least, Bloch, who was the first to note the hooklets on the scolex of tapeworms, wrote in 1782 "*Abhandlung von der Erzeugung der Eingeweidewürmer und den Mitteln wider dieselben*" [*Treatise on the Generation of Intestinal Worms and the means of their extermination*], winning the gold medal for best essay at the Copenhagen Academy of Sciences (**figures 1.8 and 1.9**).

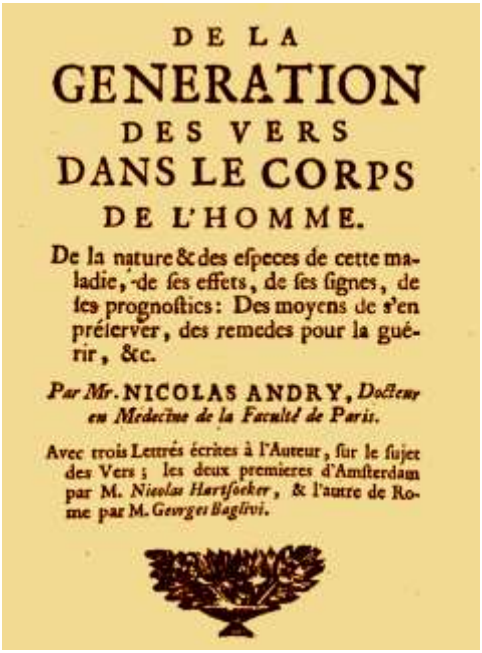

DE LA
GENERATION
DES VERS
DANS LE CORPS
DE L'HOMME.
De la nature & des eſpeces de cette maladie, de ſes effets, de ſes ſignes, de ſes prognoſtics: Des moyens de s'en préſerver, des remedes pour la guérir, &c.
Par Mr. NICOLAS ANDRY, Docteur en Medecine de la Faculté de Paris.
Avec trois Lettrés écrites à l'Auteur, ſur le ſujet des Vers; les deux premieres d'Amſterdam par M. Nicolas Hartſoeker, & l'autre de Rome par M. Georges Baglivi.

**Figure 1.7** Cover of Andry's "*De la génération des vers dans le corps de l'homme*", re-published in 1750.

**Parasitology in the nineteenth century.** Several outstanding parasitologists arose in the scientific circles of the century. Probably the most prominent of them was Carl Rudolphi (1771-1832). The Swedish scientist wrote two monumental works "*Entozoorum sive vermium intestinalium historia naturalis*" from 1808 and "*Entozoorum synopsis cui accedunt mantissa duplex et indices locupletissima*" from 1819 which substantially increased the number of known species of parasites.

The French parasitologist Félix Dujardin (1801-1860) was the first to understand that the life cycle of trematodes and cestodes involve an intermediate host. In 1845 he published his most important work: "*Histoire naturelle des helminthes ou vers intestinaux*" [*Natural history of helminthes or intestinal worms*] (**figure 1.10**).

As mentioned above, basically all published parasitological works of the time originated from mainland Europe. First English texts appeared as translations from German, French or Latin. The first book of parasitology written by a British scientist was "*Entozoa, an Introduction to the Study of Helminthology*" in 1864 by Thomas S. Cobbold (1828-1886).

The end of the nineteenth century marked a shift in the concepts of parasitology. Step by step, scientists understood the medical importance of parasites in humans and animals. Thus, from a branch of zoology, parasitology was more and more viewed as a medical science.


D. Marcus Elieser Bloch's,

Abhandlung

von der

Erzeugung der Eingeweidewürmer

und den

Mitteln wider dieselben.

Eine

von der Königlich Dänischen Societät der Wissenschaften

zu Copenhagen

gekrönte Preisschrift.

Mit 10 Kupfertafeln.

Berlin,

**Figure 1.8** Cover of the first edition of Bloch's gold medal essay.

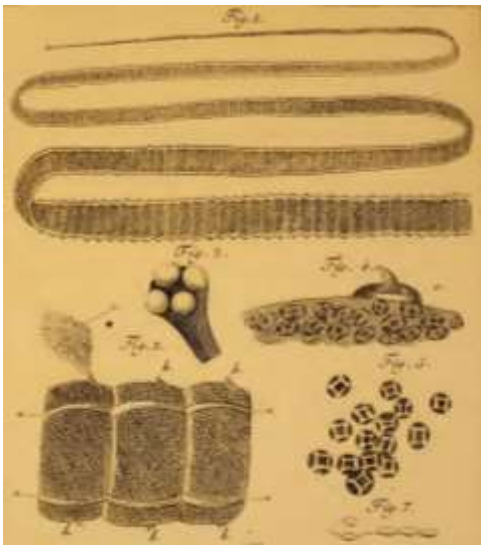


**Figure 1.9** One of the ten plates of Bloch's book.


HISTOIRE NATURELLE

DES

HELMINTHES

OU

VERS INTESTINAUX

PAR

M. FÉLIX DUJARDIN

Ouvrage enrichi de douze Planches.

PARIS

LIBRAIRIE ENCYCLOPÉDIQUE DE RORET

1845

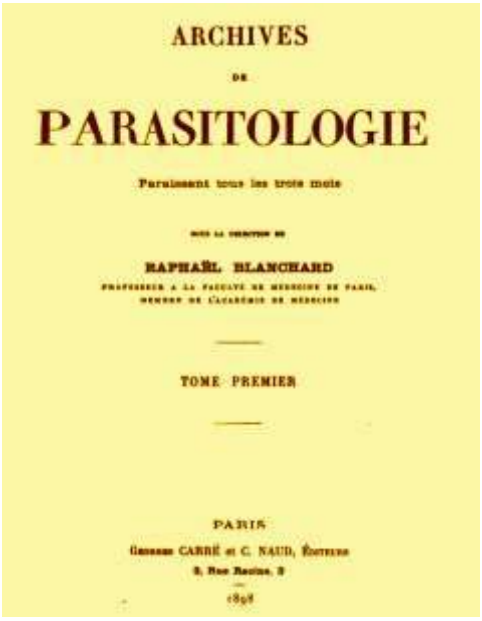
**Figure 1.10** Cover of the first edition of Dujardin's major work.

The first journal entirely devoted to parasitology, "*Archives de Parasitologie*" had its first volume published in 1898 (**figure 1.11**) by Émil Raphaël Blanchard (1819-1900). However, after 16 volumes and a break during the WW1, the publication was suspended in 1919. Overseas, the father of American parasitology was a paleontologist, Joseph Leidy (1823-1891).

**Modern parasitology.** Definitely, the most dramatic shift in modern parasitology as in many other natural sciences was the discovery of nucleic acids and of molecular biology

techniques. In the last decades, molecular tools became almost ubiquitous in biological or medical parasitological research.

But there is still a long way to go. Many parasitic diseases are becoming emergent. Some, otherwise harmless parasites are killing immune compromised hosts (i.e. HIV positive). Despite enormous amount of research and money, there is still no vaccine for malaria, the deadliest parasitic disease on Earth. And the list can continue….


ARCHIVES

DE

PARASITOLOGIE

Paraissent tous les trois mois

RAPHAËL BLANCHARD

TOME PREMIER

PARIS

**Figure 1.11** First volume of *Archives de Parasitologie*.

## 1.5 Types of parasites

Except the taxonomic approach, there are several other criteria to classify parasites, all of them conventional, as they do not always properly reflect the complex interactions with the host. Moreover, the categories frequently overlap. Like in many other areas of science, classifications are used mainly for scholastic purposes. The main criteria used for categorizing parasites are:

**(1) Location of parasites within the host**

- ***Ectoparasites*** are organisms living parasitically on the outside of their host. Although this broad definition seems clear enough, some comments are required. Certain parasites are normally living on the skin (integument), hair, feathers or scales of their hosts. These are the typical ectoparasites (i.e. ticks, some mites, fleas, lice, biting insects etc.). Other arthropods are living within the structures of the skin (i.e. *Demodex* in the hair follicles; mange-causing mites in the dermis etc.) but are customary considered ectoparasites. Larval forms of *Hypoderma* species migrate through various parts of the hosts' body and finally they stop subcutaneously (**figure 1.12**), hardly being considered ectoparasites. Parasites also inhabit various external mucosae of their hosts (i.e. *Thelazia* in the conjunctiva; *Oestrus* the nasal cavities; monogeneans or ciliates on the gills of fish (**figure 1.13**); some trichomonadids within the buccal or genital mucosa; *Otodectes* mites in the ear canal etc.) Their classification as ectoparasites is debatable.
- ***Endoparasites*** are those which inhabit the internal organs of their hosts.

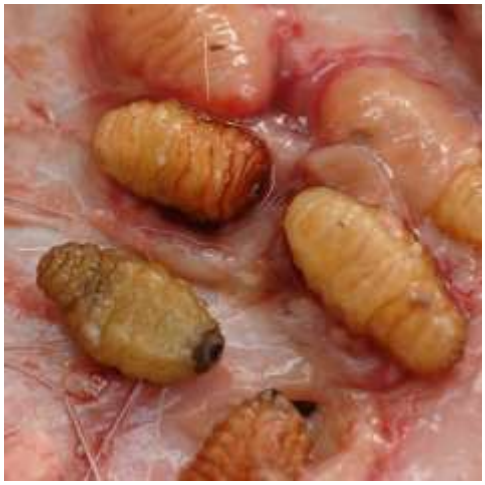


**Figure 1.12** Larvae of *Hypoderma diana* are commonly found in the subcutaneous tissue of roe deer. *(Photo Andrei D. Mihalca)*

**Figure 1.13** Ciliated protozoa (*Ichthyophthirius multifiliis*) parasitic on the gills of common carp. These parasites also infect the integument. *(Photo Andrei D. Mihalca)*

Also called internal parasites, they can inhabit all organ systems: digestive tube, liver, respiratory tube, urinary system, brain, muscles, tendons, heart, blood vessels, serosae etc. Moreover, some unicellular organisms (i.e. protists) are intracellular parasites (intracytoplasmatic or intranuclear). As shown in the previous paragraph, some ectoparasites could be arguably considered endoparasites.

**(2) Size of parasites**

- ***Microparasites*** are those not visible by humans with naked eye. We include here protists, larval stages of most helminthes, most of the monogeneans, mange causing mites etc.
- ***Macroparasites*** are visible to the human eye without the aid of the microscope. Although some parasites like *Dermanyssus* mites of birds (**figure 1.14**) or *Strongyloides* nematodes from the intestine of various vertebrates are hardly visible macroscopically, they are considered macroparasites. On the other hand, there are enormous-sized parasites. For instance, many cestodes frequently reach lengths of several meters (i.e. *Diphyllobothrium latum*, a tapeworm of fish-eating mammals, including dogs and humans can reach up to 12 meters in length). The largest known trematode, *Nematobibothrioides histoidii* from the muscles of the sun fish can reach a length of 12 meters. The largest known parasitic nematode is *Placentonema gigantissima* from the placenta of female sperm whales, which can reach up to 8.5 meters in length.

**Figure 1.14** The red poultry mite, *Dermanyssus gallinae*, parasitic on chicken are visible with the naked eye, but a careful examination should be performed. *(Photo Cristian Magdaș)*

**(3) Host specificity**

- ***Monoxenous*** parasites are limited to a single host species in certain life stages of their development. Many *Eimeria* species are strictly host-specific and could be included here.
- ***Oligoxenous*** parasites have a small host range (2-5 species). Adults of *Echinococcus granulosus* are typical examples. However, larval forms of the same parasite have a broad range of hosts (polyxenous).
- ***Polyxenous*** parasites have a broad range of hosts (low or no host specificity). For instance, *Toxoplasma gondii* is a polyxenous parasitic apicomplexan.

**(4) Number of hosts required for the completion of life cycle**

- ***Homoxenous*** (Greek: *homos* = identical; *xenos* = host) parasites include those species which require a single host category for the completion of their life cycle. Homoxeny defines parasite transmission through hosts of same ontogenetic category. These hosts should not necessarily be one and the same species. A homoxenous parasite, therefore, can be monoxenous, oligoxenous or polyxenous. Homoxeny is also called direct life cycle. Some examples of homoxenous parasites include: apicomplexans from genera *Cryptosporidium* and *Eimeria*; monogeneans; certain nematodes of domestic animals or humans (*Ascaris*, *Strongylus*, *Oxyuris*, *Strongyloides*, etc.). The vast majority of parasitic arthropods also have homoxenous life cycles (mange causing mites, fleas, lice etc). A particular type of homoxenous development was described in *Sarcocystis gallotiae* parasitizing lizards. For this species of apicomplexan parasite, transmission occurs by cannibalism, so both the definitive and intermediate host are different individuals from the same species (dihomoxenous life cycle). In some parasites with typical homoxenous life cycle, occasionally, in order to cross some trophic boundaries, they use an additional non-obligate host. They are called facultative heteroxenous parasites and examples include *Toxocara* (an

ascarid nematode of dogs and cats), *Syngamus* (a respiratory strongyle of galliforme birds) and many others.

- ***Heteroxenous*** (Greek: *heteros* = different; *xenos* = host) parasites require transmission via alternation of hosts of different ontogenetic categories. This type of development is also called indirect life cycle. According to the number of hosts, these parasites are called diheteroxenous (i.e. *Fasciola hepatica*), triheteroxenous (i.e. *Dicrocoelium dendriticum*) or tetraheteroxenous (i.e. *Alaria alata*). The term polyheteroxenous is also used to refer to parasites requiring more than two hosts.

**(5) Obligativity of parasitic life**

- ***Obligate parasites*** are those which need a host for survival, development and/or reproduction during at least one of their life stages. In some species of parasites, all developmental stages are found associated with only one host (i.e. *Trichinella*, lice etc.). In some others, only certain stages are obligatory parasitic. For instance, in many nematodes, the first larval stages are free-living, while the later stages and adults are *obligatorily parasitic (i.e. Strongylus nematodes of horses).*

- ***Facultative* parasites** are generally free-living species, which may accidentally become parasitic. The nematodes of genus *Strongyloides* can undergo two types of development. In certain cases, all life cycle takes place as free-living stages; in other cases, adult females become parasitic. Another example of facultative parasites is the case of larval stages of Calliphoridae flies. They are opportunistic and have the ability to exploit living tissue, although characteristically they are carrion feeders.

**(6) Duration of parasitism**

- ***Temporary parasites*** are in contact with their host for short periods during a certain stage of their life cycle. Mosquitoes or leeches are typical examples. If a temporary parasite visits its host several times during a particular life stage it is called a **periodic parasite**.

- ***Permanent parasites*** infect their host for longer times. All adult stages of trematodes and cestodes are associated with their definitive host during their entire adulthood.

**(7) Parasitic life stage**

- ***Pre-imaginal parasites*** are parasitic only during their immature life stages, while adults are free-living. All myiasis causing flies are pre-imaginal parasites. In these species, usually the adult stage is short living and many times it doesn't even feed. In the representatives of phylum Nematomorpha, the larval stages are always obligatory parasites while the adults are free-living.

- ***Imaginal parasites*** infect their hosts only during their adult stage, while immature stages are free-living. Fleas of genus *Ctenocephalides* (**figure 1.15**) parasitic on dogs and cats are imaginal parasites.

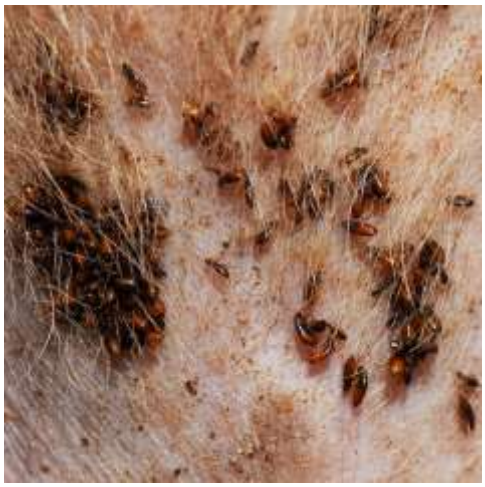


**Figure 1.15** Heavy infestation with the flea, *Ctenocephalides canis* on a dog. Only the adults are parasitic; larvae and nymphs are found in the dog's environment. *(Photo Andrei D. Mihalca)*

- *Note*: in many parasites, immature and adult stages are both parasitic in the same or in different hosts. The term to include these cases is not well-defined, but permanent parasite can be a feasible option, although it overlaps with the previous criteria.

## 1.6 Types of hosts

The ecological concept of "host" would not exist without the concepts of symbiosis in general and parasitism in particular. Basically, a host is any living organism which harbors another one, parasitic. The following terminology used for host classification is not based on single criteria.

***Definitive host*** (or final host) is traditionally defined as the host where the parasite reaches sexual maturity or as the host which harbors the adult parasites. Even if this definition looks clear and simple, due to the complexity and diversity of parasites and parasitic interactions, some comments are required. Most homoxenous metazoan parasites reach sexual maturity in the host, thus this should be called definitive host. However, as this host is singular, and no other organism is required in the development of homoxenous parasites (i.e. intermediate host), it is easier to use just the term "host". Moreover, in some homoxenous parasites, only the immature stages are parasitic, while the adults are free-living, thus, the term "definitive host" would not fit to the generally accepted definition. On the other hand, there are parasites with facultative heteroxenous life cycles. In this case, it would be useful to use the term "definitive host" to differentiate them from the eventual facultative intermediate or paratenic hosts. Moreover, in parasitic protozoa there is no such stage as "adult" or concept of "sexual maturity". Conventionally a definitive host for heteroxenous parasitic protozoa is the host in which sexual reproduction occurs. However, in some parasitic heteroxenous protozoans the life cycle does not include any sexual reproduction; hence the definition of definitive host in this case is arguable.

Asexual reproduction is also known in adult females of homoxenous nematodes of genus *Strongyloides*, so the traditional definition should be reconsidered.

***Intermediate host*** is any host involved in heteroxenous life cycles which is not definitive and in which the parasites undergo some developmental and morphological change. In polyheteroxenous life cycles, intermediate hosts are customarily numbered according to their consecutiveness in the ontogeny of the parasite (i.e. intermediate host 1, intermediate host 2, etc.).

***Paratenic host*** (or transport host) is used by some parasites to bridge a trophic gap. Ontogenetically, they are not obligatory in the life cycle of parasites, but ecologically they are very important. The most accepted definition considers the paratenic host as an organism which serves to transfer a larval stage or stages from one host to another but in which little or no development takes place. The term is rather appropriate in helminthology than in protozoology.

***Reservoir hosts*** are those organisms which are responsible to maintain the parasite populations in certain ecosystems.

***Vector hosts*** are defined as organisms which transmit certain pathogens from one host to another. However, definitions widely vary according to the vectored pathogen.

***Dead-end hosts*** are usually intermediate or paratenic hosts which are not able to transmit the parasites to further hosts. Limitations are most commonly ecological or trophic. For examples, humans are dead-end host for many parasites (i.e. *Echinococcus*, *Trichinella*, etc) as it is unlikely that definitive carnivore hosts will prey on humans.

*Notes*: (1) In some cases, a single individual can be definitive and intermediate host for the same parasite. For example, a cat which harbors the gametogonic (sexual) stages of *Toxoplasma gondii* in its intestines is a definitive host. However, cats can also develop systemic infection with merogonic stages of *T. gondii* making them intermediate hosts. Often, the same cat can harbor both stages, being in the same time intermediate and definitive host, but in different life cycles. (2) Another unusual situation is encountered in the life cycle of the nematodes of genus *Trichinella* (**figure 1.16**). The same individual acts first as definitive and later on as intermediate host in a particular heteroxenous life cycle.

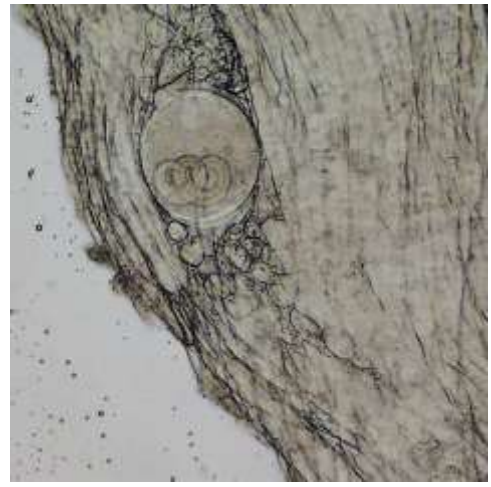


**Figure 1.16** Larva of *Trichinella britovi* parasitic in the skeletal muscles of red fox. *(Photo Călin M. Gherman)*

## 1.7 Life cycle of parasites

Every living organism has a life cycle (also known as developmental cycle or life history). Life cycle is a series of developmental stages through which an organism goes through. Always, the last stage from a life cycle must be able to produce the initial stage from the subsequent cycle. In parasitic organisms, the life cycle is extremely complex and it is the results of coevolution with their hosts. Regardless of the taxonomic group, development of parasitic species most often comprises an alternation of free-living and parasitic stages.

In order to complete their life cycle, parasites have to overcome three critical steps:

- the immune system of the host(s);
- the adverse environmental factors;
- the ecological requirements for host-to-host transmission.

Despite all odds, an impressive number of parasitic species succeeded through the caudine forks of evolution.

### 1.7.1 Types of parasitic life cycles

As shown above (Chapter 1.5), there are two main types of parasitic life cycles: homoxenous and heteroxenous. Each species has a characteristic life cycle which will be detailed in the corresponding section of this textbook, but some general aspects should be discussed further on.

In ***homoxenous life cycles*** parasites require a single host to complete their development. This way of development is encountered in some parasitic protozoans, in all monogeneans and in various nematode species. There are three different possible situations:

- In most homoxenous parasites, there is an alternation of stages in the environment with parasitic stages (**figure 1.17**). The host acquires the parasite from the environment via various routes, most commonly by ingestion. Typical examples for this case are apicomplexan protozoans of genus *Eimeria*. In some other cases, infective stages from the environment actively enter the host, by penetration of skin or mucosae (i.e. *Ancylostoma*, *Bunostomum*, etc.).

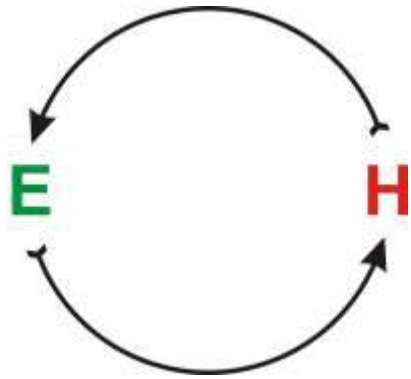



**Figure 1.17** Typical homoxenous life-cycle (E = external environment; H = host).

- Although not common, in certain homoxenous parasites the life cycle can be completed without stages in the external environment. This is

possible due to autoinfection of the single host (**figure 1.18**). For instance, the parasitic females of the nematodes of genus *Strongyloides* typically lay embryonated eggs which pass through the host's feces to the external environment where they hatch. Subsequently, infective larvae will penetrate through the skin of a new host. However, in some cases, eggs are able to hatch while still inside the host's intestine, so larvae will be autoinfective to the same host. Another possibility herein, involving two different individuals is the sexual transmission of some protozoans (*Trypanosoma equiperdum*, *Tritrichomonas foetus*).

- The third possibility for homoxenous life cycle was described for certain species of genus *Sarcocystis* parasitic in lizards. These parasites are transmitted from host to host by cannibalistic behavior during which, lizards from the very same species eat each other's tails. This particular life cycle (**figure 1.19**) was denominated as dihomoxenous and sometime even the same individual lizard can act as both definitive and intermediate host.

***Heteroxenous life cycles*** are very complex and ecologically challenging pathways in the development and host-to-host transmission of parasites. Several groups of parasitic organisms have exclusively heteroxenous development. All trematodes, all cestodes and all acanthocephalans are included here. Many protozoans and nematodes also embrace heteroxeny.

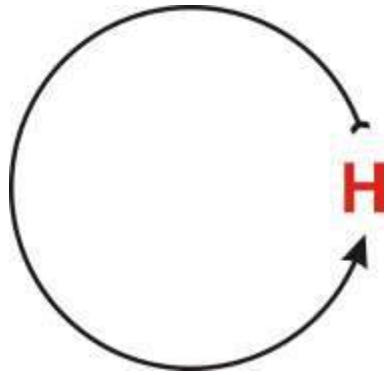



**Figure 1.18** Homoxenous life-cycle in autoinfective parasites (E = external environment; H = host).

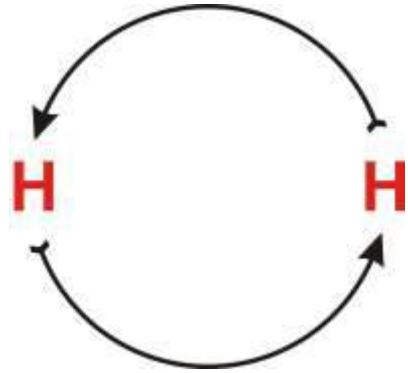



**Figure 1.19** Dihomoxenous life-cycle (H = host)

Heteroxenous development comprises of a definitive hosts and one or more intermediate hosts. Although the possibilities and variations in the alternation of hosts and stages in the external environment are multiple, the following situations will be considered:

- Most of the so called vector-borne parasites (i.e. *Babesia*, *Theileria*, *Trypanosoma*, *Leishmania*, *Dirofilaria*, etc.) have a typical diheteroxenous life cycle with direct host-to-host

transmission and with no stages in the external environment (**figure 1.20**). Transmission occurs both ways through hematophagy. Another interesting diheteroxenous life cycle with no external stages has been described in hemoparasites of genus *Hemolivia*. The definitive hosts are ticks which acquire the infection by hematophagy from tortoise intermediate hosts. Remarkably, the tortoises get the infection by ingesting infected ticks.

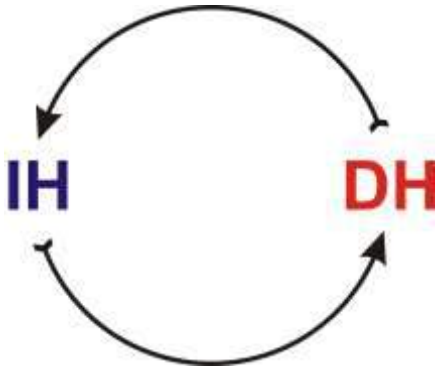



**Figure 1.20** Diheteroxenous life-cycle with no external stages (DH = definitive host; IH = intermediate host)

- Many diheteroxenous parasites - like most of the cestodes and acanthocephalans, but also some apicomplexan protozoans (i.e. *Sarcocystis*, *Toxoplasma*, *Neospora*, etc.) - are characterized by indirect transmission through the external environment from the definitive host to the intermediate host and direct transmission through predatorism from the intermediate host to the definitive host (**figure 1.21**).

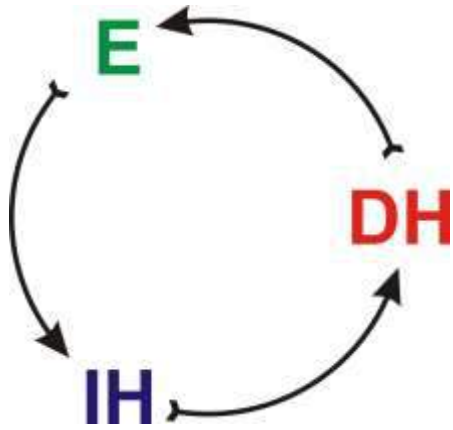



**Figure 1.21** Diheteroxenous life-cycle with single indirect transmission (E = external environment; DH = definitive host; IH = intermediate host)

- In other groups of diheteroxenous parasites there is no direct interaction between the definitive and the intermediate host, and transmission from one host to another always occurs through some external stages (**figure 1.22**). For instance, in the trematode *Fasciola hepatica*, definitive hosts shed parasitic eggs to the pasture where the larval stage (miracidium) hatches and penetrates a snail intermediate host. After several asexual reproductions, cercariae actively leave the snail and encyst as metacercariae on vegetation. A new definitive host will ingest them and a new life cycle begins.

  Most trematodes have a typical triheteroxenous development, involving very diverse possibilities. Considering a single situation (**figure 1.23**), we will illustrate the life cycle of *Dicrocoelium dendriticum*.

Ruminant definitive hosts are shedding the eggs of this parasitic liver trematode, through their feces to the external environment.

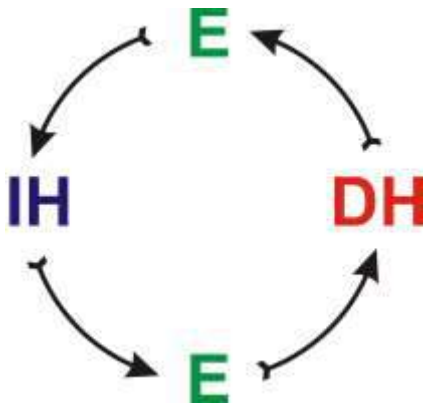



**Figure 1.22** Diheteroxenous life-cycle with double indirect transmission (E = external environment; DH = definitive host; IH = intermediate host)

The first intermediate host ($IH_1$), in this case a terrestrial snail will ingest these eggs and inside its body the miracidium will hatch and multiply by asexual reproduction. When reaching to the cercarial stage, they actively emerge from the snail's body and are ingested by a second intermediate host ($IH_2$) which is an ant. Inside the ant's body, the cercariae will encyst into a metacercariae. Further transmission to a new definitive host implies accidental ingestion by ruminants of infected ants. The chances for this apparently hazardous event are increased by the pathogenic effect of the metacercarial stage on ants, causing them impaired motility. Another similar example of life cycle but involving different hosts is known to occur in the cestode *Diphyllobothrium latum* infecting humans and other piscivorous mammals.

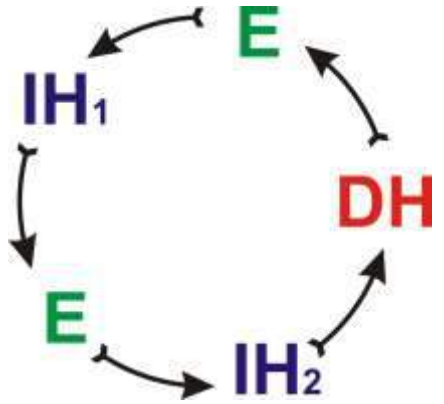



**Figure 1.23** Triheteroxenous life-cycle (E = external environment; DH = definitive host; IH = intermediate host)

### 1.7.2 Stages in the external environment

In the very previous chapter we showed that in many parasitic life cycles, transmission from a host to another requires passage through the external environment. For an organism which is primarily adapted to a parasitic lifestyle, the contact with environmental factors can seriously affect its survival. On the other hand, parasitism as a way of life probably evolved in previously free living organisms as an adaptation to avoid these factors.

An interesting situation of organism which is able to opt for a free-living or a parasitic lifestyle is known for nematodes of genus *Strongyloides*. In these nematodes, only females are parasitic in various parts of the digestive tube of their

host. By parthenogenesis, parasitic females of *Strongyloides* produce eggs which embryonate and first-stage larvae hatch. In the external environment larvae either develop into female infective larvae or grow and molt four times to produce a single free-living generation consisting of both males and females. What factors determine whether larvae will develop into free-living stages or into infective forms is still not fully known, but it is believed they may depend on environmental conditions such as pH, pO2, pCO3, consistency of substrate, temperature and level of nutrients.

Parasites use various strategies to survive or to avoid environmental conditions while being in their journey from a host to another.

Many protozoans are able to secrete a resistant covering and enter a latent stage called cyst. Encystment is very common among free-living protozoa during harsh environmental conditions but also in most parasitic protozoa when they are outside their host, in the environment. The encystment provides protection against unfavorable environmental conditions but also provides a biological background for nuclear division. The triggers for encystment include lack of nutrients, water loss, decreased oxygen concentration or changes in the temperature or pH. During encystment in protozoans, a cyst wall is produced, movement organelles (cilia or flagella) are lost and food reserves (i.e. starch, glycogen) are stored within the cell. In certain types of parasitic protozoans, the environmental cystic stages suffer complex developmental changes, essential for their further transmission. For instance, in coccidia, the cysts are called oocysts, which in most cases when shed through the feces of the host are not sporulated, hence non-infective to a new host. During its environmental life, if the physical conditions are proper, oocysts sporulate and become infective. However, in other protozoans (i.e. *Giardia*) the cyst is mainly important as a resistance stage, and excystation usually occurs when they reach in their typical parasitic habitat in a new host. More complex situations are known for parasites with a certain degree of host-specificity, when only the contact with a suitable host triggers excystation. Mechanisms for excystation include rehydration and action of host digestive enzymes on the cyst wall.

Encystment as a survival strategy is also known for trematodes (flukes). Typically, trematodes have a three-host life cycle. The definitive host is always a vertebrate, the first intermediate host is always a mollusk and second intermediate hosts are various metazoans. In few cases, the life cycle comprises only two hosts: the vertebrate and the mollusk. Nevertheless, regardless of the number of hosts, trematodes always have two stages in the environment. The definitive vertebrate host passes through the feces the eggs of the fluke. If the life cycle involves aquatic mollusks, the first larval stage of the trematode (called miracidium) hatches from the egg and it actively swims foraging for its mollusk host. As a survival strategy in terrestrial cycles, miracidia do not hatch but remain inside the egg. The next free living stages of trematodes in ontogenetic order are cercariae. They

actively leave the mollusk's body and either forage for the second intermediate host or become encysted on vegetation.

In tapeworms (cestodes) the usually complex life cycle requires at least one stage in the external environment. The definitive host (always a vertebrate) which hosts the adult stages in its intestines sheds the eggs through the feces. In certain groups of cestodes (i.e. Pseudophyllidea) the life cycle is related to aquatic environment; hence the eggs are adapted to float to enhance their chance to be swallowed by a suitable intermediate host. In other species of "aquatic" tapeworms, larval stages called coracidia (singular, coracidium) hatch from the eggs and swim waiting to be ingested by a crustacean. These aquatic stages are usually not very resistant (i.e. coracidia can survive only 24-36 hours) and normally do not feed. On the other hand, in other groups of cestodes (i.e. Cyclophyllidea), eggs containing embryos (oncospheres) are very resistant in the environment. They can easily survive several months before being ingested by a suitable vertebrate host.

In nematodes, environmental stages are known for most of the groups. Notable exceptions are species of genus *Trichinella* which are transmitted from a host to another by predatorism or vector-borne nematodes (i.e. *Dirofilaria*). However, typical nematodes pass from a host to another through the external environment. The diversity of life cycles in nematodes makes it very hard to outline some general developmental patterns. Nevertheless, in most of the cases, the infective stage is a larva. Some larvae stay inside the eggs to avoid environmental factors (i.e. in ascarids or pinworms). In other cases, larvae hatch and are infective for the definitive host. For instance, third stage larvae (L3) of Strongylidae parasitic in horses are swallowed together with the grass. They hatch as L1 and remain in the environment where they undergo two molts before turning into infective L3. During this time, they avoid drying out by being active during cooler periods of the day (morning, dusk, dawn) and hiding in shade over sunny days. In certain groups of nematodes, after L1 hatch, they must immediately get into an intermediate host to avoid the improper environmental factors (i.e. *Protostrongylus*).

Most arthropods are external parasites so they are exposed to environmental factors all the times. Additionally, many arthropods are temporary parasites, hence most of the time they spend away from the host. Biting insects like mosquitoes or sandflies are parasitic only for very short time, when they are feeding. Ticks spend a great part of their life in the search of a suitable host, dwelling in the vegetation of burrows. In other species, only larvae are parasitic and adults are typically free-living creatures (i.e. myiasis-causing insects). Other insects (fleas for instance) are parasitic only as adults while imago stages are free-living and relying on environmental food sources. In mosquitoes, larval stages develop in the water. This is why successful campaigns against mosquito-borne diseases are focused on desiccations. Other parasitic

arthropods however, lack environmental stages (lice).

### 1.7.3 Getting in/on the host

One of the most crucial milestones in the life cycle of a parasitic organism is the successful detection of a suitable host. Parasites have evolved many different strategies to increase the chance of successfully finding a host. One of the most common strategies used by parasites is the production of an enormous number of offspring. Most of the offspring will be unsuccessful and only a limited number will encounter a suitable host.

Parasites can use passive dispersion (random chance to contact a host) or active host finding (when mobile parasite stages are actively searching for the host using various sensorial features). In active strategies host detection is essential. To detect the proximity of a potential host, parasites use complex sensory organs. The first larval stages of digenetic trematodes are called miracidia. They are able to swim at a rate of about 2 mm per second and use their chemoreceptors to find a suitable snail host. After locating it, the miracidium attaches to the snails integument and using cytolytic enzymes it embeds deeper and deeper into the snail's body. In questing ticks (**figure 1.24**), the detection of the host is based on complex sensorial organs called Haller organs, which are able to perceive $CO_2$ from the breath of hosts but also on temperature and movement detection.

Transmission strategies are extremely diverse in the world of parasites. Some of them are remarkable examples of (co)evolution and natural selection of successful traits.

**Figure 1.24** Many important tick species spend the majority of their lifetime in the wait of their host, on vegetation. This host-finding behavior is called questing. *(Photo Andrei D. Mihalca)*

In homoxenous life cycles, transmission of parasites from a host to another is direct. The host usually sheds stages to the environment. In most of the cases these stages are not yet infective for a new host, but they spend some time and undergo some biological changes becoming infective. They will enter the new host passively (i.e. ingestion of infective eggs, cysts, oocysts, larvae) or actively (i.e. skin penetration by larval hookworms or schistosome cercariae).

Particular situations of direct transmission with no stages in the external environment are known. Some

situations include direct transmission through sexual contact. *Trichomonas vaginalis* in humans or *Trypanosoma equiperdum* in equines are examples of sexually transmitted parasitic diseases. Skin contact between an infected and a healthy individual is the way how scabies mites (*Sarcoptes*) get to a new host (**figure 1.25**).

Transmission strategies for parasites with heteroxenous life cycles are more complex. Transmission from a host to another can be via the environment, where there is no direct contact between definitive and intermediate hosts.

**Figure 1.25** Sarcoptic mange in a camel. Parasite transmission occurs by direct host-to-host contact. *(Photo Andrei D. Mihalca)*

In this case, detection of hosts follows the same principles as for homoxenous parasites. When the transmission from the definitive host to the intermediate host requires direct contact between the hosts, strategies are sometimes intriguing and complex. Many parasites are transmitted from a host to another by predation. One of the typical models is the life cycle of cestodes in Taeniidae family. Typical definitive hosts for these tapeworms are carnivorous mammals which acquire the infection after preying on infected intermediate hosts. Larval taeniids which infect these intermediate hosts usually induce severe lesions to make them more susceptible to be predated. This adaptation enhances their chance to get to the definitive host. Similar examples (**figure 1.26**) are known for various nematodes (*Eustrongylides* in fish intermediate hosts) or trematodes (*Dicrocoelium* in ants).

**Figure 1.26** Fish infected with the nematode parasite *Eustrongylides excisus* are easier preys to dice snakes, *Natrix tessellata*. *(Photo Andrei D. Mihalca)*

There is a significant number of parasites which infect their host through hematophagy by other parasites. These are the so called vector-borne parasitic

infections. The vectors are usually blood eating arthropods (ticks, mites or insects) and they play the major role for inoculating various parasites to the vertebrate host. Probably the most well-known examples are among protozoans. *Plasmodium* species causing malaria are vectored by certain mosquitoes.

Another tropical disease, the sleeping sickness is caused by various species of *Trypanosoma*. Their transmission is done by tsetse flies. Blood sucking insects are also responsible for vectoring metazoan parasites. Many filarial nematodes are injected as larvae to their vertebrate host by mosquitoes or flies (i.e. *Dirofilaria*, *Loa*, *Onchocerca*, *Wuchereria*)

Except the aforementioned possibilities of host infection, there are also some particular situations. A form of autoinfection known as retrofection has been described for pinworms (Oxyuridae). The eggs of these nematodes hatch on the anal skin and mucosa and the larvae migrate up the bowel to the cecum. Another type of autoinfection is known in the hydatid disease where the protoscolices of *Echinococcus* are able to infect other tissues in the same host individual by metastasis.

Last but not least, vertical transmission of parasites from the mother to the offspring is another strategy used by some parasites. The zoonotic protozoan *Toxoplasma* is one prominent model. Roundworms of genus *Toxocara* are also known to transplacentally pass from mothers to fetuses during pregnancy.

### 1.7.4 Migration and development in the host

Parasites are adapted to a multitude of habitats within the host. However, the site where parasites gain access to the host is different than the target organ/tissue. So the parasites have to move from the site of infection to the site of predilection within the host's organism. In some other cases, the site of infection is the same as the typical site for the final stage in that particular host, but parasites need to undergo some development in order to survive there. For this development they might migrate through various tissues before returning to the initial site.

Most parasites enter the host via the digestive tube after being ingested (see Chapter 1.7.3 for details). Some of these parasitic species will need to get to their typical habitat within the host. As situations are quite diverse, we will use some examples instead of drawing a general picture.

Herbivores acquire the infection with the liver fluke *Fasciola hepatica* after eating grass with encysted cercariae. In the intestine of the herbivores, cercariae excyst and start their journey towards the bile ducts from the liver. Their migration can follow three pathways: (1) some travel directly through the intestine wall, penetrating the peritoneum, the liver capsule and hepatic tissue; (2) others will use the common bile duct or (3) after penetrating the intestinal wall they will enter the blood stream and through the hepatic portal venous system will reach the liver.

If in *Fasciola hepatica* there are multiple possible routes, most helminthes which enter the host's body through the digestive system use the blood or lymphatic stream for migration. The main gate for parasites to enter the circulatory system from the intestine is the hepatic portal system. This remarkable venous system drains the blood with nutrients absorbed by the intestinal mucosa and transports it to the liver. Together with these molecules, microscopic parasites make their way to the liver and further on, through the posterior vena cava to the right atrium of the heart. From the right atrium they pass to the right ventricle and continue their travel via the pulmonary arteries to the lungs. In the lungs they either leave the blood stream entering the respiratory ducts or they continue their blood adventure and return to the heart through the pulmonary vein to the left atrium and left ventricle.

The best group to illustrate the diversity of migrations is represented by ascarid nematodes. Ascarids of horses, pigs or humans (genera *Ascaris* and *Parascaris*) are within the first type, leaving the vascular system in the lungs. Subsequently they migrate through the bronchi and trachea until the pharynx from where they are swallowed and reach again the digestive tube. During this migration they undergo several molts and they grow in size accordingly. This type of migration is called entero-pneumo-tracheo-enteral. Ascarids of genus *Toxocara* (parasites of carnivores and cattle) use even more complex migration pathways. If larvae of *Toxocara canis* (parasitic in canids) infect adult dogs, the larvae use the same migration route until they reach the lungs, but instead of leaving the blood they continue their journey back to the heart via the pulmonary veins. After they reach the right atrium they pass into the right ventricle and from there, through the aorta the get to various tissues (muscles, brain, etc.). This migration pattern is called entero-pneumo-somatic.

Another nematode, *Trichinella spiralis* enters its carnivorous host after this feeds on the infected meat of another host. After completing its life cycle in the intestine, newborn larvae use several pathways to get to the skeletal muscles. These include direct invasion of capillaries and lymphatic vessels in the intestine as well as migration through the intestinal serosa to the peritoneal cavity or via the hepatic portal vein blood to the general circulation.

Among tapeworms (Cestoda) we take into discussion again the family Taeniidae. Their eggs, if ingested by a suitable intermediate host will hatch in its intestine. The newborn embryo will migrate via the circulatory system to various organs, depending on the tapeworm species. Eggs of *Taenia solium* from human feces, if ingested by a pig, will hatch in its intestine. The embryos will migrate via the blood and will spread systemically to skeletal muscles of pigs. Similar migration route is known for *Taenia saginata* but intermediate hosts in this case are bovines. For both species, humans are the definitive hosts and they acquire the infection after eating raw or undercooked meat from the respective intermediate host.

In unicellular parasites, the mechanism of spreading in the host's body is usually involving intracellular parasitism in transport cells. Protozoans like *Toxoplasma gondii* enter the host by the digestive route. In the intestine they enter macrophage cells and move throughout the body spreading to various tissues and inducing a systemic infection.

Other parasites use different entry routes to the body. Nematodes from the family Ancylostomatidae penetrate the skin or oral mucosa of their host. However, the migration is hematogenous as well.

It is beyond the scope of this general chapter to exemplify all the parasites and their migration patterns. Though, one idea is evident: most parasites infect the host via the digestive route and use the circulatory system of the host to get to the predilection tissue/organ. During this migration, most of them undergo complex changes, some of them aimed to evade the host's immune system.

Development in the host is extremely varied according to the taxonomic group and will be discussed in more detail in the respective chapters.

### 1.7.5 Biological background for host specificity

Parasites can infect variable numbers of host species. Specialized parasites infect a narrow spectrum of host species while generalist parasites infect a wide range of host species. Medically and ecologically, the degree of host specificity is one of the most important characteristics of a parasite. An ecologic approach might state that a parasite with a narrow host range depends on its host in a greater degree than one with a broader host range. Medically, the importance of host specificity resides in the possibility of the generalist parasites to jump on to a new host species and the possibility of development of a new, emerging disease. Moreover, some of these parasites are transmissible from vertebrate animals to humans often producing important conditions known generically as zoonoses.

Several hypotheses try to explain the mechanism for host specificity. However, none of them is fully explaining the complexity of parasite-host interaction in nature. All of them have limitation and probably the mechanism is a combination of these factors. They were reviewed recently by Schmid-Hempel in his excellent monograph on Evolutionary Parasitology. Below is a synthetic account of these theories.

**(1) Host range is limited by phylogenetic constrains:** some parasites tend to have more host species when the hosts belong to a species-rich taxonomic group (many similar enough hosts to be infected); for instance, microsporidia are typical parasites of invertebrates and rarely of worm-blooded vertebrates; one reason is that microsporidia do not tolerate high temperatures.

**(2) Host range depends on the phylogenetic age of the parasite group**: during the evolutionary history of a parasite group, the host range expands as the parasites evolve. For example in some genera of fleas parasitic in small

mammals, host specificity is low as compared to other flea taxa.

**(3) Host range depends on transmission mode**: some parasites with active transmission (i.e. with mobile, free-living stages) can be more selective regarding the host than parasites with passive transmission (i.e. transmitted by direct contact between hosts or by unspecific vectors).

**(4) Host range depends on the stages of the parasitic life cycle**: in many heteroxenous parasites the larval stages have low specificity for the intermediate host while the adult parasites are more specialized to a narrow range of definitive hosts. When the parasitic stage is a encysted form (hypobiotic) the selection is probably weaker on cysts than on the active adult forms. A good example to support this theory is the tapeworm *Echinococcus granulosus*. Larval stages (known as hydatids) infect virtually almost all mammal species while adults infect only canids.

**(5) Host range depends on the virulence of the parasites**: if a parasite is more virulent the host specificity should be broader; if the host range of a virulent parasite is narrow, the number of susceptible individuals in the receptive population might be decreased by the parasite, leading to co-extinction.

**(6) Host range depends on parasite geographic distribution**: parasites with a wider geographical distribution tend to encounter a larger range of likely hosts than parasites with a more territorially restricted distribution.

**(7) Host range depends on immune defenses**: Although still not fully understood, the immune mediated host specificity can be synthesized in the following sentence: the host range is given by the parasite's capacities to evade the host's immune system.

## 1.8 Host-parasite interactions

Life-cycle of parasites is usually an alternation of free living and parasitic developmental stages. During this parasitic phase, stages of the parasites are located within various tissues of their host. The interactions between parasites and their hosts are complex and not always fully understood. As for all living beings, natural selection shapes the evolution of both, the parasite and the host (independently or together), through the same general mechanisms.

Usually, parasite-host interactions are long term relationships, resulting in a non-lethal coexistence of both partners. However, in certain cases, parasites can seriously impair the homeostasis of their hosts, sometimes resulting in the death of the later. In other cases, the immune system of the host is able to keep parasite development under control and even eliminate it completely.

The parasite-host interaction has been commonly described as an antagonistic relation, where both organisms are in a permanent struggle for survival. This antagonistic state might be easily questionable from evolutionary point of view, but medically, it eases the understanding of parasitism as such.

In this short chapter we will summarize the host-parasite interactions independently. First approach will include the actions and effects of parasites on their host, mainly from medical point of view (i.e. how parasites produce disease). In the second part, the reaction of host will be discussed, with emphasis on its immunologic strategies.

### 1.8.1 Pathogenicity of parasites

To the inexperienced reader, it seems strange to find out that most of the wild animals (invertebrates or vertebrates) harbor parasites. We can say with almost no chance to be wrong, that each single animal burdens at least one parasite at a certain time. The situation in domestic animals is not very much different. However, the clinical effects are not present all the time; on the contrary, the onset of the disease is the exception for most parasite-host associations.

By bearing in mind the definition of parasitism we can easily conclude that parasites are supposed to induce some pathology to their host. And this is true. Nevertheless, these lesions are in most situations minor and not reflected in the general health status of the infected host. In wild animals (maybe even in domestic), the "non-clinical" parasitism is likely to influence in a bigger or smaller extent the overall fitness of the host. Although "fitness" is hard to be evaluated, there are multitudes of examples in this direction.

The aim of this section is not to discuss the ecological effect and influence of parasites on their host but rather to be focused on its medical and veterinary side. Hence, in the next paragraphs, we will approach the pathogenic effect of parasites on their host, or, to put it in other words, how parasites are able to produce diseases.

Certainly there are many factors influencing the pathogenicity of parasites. Some of them are related to the host, some others to the parasite. The factors related to the host include: species, breed, age, sex and individual immunity. The ***species*** is very important when considering pathogenicity. Some species are very prone to develop clinical signs when infected by certain parasites, while others are infected but evident symptoms are absent. For instance, humans are very sensitive to the infection with the nematode *Trichinella spiralis* and develop a severe disease, often lethal if not treated. On the other hand, infected pigs or carnivores can harbor immense number of larvae in their muscles without any sign of disease. Even within the same host species, there might be variations between different ***breeds***. Usually, highly specialized breeds are more sensitive to parasitic infections than local breeds. Probably the most prominent example is the existence of the so called trypanotolerant breeds of cattle, sheep and goat, very resistant to the infection with the otherwise deadly agents of Nagana in Africa (**figure 1.27**). When colonists introduced highly productive European cattle breeds in Africa with the hope of huge profits, their efforts were soon vanished by massive die-offs due to the tsetse fly transmitted trypanosomoses. Another significant

host-related factor which influences pathogenicity is the ***age***. Parasites are usually able to infect all age groups of their host. However, in many situations, only the young ones develop (severe) clinical diseases. Coccidia of genus *Eimeria* are able to produce epidemic mortality in chicken or young domestic rabbits, but adults are usually infected without showing clinical signs. This is particularly important mainly from epidemiologic point of view, when adults with undetected infections are the main source of infections for the young offspring.

**Figure 1.27** Some local African cattle breeds are resistant to trypanosome infections. *(Photo Andrei D. Mihalca)*

***Sex*** of the host is also able to influence the clinical course of the disease. The human genital parasitic protozoan *Trichomonas vaginalis* is commonly producing clinical infection in females but males are often asymptomatic carriers. Last but not least, ***individual resistance***, mostly related to immunity is a key factor when considering parasite pathogenicity. For causes not so evident, in certain host groups with similar populational features (same species, breed, age or sex), some individuals develop a more severe parasitic diseases than others.

The second group of factors influencing the pathogenicity comprises those related to the parasite. Maybe the most important in this category is the ***intensity*** of the infection. Usually the higher the number of parasites within a host individual is, the more severe the clinical signs are. The ascarid nematodes infect a wide variety of hosts. When only few nematodes are present in the intestine, the clinical effect is usually absent. However, when the infection intensity is of tens or hundreds of individual parasites, severe symptoms or death due to intestinal obstruction may occur. Another important factor is the ***strain*** within the parasitic species. Some strains may be more pathogenic than others or may have different host affinities.

The mechanism by which parasites are pathogenic can be grouped in five main categories: physical damage, spoliation, toxin production, inoculation effect and interactions with the host's defense mechanism. Most parasites fit into several of these categories, if not in all together.

***Physical damage*** can take various forms, depending on the organ affected or the parasite species or stage involved. Some parasites, due their large size or high number in the host's tissues and organs can induce severe mechanical trauma. Bladder worms (vesicular structures of

larval cestodes) located in parenchymatous organs are compressing the surrounding tissues inducing ***atrophy***. This is highly evident in case of the "coenurus" type larvae of *Taenia multiceps* located in the brain of small ruminants. For large parasites or large parasite groupings in luminal organs (i.e. intestine, bronchi etc.) one of the most common extreme effects is ***obstruction***. The most common parasites responsible for intestinal obstruction in dogs, horses and humans are ascarid nematodes (**figure 1.28**). Parasites are also responsible for ***direct tissue destruction***. Parasites can destroy the tissues by several ways. Migrating parasites (see Chapter 1.7.4) are responsible for important traumatic lesions in various organs. Other situations include direct tissue damage during feeding or attachment. Most parasites possess various adherence structures to avoid being eliminated by the host. These structures (i.e. hooks, spines, suckers etc.) are highly irritating to the host's tissues, causing local destruction at the site of parasite fixation.

***Spoliation*** (the act of plundering) or nutritional robbing is common among intestinal parasites. The parasites utilize the same food resources as their hosts do. Large parasites or large parasite groupings are able to use huge amounts of certain nutrients ingested by the host. The fish-borne tapeworm *Diphyllobothrium latum* absorbs large amounts of vitamin B12 from the host's intestine inducing systemic deficit which results in anemia. Other parasites are responsible for unspecific spoliation, resulting in general malnutrition and retarded growth. With effects similar to spoliation, the malabsorption caused by intensive intestinal mucosal damage is also a common pathogenic feature of parasitic infections.

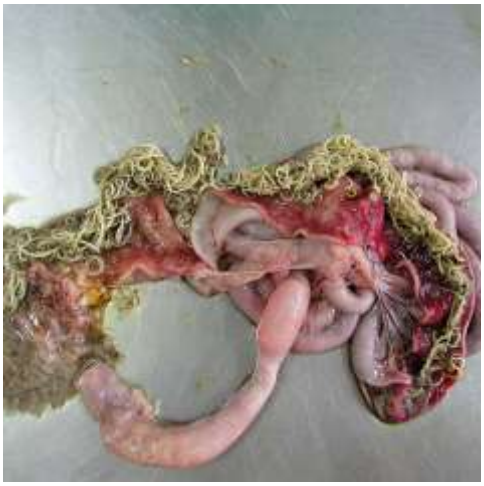


**Figure 1.28** Ascarid nematodes like *Toxocara canis* in dogs are often obstructing the host's intestine. *(Photo Andrei D. Mihalca)*

***Toxin production*** by parasites can have local or systemic effect. The toxins can result either as parasite waste products or due to massive destruction of parasites. The agents of human malaria (apicomplexans of genus *Plasmodium*) are producing a toxin called hemozoin, responsible of an overall reduced phagocytic performance by host's white blood cells. The saliva of ticks contain various products, which, when injected into the host may induce general paralysis. Following the death of large numbers of individuals of *Toxocara canis*, the post-mortem release of toxins

induces nervous signs similar to epilepsy in puppies.

The ***inoculation effect*** refers to situations when parasites facilitate the invasion of other microorganisms inside the hosts body, tissues or organs. Several bacteria are commonly found in the intestine where they are harmless. However, if they are carried by parasites in other organs or tissues (liver, brain, peritoneum, etc.) they are able to produce severe infections. In other cases, parasites induce lesions to the mucosa of the intestine or respiratory ducts, allowing pathogenic bacteria or viruses to produce the infection which is otherwise unlikely through unharmed epithelium. One of the most well-known inoculation effects of parasites is the case of vector-borne infections, when hematophagous arthropods are transmitting various pathogens to their hosts (i.e. ticks, tsetse flies, mosquitoes, sand flies, biting midges etc.).

The most severe and complex pathogeneses in parasitic infections are caused by the ***altered immune response*** of the host. The host responds to the presence of parasites by inflammation. Severe granulomatous lesions or strong inflammatory reactions are produced by migrating nematode larvae in various tissues. Parasites are also able to induce changes on the surface of various cells, cheating the host's immune system and producing autoimmune responses. The red blood cells infected with *Babesia* are recognized as non-self and destroyed by the host's own immune system resulting in severe hemolytic anemia.

This section can hardly be more detailed than this, but pathogenesis will be discussed individually for each parasite in the chapters to follow.

### 1.8.2 Immunity of host to parasites

Usually, the immune system of the host is able to eliminate or to stop the parasitic invasion. Most hosts are hence resistant to the majority of parasitic infections. Some parasites which are host specific are able to infect individuals from a single host species, while all the other host organisms are able to stop the parasite invasion. This gives the so called host susceptibility or resistance to certain parasites. However, ***resistance*** is not synonym to ***immunity***. Immunity refers to those mechanisms by which specialized cells or tissues of an organism are able to recognize foreign (non-self) structures and eventually protect against potential invasions. The immune system is present in various degrees of complexity in all animal organisms, invertebrate or vertebrate.

Most vertebrate animals possess in general two types of immunity: the innate immunity and the acquired immunity.

The ***innate immunity*** (also known as non-specific immunity) includes various inborn defense mechanisms known in all plants and animals. There are certain physical or chemical barriers which prevent invasion by pathogens. In vertebrates, the skin together with mucosal layers lining the inner lumen of respiratory ducts and digestive tube are the first obstacle for most pathogens, including parasites. Except these

anatomical barriers, the innate immune system includes important components: the cytokines, the complement system or a range of specialized cells like mast cells, phagocytes (macrophages, neutrophils, dendritic cells), basophils, eosinophils, natural killer cells or gamma-delta T cells.

The ***acquired immunity*** (also known as adaptive or specific immunity) is known only in vertebrates. Nevertheless, the specific immune response is activated by the innate components. The adaptive immunity is responsible for protecting the organism specifically against pathogens and to "remember" specific antigens (immune memory). The main factors of the acquired immunity are antibodies, produced by B lymphocytes. T lymphocytes are also part of the acquired immunity. There are several types of T cells know, each of them with specific functions: helper, cytotoxic, memory, regulatory, natural killer, gamma-delta etc. Acquired immunity can be active (post-infection or post vaccination) or passive (maternal transfer or after immunoglobulin administration).

Compared to prokaryotic pathogens (viruses and bacteria), traditional parasites (protists, helminthes, arthropods) are much larger in size and have a much more complex antigenic surface. Moreover, some of the parasitic antigens are excretory antigens, not surface antigens, and are produced intermittently. There are several components of the immune system which act in the defense against parasitic protozoa, mostly antibodies and T cells. In the case of helminthes, the infections are usually associated with hypereosinophilia, increased immunoglobulin E (IgE) production, mastocytosis and goblet cell hyperplasia. Immunity against biting arthropod saliva has been also described in detail.

Despite the complex immune system involved in the protection of host, parasites are often able to ***evade*** all these mechanisms and to produce infection or even severe disease or death. The avoidance strategies are both complex and interesting. One common mechanism is ***antigenic variation*** when the parasite is one step ahead the immune system of the host. By the time the antibody is produced, the surface of the parasite has a completely new antigenic structure so the initial antibodies are useless. This strategy is used by many important protozoan parasites (i.e. *Plasmodium falciparum*, *Trypanosoma brucei*) or molting nematode larvae. Another interesting avoidance mechanism is ***molecular mimicry***, when the parasite is able to pass undetected (i.e. *Plasmodium falciparum*). Some parasites are able to produce ***immunoglobulin cleaving proteases*** (i.e. *Dirofilaria immitis*, *Fasciola hepatica*) which are destroying all adherent antibodies. Others are producing ***prostaglandin E2*** (i.e. *Brugia malayi*, *Taenia taeniaeformis*) which has a strong anti-inflammatory effect. Many helminth or protozoan parasites are able to ***interfere with the complement cascade***, blocking certain steps in its activation (*Echinococcus granulosus*, *Taenia solium*, *T. taeniaeformis*, *Trypanosoma brucei*, *T. cruzi*, *Entamoeba histolytica*, *Leishmania* spp.). These are only few of the extremely various known molecular mechanisms.

Except these intimate mechanisms, there are also some general strategies which allow parasites to avoid host's immune system. Most parasites are located in the intestinal lumen. This can be evolutionarily explained by the low amounts of immune effectors present at the surface of the intestinal mucosa. The only immunoglobulin normally present in the intestinal lumen is IgA, but this has almost no effect against helminthes. Other parasites simply hide from the immune effectors. Larval cestodes (bladder worms) are isolated within a cystic membrane and some unicellular parasites develop inside various cells of the host organisms (i.e. *Toxoplasma*, *Babesia*).

Detailed studies of immunology are available for many parasites. A practical use of this knowledge is the possibility of immunodiagnostic of parasitic infections by detection of circulating antibodies or antigens using various laboratory or clinical tests: intradermal allergy test (IDR), indirect hemagglutination (IHA), indirect fluorescent antibody (IFA), complement fixation (CF), enzyme-linked immunosorbent assay (ELISA), western blot (WB) etc.

## 1.9 Classification of parasites

Definitely, taxonomy is not the most important part of medical parasitology. Most veterinary students consider taxonomy boring and very difficult to learn and remember. Moreover, they do not understand why it is important to know basic taxonomy of parasites. On the contrary, others tend to overestimate its importance and learn by heart the taxonomic position of each parasite. So the most important objective of this small section is to make scholars understand the practical importance of taxonomy in the process of teaching and learning.

All parasite species are grouped in systematic assemblages called taxa based on certain morphological and biological features. For instance, genera like *Sarcocystis*, *Toxoplasma*, *Neospora*, *Hammondia* and *Besnoitia* are all included in the family Sarcocystidae. It means that all members of these genera share ***common characteristics***, the ones of the family grouping all of them. One of these characteristics is that all species in the family Sarcocystidae are heteroxenous. So if one knows that any of the genera above is part of Sarcocystidae, it also knows that its life cycle is heteroxenous.

### 1.9.1 Principles of zoological taxonomy

Zoological nomenclature is the system of scientific names applied to taxonomic units of extant or extinct animals. These units are called ***taxa*** (singular: taxon). Parasites, as all animal taxa, are classified according to the rules of the International Code of Zoological Nomenclature. The species is the basic unit and taxonomical rank in biological classification. Except species, there are seven main taxonomic ranks: Domain, Kingdom, Phylum, Class, Order, Family and Genus.

The taxa are hierarchically arranged so that always a higher taxon includes usually several lower ones (i.e. one

kingdom includes several classes, one family includes several genera, one genus includes several species). To face the increasing diversity of described life forms, taxonomists introduced intermediary ranks for further divisions. For instance a class might contain several subclasses, a subclass could include several superorders or more families could be all included in the same superfamily.

Each taxon above the rank of species gets a scientific name in one word (uninominal name), always spelled with capital letter. A species always has a binomial name (composed of two words). The first word is always spelled with capital letter and represents the name of the genus. The second word is called the specific epithet and is spelled with lower case (i.e. *Homo sapiens*, *Canis lupus*).

Sometimes, especially when in a text the species name was written in full, the next occurrences are abbreviated (i.e. *H. sapiens*, *C. lupus*). As a rule, the names of genus and species are written with italics. There are also typical terminations for various supraspecific taxonomical ranks (**table 1.3**), although exceptions are known. Biological taxonomy is probably the most dynamic science. Entire taxa are permanently reordered (reclassified) according to molecular phylogenetic studies. Hence, it is very difficult to put down on paper a kind of "officially" recognized taxonomical hierarchy. Moreover, different authorities have different opinions.

**Table 1.3** Scientific names of metazoan parasites

| Rank | Termination |
|---|---|
| Order | -ida |
| Superfamily | -oidea |
| Family | -idae |
| Subfamily | -inae |

**Table 1.4** Main taxa of parasites

| Phylum | Class* | Common name |
|---|---|---|
| Euglenozoa | Kinetoplastea | **Flagellates** |
| Parabasalia | Tritrichomonadea | |
| | Trichomonadea | |
| | Hypotrichomonadea | |
| Fornicata | Retortamonadea | |
| | Trepomonadea | |
| Apicomplexa | Coccidia | **Coccidia** |
| | Cryptosporidea | **Cryptosporidia** |
| | Haematozoea | **Piroplasms** |
| Ciliophora | Litostomatea | **Ciliates** |
| Platyhelminthes | Trematoda | **Flukes** |
| | Cestoda | **Tapeworms** |
| Nematoda | Secernentea | **Roundworms** |
| | Adenophorea | |
| Acanthocephala | Archiacanthocephala | **Thorny-headed worms** |
| Arthropoda | Pentastomida | **Tongue-worms** |
| | Insecta | **Insects** |
| | Arachnida (Subclass Acari - order Ixodida) | **Ticks** |
| | Arachnida (Subclass Acari - except Ixodida) | **Mites** |

**Only classes of veterinary significance are included*

### 1.9.2 Major parasitic taxa

All species of parasites are included in domain Eukaryota. The unicellular heterotrophic mobile species are included in a paraphyletic group referred to as Protista. Protista includes the heterotrophic Protozoa, which groups animal-like unicellular organisms. They include the following phyla with representatives parasitic in domestic animals: Euglenozoa, Parabasalia, Fornicata, Apicomplexa and Ciliophora. A group formerly regarded as protists are Microsporidia which now are classified within Fungi. All the other parasites are members of Kingdom Animalia. **Table 1.4** lists the major phyla and classes of parasites. The right column from the table lists the most widely used terms in English.

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# 2 PROTOZOA

## 2.1 General considerations

Recent phylogenetic studies brought apparent chaos to traditional systematics of living organisms. Through time, unicellular eukaryotic organisms were included in various taxonomic groups. Currently, they are all referred to with the name Protista. However, this term has no taxonomic ranking. Within protists we include autotrophic, heterotrophic and phototrophic organisms. Traditionally, all heterotrophic protists are included in the group known as Protozoa.

Protozoa are found in all possible habitats and include free-living or symbiotic forms. Among the later, parasitic protozoa can be associated with all types of hosts, from plants to animals.

Protozoans are a group of very old and divers unicellular organisms, hypothetically originating from the long coevolution of two or more symbiotic prokaryotic cells. With this view, the mitochondria and the chloroplasts from the eukaryotic cells were originally prokaryotic endosymbionts of larger cells. All protozoans have a typical eukaryotic structure of the cell. The cell membrane may be naked or covered with locomotion structures like cilia or flagella. Within the cytoplasm, the organelles include mitochondria, Golgi apparatus, lysosomes, ribosomes, rough and smooth endoplasmatic reticulum and a nucleus with the genetic material organized in chromosomes. The nucleus is separated by the rest of the cytoplasm by a nuclear membrane, hence the name eukaryote (Greek: *eu* = true; *karyon* = nucleus). The number, structure and position of these organelles within each cell are highly dependent on the taxonomic group.

Parasitic protozoa can inhabit virtually all organ systems and tissues of their host, with both intracellular (i.e. apicomplexans) or extracellular (i.e. flagellates, ciliates) locations. Some of the parasitic protozoa cannot survive in the environment (i.e. flagellates) and therefore are transmitted from host to host by direct contact or using living vectors (i.e. arthropods). In some other groups (i.e. apicomplexans) when eliminated by the hosts to the environment, they transform into resistant stages, like cysts or oocysts.

Parasitic protozoans obtain their food from their hosts. Mechanisms used for uptake of nutrients include phagocytosis, pinocytosis, osmosis or active ingestion via a cell "mouth" called cytostome. Maybe the most heterogenic process in protozoa is reproduction. Some groups use only asexual reproduction (binary fission or cell division). All flagellates use

this multiplication mechanism. Some other groups employ also sexual reproduction. In apicomplexans, the fecundation of micro- and macrogametes is a typical phase of the life cycle. Ciliates use conjugation to exchange genetic information during multiplication.

Protozoans are extremely important from medical point of view. In humans, diseases like malaria, sleeping sickness or Chagas' disease are responsible for millions of fatalities every year. In domestic animals protozoans cause massive economic losses especially in tropical areas (i.e. Nagana of livestock) but also worldwide (i.e. eimeriosis of poultry). Last but not least, several parasitic protozoans are transmissible from animals to humans. These zoonotic conditions include toxoplasmosis, sarcocystoses or leishmaniosis. All major groups will be detailed in the following sections.

## 2.2 Diversity of parasitic Protozoa

Several classifications of protozoans are available in the literature, and apparently none is generally accepted. Several complex phylogenetic approaches gave birth to new clades, but these will not be considered here. Herein, we chose an adaptation of the revised classification of eukaryotes by Adl et al 2012.

The protozoans of veterinary importance are grouped in five phyla (see text below). Other higher ranked taxonomic groups, previously included within Protozoa were shown to be distinct groups. Myxozoa are currently included within the metazoans while Microsporidia is within Fungi.

## 2.3 The flagellated protozoa

Flagellates are motile protozoans, many of them free-living, but some pathogenic to humans and animals. The locomotory structures are known as kinetids. The genera of medical importance are currently included into three phyla (**table 2.1**).

They infect almost all animal phyla occupying various habitats within their host, with either extra- or intracellular location. Most of the species feed by osmosis and reproduce by binary fission.

**Phylum Euglenozoa** includes a single class of medical importance, Class Kinetoplastea (**table 2.1**). The class comprises of organisms with a kinetoplast, a cell organelle containing a particular type of mitochondrial DNA. The kinetoplast is located close to the basal body of the flagellum, and is easily visible at Giemsa staining as a deep purple dot. Several genera are included here, but only two will be considered in this book, as the others are parasitic mostly in invertebrates. All diseases produced by members of this phylum are treated under Chapter 2.3.1 and the members will be generically called kinetoplastids.

**Phylum Parabasalia** includes organisms which have a parabasal apparatus. The kinetid consists of four kinetosomes. Three classes of veterinary significance are included here (**table 2.1**). Most genera of medical importance in this

phylum were formerly known as trichomonads. In general, they possess a variable number of flagella, according to genus. In most of the cases, they have a group of flagella at their apical pole and an additional recurrent flagellum which runs backward, along an undulating membrane. Each flagellum originates in a basal body. Another common feature of species from this group is the presence of an axostyle. This structure plays the role of a cytoskeleton. Generally, this group lacks mitochondria. All the species in this group will be referred to as trichomonads. They are responsible for a group of diseases medically known as trichomonoses and will be discussed in Chapter 2.3.2.

**Phylum Fornicata** includes species with single or one pair each of kinetids and nuclei. They usually possess a feeding groove or a cytopharyngeal tube associated with each kinetid. The phylum includes two classes of medical importance (**table 2.1**). Although genus *Chilomastix* is currently included here and not considered to be related to trichomonads, it will be listed under the chapter referring to them (2.3.2). Genus *Giardia* and giardiosis will be discussed separately in chapter 2.3.3.

**Table 2.1** Current classification of flagellates parasitic in domestic animals (adapted after Adl et al. 2012)

| Phylum | Class | Genera | Disease (Chapter) |
|---|---|---|---|
| Euglenozoa | Kinetoplastea | *Trypanosoma* | Equine dourine (2.3.1.1) |
| | | | Vector-borne trypanosomoses in domestic animals (2.3.1.2) |
| | | *Leishmania* | Canine leishmaniosis (2.3.1.3) |
| | | | Feline leishmaniosis (2.3.1.4) |
| Parabasalia | Tritrichomonadea | *Tritrichomonas* | Genital trichomonosis in cattle (2.3.2.1)<br>Intestinal trichomonosis in birds and mammals (2.3.2.4, 2.3.2.5) |
| | | *Histomonas* | Histomonosis of poultry (2.3.2.6) |
| | Trichomonadea | *Trichomonas* | Anterior digestive trichomonosis in birds (2.3.2.3) |
| | | *Tetratrichomonas* | Buccal trichomonosis in dogs and cats (2.3.2.2)<br>Intestinal trichomonosis in birds and mammals (2.3.2.4, 2.3.2.5) |
| | | *Pentatrichomonas* | Intestinal trichomonosis in domestic mammals (2.3.2.5) |
| | Hypotrichomonadea | *Trichomitus* | Intestinal trichomonosis in domestic mammals (2.3.2.5) |
| Fornicata | Retortamonadea | *Chilomastix* | Intestinal trichomonosis in birds (2.3.2.4) |
| | Trepomonadea | *Giardia* | Giardiosis in domestic animals (2.3.3.1) |

### 2.3.1 Kinetoplastids

**Introduction.** Kinetoplastids include several genera parasitic in invertebrates, vertebrates and plants. Only two genera (*Trypanosoma* and *Leishmania*) are of veterinary importance. The main feature of Trypanosomatidae is the presence of a single flagellum and a kinetoplast. Based on their life cycles, members of this family are either homoxenous or heteroxenous. All species parasitic in domestic animals are heteroxenous, with stages alternating between the invertebrate and the vertebrate host. However, a single species, namely *Trypanosoma equiperdum* is transmitted directly by coitus and does not require an additional host.

**General morphology.** The morphology of trypanosomatids is heterogenic during different stages of the life cycle. The main feature distinguishing the morphological stages of Trypanosomatidae is the position of the flagellum. The most common morphological types (**figure 2.1**) are: ***amastigote*** (rounded or elongated forms lacking flagellum); ***sphaeromastigote*** (rounded forms with a free flagellum); ***promastigote*** (elongated forms with antenuclear kinetoplast and flagellum arising near it but emerging from the cell at the anterior end); ***epimastigote*** (elongated forms with juxtanuclear kinetoplast and flagellum arising near it but emerging from the side of the body); ***trypomastigote*** (elongated forms with postnuclear kinetoplast; flagellum arising near it, emerging from the side of the body and running forward along an undulating membrane).

The trypomastigote (**figure 2.2**) is the most important stage from diagnostic point of view, as it is the most frequently encountered in the blood of the vertebrate host. Its trepan (= drill) shape gave the name to the genus. The trypomastigotes are usually lanceolate in shape, like a flat elongated blade, oval or elliptic in transverse section and with tapering ends. Conventionally, the anterior end is considered the one directed forwards during locomotion. The body surface of trypanosomes is covered with a periplast. The main cell organelles are the nucleus, the kinetoplast and the locomotion system (**figure 2.1**).

The kinetoplast, defining structure of all members of order Kinetoplastida (hence the name), is always located in the close vicinity of the basal body. The locomotion system (also known as the mastigont system) is represented by the flagellum and the basal body. The single flagellum originates from the posterior end, then runs forward along an undulating membrane, and freely terminates at the anterior end of the cell. At the starting point of the flagellum stays the basal body (also called blepharoplast). Most stages are mobile, but mobility is seldom observed, as most morphological examinations require fixation and staining.

**Ecology and transmission.** With the exception of *T. equiperdum*, all species of Trypanosomatidae parasitic in vertebrates have heteroxenous development. Vertebrates are considered

definitive hosts and invertebrates are the intermediate hosts, also referred to as vectors. For all species parasitic in mammals, the intermediate hosts are insects from orders Hemiptera (true bugs), Diptera (true flies) and Siphonaptera (fleas). The insects take up the bloodstream forms of the parasites when feeding on infected mammals. In the insect intermediate hosts, they undergo a cycle of development with the final production of special infective forms called metacyclic (Greek: *meta* = after) trypanosomes. These are transmitted to a new definitive vertebrate host by various ways, according to the location of the final developmental stage within the vector. Transmission from vector to the mammal occurs only after the trypanosomes have completed their entire cycle of development in the invertebrate host. This cyclic (indirect) transmission is the typical one for heteroxenous trypanosomes. In some cases, direct transmission from mammal to mammal was reported, when blood stages are mechanically passed by hematophagous insects or by syringe inoculation of infected blood. However, the ability of vectors to mechanically transmit the disease is measured in minutes, while the typical cyclic transmission equals sometimes the whole life duration of the insect.

**Medical importance.** Diseases caused by members of genus *Trypanosoma* have the generic name of trypanosomoses (singular: trypanosomosis). Most of the infections occur in tropical areas of the world where they cause severe, often lethal conditions in humans and animals as well.

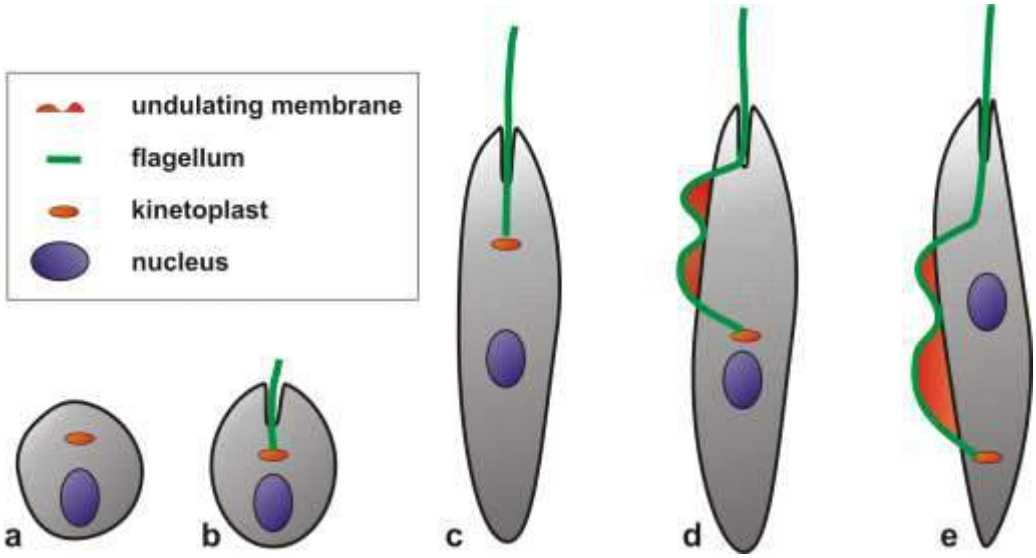


**Figure 2.1** Main stages of trypanosomes: a - amastigote; b - sphaeromastigote; c - promastigote; d - epimastigote; e - trypomastigote.

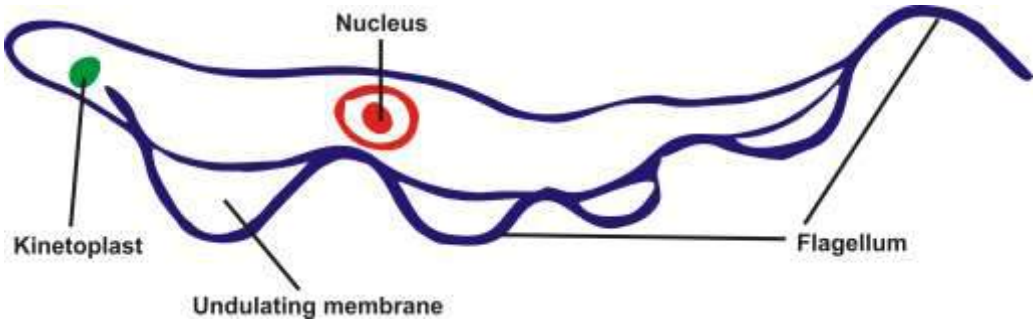


**Figure 2.2** General structure of a trypomastigote, the blood stage of trypanosomes.

The only pathogenic species occurring in temperate areas seems to be *T. equiperdum*, the agent of equine dourine. Many other trypanosomes are not pathogenic, although infections are common worldwide. Genus *Leishmania* is responsible for several infectious conditions in humans and animals worldwide. Among domestic species, dogs and cats are the only common hosts to these parasites.

#### 2.3.1.1 Equine dourine

Dourine (Arabic: *darina* = mangy, dirty), also known as the covering disease, is a chronic protozoal disease, with venereal transmission, naturally occurring in equids. It is eradicated in most of the countries but is still present in parts of Africa and Asia. Main clinical signs include edematous lesions of the genitalia, typical skin plaques and paralytic nervous signs, usually followed by death.

**Historical notes.** Ancient Arab texts mention a disease in horses with similar signs to what we know today as dourine. Apparently, the first description belongs to the Byzantine veterinarian Chiron in his book on the diseases of horse, "*Mulomedicina Chironis*" (~ 400 AD). Dourine is mentioned also in a treatise of veterinary medicine published in the 12th century by the Arabian, Ibn-al-Awan in Seville. The first description of the disease in Europe was done in a Prussian horse by Ammon and Dirkhausen in 1796. The causative agent was seen for the first time by Rouget in 1894, who demonstrated its presence in the blood of an Algerian horse. In 1900, Buffard and Schneider reproduced dourine in a horse after they subcutaneously injected the parasite isolated from a naturally infected horse. One year later, in 1901, Doflein described and named the causative agent *Trypanosoma equiperdum*.

**Etiology.** In classical parasitology textbooks, the agent of equine dourine is considered to be *Trypanosoma equiperdum*. Recent molecular analysis of different laboratory strains originating from endemic areas, brought controversy

on the validity of this species. *Trypanosoma equiperdum* is very closely related to several subspecies of *Trypanosoma brucei*: *T. brucei brucei*, *T. brucei gambiense* and *T. brucei rhodesiense*, all agents of African trypanosomoses in a variety of hosts, but also to *Trypanosoma evansi*, which causes a disease called Surra. Some authors suggest considering the agent of dourine as a subspecies of *T. brucei* (*T. brucei equiperdum*). Until the status of the species will be clarified, the conventional name *T. equiperdum* will be used herein.

Several natural strains were described over time, varying mainly in pathogenicity. Even if in the last decades no single strain was isolated from natural infections, at least 12 laboratory strains are available worldwide (**table 2.2**).

**Table 2.2** Available laboratory strains of *T. equiperdum**

| Code | Origin | Host |
|---|---|---|
| BoTat 1.1 | Morocco, 1924 | Horse |
| STIB 818 | China, 1979 | Horse |
| OVI | South Africa, 1977 | Horse |
| ATCC 30019 | France, 1903 | Horse |
| ATCC 30023 | France, 1903 | Horse |
| American stabilate | America (?) | Horse |
| Canadian stabilate | Canada (?) | Horse |
| Alfort | (?), 1949 | Horse |
| AnTat 4.1. | (?) | (?) |
| Hamburg | (?) | (?) |
| SVP | (?) | (?) |
| TREU 2259 | (?) | (?) |

* - modified from Claes et al. (2005)

**Morphology.** *T. equiperdum* is a monomorphic species of trypanosome, morphologically indistinguishable from *T. evansi*. The nucleus is usually located in medial position. The length of *T. equiperdum* ranges from 15.6 to 31.3 μm in Asian strains and from 22.3 to 29.0 μm in Russian strains.

**Life cycle.** *T. equiperdum* is the only trypanosome not transmitted by an invertebrate vector. Transmission occurs mainly during mating from stallions to mares and vice versa. There are some reports stating that foals from infected mothers can get the infection through their conjunctival mucosa during birth or even through milk contaminated from lesions of the udder. Transmission by the means of unsterilized instruments used for artificial insemination has also been cited.

Unlike other species of the genus, *T. equiperdum* is primarily a tissue parasite which rarely invades blood. They are extracellular parasites, typically inhabiting the surface of the mucosa or between the epithelial cells. In stallions, they are found also in the seminal fluid. Later in the course of infection they invade also surrounding tissues or even blood.

Nutrition takes place by osmotic absorption of dissolved substances from the host's tissues, through the body pellicle.

Experimental infection of laboratory animals is possible, but difficult. In order to establish the infection in murine rodents, first inoculation using a natural strain should be done in splenectomized animals. Once the strain becomes adapted, it can be subsequently passed virtually for unlimited times. Wild strains can be adapted after several passages to

laboratory animals (mice, guinea pigs, rabbits, dogs), but they change their pathogenic properties. However, first inoculation form naturally infected horses typically fails. Attempts to infect domestic ruminants or pigs resulted in inapparent disease and low or no detectable parasitemia.

**Epidemiology.** Since the 19th century, dourine has occurred sporadically in Europe. Around 1918, the disease was reported only in Russia, Turkey, Hungary and Spain. During World War II, the disease was spread by army into Western Europe. After the war, dourine was eradicated from Western Europe by systematic screening and control, including stamping out.

Currently, natural infections occur only in horses, donkeys and their hybrids in Africa, Asia and parts of Russia. Occasional outbreaks are known sporadically from Europe, following international trade with horses. Official OIE reports state that the only countries where dourine occurred in the last years are: Botswana, Mongolia, Ethiopia, Kyrgyzstan, Namibia, Pakistan, Russia and South Africa. This officially reported distribution of the disease may not be accurate because testing of horses in many countries is not being done routinely.

All equines are theoretically susceptible to the infection with *T. equiperdum*. However, the donkeys and mules are more tolerant than horses. Even among horses, there is an evident difference in sensibility, with thoroughbred breeds more susceptible than native ones.

**Pathogenesis.** All the lesions and symptoms of dourine are related to the histotropism of *T. equiperdum* for the epithelial tissues of the genital mucosae or skin. After the onset of the infection, the flagellates invade also surrounding tissues. It is questionable if they are able to penetrate intact mucosae or they priory need some degree of abrasion. However, it seems they invade local capillaries.

The local effect is considered to be induced by a toxin secreted by the parasite which causes vasomotor disturbances with exudation of the plasma and inflammatory reaction in the invaded tissues. The nervous damages are considered to be also the result of the toxin, carried systemically by the blood stream. If all the motor and sensory alteration can be attributed to nerve damage, the emaciation is due to the secondary atrophy of the muscles. However, the toxin was never isolated, but other proof which sustains the toxin hypothesis is the sudden death of laboratory rodents infected with a high number of parasites.

Not all strains of *T. equiperdum* invade the blood stream of horses. Parasitemia is more common in laboratory rodents where trypanosomes invade the blood 2-3 days after the inoculation.

**Immunology.** As some species of equids or some breeds of horses are naturally resistant to infections, there is certainly an inborn immunity acquired through a long parasite-host coevolution. The immune factors responsible for the defense against the agent of dourine include both humoral and cellular types,

with an important role of phagocytosis. There are some evidences that passive immunity can be transmitted from immune mares to foals during pregnancy.

Despite many other similarities, there is no cross immune protection between *T. equiperdum* and *T. evansi*. Moreover, *T. equiperdum* is able to periodically shift its surface glycoprotein antigens, allowing chronic, persistent infections.

**Clinical signs.** Symptoms vary considerably, depending mostly on the infecting strain. Those originating in northern Africa, Europe and Asia seem to be more virulent than those from southern Africa and from the former American strains. The nutritional status of the animal and other stress factors can influence the severity of symptoms.

The incubation period is variable. Clinical signs usually appear within a few weeks of infection but may not be evident until after several years. Although acute, asymptomatic or latent infections are known, the most common character of the dourine in horses is chronic. The duration of the disease in mild chronic cases may persist for 1-2 years, and occasionally up to 4-5 years. Experimentally, horses infected with laboratory have survived for up to 10 years after infection with these strains. In more severe chronic cases, animals die after several months. In ***acute*** forms, disease lasts for 1-2 months, or, exceptionally one week. As a rule, dourine is a fatal disease and the average mortality is about 50%. Recovery can occur spontaneously, especially in stallions.

The ***chronic*** disease is conventionally divided into three phases. During the ***first phase*** of the infection, the common lesions are localized within external genitalia. In mares, the first usual sign of infection is a small amount of vaginal discharge. Swelling and edema of the vulva develop later and extend along the perineum to the mammary glands and ventral abdomen, accompanied by vulvitis, vaginitis, polyuria and elevated tail. These last signs mimic heats, and usually mares are receptive to males. If pregnant, abortion may take place when the infection is with more virulent strain. In stallions, the initial signs are edema of the prepuce and glans penis, of variable degree which may spread to the scrotum, perineum, ventral abdomen and thorax. Paraphimosis may is not uncommon. In both sexes, the swelling may resolve and reappear periodically. Following edema, vesicles and ulcers usually appear on the genitalia. They may heal and leave permanent white scars, called leukodermic patches. In some outbreaks, conjunctivitis and keratitis are common ocular signs in infected animals. ***The second stage*** of chronic dourine is more or less pathognomonic. Typical cutaneous plaques or skin thicknesses can occur, with sizes ranging from extremely small to several centimeters. Interestingly, these plaques have also been observed sporadically in animals infected with *T. evansi*. To complicate the understanding of the taxonomic status of the causative agent, in the case of certain strains of *T. equiperdum* these typical skin lesions do not occur. ***The third phase*** usually onsets after several month from the infection and is characterized by progressive

anemia and disorders of the nervous system. Initially these signs consist of restlessness and the tendency to shift weight from one leg to another followed by progressive weakness and incoordination. Paralysis of the hind legs and paraplegia and ultimately recumbency and death are the final stages of infection.

All these symptoms are characterized by periods of exacerbation and relapse that may vary in duration and occur several times before death. Recovery is also possible, especially in infection with less virulent strains. The disease caused by more virulent strains is often acute and the mortality rate is higher. In other equids (i.e. zebras) animals can be positive and show no clinical signs.

**Pathology.** If the outcome of the disease is death, anemia and cachexia are the most evident lesions of gross necropsy. Signs of early disease like edema can be found as indurated areas on the genitalia or ventral parts of abdomen and thorax. Internal lymph nodes are hypertrophied. If nervous signs occurred before the death of the animal, perineural connective tissue is infiltrated with edematous fluid and a serous infiltrate may surround the spinal cord, especially in the lumbar or sacral regions.

**Diagnosis.** Most commonly, the diagnosis is based almost exclusively on clinical signs. As the isolation of the parasite from infected tissues or blood is rather difficult, other laboratory methods were used through time for etiological diagnosis. The use of complement fixation test (CFT) was widely spread in implementation of eradication programs in North America and Europe. Also xenodiagnosis is considered feasible.

***Direct*** parasitological diagnosis by observing the flagellates in samples is achievable in the first 4-5 days of the infection. Scrapings of the vaginal mucosa in mares or urethral washings in stallions are recommended in this case. In the later stages of the infection the parasites may be found in aspiration of fluids from edema and cutaneous plaques, especially shortly after eruption.

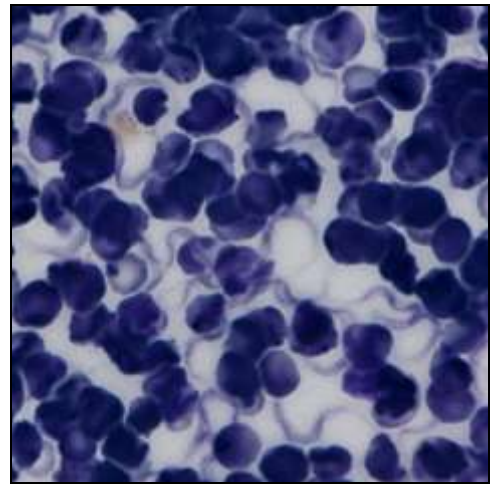

**Figure 2.3** *Trypanosoma equiperdum* in a blood smear from an artificially infected rodent. *(Photo Andrei D. Mihalca)*

Detection in blood smears from naturally infected horse is exceptional, but possible in laboratory rodents (**figure 2.3**). In countries where other trypanosomoses than dourine occur in horses, the morphological differentiation of *T. equiperdum* from other species (i.e. *T. evansi* and *T. brucei*) is impossible.

***Xenodiagnosis*** (inoculation of suspicious samples to laboratory animals) was successfully achieved in splenectomized animals or after intratesticular injections in rabbits.

***Serology*** has been used in combination with clinical diagnosis. The ***complement fixation test*** (CFT) is the recommended test for international trade. Uninfected equids, mainly donkeys and mules, often show false positive results due to anticomplementary effects in horse serum. Indirect fluorescent may help to resolve these cases. Other serologic tests include ***ELISA***, ***radioimmunoassay***, ***counter immunoelectrophoresis***, agar gel immunodiffusion (***AGID***) and ***card agglutination***. However, cross-reactions can occur, especially with *T. brucei* and *T. evansi,* but correlations with clinical signs might be helpful. A test that can distinguish equine piroplasmosis, dourine and glanders by immunoblotting has been developed in USA but it is not commercially available.

***Differential diagnosis*** is done against coital exanthema, contagious equine metritis, Surra, Nagana, anthrax, equine viral arteritis, equine infectious anemia, purpura hemorrhagica, malnutrition, helminth infestations etc.

**Treatment.** Although reported in some endemic areas, treatment is not recommended as asymptomatic infected carriers may result. If attempted, treatment should be done with trypanocidal drugs. Suggested treatments include:

- diminazene aceturate (i.e. Berenil): 7 mg/kg bw, as a 5% solution injected im with a second injection after 24 h at half dose;
- suramin: 10 mg/kg bw, given iv for two or three treatments at a weekly interval;
- quinapyramine dimethylsulfate: 3-5 mg/kg bw in divided doses injected sc.

**Control.** As no immune prophylaxis measures are available, the most effective way for preventing and controlling the disease are systematic surveys for identifying positive animals by serology. CFT has been used successfully for eradication programs in North America and Europe. Infected animals should be euthanized or castrated to prevent further transmission. All equids in areas where dourine is diagnosed should be quarantined and breeding should be stopped for until no new positive cases are found. To avoid accidental transmissions in endemic areas, during artificial breeding, all instruments should be sterilized properly. In dourine free countries, importation of horses from endemic countries should be prohibited.

### 2.3.1.2 Vector-borne trypanosomoses in domestic animals

Vector-borne trypanosomoses include mainly tropical infections of humans and animals caused by several species of genus *Trypanosoma* (**table 2.3**). Two severe diseases affect humans: the sleeping sickness in Africa and the Chagas disease in South America.

**Table 2.3** Main vector-borne trypanosomoses of animals and men*

| (Sub)Species | Main vertebrate host | Disease | Vector | Transmission | Distribution |
|---|---|---|---|---|---|
| *T. theileri* | cattle, antelopes | Nonpathogenic | Tabanidae | Feces | Worldwide |
| *T. rangeli* | humans, wide range of domestic and wild mammals | Nonpathogenic | Reduviidae | Bite | South America |
| *T. lewisi* | rats, humans (?) | Nonpathogenic (?) | Rat fleas | Feces | Worldwide |
| *T. cruzi* | humans, virtually all mammals | Chagas' Disease | Reduviidae | Feces | South America |
| *T. evansi* | camels, equines, bovines, goats, dogs and wild animals | Surra | Tabanidae *Stomoxys* spp. | Mechanical | Asia, Africa, Australia, South and Central America |
| *T. vivax* | ruminants, equines, camels | Nagana (Souma) | *Glossina* spp. Tabanidae | Bite Mechanical | Africa, South America |
| *T. congolense* | bovines, equines, sheep, goats, camels, pigs, dogs | Nagana | *Glossina* spp. | Bite | Africa |
| *T. simiae* | pigs, camels, horses, cattle | Nagana | *Glossina* spp. | Bite/Mechanical | Africa |
| *T. suis* | pigs | Nagana | *Glossina* spp. | Bite | Africa |
| *T. brucei brucei* | domestic animals, camels, antelopes, carnivores | Nagana | *Glossina* spp. | Bite | Africa |
| *T. brucei gambiense* | humans, pigs, sheep | Sleeping sickness | *Glossina* spp. | Bite | Africa |
| *T. brucei rhodesiense* | humans, cattle, pigs, goats, dogs, primates, various wild animals, including antelopes | Sleeping sickness | *Glossina* spp. | Bite | Africa |

* Compiled from Maudlin et al. (2004)

Trypanosomoses bear different names in animals, according to their geographical distribution and etiologic agent. However, some vector-borne trypanosomoses are also present in temperate areas (i.e. the *T. theileri* asymptomatic infections of cattle worldwide).

**Historical notes.** Probably the stages of the first seen trypanosome were those of *T. theileri* from the gut of horse flies, by Antonie van Leeuwenhoek (1632-1723) in 1680. However, the first accurate description of a blood stage was done in 1841 by Gabriel Valentin (1810-1883) in blood smears from trouts. The genus *Trypanosoma* was erected in 1843 by David Gruby (1810-1898) for hemoflagellates found in the blood of frogs.

The first major discovery from medical point of view came only in 1880, from Griffith Evans (1835-1935). While working as a veterinarian in India, he

discovered the agent of Surra, a disease of local horses and camels. Later, the species was named in his honor, *T. evansi.* Another major milestone in the history of trypanosome research was represented by the works of David Bruce (1855-1931). Between 1894 and 1897 when he was working in southern Africa, he proved that the disease of livestock known as Nagana was also caused by a trypanosome. As a tribute for him, this species was later named *T. brucei*. Bruce was the first scientist to prove the vector-borne nature of trypanosomoses. He demonstrated that Nagana is transmitted from wild to domestic animals by the bite of tsetse flies. Interestingly, the same hypothesis, though not proved, was suspected 40 years before Bruce's demonstration by the famous British explorer of equatorial Africa, David Livingstone (1813-1873). Joseph Dutton (1879-1905) was the first to describe the agent of sleeping sickness in humans, *T. gambiense*. He died three years later by the disease he had been studying.

**Etiology.** There are several species of genus *Trypanosoma* involved in the etiology of vector-borne trypanosomoses of domestic and wild mammals (**table 2.3**). Traditionally, members of genus *Trypanosoma* parasitic in domestic animals are divided into "Stercoraria" and "Salivaria".

The stercorarian trypanosomes (Latin: *stercoralis* = living in feces) comprise species in which the development of the metacyclic stages in the vector takes place in the fecal medium of the hind gut ("posterior station") and transmission is contaminative. Among species of medical importance, *T. theileri*, *T. rangeli, T. lewisi* and *T. cruzi* are included in this group. They always possess a free flagellum. The kinetoplast is large and it is never located terminally. The posterior end of the body is pointed. Reproduction in the mammalian host is discontinuous, typically taking place in the amastigote or epimastigote stages. With the exception of *T. cruzi*, the other species are not pathogenic.

The salivarian trypanosomes include those species in which the metacyclic trypomastigotes develop in the "anterior station" (proboscis and salivary glands) and the transmission is inoculative. Species from this group include: *T. vivax*, *T. congolense*, *T. simiae*, *T. brucei*, *T. evansi* and *T. suis*. The free flagellum may be present or absent and the kinetoplast is always located subterminally or terminally. The posterior end of the body is usually blunt. Reproduction in the mammalian host is continuous and takes place in the trypomastigote stage. Most of the species are pathogenic to mammals.

**Morphology.** Specific morphological details for the stages in the blood of vertebrates of each species were given above. Nevertheless, morphologic identification to species level is not generally feasible, and new molecular techniques tend to replace the traditional microscopy for this goal. All morphological characters given below refer to the stage from the blood of the vertebrate host. *T. theileri* (**figure 2.4**) is a common, non-pathogenic parasite inhabiting the blood of various domestic and wild ruminants worldwide. It is one of the largest mammalian trypanosome

(60-70 μm in length). The posterior end of the body is pointed. The kinetoplast is located near the nucleus, the later being positioned centrally in the cell. The undulating membrane is well developed and the free part of flagellum relatively long.

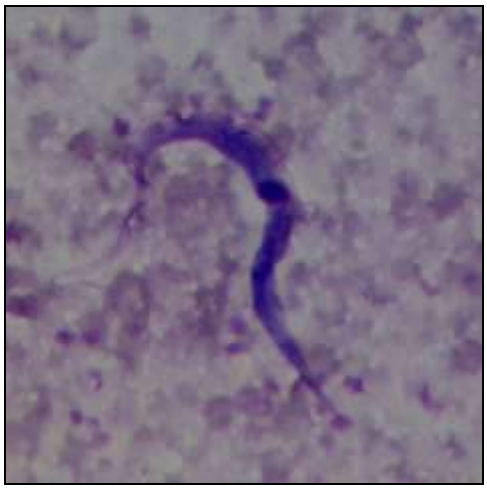

**Figure 2.4** *Trypanosoma theileri* in a blood smear from a naturally infected cow. This species does not seem to be pathogenic for their bovine or wild hosts *(Photo Viorica Mircean)*

*T. rangeli* is a non-pathogenic species infecting humans and a multitude of wild and domestic mammals. The trypomastigotes are slender and large (26-34 μm). The kinetoplast is subterminal and relatively small.

*T. lewisi* is parasitic in rats worldwide. The body is curved and pointed at the posterior end, with a mean length of 21-37 μm. The well-developed free flagellum delimitates a more or less developed undulating membrane. There is a single report from Malaysia, where the species has been isolated from a human clinical case.

*T. cruzi* has a wide host range. More than 150 species of mammals were reported to be infected with this species, and virtually all mammals are considered to be susceptible. It causes the Chagas Disease, a severe condition of humans from several South American countries. The species is a small, "C" shaped trypanosome, measuring 16.3-21.8 μm in length. The large kinetoplast is located near the posterior end of the cell.

*T. evansi* is probably the widest distributed pathogenic trypanosome, causing a disease called Surra. Infection occurs in many hosts, but the most important include dromedaries, equines and dogs. The trypomastigotes (16.8-24.9 μm) are usually slender, with a long free flagellum and a rounded posterior end. However, the species is quite polymorphic, and stumpy or intermediate forms have been described.

There are several species of the genus *Trypanosoma* listed as the agents of a group of diseases of livestock from Africa, collectively called Nagana: *T. vivax*, *T. congolense*, *T. simiae*, *T. suis* and *T. brucei*.

*T. vivax* causes a disease called Souma in west, central, east and South Africa. Its hosts are various ungulates (cattle, sheep, goats, horses, camels, antelopes). It was also introduced to West Indies, Central and South America together with cattle imported from Africa.

In the New World, the disease got the various names (Secadera, Huequera, Cacho Hueco) and interestingly, became

transmitted mechanically by non-tsetse vectors (horse flies and stable flies). Mean length is between 21.0 and 25.4 μm. There are two recognized subspecies: *T. vivax vivax* (in Africa) and *T. vivax viennei* (in the New World).

*T. congolense* infects a broad spectrum of domestic hosts. It is a small species, with a mean length between 11.5 and 14.0 μm. There are several strains with no taxonomic status which differ in certain morphological feature but also virulence.

*T. simiae* is primary a parasite of pigs, with high pathogenic importance, though the name is misleading, meaning "of monkeys". Morphologically, the species resembles *T. congolense* but it is more motile. The average length is 14.9-19.0 μm, with the kinetoplast typically occupying a marginal position near the posterior end of the body.

*T. suis* parasitize suids in several regions of Africa, being relatively pathogenic. It exhibits slow movements. The body is short and stumpy (14.0-16.0 μm), with a small kinetoplast in subterminal position.

*T. brucei* (**figure 2.5**) includes several strains, some of them assigned to subspecies. *T. brucei gambiense* and *T. brucei rhodesiense* are responsible for human sleeping sickness.

Another subspecies, *T. brucei brucei* completes the list of etiologic agents of Nagana of livestock in Africa. The trypomastigotes of the later subspecies are polymorphic, with slender, intermediate and stumpy forms with wide variation of the cell's average length, between 11.0 and 42.0 μm.

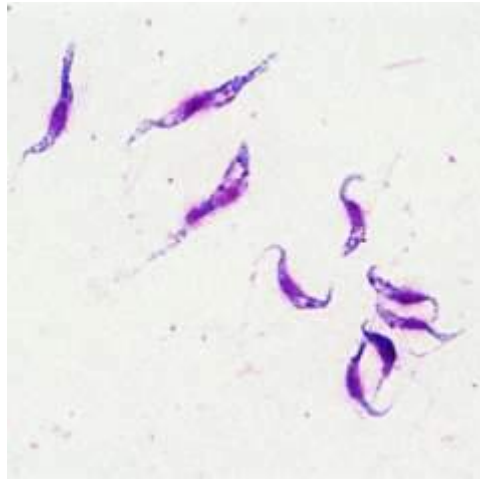

**Figure 2.5** *Trypanosoma brucei* from laboratory culture. *(Photo Andrei D. Mihalca)*

**Life cycle.** General aspects regarding development and biology of trypanosomatids were given above. Yet, each species has its own peculiarities.

The metacyclic trypanosomes of the **stercorarian** species (*T. theileri*, *T. rangeli*, *T. lewisi*, *T. cruzi*) develop in the posterior part of the vector's gut and are discharged through the feces of the insect. The metacyclic stages then invade the vertebrate host through the wound caused by the insect bite, through skin abrasions or other wounds. If infected insects are swallowed by vertebrates, trypanosomes may enter the bloodstream through various contact mucosae. Inside the mammalian body, development of trypanosomes varies according to species.

In *T. theileri*, *T. lewisi* multiplication is by binary or multiple fission of epimastigotes in the plasma of blood. In *T. cruzi* binary fission of amastigotes

takes place inside the reticuloendothelial cells (i.e. macrophages) from the liver, spleen or bone marrow but also in myocardial tissue.

Various intermediate hosts were reported for stercorarian trypanosomes:

- For *T. theileri* the vectors are tabanids (horse flies). Several species were proven to transmit the parasites to cattle: *Tabanus glaucopis*, *T. striatus* and *Haematopota pluvialis*. Some authors suggested that also hippoboscids are feasible vectors for *T. theileri*.
- For *T. rangeli* the intermediate hosts are bugs of subfamily Triatominae (family Reduviidae). The main vectors are *Rhodnius prolixus*, *R. pallescens*, *Triatoma infestans*, *T. dimidiata* and many other experimental insect hosts.
- *T. lewisi* is transmitted by rat fleas. In temperate areas, the main vector is *Nosopsyllus fasciatus* while in tropical and sub-tropical regions the natural vector is *Xenopsylla cheopis*. Many other flea species were infected experimentally and were able to transmit the trypanosomes.
- *T. cruzi* is using as vectors bugs of Triatominae subfamily. Many species were reported as insect hosts, the most important genera being *Triatoma*, *Rhodnius*, *Panstrongylus* and *Eratyrus*.

In the other group, **Salivaria**, the metacyclic trypanosomes develop in the salivary structures of insects. The main vectors are tsetse flies from genus *Glossina* (**figure 2.6**). Transmission from the vector to the vertebrate host is inoculative, through the saliva.

In *T. vivax*, trypomastigotes from the blood of the vertebrate host multiply by binary fission. When ingested by tsetse flies, they become epimastigotes in the esophagus, than multiply and migrate to the pharyngeal region where they transform to metacyclic trypanosomes. These are the infective stages and are inoculated to a new vertebrate host. There are several species of *Glossina* reported as vectors for *T. vivax*: *G. morsitans*, *G. pallidipes*, *G. longipalpis*, *G. swynnertoni*, *G. austeni*, *G. palpalis*, *G. fuscipes*, *G. tachinoides*, *G. vanhoofi*, etc. In geographical areas where tsetse flies are not present (parts of Africa, South America), tabanids are able to transmit the disease mechanically, by bite.

**Figure 2.6** *Glossina* sp. feeding on human host. *(Photo Andrei D. Mihalca)*

In *T. congolense* the aflagellated trypomastigotes multiply in the blood continuously and are transmitted to tsetse flies. After ingestion, they arrive to the midgut of the flies, change shape to longer and slender trypomastigote forms which migrate to the pharynx where they become epimastigotes. Finally the epimastigotes transform to aflagellate infective metacyclic trypomastigotes. The main vectors for *T. congolense* are: *G. morsitans*, *G. palpalis*, *G. pallidipes*, *G. longipalpis* and *G. austeni*.

*T. simiae* has at least ten species of competent vectors in genus *Glossina*. However, mechanical transmission by other blood-sucking dipterans was reported commonly. Experimental work, showed that the complete cyclic development of *T. simiae* in *G. brevipalpis* takes 20 days.

Only two vector species are known for *T. suis*: *G. brevipalpis* and *G. vanhoofi*. The cycle in the tsetse fly takes about 28 days.

All subspecies of *T. brucei* develop in the midgut, proboscis and salivary glands of various *Glossina* species. In the case of *T. b. brucei*, mechanical transmission by tabanids (horse-flies) or *Stomoxys* has also been described.

**Epidemiology.** The geographical distribution of animal vector-borne trypanosomoses is closely related to the distribution of their vectors. African animal trypanosomoses occur where the tsetse fly vector exists in Africa, between latitude 15°N and 29°S (**figure 2.7**). As some species of *Trypanosoma* can also be transmitted mechanically by biting flies, the distribution range of Nagana is also in parts of Africa free or cleared of tsetse, and parts of Central and South America. According to OIE, Nagana is spread over the territory of 37 countries (mostly sub-Saharan African) on 10 million square kilometers. It is the most important disease of cattle in Africa. At least 30 species of wild mammals are known as natural reservoirs for Nagana-causing trypanosomes, although they do not show clinical signs of infections. Nagana is widespread also because of the high number of competent biological vectors. About 23 species of tsetse flies (genus *Glossina*) are competent vectors for trypanosomes. Moreover, an infected fly remains infective for all its life.

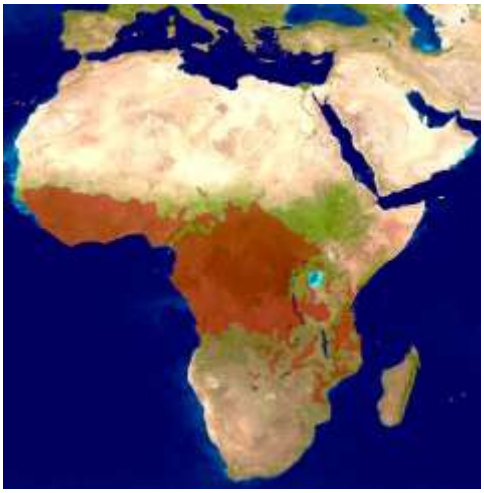

**Figure 2.7** Distribution of genus *Glossina* is shown with red shading (redrawn, from Leak, 1999)

Virtually all domestic mammals are susceptible to clinical infection with African trypanosomoses. However, there are several livestock breeds considered to be resistant (known as trypanotolerant

breeds). These include West African indigenous cattle breeds (N'Dama, Baoule, Muturu, Laguna, Somba and Dahomey), East African zebu breeds (Orma Boran and Maasai zebu) and African indigenous breeds of small ruminants (West African dwarf sheep and goats, and East African goats).

The infection sources are blood, lymph and other fluids of infected animals.

**Pathogenesis.** After the transmission by tsetse flies, trypanosomes undergo a period of multiplication in the dermis and subdermis where they enter the afferent lymphatic vessels.

At the inoculation site, a lesion called chancre will develop 4-10 days after the tsetse bite. This cutaneous lesion precedes the detection of trypanosomes in the blood by 4-6 days. The development of the chancre is depended on the infective dose and the rhythm of parasite multiplication but generally its cellular population consists of neutrophils, lymphocytes, macrophages and mast cells, associated with edema and congestion.

After a period of multiplication in the skin, trypanosomes appear in very high numbers in the afferent lymph that drains from the site of vector inoculation. Subsequently, the draining lymph nodes become significantly enlarged because of B-cell proliferation and local migration of other leucocytes. Through the lymphatic system, the trypanosomes finally gain access to the main blood stream and to the all the internal organs. Detection of trypanosomes in the blood is possible usually during the second week after the infection with the first peak at 14-21 days of infection. This first peak corresponds to the first presence of specific antibodies.

Lymphocyte sequestration and increased macrophage population are responsible for the splenomegaly. The same applies to the liver, which might be increased and congested due to increased phagocytic activity by Kupffer cells. Trypanosome infection is also responsible for a more or less severe pancytopenia. The resulting anemia is responsible for further pathology in the myocardium and blood vessels irrigating the heart, finally inducing cardiac decompensation. In female cows, chronic infection leads to infertility, endometritis and abortion.

Blood biochemistry changes in infected animals consist of: decreased cholesterol and lipid concentration, reduced total serum lipids, decreased serum albumins and increased globulin. There is no change in the level of total proteins, calcium, iron or fibrinogen. During parasitemic phases, the animals are hypoglycemic. Hematology is characterized by leucopenia, anemia, and thrombocytopenia.

**Immunology.** The primary immune response is targeted mainly on the variable surface glycoproteins (VSG), which cover the surface of the parasites. Based on the type of VSG, trypanosomes are grouped in (sero)demes. Reinfection of the animals with trypanosomes from the same deme is usually associated with a reduced chancre development. The antigenic variability in *T. congolense* and *T. b. brucei* is huge; hence development of immunity against all demes is virtually

impossible. On the other hand, the number of VSG types in *T. evansi* and *T. vivax* is more limited.

**Clinical signs.** Despite the diversity of agents involved in the etiology of ***Nagana***, many clinical features are common to all domestic animals, regardless the host or trypanosome species. However, there are particular pathogenic characteristics, listed in **table 2.4**.

**Table 2.4** Pathogenicity of *Trypanosoma* species causing Nagana in different hosts*

| (Sub)Species | Degree of severity | | | | | | |
|---|---|---|---|---|---|---|---|
| | Cattle | Sheep, goats | Horses | Donkeys | Camels | Pigs | Dogs |
| *T. vivax* | 3 | 2 | 2 | 1 | 2 | 0 | 0 |
| *T. congolense* | 3 | 2 | 2 | 2 | 3 | 1 | 2 |
| *T. simiae* | 0 | 1 | 0 | 0 | 0 | 3 | 0 |
| *T. suis* | 0 | 0 | 0 | 0 | 0 | 3 | 0 |
| *T. brucei brucei* | 1 | 2 | 3 | 3 | 3 | 1 | 3 |

0 - nonpathogenic; 1 - mild; 2 - moderate; 3 - severe.
* - from Maudlin et al. (2004)

As shown in table 2.3, Nagana is a group of diseases caused by various species of *Trypanosoma*, which affects a wide range of domestic animals in Africa and South America: large and small ruminants, camels, horses, donkeys, pigs and carnivores.

There are many descriptions on the clinical course of the disease originating in observation on natural or experimental infections. The prepatent period is 1-3 weeks. In some (but not all) animals, at the site of the tsetse fly bite an inflammatory reaction called chancre may develop. This might appear as painful swelling of the skin, of variable size. The symptoms in the ***acute phase*** include high fever, which subsequently is correlated with the fluctuating parasitemia, anemia of variable severity (depending on the breed and age of the infected animal), enlarged lymph nodes, enlarged spleen, weakness and lethargy. Abortion or birth of weak offspring and high neonatal mortalities are also common. In 1-4 weeks after the infection, the animals are unable to rise and death may occur. If infected animals survive, they usually go into the ***chronic phase***. This is characterized by a persistent anemia, stunted growth, decrease of productions (i.e. milk) and infertility. However, there is no loss of appetite. The chronic phase might last even for years, before the death of the animals.

There are various clinical differences of Nagana which are dependent on the host species. In local ***cattle*** from Africa, Nagana is usually chronic, although hyperacute forms are also known. The severe drop in the packed cell volume is correlated with decrease in milk production. The anemia in this stage must be differentiated from other types of infectious or parasitic anemia (i.e. babesiosis, anaplasmosis, strongyles or heavy tick-infestations). ***Small ruminants*** usually display fewer and less severe symptoms than bovines. However, in both sheep and goats, the milk production is significantly decreased and the infection is associated with low reproductive performance and neonatal mortality. In ***camels***, the disease is usually chronic, rarely lethal. ***Horses and donkeys*** are very sensitive to *T. b. brucei* Nagana and they usually develop an acute

or hyperacute disease, with subcutaneous edema, keratoconjunctivitis, ataxia and paralysis. If affected by *T. vivax* or *T. congolense*, equids are more resistant, and they develop chronic or asymptomatic infections. In ***pigs***, infections are rare, and clinical, severe, acute forms are likely to be caused by *T. simiae*. The other Nagana-causing species are responsible for reproductive disorders in pigs. In ***dogs***, the infection with *T. b. brucei* produces and acute disease, with high fever, generalized edema, keratitis and rabies-like symptoms, followed by death.

The other animal trypanosomosis, ***Surra***, is more widely distributed (Africa, Asia, South America) than Nagana and its specific pathogenicity is different. The agent, *T. evansi* is producing severe syndromes in camels, horses and dogs, with significant mortalities. In ***camels***, Surra is usually acute in young camels and pregnant females and it evolves with high fever, anemia, extreme weight loss, subcutaneous edema, kerato-conjunctivitis, hypertrophy of lymph nodes, neurological signs, abortions and death. In ***horses*** from Africa and Asia, the disease is similar to Nagana. In South American horses and donkeys, Surra is rather chronic, with less severe clinical signs. Dogs in Asia and South America are sensitive and develop the acute disease with signs similar to Nagana. ***Cattle*** are more resistant to Surra.

The cause of death in animal trypanosomoses is usually **congestive heart failure**, caused by the persistent anemia, myocardial damage and alteration of vascular permeability.

**Pathology.** Four types of lesions are associated with trypanosomoses in animals: inflammatory, congestive, hemorrhagic and degenerative affective various organs: skin, lymph nodes, spleen, liver, heart, central nervous system, eyes, testes, ovaries etc.

The characteristic skin lesion is called chancre, and if it is visible macroscopically it appears as a 2-5 cm swelling at the site of vector bite. Histological sections show edema and mast cell degranulation at the site of trypanosome initial development in the skin.

The gross appearance of lymph nodes is a marked increase in size. This enlargement is evident mainly in the nodes draining the lymph from the site of the infected tsetse bite. Histologically, this enlargement corresponds to a proliferation of B cells, with expansion of lymphoid follicles in the cortical and medullar areas and reduced paracortex.

The spleen is increased in size during the acute phase. The associated histopathology consists of development of secondary lymphoid follicles and expansion of the red pulp. The hepatomegaly and hepatic congestion are also common lesions in the acute trypanosomoses.

In histological sections, the main lesions consist in hyperplasia of the Kupffer cells, periportal mononuclear cell infiltration and centrolobular necrosis. Severe lesions are found in the heart. The blood vessels irrigating the heart congested, with swollen and vacuolated walls and

perivascular edema. The most severe cardiac lesion consists in myocardosis.

Other lesions observed in animals suffering of trypanosomoses are: pituitary necrosis, orchitis or testicular degeneration, cystic ovaries, endometritis, meningoencephalitis,

**Diagnosis.** Although laboratory diagnosis is more or less essential, in the field conditions where vector-borne trypanosomoses occur are hardly available or quasi-absent. So veterinarians and animal health officers should rely to a great extent on ***symptoms***. Although clinical signs are not considered to be characteristic, they should not be disregarded. Cattle with anemia, fever, weight loss, enlarged lymph nodes, lacrimation, abortion and rough hair coat should be at least suspected as acute Nagana. In hyperacute cases animals are found dead and systemic hemorrhage is dominating the pathological picture. If symptoms progressively remit following administration of trypanocidal drugs, the diagnosis is confirmed.

If animals die and ***necropsy*** is performed the atrophy of fat, enlarged lymph nodes, spleen and liver, subcutaneous edema and hemorrhagic lesions can be also indicative of trypanosomoses.

However, ***laboratory techniques*** are the only ones which can confirm the etiological diagnosis. They can be divided in direct methods (identification of the parasites, parasitic antigens or parasite DNA) and indirect methods (serology).

***Direct methods*** include:

- The simplest methods to confirm the diagnosis are ***parasitological methods*** by which parasites can be seen under a microscope. The most useful sample to detect the parasites is blood. This can be examined directly (fresh or stained smears) or using concentration methods. Direct detection in ***smears*** is not considered being sensitive enough, mainly because of the low numbers of trypanosomes in the blood. This can be partly overcome by collecting blood early in the morning and from peripheral capillaries (i.e. tail or ear). Smear can be examined fresh (wet blood films) enabling the detection of live, motile trypanosomes. Stained smears (thick or thin) are also useful but with limited value, mainly in the chronic phase. However, if no centrifuge is available, this can be a simple option.
- Two ***blood concentration methods*** are available: the microhematocrit centrifugation technique (Woo method) and dark-field/phase-contrast buffy-coat technique (Murray method) (OIE, 2012).
- The ***Woo method*** is based on the separation of the blood components depending on their specific gravity. After the blood is collected into a heparinised capillary tube and one end is sealed, the tubes are centrifuged in a microhematocrit centrifuge at 9000 g for 5 minutes. After centrifugation, the tube is examined under the microscope at the separation level of the plasma-

cell interface (buffy coat) using the 40 objective. The Woo method is more sensitive than direct examinations techniques. According to OIE (2012), in the case of *T. vivax*, the sensitivity of this method is 100% when the parasitemia is >700 trypanosomes/ml blood and it decreases to 50% when parasitemia is between 60 and 300 trypanosomes/ml blood. When parasitemia is lower than 60 trypanosomes/ml blood the Woo method usually fails to detect the infection.

- The ***Murray method*** is similar to Woo method, but the buffy coat is extracted from the tube on a microscope slide (after the tube is cut) and examined under a dark-field or contrast-phase microscope.
- Other concentration techniques (used mostly in the diagnosis of human trypanosomoses) include the ***anion exchange method*** (using the miniature anion-exchange chromatography technique) or ***in vitro cultivation***.
- Identification of the parasite can be achieved also using ***animal inoculation*** (using mice and rats) followed by examination of their blood.
- Another direct method which is aiming this time ***parasitic antigens*** but used with inconsistent results is ***ELISA***.
- The most sensitive methods which are more and more routinely used in laboratories worldwide are ***molecular techniques*** to identify the parasite's DNA. These include PCR, Real-Time PCR and Restriction Fragment Length Polymorphism (**RFLP**).

***Indirect methods*** (serological methods) include:

- Indirect Fluorescent Antibody Test (***IFAT***)
- Enzyme-Linked Immunosorbent Assay (***ELISA***), targeted on detection of anti-*Trypanosoma* antibodies.
- Card Agglutination Test (available for *T. evansi*).
- Immune Trypanolysis Test (available only for *T. evansi*).

The main problem with highly specific and highly sensitive laboratory tests (molecular methods or serology) is their price and the need for sophisticated equipment which is rarely available in the countries which really need them.

Acute Nagana ***differential diagnosis*** in cattle includes in the acute phase babesiosis, anaplasmosis, theileriosis, anthrax and acute pasteurellosis. In the chronic stage, various helminthosis and malnutrition must be considered. In horses infected with *T. evansi* the differential diagnosis is made with African horse sickness (a vector-borne viral infection), equine viral arthritis, equine infectious anemia or dourine. In camels, the *T. evansi* infection shows similar signs anthrax and in dogs rabies should be also considered, mainly because both diseases are endemic in many tropical countries.

**Treatment**. The number of drugs used in the treatment of animal trypanosomoses is limited and most of them are available for almost 50 years (**table 2.5**). The treatment is carried out based on certain plans: routine or strategic block treatments (with prophylactic drugs like isometamidium chloride), treatment of infected animals (diminazene aceturate) or treatment of clinical cases (any other trypanocide drug).

**Table 2.5** Drugs used in the treatment of animal trypanosomoses*

| Drug | Trade name(s) | Dose (mg/kg) | Route | Target parasites | Species |
|---|---|---|---|---|---|
| Diminazene aceturate | Berenil, many others | 3.5-7 | i.m | *T. congolense*<br>*T. vivax*<br>*T. brucei*<br>*T. evansi* | Cattle<br>Sheep<br>Goats<br>Dogs<br>Horses<br>Donkeys |
| Homidium chloride<br>Homidium bromide | Novidium<br>Ethidium | 1 | i.m. | *T. congolense*<br>*T. vivax* | Cattle<br>Sheep<br>Goats<br>Pigs<br>Horses<br>Donkeys |
| Isometamidium chloride | Samorin,<br>Trypamidium,<br>Veridium | 0.25-0.5 | i.m. | *T. congolense*<br>*T. vivax*<br>*T. brucei*<br>*T. evansi* | Cattle<br>Sheep<br>Goats<br>Horses<br>Donkeys<br>Camels |
| Quinapyramine dimethylsulphate | Trypacide sulphate | 3-5 | s.c. | *T. congolense*<br>*T. vivax* | Camels |
| Quinapyramine dimethylsulphate:chloride | Trypacide Pro-salt | 3-5 | s.c. | *T. vivax*<br>*T. brucei*<br>*T. simiae*<br>*T. evansi* | Camels<br>Horses<br>Donkeys<br>Pigs<br>Dogs |
| Suramin | Naganol | 7-10 g/animal | i.v. | *T. evansi* | Camels<br>Horses<br>Donkeys |
| Melarsomine | Cymelarsan | 0.25 | s.c./i.m. | *T. evansi* | Camels |

* compiled from Maudlin et al. (2004)

Usually only animals with severe acute forms are treated. Otherwise they are left to develop immune response which eventually protects them during subsequent infections.

**Control.** No vaccines are available. However, general prevention measures (i.e. control of the vector populations, animal husbandry practices, selection of trypanotolerant breeds) and chemical prophylaxis are known to work. For the control of tsetse fly populations various methods have been used over time but most of them were aborted (i.e. spraying of land with insecticides, clearing of bush). Nowadays, the commonly used methods include application of synthetic ***insecticides*** on the animals or ***biological control*** using sterile male flies (as females tsetse flies reproduce only once

in their life) or fly ***traps*** baited with pheromones. A proper farming ***management*** should reduce the contact of animals with the vectors. Selection of ***trypanotolerant crossbreeds*** is an important strategy. These include West African indigenous cattle breeds (N'Dama, Baoule, Muturu, Laguna, Somba, Dahomey), East African zebu breeds: (Orma Boran, Maasai zebu) and indigenous breeds of small ruminants: (West African dwarf sheep and goats, and East African goats) (OIE, 2012).

***Chemical prophylaxis*** is achieved using mainly Isometamidium.

### 2.3.1.3 Canine leishmaniosis

Canine leishmaniosis is a severe zoonotic disease, affecting primarily dogs, but also humans and other mammals, transmitted by hematophagous vectors (sandflies).

**Historical notes.** All early notes and the discovery of *Leishmania* are related to human diseases. The first written documents on the Old World cutaneous leishmaniosis (oriental sore) are known from the tablets from the library of King Ashurbanipal from the 7th century BC. Avicenna and other Arab physicians also mention the disease as early as the 10th century by the name of Balkh. The Old World visceral leishmaniosis (kala azar was first mentioned in India in 1824. However, the first observation of the parasite came in 1885 when a Russian military surgeon, Borovsky saw unknown forms in the blood. A Scottish army physician, William Leishman and a physiology professor from India, Charles Donovan are credited with the discovery and description of the parasite. The vectors were identified to be the sandflies in 1921 by two brothers, Edouard and Etienne Sergent.

The New World leishmaniosis is known from, pre-Incan pottery as early as 1st century AD in Peru and Ecuador and from record of Spanish missionaries from the 16th century. The agents, which initially were considered to be similar to the Old World, were accurately described as new species in 1911 by Gaspar Vianna and the vectors were recognized as flies from genus *Lutzomyia* in 1922. The first case of canine leishmaniosis was described in 1903. In 1940, it is estimated that 40% of the dogs in Rome were infected with *Leishmania*.

**Etiology.** More than 30 species are currently recognized in genus *Leishmania*, all parasitic in mammals. The genus is divided in two subgenera *Leishmania* and *Viannia*, based on the site of development in the sandfly host. Subgenus *Leishmania* develops in the anterior alimentary tract of sandflies while *Viannia* develops in the midgut and hindgut. Subgenus *Leishmania* is distributed in the Old World (*L. aethiopica*, *L. donovani*, *L. infantum*, *L. major*, *L. tropica*) and the New World (*L. amazonensis*, *L. infantum*, *L. mexicana*, *L. pifanoi*, *L. venezuelensis*). Subgenus *Viannia* is restricted the New World (Central and South America) and includes several medically significant species: *L. braziliensis*, *L. guyanensis*, *L. panamensis* and *L. peruviana*. All of the species listed above cause infections in humans, often

with severe syndromes, life-long disabilities or death.

The most important species responsible for canine leishmaniosis is *L. infantum*. However, several other species of *Leishmania* were reported to infect dogs in various geographical regions: *L. donovani*, *L. tropica*, *L. braziliensis*, *L. peruviana*, *L. panamensis*, *L. amazonensis*.

**Morphology**. *Leishmania* has two developmental forms: the motile extracellular promastigotes (in the sandfly) and the amastigotes (in the vertebrate host).

The promastigotes (15 µm in length) are elongated and have a conspicuous free flagellum (**figure 2.8**). The amastigotes (**figure 2.9**) are usually found in vacuoles within the infected macrophages of the dogs. They are round or oval, lack a free flagellum and they measure 2.5-5 x 1.5-2 µm. They typically have one large basophilic nucleus.

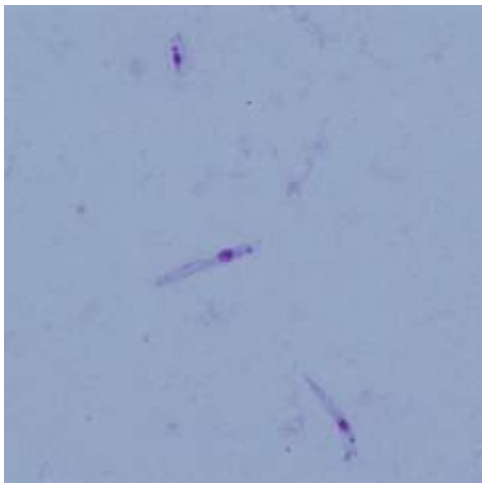

**Figure 2.8** Promastigotes of *Leishmania* from laboratory culture. *(Photo Andrei D. Mihalca)*

**Life cycle**. The life cycle is heteroxenous without free living stages. As there is no direct evidence of sexual reproduction in *Leishmania*, defining the intermediate and definitive host is rather arbitrary. Hence, as most parasitology resources consider the sandfly vectors are the intermediate hosts and vertebrates are the definitive hosts, we will also follow this concept.

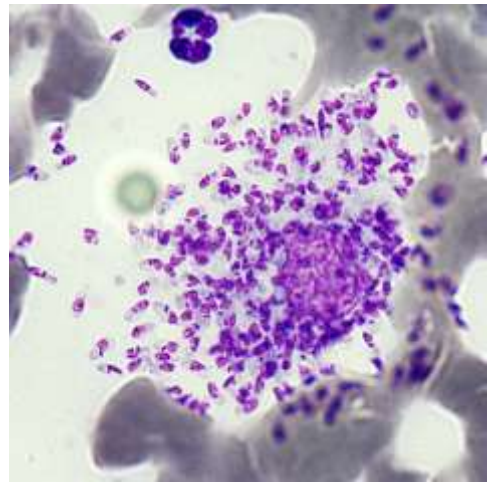

**Figure 2.9** Amastigotes of *Leishmania* in the macrophages of an infected dog. *(Photo Andrei D. Mihalca)*

In the Old World (Europe, Asia, Africa) the vectors are represented by sandflies in genus *Phlebotomus* (**figure 2.10**) while in the New World (the Americas) by genus *Lutzomyia*. Sandflies are small (cca. 3 mm) blood sucking insects, with crepuscular and nocturnal activities. From more than 500 known species of sandflies (Phlebotominae), around 30 have proven vectorial capacity and other more than 40 are suspected as probable vectors. Other hematophagous

arthropods (ticks, fleas) have been suspected to act as competent vectors for *Leishmania*, but experimental results are scarce and hence inconclusive.

**Figure 2.10** *Phlebotomus papatasi*, a vector for *Leishmania infantum*. *(Photo David Modrý)*

In the sandflies, *Leishmania* develops in the extracellular environment while in mammals, development is intracellular.

When uninfected female sandflies feed on the blood of an infected host, they acquire the amastigotes. The amastigotes are released from the host macrophage in the insect's gut and they undergo a series of transformations and become flagellated. This initial stage in the sandfly is called procyclic promastigote. They start to replicate and ultimately they detach from the intestinal surface and migrate to foregut and mouthparts of the vector. They are known as metacyclic promastigotes and they are infective for the vertebrate host.

When the infected female sandfly feeds again, the promastigotes are injected into the vertebrate host. Here, the promastigotes are phagocytized by the macrophages, they lose their flagellum and become amastigotes.

Amastigotes multiply intracellularly by binary fission and increase in number until they rupture the macrophage. Subsequently, they invade other cells, and are disseminated systemically.

**Epidemiology.** Canine leishmaniosis is endemic to several areas of the world, usually in Mediterranean, subtropical and tropical climates. The disease in endemic in the countries bordering the Mediterranean Sea (Southern Europe and Northern Africa), Asia Minor, parts of Central Asia and Western China, South America (mainly Brazil), Eastern North America and parts of Sub-Saharan Africa. However, sporadic cases of canine leishmaniosis are diagnosed also in non-endemic countries, mainly because of intensive travelling and importation of dogs.

It is estimated that millions of dogs are infected in the endemic areas. Dogs are important reservoirs for the human infection, mainly in the Old World. The main diagnosis puzzle in these areas, are dogs with subclinical infections which are a natural source of infection for sandflies (and indirectly to humans). They remain undiagnosed, unless serological surveys are carried out.

There are several factors associated with a higher risk of clinical disease. Age has been shown to be among the most important. Young (2-4 years) and old

(more than 7 years) dogs seem to be more commonly with symptomatic infections. The breed has also been incriminated as a risk factor. Certain dog breeds are more susceptible to disease (German shepherd, Rottweiler, boxer, cocker spaniel) while others are resistant because of co-evolution with the pathogen (Ibizan hound).

Even in the endemic areas, the higher risk of infection is present in rural areas. This is caused by various factors, including more suitable sandfly habitats and availability of other reservoir hosts that dogs. Various domestic or wild animals are known to harbor the infection, clinically or not: cats, horses, pigs, wild canids, rodents, bats, seals etc. However, only few of these are able to transmit the infection to sandfly vectors feeding on them.

Although the main route of infection is via the bite of the vector sandfly, other mechanisms have been suspected or incriminated: transplacental, venereal and blood transfusion.

**Pathogenesis.** Not all infected dogs develop clinical signs. The clinical outcome is dependent on various factors. Some of them are related to the vector (sandfly species, number and duration of the infective bites), some others to the pathogen (strain-dependent) and others to the host (genetic, age, breed, immune status).

After the infectious bite by the sandfly, when metacyclic promastigotes invade the dog's body, they typically start to multiply in the macrophages. If the dog is resistant, it is able to limit the infection to the skin and lymph nodes. Otherwise, the disease becomes systemic (lymph nodes, bone marrow, spleen, liver, etc).

The incubation period is between 3 months and 7 years. The main pathogenetic mechanism is related to the immune response. Generally, *L. infantum* is responsible for the depletion of T lymphocytes and a proliferation of B cell regions in the lymphoid organs. This, together with the proliferation of other cellular populations (plasma cells, histiocytes, macrophages) results in a significant and systemic lymphadenomegaly, splenomegaly and hyperglobulinaemia. However, the increased immunoglobulin response does not offer protection. Adversely, they are associated with supplementary detrimental effects like immune-mediate thrombocytopenia and glomerulonephritis. In addition, the large amount of circulating immune complexes (CIC) is responsible for vasculitis, uveitis and glomerulonephritis. Hence, usually the cause of death in dogs with clinical leishmaniosis is renal failure. The immune-mediated vasculitis is responsible for tissue necrosis in the skin, internal organs and in the eye. Another interesting pathogenic mechanism involves the formation of cryoglobulins which precipitate when exposed to cold and result in ischemic necroses of extremities exposed to low temperatures.

The genetic basis for susceptibility or resistance to canine leishmaniosis has been shown to be related to certain mutations and polymorphisms in the *Slc11c1* gene. This gene is responsible for encoding an iron transporter protein,

involved in the macrophage activation and development of parasites inside the phagolysosomes.

**Immunology.** Both components of the immune system are involved in the defense against *Leishmania* in dogs. However, in dogs susceptible for clinical infections, *Leishmania* is able to evade them, or use it in its own favor.

There are several mechanisms by which *Leishmania* escapes the innate, non-specific immunity. The most important is the ability of amastigotes to survive and even replicate inside the macrophages. This is possible due to lipophosphoglycans produced by the parasite which inhibit the maturation of the phagolysosomes.

The role of the specific (acquired) immune system in leishmaniosis has been intensively studied mostly on experimental models in laboratory rodents. How and if the results could be extrapolated to dogs remains uncertain. The protective immunity in canine leishmaniosis is based on the T lymphocytes and macrophages. Activated T cells produce IFN-γ, IL-2 and TNF-α which induce the anti-leishmanial activity of macrophages. The macrophages produce nitric oxide which is ultimately responsible for the intracellular killing of the amastigotes. Another line of specific immune defense are CD8+ and CD4+ cytotoxic T cells which are responsible for the destruction of macrophages infected with *L. infantum*.

All these cellular processes are inhibited in dogs which develop clinical signs. Alternatively, they develop an increased, but non-protective IgG response against *Leishmania*. Overall, there are strong proofs towards a cell-mediated anti-*Leishmania* immunity in the so called resistant dogs and a rather humoral immune response in dogs with clinical signs. For instance, resistant dogs develop a strong delayed-type hypersensitivity response (indicative of cell-mediated immunity) when inoculated intradermally with *Leishmania* antigens.

**Clinical signs.** Infected dogs may display a great variety of clinical signs, from asymptomatic infection to severe clinical syndrome. When the infection is clinical, the diversity of symptoms might be high (**table 2.6**).

**Table 2.6** Frequency of symptoms in dogs with clinical leishmaniosis*

| Symptom | Percentage |
|---|---|
| Lymphadenopathy | 93.5 |
| Onychogryphosis | 75.0 |
| Cutaneous lesions | 58.7 |
| Weight loss | 26.1 |
| Cachexia | 23.9 |
| Locomotory abnormalities | 22.8 |
| Somnolence | 21.7 |
| Conjunctivitis | 18.5 |
| Anorexia | 16.3 |
| Polydipsia | 13.0 |
| Polyphagia | 13.0 |
| Onychorrhexis | 10.9 |
| Epistaxis | 8.7 |
| Diarrhea | 6.5 |
| Sickness | 2.2 |
| Cough | 1.1 |

* - from Semiao-Santos (1995)

Incubation period is considered to be between 2-8 months, but sporadic records extend this period to 15 months or even several years. There are several

classifications of canine leishmaniosis. Some authors divide the disease in phases: acute, subacute, chronic and latent. In other texts, three types of leishmaniosis are recognized: asymptomatic, oligosymptomatic and polysymptomatic. However, the most useful classification is the one based on the chronology of the clinical signs (*early symptoms, patent period and final period*), accurately described based on experimental infection trials.

***Early symptoms*** consist of significant weight loss, asthenia and apathy. Three month after the infection, cutaneous signs may be visible (periorbital and auricular alopecia), accompanied by conjunctivitis, and renal pain on palpation. Hemorrhagic signs may also be present in this early stage.

***Patent period*** may vary according to dog's immune status and pathogen strain involved, but also to other factors. Combinations of non-specific symptoms (39-40°C hyperthermia, apathy, asthenia, loss of appetite, polydipsia, loss of weight) may appear. One of the most present signs is lymphadenopathy, easily detectable by superficial palpation of popliteal, prescapular and submaxillar lymph nodes. Ultrasound may reveal in this phase marked hepatomegaly and splenomegaly.

In general, the principal symptoms of clinically ill dogs include: skin lesions, enlarged lymph nodes, weight loss, lethargy, ocular lesions, digestive signs (vomiting, diarrhea) or lameness.

Skin lesions are also common in infected dogs. They can appear as non-prurigenic, alopecic patches covered by abundant squamous debris (**figure 2.11**), seborrhea and ulcers (**figure 2.12**). The onset of the cutaneous signs is usually around the orifices of the head and subsequently spread all over the body. The most severe skin lesions are at the level of prominent bones. The apparent similarity with human cutaneous leishmaniosis is restricted to the location of lesions. In humans the pathological changes of the skin are localized, while in dogs, the cutaneous phase is also accompanied by visceral pathology. Loss of hair on the tail ("rat tail") (**figure 2.13**) or nasal hyperkeratosis (**figure 2.14**) have also been reported in infected dogs.

Ocular involvement is frequent in infected dogs with mucous or mucopurulent conjunctivitis, keratitis, corneal ulceration and subsequent blindness. Other symptoms include: nasal discharge, uni- or bilateral epistaxis, progressive muscular atrophy, perionyxis, onychogryphosis (**figure 2.15**), onychorhexis, tremor, paralysis of the hind limbs, arthritis etc. Prior to death, dogs show severe cachexia (**figure 2.16**).

**Pathology.** As explained in the previous section, the lesions characteristic for leishmaniosis are caused primarily by the altered immune response. These include mainly necrotic lesions in the skin, eye and internal organs.

The skin lesions are usually generalized, and microscopically they involve the decrease of collagen type I and the decrease of collagen type III fibers.

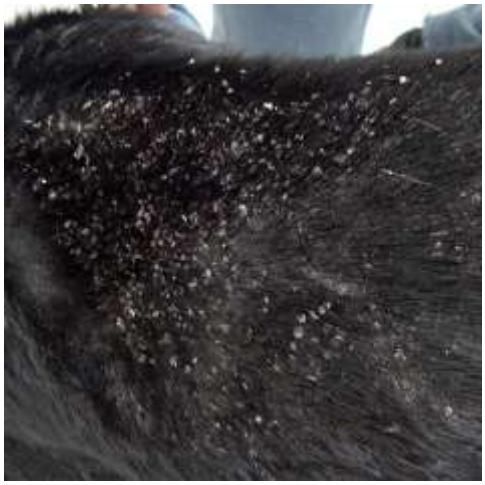

**Figure 2.11** Exfoliative dermatitis in a dog infected with *L. infantum*. *(Photo George Popa)*

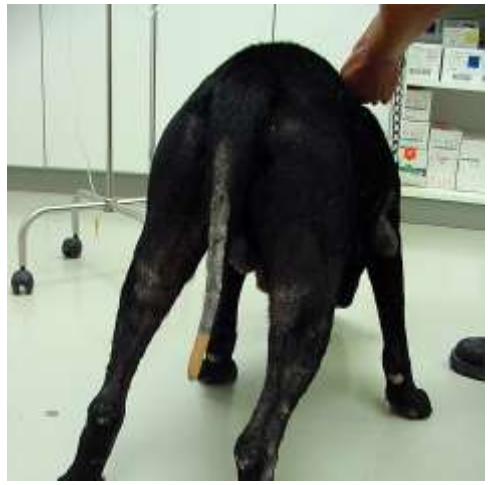

**Figure 2.13** "Rat tail" lesion in a dog infected with *L. infantum*. *(Photo George Popa)*

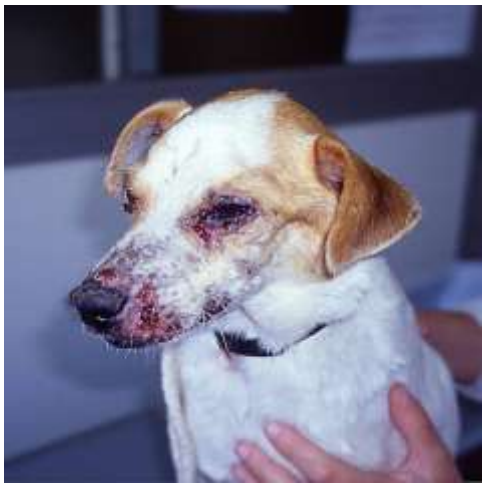

**Figure 2.12** Cutaneous ulcers and erosions in a dog infected with *L. infantum*. *(Photo George Popa)*

**Figure 2.14** Nasal hyperkeratosis in a dog infected with *L. infantum*. *(Photo George Popa)*

The ocular lesions include: conjunctivitis, blepharitis and anterior uveitis. In some cases, because of the retention of lacrimal secretion due to adjacent inflammation, dogs exhibit keratoconjunctivitis sicca.

Other lesions associated with the altered immune response and CIC include mononuclear myositis, neutrophilic vasculitis, hemorrhages in internal organs, granulomatous rhinitis, epistaxis and anemia.

**Figure 2.15** Onychogryphosis in a dog infected with *L. infantum*. *(Photo George Popa)*

**Figure 2.16** Severe cachexia in a dog infected with *L. infantum*. *(Photo George Popa)*

Usually the cutaneous lesions are generalized. Several types are mentioned in the literature: exfoliative dermatitis with alopecia, ulcerative dermatitis, nodular dermatitis, mucocutaneous proliferative dermatitis, or popular dermatitis. The histopathologic lesions in the skin are pyogranulomatous with hyperkeratosis.

Gross lesions include: generalized lymphadenopathy, splenomegaly and hepatomegaly, granulomatous nodules in various internal organs.

**Diagnosis.** Clinical signs are not enough for a positive diagnosis of canine leishmaniosis. Basically, for a certain diagnosis, the presence of the parasite must be demonstrated directly (PCR, cytology, histology, culture) or indirectly (antibody detection).

Direct evidence of the amastigotes should based on microscopic observation of the parasitic stages in biopsy from lymph nodes, spleen, bone marrow or skin. Although this method is 100% specific, its sensitivity is maximum 80%, depending on the experience of the examiner and examination effort. However, direct identification of *Leishmania* amastigotes is not always achievable, even in dogs with clinical infection. Amastigotes can be observed also in tissue sections by histology or immunohistochemistry. Although not commonly used as routine diagnostic methods, cultivation (Novy-MacNeal-Nicolle medium) and experimental infection (hamsters) are also possible.

Probably the most sensitive methods for the detection of *Leishmania* infection are molecular biology techniques. PCR has been used routinely for the detection of *Leishmania* DNA in various tissues (bone marrow, lymph nodes, spleen, skin), blood (whole blood or buffy coat) or body fluids (urine).

The easiest diagnosis methods however refer to the detection of anti-*Leishmania* antibodies in the serum of dogs. Various methods have been developed, some of the using whole parasite extracts (more sensitive, less specific), some others recombinant protein antigens (more specific, less sensitive). Whole parasite extract can cross-react with other kinetoplastids. Usually, it is considered that high antibody titers in dogs with compatible symptoms are indication of clinical leishmaniosis. If clinical signs are present but antibody titers are low, additional methods are recommended (cytology, histology, PCR).

**Treatment.** Treatment of leishmaniosis does not necessarily eliminate the parasite form the organism and this is one of the causes of the frequent clinical relapses. There are several drugs used for the treatment of canine leishmaniosis. The most commonly used are pentavalent antimonials. Their mechanism of action is by inhibiting the enzymes responsible for oxidation of fatty acids and glycolysis. The most commonly used antimonial is meglumine antimoniate which is given by subcutaneous injection, for 4-8 weeks, daily, at 75-100 mg/kg body weight. Besides the side effects (local reactions, nephrotoxicity), there are several reports of resistant *Leishmania* strains in Europe.

Another compound used in the treatment of canine leishmaniosis is allopurinol. This drug was originally developed for the treatment of gout (hyperuricemia) in human patients. Its efficacy against *Leishmania* is explained by its capacity of being metabolized by the parasite into an analogue of inosine (a common nucleoside in the structure of tRNA). This analogue is incorporated into the structure of the *Leishmania* RNA, causing an altered translation and inhibiting parasite multiplication. Unlike the previous drug, allopurinol is given orally (10 mg/kg body weight), twice a day, for month or even years. Despite the fact that the drug has little adverse effects, the discontinuation of treatment often results in clinical relapses. Hence, dogs may require life-long treatment. One of the most common side effects is hyperxanthinuria, resulting in urolithiasis. However, the most effective therapeutic protocol is the combination of meglumine antimoniate with allopurinol.

Several other drugs have been used for the treatment of canine leishmaniosis: miltefosine (2 mg/kg body weight, orally, once per day, for four weeks), amphotericin B (causes renal toxicity).

Except specific treatment, a symptomatic therapy must be conducted.

**Control.** None of the several control measures used is fully effective. The euthanasia of infected dogs is the most controversial and its efficacy is doubtful because of the persistence of reservoirs in wildlife. Environmental control of sandflies (spraying, destruction of breeding habitats) has been also shown to be little effective. Moreover, there is no prophylactic drug available. However, owners can reduce the sandfly bites on their dogs by avoiding outdoor access during maximum activity of the vectors (overnight, warm season) or by using prophylactic insecticides (spot-on, collars, sprays).

Currently, there is a commercial vaccine for dogs, but its availability is variable from country to country. This commercial vaccine contains a glycoprotein from *L. donovani* (GP63).

#### 2.3.1.4 Feline leishmaniosis

Unlike canine leishmanioses, the disease in cats is reported only sporadically. As most aspects have been already presented in the chapter on canine leishmaniosis, only specific aspects will be given here.

**Historical notes.** The first case of feline leishmaniosis was described in Algeria in 1912, in a kitten housed together with an infected dog and an infected child.

**Etiology.** Several species have been reported from cats: *L. infantum* (Europe, South America), *L. mexicana* (USA), *L. venezuelensis*, *L. braziliensis* and *L. amazonensis* (South America).

**Epidemiology.** The disease in cats is sporadic, and all cases were reported from areas endemic to canine leishmaniosis: Mediterranean Europe, North Africa, Middle East, and the Americas.

**Clinical signs.** In immunologically competent cats, the predominant symptoms of *L. infantum* infection are cutaneous (ulcers, nodular dermatitis, scaling, alopecia) with the lesions located mainly on the head. The visceral involvement seems to be limited to cats coinfected with FIV (Feline Immunodeficiency Virus) or FeLV (Feline Leukemia Virus). American species produce also cutaneous signs, mostly located on the ear pinna.

**Treatment.** Despite the lack of extensive clinical studies, the few reported treated cases suggest that the same protocols which is used in dog can be successfully applied also in cats.

### 2.3.2 Trichomonads

**Introduction.** The trichomonads group several genera of medical and veterinary importance (**table 2.7**).

The morphological differences between species (and even genera) are not very easy to discern, mainly if using regular direct examination. They are unicellular organisms, lacking mitochondria with a typical anaerobic metabolism. They include mostly symbiotic species, but also few free-living organisms.

**General morphology.** Although there are slight morphological differences between different genera and species, generally, all trichomonads have a group of three (*Tritrichomonas*), four (*Tetratrichomonas*, *Trichomonas*) or five (*Pentatrichomonas*) anterior free flagella, an additional recurrent flagellum which delimitates an undulating membrane. The axostyle, a rigid rod-like structure runs medially through the cell. The axostyle is usually longer than the cell. Due to these structures, trichomonads show a very characteristic movement, making them easily recognizable in fresh preparations

**Ecology and transmission.** The vast majority of the Trichomonads are parasitic, mutualistic or commensals.

**Table 2.7** Most important species of trichomonads

| Genus | Species | Hosts | Location |
|---|---|---|---|
| *Trichomonas* | *T. gallinae* | birds | anterior digestive system |
| | *T. vaginalis* | humans | genital |
| *Tritrichomonas* | *T. foetus* | cattle | genital |
| | | cats | large intestine |
| | *T. suis* (=*T. foetus*) | pigs | digestive, nasal |
| | *T. enteritis* | cattle | colon |
| | *T. caviae* | cavies | cecum |
| | *T. muris* | rodents | large intestine |
| | *T. minut* | rodents | large intestine |
| | *T. wenyoni* | rodents | large intestine |
| | *T. eberthi* | birds | cecum |
| *Tetratrichomonas* | *T. canistomae* | dogs | mouth |
| | *T. felistomae* | cats | mouth |
| | *T. ovis* | sheep | cecum, rumen |
| | *T. buttreyi* | ungulates | large intestine |
| | *T. pavlovi* | cattle | cecum |
| | *T. microti* | rodents | large intestine |
| | *T. gallinarum* | birds, humans | cecum |
| | *T. anatis* | ducks | intestine |
| | *T. anseris* | geese | cecum |
| *Pentatrichomonas* | *P. hominis* | primates, dogs, cats, rodents | large intestine |
| *Trichomitus* | *T. rotunda* | pigs | large intestine |
| *Chilomastix* | *C. gallinarum* | birds | cecum |

They inhabit the digestive or reproductive tracts of vertebrates or invertebrates. Few species are free-living. The life-cycle is always homoxenous and generally, they are considered to be host-specific.

Feeding occurs by phagocytosis of fluids, leukocytes or bacteria from the in-host habitats. Reproduction is by longitudinal binary fission, with the formation of large numbers of trophozoites.

As there are no cystic stages known, transmission between hosts is always by direct contact.

**Medical importance.** Certain trichomonads are important human and veterinary pathogens. Most of the species are located in the digestive tract (mouth, gut), others in the genital system. Most veterinary important species are responsible for digestive symptoms in animals, usually young ones. One single species (*Tritrichomonas foetus* in cattle) is producing genital infection. In humans, several species are known, some of them producing digestive signs, some others genital symptoms.

#### 2.3.2.1 Genital trichomonosis in cattle

**Introduction.** Genital trichomonosis is a venereal disease of cattle, with long-term impact on fertility in females and asymptomatic carrier state in bulls, with worldwide distribution, caused by *Tritrichomonas foetus*. The disease is mainly important from its economic perspective.

**Historical notes.** The disease was reported and described for the first time in 1888 by Kunstler in France and later, in 1890, by Mazzanti in Italy as isolated cases of infertility. Later reports from 1924-1929 are from Germany, where enzootic outbreaks were described. The first cases of bovine genital trichomonosis in the United States were found in 1932.

**Etiology.** The causative agent, *Tritrichomonas foetus* has been subject to several molecular taxonomy approaches, and its synonymy with *T. suis* has long been debated. Most recent taxonomical papers list them as synonyms.

Three serotypes have been found using seroagglutination. The "belfast" strain (common in Europe, Africa and USA), the "brisbane" strain (in Australia) and the "manley" strain (the most rare one).

**Morphology.** *Tritrichomonas foetus* (**figure 2.17**) is a spindle to pear shaped unicellular organism, 10-25 x 3-15 µm, with four flagella. Three anterior flagella are free and the fourth, called the recurrent flagellum, runs backward and delimitates an undulating membrane, with 3-5 waves. The axostyle is thick and it protrudes in the posterior part of the cell.

**Life cycle.** The typical habitat for *T. foetus* is at the level of genital mucosae of cattle, in both sexes. In bulls, they are found in the preputial cavity, penis mucosa, distal urethra, epididymis, testes and seminal vesicles. In cows, they infect the vaginal mucosa, the uterus and the fetus. Transmission between hosts is by sexual contact between infected and non infected animals. Transmission by artificial insemination has also been reported.

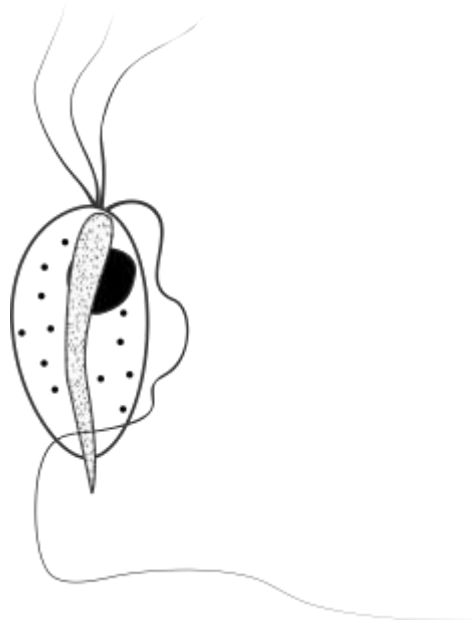

**Figure 2.17** *Tritrichomonas foetus*.

Passive transmission has also been reported. This means that, if a non-infected bull had recent coitus with an infected female, he is able to transmit infect another cow in a subsequent intercourse.

The gynecological examination of cows can also induce passive transmission, if instruments are not sterilized after being used before in an infected cow.

**Epidemiology.** The disease is distributed worldwide. Bovines are the only important infection reservoirs, with asymptomatic bulls being responsible for infection of cows mainly by natural mating. Nonetheless, experimental

infection has been established in a great variety of mammalian hosts (rabbits, laboratory rodents, dogs, small ruminants, pigs), but their epidemiological importance is practically zero. Since the taxonomic debate on the identity of *T. foetus* with *T. suis,* it is still not certain if cross infection could occur, and how. Moreover, the identification of *T. foetus* from cats with diarrhea make this puzzle even more complicated.

Both breed and age susceptibility has been reported in bulls. Bulls of some breeds (Bragus, Simmental, Charolais, and Angus) seem to be more predisposed to infection than others (Braford). The origin of the breed is likely to be the cause of different susceptibility. It has been estimated that *Bos taurus* bulls are more prone to infection than *B. indicus*. The farm management system is also important. The prevalence in bulls tends to be higher in larger farms. Similarly, a higher bull-cow ratio is directly correlated with an increased prevalence of the disease.

The resistance of *T. foetus* outside its host is very limited. Hence, contamination of new hosts is only by direct contact. However, the organism can remain infective in frozen semen.

**Pathogenesis.** As most venereal diseases of domestic animals, bovine trichomonosis is asymptomatic in males and symptomatic in females.

In bulls, the parasites live on the mucosal surface and do no invade the epithelial tissue. One interesting observation following an experimental infection trial, was that bulls older than 3 years are more prone to develop chronic infection and to become long-time, asymptomatic carriers. This is associated with a morphologic trait of epithelial crypts of the penis and prepuce, which become deeper as bulls are aging. Deeper crypts offer a better microaerophilic environment. Conversely, young bulls are only transient carriers.

In cows, immediately after the infection, the parasite produces a mild infection of the vaginal mucosa, with vaginitis. During estrus, the flagellates are able to enter the uterus through the cervix. Within 7-14 days, *T. foetus* is able to colonize the entire female reproductive tract. The endometritis is responsible for the persistence of the corpus luteum which induces pyometra.

The mechanism of fetal death is not fully understood, but cytotoxic and hemolytic effects have been incriminated. The adhesion of *T. foetus* to mammalian cells is facilitated by a surface adhesin.

After the experimental infection in heifers, the clearance of the parasite without medication is between 3 and 28 months.

**Immunology.** There is no evidence showing that after infection, cows will develop long-term or life-long immunity. However, a maximum 15 month convalescent immunity has been reported. *Tritrichomonas foetus* produces extracellular proteases which are able to digest immunoglobulins, impairing even the local immunity.

The antigenic structure of *T. foetus* includes 55-60 proteins

The surface antigens of *T. foetus* are able to activate the alternative pathway of bovine complement. The bovine complement system is able to kill *T. foetus* in the presence of specific antibodies. On the other hand, neutrophils are not efficient in killing *T. foetus*, even in the presence of specific antibodies.

Studies have shown that there is no cross-immunity between the three serotypes of *T. foetus*.

**Clinical signs.** The infection of bulls is usually asymptomatic.

In females, a mild vaginitis is the first sign of infection. If the infective mating results in gestation, the usual outcome is abortion between days 50 and 70. About one third of the abortions caused by trichomonosis occur in the first trimester. The death of the embryo or fetus during this early stage is usually followed by a longer interestrous interval. As the abortion is very early, often it goes unobserved by farm owners. Usually, all fetal membranes are passed after abortion and the cows recover quickly. Nevertheless, fetal membrane retention leads to chronic endometritis and possibly permanent sterility. Most cows are however able to bare a normal gestation and deliver normal calves. As a result of abortion about 5% of the cows develop pyometra. Detection of pyometra is usually late, and by that time the uterine mucosa is severely damaged.

Persistent infection with *T. foetus* in cows results in temporary or permanent infertility, irregular estrus, persistent abortions etc. The economical losses caused by bovine trichomonosis are significant, with losses estimated to 35% of the profit in infected farms.

**Pathology.** Following abortion, the lesions are detectable in the placenta and in the fetus. In late term abortions, the placenta shows focal or diffuse invasion of the chorionic stroma by the parasitic flagellates. The typical morphology of *T. foetus* is easily visible in histological sections stained with Bodian's silver technique but is imperceptible at routine staining methods. Additionally, monocytic infiltrate is present in the placental layers. The aborted fetuses have pyogranulomatous bronchopneumonia, interstitial pneumonia, hepatic and intestinal necrosis with the presence of the parasites in the air ducts but also in the esophagus, abomasum and intestine.

**Diagnosis.** The clinical signs and features of the disease are neither characteristic nor pathognomonic to trichomonosis. Similar reproductive problems are caused by bacterial agents (e.g. *Campylobacter foetus*, *Leptospira* spp., *Ureaplasma diversum*) or by nutritional conditions. Hence, the certain diagnosis must be based on the identification of the parasitic agent by various laboratory methods.

The following section is based on the recommendations by OIE (The World Organization for Animal Health). The samples which are used for the detection of *T. foetus* are: vaginal mucus, vaginal washing or scrapings, preputial washing or scraping, uterine washing, pyometra discharge, placental fluid, stomach content of the aborted fetus.

A correct technique and timing for the collection of samples are essential. It is important mainly to avoid fecal contamination, or if so, the examiner should be very experienced in order to differentiate intestinal flagellates from *T. foetus*. Contamination can be avoided by mechanically removing the dirty hair around the preputial orifice or around the vulva. Chemical disinfectants are nor recommended, as may inactivate *T. foetus* and reduce diagnosis sensitivity. Collection of preputial samples from bulls can be done using an artificial insemination pipette, a brush, by preputial washing or by washing the artificial vagina after seminal material collection. Samples from cows are collected by vaginal washing or by scraping the cervix with a brush or pipette.

Samples must be examined as fast as possible. If they cannot be sent to the laboratory in maximum 24 hours, they should be included in a transport medium (thioglycollate broth media with antibiotics) and kept at temperatures between 5 and 38°C, away from direct sunlight.

Samples can be examined immediately under the light microscope, or if the sample is poor in trichomonads, after enrichment on cultivation media (Diamond's trichomonad medium or other commercial media). *Tritrichomonas foetus* should be motile, with typical movements and morphology (pear-shape, presence of free flagella and undulating membrane, presence of axostyle).

More recently, new, molecular techniques have been developed for the detection of *T. foetus*. A PCR-based assay which is used has certain advantages: increased sensitivity, possibility to detect non-viable pathogens. Additionally, immunohistochemistry on tissues (placenta, fetal lungs) has been also used.

**Treatment.** For the treatment of bulls, various drugs have been used in the past: dimetridazole, ipronidazole and metronidazole. As all are nitroimidazoles, their use in livestock is banned in most countries. Hence, currently there are no therapeutic options for treating bovine trichomonosis. Treatment of bulls can be performed only under certain conditions, mainly in the case of expensive, valuable breeding animals.

**Control.** Prevention and control by herd management are the only reliable methods for reducing the economic impact of bovine genital trichomonosis. General measures include: control of the animal movement, avoid grazing on common pastures where bulls from other herds may have access, purchase only virgin bulls and heifers for restocking, purchase the animals from *T. foetus*-free farms, ask for the preputial washing result for any bull purchased, graze separately cows and bulls, keep the average age of bulls as young as possible (maximum 3 years), use of artificial insemination with tested semen.

Commercial vaccines for cows are available in certain countries. They are killed vaccines and initial vaccination must be given twice, subcutaneously, at 2-4 weeks apart. In the following years, all cows must be revaccinated, 4 weeks prior to the beginning of the breeding season. Although the vaccine does not

prevent the infection, it significantly decreases the economic impact of the disease by reducing the rate of abortions and the duration and severity of the disease.

### 2.3.2.2 Buccal trichomonosis in dogs and cats

**Introduction.** Buccal trichomonosis in carnivores are poorly known conditions, caused by flagellates in genus *Tetratrichomonas* and characterized by gingivitis usually in immunodeficient patients.

**Historical notes.** Both conditions (in cats and dogs) were described by Hegner and Ratcliffe in 1927 in United States.

**Etiology.** The species responsible for buccal trichomonosis in cats is *Tetratrichomonas felistomae* and in dogs it is *T. canistomae*. As the original description is very simple, and records of these parasites are scarce, the taxonomic status of *T. felistomae* and *T. canistomae* is uncertain. Moreover, recent studies revealed the presence of flagellates with a different morphology, puzzling even more the situation.

**Morphology.** Species in genus *Tetratrichomonas* have 4 free flagella and one more trailing free flagellum, running backwards. Trophozoites of *T. canistomae* (**figure 2.18**) have four anterior flagella, that rise in pairs from a large blepharoplast at the anterior end of the body. The recurrent flagellum starts from the blepharoplast, runs backwards along the edge of the undulating membrane and ends freely at the posterior end. The axostyle extends far beyond the posterior end of the cell. The size is 7-12 x 3-4 µm.

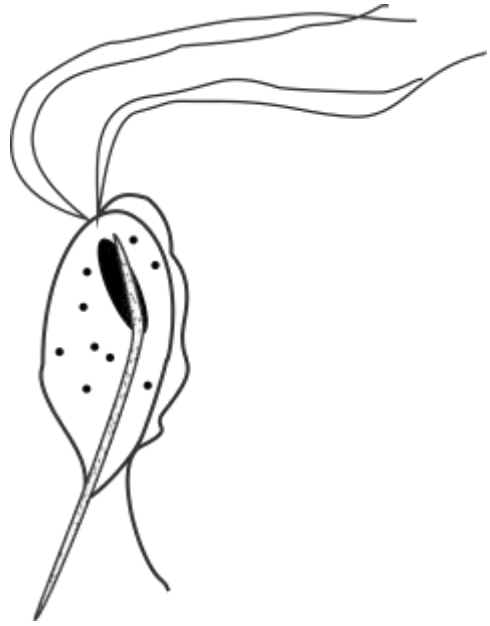

**Figure 2.18** *Tetratrichomonas canistomae*.

Trophozoites of *T. felistomae* (**figure 2.19**) have a similar morphology. Their size is 6-11 x 3-4 µm. They have a well visible axostyle and the undulating membrane has 3 waves.

**Life cycle.** Not too many aspects are known about the biology of these parasites. They are located in the mouth of dogs and cats, along the gums or associated with dental calculus. Transmission between hosts is probably by direct contact.

**Epidemiology.** In cats, the parasite have been reported only in USA, Italy and Germany. In dogs, the parasite is known from United States and various European

countries. No data about resistance in the environment are available.

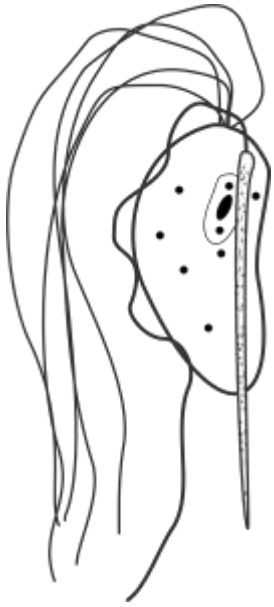

**Figure 2.19** *Tetratrichomonas felistomae*.

**Pathogenesis.** In a recent study, the trichomonads in cats were found only in individuals infected with immunosuppressive viruses: feline immunodeficiency virus, feline leukemia virus and feline infectious peritonitis virus.

**Immunology**. Unknown

**Clinical signs.** The presence of the parasites in cats was associated with gingivitis, while in dogs with dental calculus. *Tetratrichomonas felistomae* was never isolated from cats without lesions, while it seems that in dogs, *T. canistomae* can be found also in healthy patients. Hence, some authors consider it to be mostly non-pathogenic.

**Pathology.** Gingivitis in cats, dental calculus in dogs.

**Diagnosis.** Fresh wet mounts from dogs and cat with buccal lesions might reveal the presence of flagellates. In dogs, scraping the dental calculi from the lower jaw and decayed teeth yielded better results.

**Treatment.** Unknown.

**Control.** Unknown, but a proper oral hygiene with removal of dental calculi and treatment of buccal inflammations might be useful.

### 2.3.2.3 Anterior digestive trichomonosis in birds

**Introduction.** It is a worldwide distributed parasitic disease of pigeons, doves, galliformes and birds of prey, with possible severe buccal lesions mainly in young birds. The disease in pigeons is also known as canker. In poultry, the name of the disease is "roup" and in birds of prey "frounce".

**Historical notes.** The first description of the disease was in pigeons, together the etiological agent, by Rivolta in 1878 in Italy.

**Etiology.** Only one species is involved, namely *Trichomonas gallinae*. This species must not be confused with *Tetratrichomonas gallinarum*, which infects the large intestines of birds. Different strains are know, some of them more pathogenic than the others and some considered avirulent, part of the normal buccal fauna. One of the most virulent strains is Jones' Barn, which is

able to kill even adult pigeons at 8 days after the infection.

**Morphology.** Trophozoites (**figure 2.20**) are piriform to rounded, 6-19 x 2-9 µm, Four free flagella extend forward, and the fifth runs backward along the margin of the undulating membrane. Unlike in genus *Tetratrichomonas*, there is no free trailing flagellum.

**Life cycle.** The parasites inhabit the mucosal surface of the anterior digestive system (mouth, pharynx, esophagus, crop) and head sinuses in pigeons and doves. Although it seems that only Columbiformes are the natural host for *T. gallinae*, the infection was reported in many other groups of domestic or wild birds. Among domestic species, chicken and turkeys are susceptible to infections. The infection was reported in captive finches and canaries as well as in quails. Among wild avian species, the most important clinical disease was described in raptors. Moreover, many experimental trials succeeded transmitting *T. gallinae* to many other host species, showing the parasites lacks specificity.

Mammals are not susceptible to natural infection. They multiply rapidly by binary fission. Only a single stage is know, the trophozoite.

Transmission no new host is realized by direct contact between infected and non-infected birds. In pigeons, the adult birds are infecting their nestlings during the regurgitation and feeding. Birds of prey get the infection when feeding on other infected birds. Other birds get the infection by drinking contaminated water.

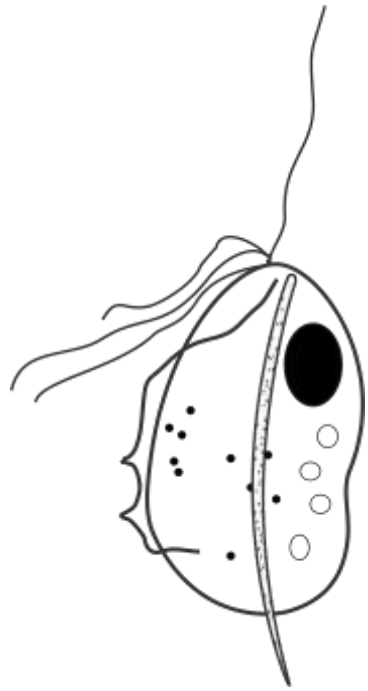

**Figure 2.20** *Trichomonas gallinae.*

**Epidemiology.** The parasite is distributed globally, with virtually all domestic and wild pigeon populations positive for the infection. In domestic fowl (chicken, turkey) the infection is rather sporadic. In birds of prey, the infection has been reported with variable prevalence in several countries where studies were performed. Nevertheless, the source if infection for the other birds are always pigeons or doves.

Young pigeons are particularly susceptible for the diseases, while adults are usually asymptomatic carriers. Certain breeds (i.e. tumbler) were shown to be more sensitive to the infection. The severity and clinical outcome of canker is also influenced by certain environmental factors: poor ventilation, overcrowding, unbalanced diet, excessive humidity.

Interestingly, the prevalence of infection is usually higher in adults than in young. The outbreaks and the prevalence are positively correlated with humid seasons. Stress is also a factor contributing to clinical outbreaks.

Although the resistance of *T. gallinae* in the environment is very limited, it survives enough time in drinking water to facilitate transmission in birds which do not feed their newly hatched offspring, like chicken or turkey.

**Pathogenesis.** The chronic infection is usually accompanied by a low number of parasites which are not able to produce the disease. When certain factors are met (see above), the parasite multiplies very fast and invades the surface of the upper digestive system, causing severe inflammation followed often by death in the acute form. In severe infection, *T. gallinae* colonizes also the sinuses and the liver.

**Immunology.** Despite the lack of targeted studies on the immune response of birds to the infection with *T. gallinae*, it seems that if the original exposure is not followed by death, sufficient immunity is developed to protect the adults against clinical disease. Non-exposed adults introduced to infected colonies develop a severe form of trichomonosis and may even die. It is not fully understood if there is cross acquired immunity against the different strains of the parasite.

**Clinical signs.** Infection may vary from asymptomatic cases to severe outbreaks with over 90% mortality, mainly in young birds. In adults mortality is rare, but extremely virulent strains can produce mortality rates up to 50%.

In the acute cases, the duration of the disease is 10-14 days. The typical clinical picture includes: partial or total loss of appetite, weight loss, ruffled aspect and listlessness. Some pigeons which still present appetite have difficulties in swallowing. Other birds may also show signs of respiratory distress. When examining the mouth, small, whitish or yellowish adherent masses are seen on the surface of the buccal and pharyngeal mucosa (**figure 2.21**). Sometimes, these caseous nodules join and become large enough to completely obstruct the pharynx (**figure 2.22**). These small nodules disappear spontaneously within few days and the bird becomes healthy. In other cases, the infection results in death. Interestingly, the severity of nodular lesions is not correlated with the severity of clinical form.

The most virulent strains produce the first oral lesions after 1 week, as yellowish areas on the mucosa of mouth and the tissue bordering the nasal cleft or palatal flaps. When nodules become big enough to obstruct the food passage, the clinical course becomes rapid, with extreme and fast weight loss and fluid accumulation in the crop which virtually can drown the bird. Death occurs in about 10-12 days from the first clinical sign (around 20 days from infection). Sometimes also diarrhea is present.

The infection with less virulent strains can be asymptomatic or can cause maximum excessive salivation and mild stomatitis.

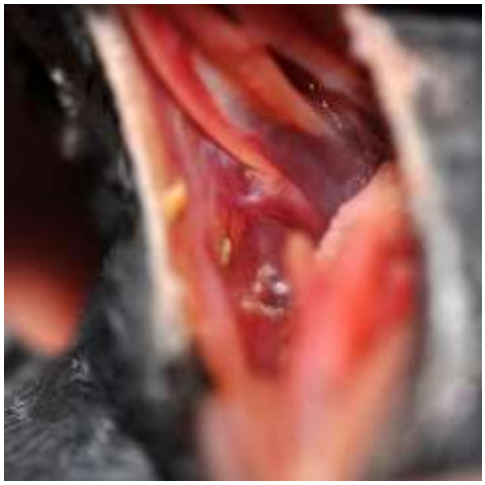

**Figure 2.21** Small, disseminated caseous nodule on the surface of buccal mucosa in a pigeon naturally infected with *T. gallinae*. *(Photo Andrei D. Mihalca)*

In birds of prey and gallinaceous birds, buccal trichomonosis is a severe condition and the clinical expression is somehow similar with the one described in pigeons. Additionally, these hosts show moderate or severe dyspnea along with nasal and oral exudation. In exotic birds (i.e. budgerigars, finches) clinical sings include also wasting, vomiting and diarrhea.

**Pathology.** The lesions are usually limited to the anterior digestive system, liver and sometimes to the respiratory system. The less virulent strains usually produce only buccal and pharyngeal lesions while more virulent strains are able to cause damage to various internal organs. Yellowish to grayish necrotic lesions are found on the mucosa lining the mouth, pharynx, esophagus, crop and proventriculus. Sometimes, the crop and the proventriculus contain a greenish or whitish fluid. The crop may be covered with a diphteric membrane which lines the digestive mucosa down to the glandular stomach.

Histology shows congestion with mononuclear inflammatory infiltration in the lamina propria of the larynx and pharynx, surrounded by area of necrosis.

The liver may show signs of congestion or areas of yellow necrosis, with peritoneal adhesions. Microscopically these correspond to hyperemia, hepatocellular necrosis, hyperplasia of the bile duct, all with heterophils and mononuclear infiltration and presence of multinucleated giant cells.

The heart may also be affected, with caseous material deposited on the apex. Similar lesions are occasionally reported in all organs found in direct contact with the liver: small intestine, spleen, pancreas, air sacs, lungs, kidneys.

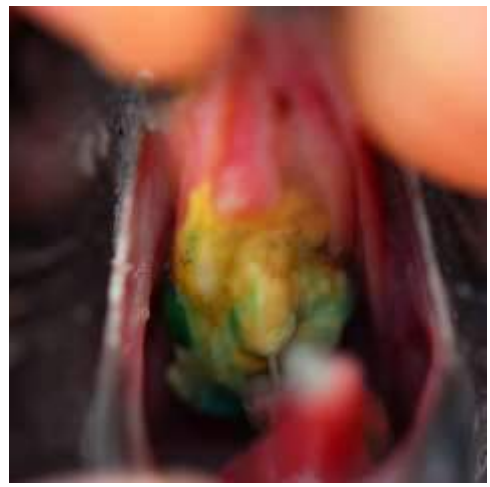

**Figure 2.22** Large caseous nodule in the palatal flaps area in a pigeon naturally infected with *T. gallinae*. *(Photo Andrei D. Mihalca)*

Microscopic lesions in the respiratory organs of pigeons infected with virulent strains are predominantly congestive and inflammatory. Severe congestion might be present in the lungs, with extensive infiltration of heterophils and mononuclear cells in the mucosa of the trachea and hyperplasia of tracheal mucous cells. If kidneys are involved, the microscopic lesions consists of mild tubular necrosis with mononuclear infiltrate.

**Diagnosis.** The diagnosis might be done in healthy birds to estimate the asymptomatic carrier status. This approach is essential in pigeon housings, to detect the infection in adults, before the reproduction season. The etiological diagnosis is of course important also in clinically ill birds.

The most reliable diagnosis is by demonstrating the presence of trichomonads by microscopic examination. Wet smears should be prepared using cotton-tipped swabs soaked in warm sterile physiological saline (**figure 2.23**). The content must be immediately examined under the microscope, using medium or large magnification in order to observe the typical morphology. When the intensity of the infection is low, the small number of parasites might go undetected. In these cases, an initial enrichment by cultivation is helpful.

A variety of cultivation can be used, but the most recommended is Diamond's medium. For more sensitive diagnosis, various PCR assays have been developed, but their use as routine examinations might be costly.

**Figure 2.23** Collection of the pharyngeal swab for the diagnosis of buccal trichomonosis in pigeon. *(Photo Andrei D. Mihalca)*

**Treatment.** Several drugs are available for the treatment of trichomonosis in pigeons: metronidazole (60 mg/kg body weight); dimetridazole (50 mg/kg body weight); ronidazole (30 mg/pigeon), all orally. As resistance to these drugs was reported in various strains of *T. gallinae*, commercial products containing combination of these (i.e. metronidazole + ronidazole) have been developed. All these products are soluble, and are given in drinking water for 5-6 consecutive days. The recommended concentration is 0.05%.

**Control.** General measures like a good hygiene, avoiding overcrowding and stress should reduce the impact of the disease. Treatment of the adult pigeons before the breeding season will certainly reduce the parasite load and mortality in squabs to come.

### 2.3.2.4 Intestinal trichomonosis in birds

**Introduction.** Is a worldwide distributed parasitic disease of various birds species, with digestive clinical signs mainly in young birds.

**Historical notes.** *Tetratrichomonas gallinarum* was described in 1911 from chickens.

**Etiology.** Several species have to be considered as etiologic agents of intestinal trichomonosis in domestic birds. However, their taxonomic status (or even validity) and pathogenic potential have been questioned by recent molecular biology works.

Although *Tetratrichomonas gallinarum* is still listed by most veterinary parasitology textbooks, its pathogenic potential is uncertain, and recent genetical insights have shown that this species might be actually a complex of cryptic species with more implications in human health than in birds. Until more clarification is available, we will follow the classical concept and consider all these species valid. *Tetratrichomonas gallinae* was reported from various domestic and wild species of birds (chicken, turkey, guinea fowl, quail, pheasant, partridge, ducks etc.) and has been recently incriminated in the etiology of severe lung disorders in humans.

*Tetratrichomonas anatis* is the cause of small and large intestine trichomonosis in ducks. *Tetratrichomonas anseris* is responsible for the disease in geese. *Tritrichomonas eberthi* and *Chilomastix gallinarum* have been reported as causing caecal infection in chicken and turkeys.

**Morphology.** All species have the typical morphology of trichomonads, but small differences in size, shape, number of flagella or internal structure allows more specific identification.

*Tetratrichomonas gallinarum* (**figure 2.24**) has a piriform body, 7-15 x 3-9 µm. Like all member of its genus, it has four free anterior flagella, and a recurrent flagellum which runs along the edge of the undulating membrane and ending with a free part. The axostyle is long, slender and pointed.

*Tetratrichomonas anatis* has an elongated body, 13-27 x 8-18 µm, with four anterior flagella, a long undulating membrane a the recurrent flagellum with a free termination. *Tetratrichomonas anseris* has the morphology is similar to *T. anatis* but it is slightly smaller.

*Tritrichomonas eberthi* (**figure 2.25**) has an elongated body, 8-14 x 4-7 µm, three free anterior flagella and a well-developed undulating membrane, lined by the recurrent flagellum which ends freely at the posterior end. The axostyle is relatively thick and extends the posterior end of the body.

*Chilomastix gallinarum* (**figure 2.26**) is piriform, 11-20 x 5-12 µm, three anterior flagella, and an additional spiraling flagellum. The undulating membrane is relatively narrow. In the case of this species, also cysts are known to be formed (7-9 x 4-6 µm).

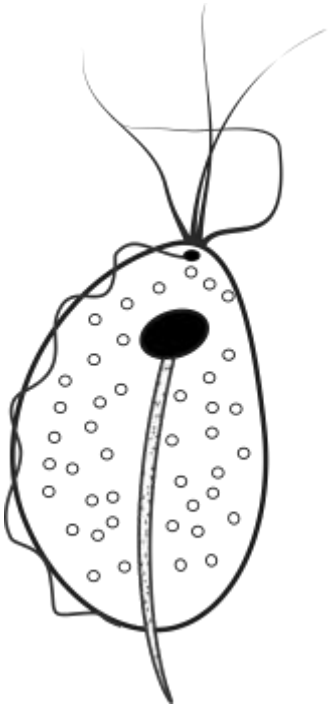

**Figure 2.24** *Tetratrichomonas gallinarum.*

**Life cycle.** All the species inhabit the mucosa of the large intestine or small intestine where they multiply by binary fission. Transmission from a host to another is via fecal-oral route, by fresh feces. In the case of *C. gallinarum*, transmission can be done through cysts.

**Epidemiology.** All the species have global distribution. The resistance in the environment is limited. Usually, young birds seem to be more affected by the clinical diseases.

**Pathogenesis.** There is serious debate on the pathogenicity of certain species. Generally, they are considered to be non-pathogenic. However, most of them have been isolated from birds with clinical signs or lesions of enteritis.

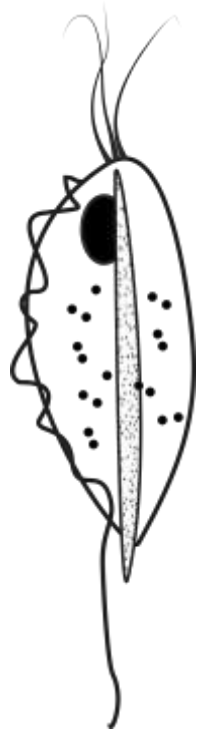

**Figure 2.25** *Tritrichomonas eberthi.*

**Figure 2.26** *Chilomastix gallinarum.*

It is a question if they are the primary agents, or they just over multiply on lesions primarily induced by bacteria or viruses.

**Immunology.** Unknown.

**Clinical signs.** With the same mentions as stated above, clinical signs associated with the presence of intestinal trichomonads vary from asymptomatic infections (in the vast majority of cases) to mild, moderate or severe diarrhea, or even sudden death (in ducks).

**Pathology.** The lesions from birds infected with intestinal trichomonads are usually fibrinous or necrotic enteritis, mainly in the cecum (typhlitis). Sometimes, necrotic lesions on the liver have been described. In ducks, extraintestinal lesions were also described: mucopurulent sinusitis, catarrhal rhinitis and tracheitis.

**Diagnosis.** In vivo, the flagellated protozoans can be identified under the microscope in wet mounts from cloacal swabs. Their presence must be interpreted with caution, as usually the underlying cause of the symptoms are more likely to be bacteria, viruses or even other protozoans (i.e. *Histomonas meleagridis*). In dead animals, if they are fresh, a wet mount from the cecal lesions may reveal the presence of mobile organisms with the typical trichomonads morphology. Otherwise, cytology or histopathology are other options.

**Treatment.** Usually the infections should not be treated, as nitroimidazoles are forbidden in animals intended for human consumption. The underlying conditions should be rather identified and eliminated.

**Control.** Hygiene measures are essential, as contamination is usually via infected water.

### 2.3.2.5 Intestinal trichomonosis in mammals

**Introduction.** Intestinal trichomonads of domestic mammals include a great variety of species, usually non-pathogenic. If disease occurs, it is a result of multiple pathogen infections.

**Etiology.** For a list of species and hosts please refer to **Table 2.7**.

**Morphology.** *Tritrichomonas enteritis* has a small body, 6-12 x 5-6 µm, with three free anterior flagella, and a medium-sized undulating membrane.

*Tetratrichomonas buttreyi* is ovoid or ellipsoidal, 4-7 x 2-5 µm, with four free anterior flagella, an undulating membrane with 3-5 waves and a recurrent flagellum. The axostyle is rather narrow.

*Tetratrichomonas ovis* has a piriform body, 6-9 x 4-8 µm, with typical number of flagella for the genus.

*Tetratrichomonas pavlovi* has a pear-shaped body, 11-12 x 6-7 µm, an undulating membrane with 2-4 waves and otherwise a similar morphology with *T. buttreyi*.

*Pentatrichomonas hominis* is piriform, 5-14 x 7-10 µm. There are five free flagella, four of which are grouped together and oriented forward, and the fifth is separate

and somehow recurrent. The sixth flagellum, the true-recurrent one, runs along the undulating membrane ending with a free portion.

*Trichomitus rotunda* has wide piriform or ovoid shape, 7-11 x 5-7 µm

**Life cycle.** Direct. No cysts stages are known.

**Pathogenesis. Clinical signs. Pathology.** The pathogenic role of intestinal trichomonads from mammals has not been clearly defined. All the species mentioned above have been isolated from clinical cases with diarrhea and/or enteritis.

**Diagnosis. Treatment. Control.** See comments from Chapter 2.3.2.4.

### 2.3.2.6 Histomonosis of poultry

**Introduction.** Known also as the black-head disease, histomonosis is a major disease causing extensive economic losses mainly in turkey farms with a very unusual way of transmission.

**Historical notes.** The disease was described for the first time in Rhode Island, USA in 1893, in turkeys. Two years later, Smith described the agent. Immediately after its discovery, *Histomonas* decimated turkey populations of USA from 11 million birds in 1890 to 3.7 million in 1920, accounting for one third of all the mortality cases in this species. The mechanism of transmission was fully understood only in 1920, by Tyzzer. In 1926, the disease was already known also in Europe, Asia and Australia. In the late 1960s, there was a major decline of the disease due to the introduction of efficient drugs. First report in chicken came in 1900 in USA.

**Etiology.** The causative agent of black-head disease in turkeys and chicken is *Histomonas meleagridis*. The same species has been reported occasionally from a great variety of birds, including ostriches and birds captive in zoos. Another species (*Parahistomonas wenrichi*) which is non-pathogenic was described more recently from turkeys and other birds.

**Morphology.** *Histomonas meleagridis* (**figure 2.27**) has several developmental forms (pleomorphism). However, all of them are trophic stages; no cyst is known. When they inhabit the lumen of the cecum or when they are cultured, the shape is irregular (or amoeboid), 5-30 µm in diameter and with a single flagellum. Sometimes, during cell division, two flagella can be observed. Even during this stage they are able of moving by pseudopodia to invade tissues. The tissue stages lack flagellum and resemble amoebae. Like trichomonads, they lack mitochondria.

**Life cycle.** The life cycle is considered heteroxenous, with poultry as definitive hosts and nematodes from genus *Heterakis* as intermediate hosts. *Heterakis gallinae* are nematodes parasitizing the ceca of domestic poultry. When they feed, they accidentally ingest the trophozoites of *Histomonas meleagridis*. In the nematodes gut, they multiply and subsequently they invade the germinative area of the female ovaries. They continue to feed and multiply and

gradually they are incorporated in the forming eggs of the nematode.

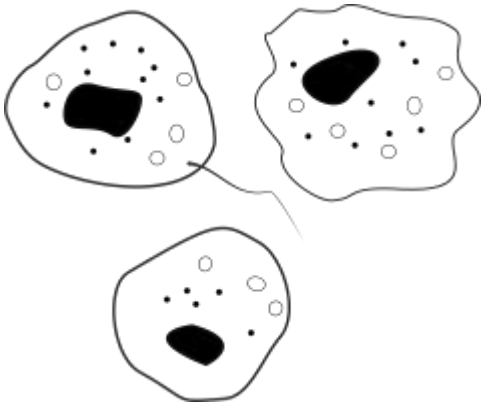

**Figure 2.27** *Histomonas meleagridis*: form in the lumen of the cecum (upper left); intermediate form (upper right); tissue form (lower).

More interestingly, it continues multiplication even after the new nematode embryo starts in ovo development, and *H. meleagridis* invades the newly formed nematode larvae, still inside its egg. The larvated egg offers a perfect shelter to *Histomonas*.

When the infective nematode eggs of *Heterakis gallinae* are ingested by a bird, nematode larvae hatch, and *Histomonas meleagridis* leaves its "Trojan horse", invading the avian host. To make things even more complicated, earthworms commonly act as paratenic hosts to *Heterakis*, and indirectly to *Histomonas*. *Parahistomonas wenrichi* has the same way of transmission, through *Heterakis gallinae*.

*Histomonas* multiplies by binary fission. No sexual stages are known.

**Epidemiology.** The distribution of histomonosis is global. It is distributed virtually everywhere where large turkey or chicken communities exist. Although the main clinical importance of *Histomonas* is in turkeys, the principal reservoirs for the infections are chicken, as asymptomatic carriers.

Infected *Heterakis gallinae* eggs can survive in the environment at least two years. However, if free trophozoites are eliminated in the environment directly with the feces of the birds they survive for very short periods of time. Hence, most transmission from host to host is relying on *Heterakis gallinae*. Nevertheless, direct contamination is also possible and it is assumed that this situation is responsible for sudden epizootics in dense flocks of turkeys.

Young birds are the most susceptible and the most infected age group is in turkeys up to 14 weeks, with the higher frequency of clinical cases between 3 and 12 weeks. Mortality can reach 100%. The main reservoirs for infection (directly for turkeys if kept together, or indirectly, by infecting the vector nematodes) are chicken, which are often asymptomatic carriers.

**Pathogenesis.** After *Histomonas* trophozoites emerge from the carrier nematode hosts, they invade the ceca and from there, via blood they are carried to various other internal organs (liver, kidney, bursa of Fabricius). During the infection, the xanthophylls from the blood decrease and methemoglobinemia increases, resulting in a dark coloration of the skin (hence the name black head disease). The symptoms are very

commonly exacerbated by the concurrent infection with *Escherichia coli* or *Clostridium perfringens*.

**Immunology.** As soon as histomonosis has been identified as a severe economical problem in farmed turkeys, researchers have tried to figure out a way for making them immune to the infection. Infection does not confer immunity. Moreover, reinfections can be as severe as any infection and may even cause death. Nevertheless, young turkeys are more sensitive than adult ones, but it was not shown if immunity plays a certain role in this. An interesting observation is that birds with some degree of immunity against *Heterakis gallinae* are more resistant to the infection with *Histomonas meleagridis*.

**Clinical signs.** The most common form of the disease, mainly in young turkeys is acute. Infected turkeys have ruffled feathers, hanging wings and tail with yellowish (sulfur-colored) diarrhea. The skin of the head might become dark colored, almost black, hence the name of the diseases. If not treated, most birds die in 1-2 weeks. In older turkeys, the disease is more often asymptomatic or chronic, but virulent strains can induce even in them an acute form. In chicken, the infection is usually mild or asymptomatic.

**Pathology.** The main sites of lesions are the liver and the ceca. The hepatic lesions in turkeys are considered to be very characteristic or even pathognomonic. They consist of round, 1-2 cm areas of necrosis on the liver surface, yellowish and crater-like, with well-defined margins (**figure 2.28**).

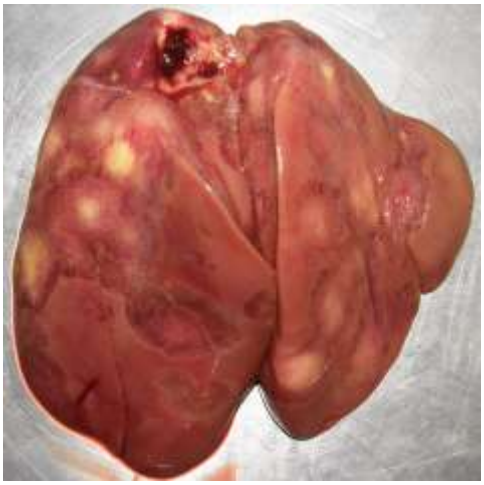

**Figure 2.28** Liver of a turkey naturally infected with *Histomonas meleagridis* showing typical circular necrotic lesions. *(Photo Cristian Magdaș)*

On gross section, they extend also into the internal parts of the hepatic tissue. In chronic cases, these lesions might extent to contiguous organs like kidneys or lungs. These hepatic lesions may be small and multiple or large and confluent. In other cases, they have a tumour like appearance (differential diagnosis with leucosis). Histology shows lymphocytic and macrophage infiltration with the presence of multinucleated giant cells in these. The parasitic organisms appear in clusters. The cecal lesions in turkeys are also necrotic, with abundant white fibrinous material filling the entire lumen (**figure 2.29**). The mucosa shows also small, hemorrhagic ulcers (**figure 2.30**).

The ceca are often enlarged. Cecal perforation may occur. In chicken, the cecal lesions can be necrotic, like in turkeys or hemorrhagic, resembling *Eimeria tenella* coccidiosis.

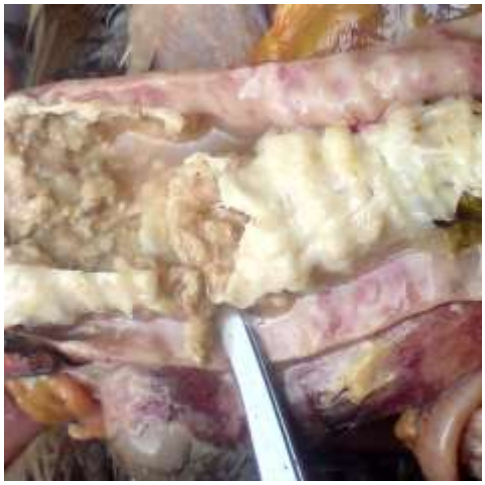

**Figure 2.29** Cecum of a turkey naturally infected with *Histomonas meleagridis* showing necrosis of the mucosa and fibrinous content in the lumen. *(Photo Cristian Magdaș)*

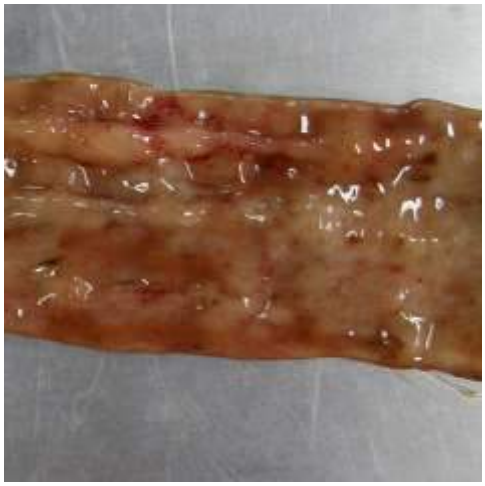

**Figure 2.30** Cecum of a turkey naturally infected with *Histomonas meleagridis* showing multiple ulcerations of the mucosa. *(Photo Cristian Magdaș)*

**Diagnosis.** The most common diagnosis is based on gross lesions. Circular liver necrosis are considered pathognomonic. From the hepatic and cecal lesions, the direct microscopic examination (wet mount) can demonstrate the presence of live, highly motile *Histomonas*.

Alternatively, in cytological examination or histological section the parasite is also well visible and identifiable.

There are techniques available for the in vitro cultivation of *Histomonas meleagridis*. Several cultivation media can be successfully used: Egg slant medium, Laidlaw and Devolt medium or Dwyer medium.

Recently, primers for the PCR diagnosis were designed and employed for research, but their use in the routine tests is not yet widely introduced.

**Treatment.** Despite of many successfully in vitro tested compounds, no single drug is available for in vivo use.

Most of them have high toxicity and their use is forbidden due to human health risks. Such products include: arsenical compounds, nitroheterocyclic compounds etc. The only drugs which are allowed are those against the vector host, the nematode *Heterakis gallinae* (albendazole, fenbendazole).

**Control.** As no drugs for the treatment are available, the only real control measure is prevention. In general, avoiding housing together turkeys and chicken, pasture rotation if grazing systems are used, avoiding of overcrowding are some measure which are somehow effective.

Periodic treatment and chemoprophylaxis against nematodes are also very important.

Several attempts have been made to obtain a commercial vaccine. Live attenuated strains used during the early days offer some protection. However, they cannot be used, as prolonged culture induces the loss in their colonizing capacity. After the discovery of the second, non-pathogenic species, *Parahistomonas wenrichi*, it was believed that it will induce cross immunity. However, the results were inconsistent. There were some moderate results following chemo-immunization (infection followed by treatment to stop the disease before serious lesions appear).

### 2.3.3 Diplomonadids

**Introduction.** The family Hexamitidae is a member of order Diplomonadida. The order includes flagellates with two separate nuclei, a simple cytoskeleton, no plasmids and no mitochondria.

The family Hexamitidae includes five genera. There are two medically important genera, *Giardia* and *Hexamita*, the later not detailed in this textbook due to its limited importance in domestic animals.

**General morphology**. The most striking aspect is the apparent bilateral symmetry (two symmetric nuclei, the position of the even number of flagella). More aspects will be discussed for members of genus *Giardia*.

**Ecology and transmission.** Some authors regard Hexamitidae as commensals inhabiting the digestive tract of a variety of vertebrate species. Transmission is via fecal-oral route.

**Medical importance.** Genus *Giardia* is an important pathogen of domestic animals and humans, with zoonotic potential, causing digestive problems mainly in young animals and children. Genus *Hexamita* is responsible for enteric lesions in colonies of laboratory rodents, in turkeys and pigeons. Both genera are considered opportunistic.

#### 2.3.3.1 Giardiosis

**Introduction.** Giardiosis is a worldwide distributed zoonotic disease caused by *Giardia duodenalis*, affecting hundreds of mammal species, including domestic animals and humans, responsible for diarrheal outbreaks and malabsorption mainly in children and young or immunosuppressed animals.

**Historical notes.** *Giardia duodenalis* has a very important place in history. It was one of the first microscopic organisms to be ever seen by the human eye. It was observed for the first time by the Antonie van Leeuwenhoek with one of the first microscopes, built by himself. He saw *Giardia* in his own feces in 1681. For more than 200 years the parasite was forgotten. In 1902, Stiles associated the presence of *Giardia* with diarrhea in humans. His assumptions were correct, as in the World War 1, many soldiers suffering from diarrhea were passing in their stools *Giardia* parasites.

**Etiology.** The taxonomy of genus *Giardia* is still a controversial issue in systematic parasitology. We will follow the opinion of the latest reviews which list 6 valid species: *G. agilis* (from amphibians), *G.*

*ardeae* and *G. psittaci* (from birds), *G. muris* and *G. microti* (from rodents) and *G. duodenalis* (from various mammals).

The only species with veterinary and zoonotic importance is *G. duodenalis*. All previous names used for this species in the past, some of them extensively (i.e. *G. lamblia*, *G. intestinalis*) must be regarded now as invalid and considered synonyms. Today, *G. duodenalis* is divided into 7 assemblages, named from A to G:

- assemblage A (zoonotic): primates (including humans), livestock, cats, dogs, beavers;
- assemblage B (zoonotic): primates (including humans), dogs, beavers;
- assemblage C (non-zoonotic): dogs;
- assemblage D (non-zoonotic): dogs;
- assemblage E (non-zoonotic): cattle, sheep, pigs;
- assemblage F (non-zoonotic): cats;
- assemblage G (non-zoonotic): rodents

There are some attempts to use binomial specific names for each of these assemblages: *G. canis* (for C and D), *G. simondi* (for G), *G. cati* (for F), *G. bovis* (for E), *G. enterica* (for B) and *G. duodenalis* (for A). As they are not widely accepted, their further use in this textbook will be avoided.

**Morphology.** Species of genus *Giardia* have two developmental stages: the endogenous stage called trophozoite and the exogenous stage, known as cyst. The trophozoites of *Giardia* (**figure 2.31**) are piriform, with round and broad anterior end and pointed posterior end. The body is flattened dorsoventrally, and has a convex shape towards the dorsal part (spoon-like aspect). On the ventral side of its surface, *Giardia* possesses an adhesive disk. The adhesive disk (which is rigid due to the presence microtubules) is used for adherence to the host cells.

The trophozoite bears four pairs of flagella. One pair (known as ventral flagella) is located in the ventral groove. Each flagellum originates from an organelle called kinetosome. Posterior to the adhesive disk, *Giardia* has two structures with unknown function, called median bodies. *Giardia* has no mitochondria, no Golgi apparatus and no axostyle.

The cysts (**figure 2.32**) are 8-12 x 7-10 µm, with 2-4 nuclei and inner structures corresponding to axonemes of the flagella and median bodies.

**Life cycle.** The infective elements are cysts from the environment (usually from contaminated drinking water).

After ingestion, the excystation is triggered by the low gastric pH and pancreatic enzymes. From each cyst, usually two trophozoites are emerging in the duodenum. They swim by rotational movements towards the mucosal surface where they attach using the adhesive disks. Trophozoites inhabit the surface of the intestinal epithelium. Despite the trophozoites of *Giardia* are highly mobile, they prefer to stay attached rather than swim.

They multiply by binary fission. Three cellular divisions take place before the trophozoites are mature.

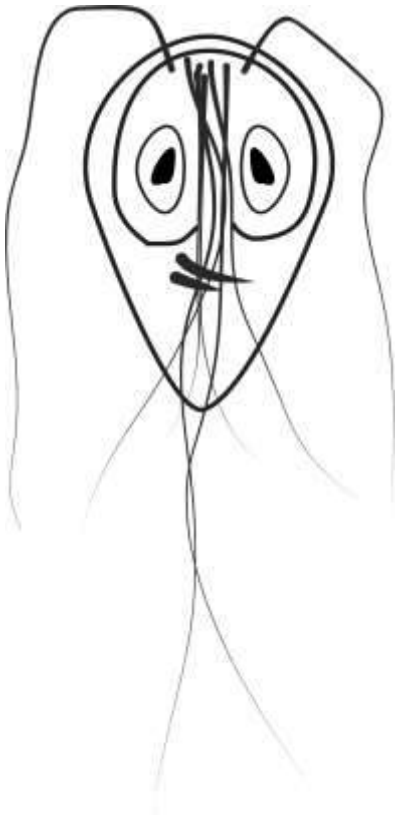

**Figure 2.31** Trophozoite of *Giardia duodenalis*.

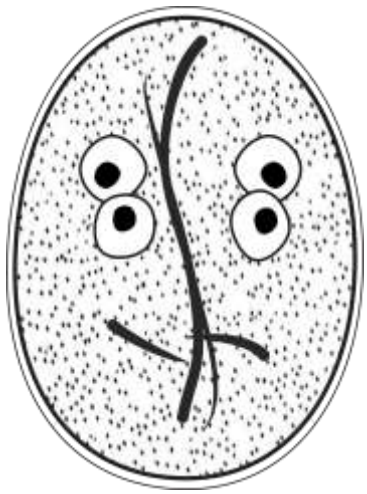

**Figure 2.32** Cyst of *Giardia duodenalis*.

The speed and amount of multiplication in *Giardia* is amazing. Estimates say that diarrheic stools from infected humans can contain around 14 billion *Giardia* individuals.

As trophozoites move to the colon and the intestinal content becomes dehydrated, *Giardia* begins the encystation process. They are eliminated through the feces of the infected host in the environment where they are immediately infective to a new host.

The metabolism of *Giardia* is anaerobe. However, it tolerates well the presence of oxygen. Trophozoites feed on the mucus produced by the host cell.

**Epidemiology.** Although not a severe or life threatening disease, giardiosis is among the most widespread infection of humans and animals. The geographical wide distribution, the large spectrum of hosts, the high prevalence in certain areas makes giardiosis an important public health problem. This large scale importance is probably caused by the relatively high resistance of cysts in the environment and by the great variety and ease of transmission mechanisms. The transmission route is always fecal-oral, and the possible mechanisms, direct or indirect are: human to human, animal to animal, animal to human or human to animal. The infection sources are represented in all cases by: contaminated water (drinking or recreational), contaminated food (from water used in its preparation or from food handlers).

The most common source of infection for humans is drinking water from various sources, including public systems. They

are usually caused by severe deficiencies in the water filtration process. The contamination of water is through the discharge of human sewage or drainage of animal feces, mainly from farms (cattle, sheep, goats, pigs and horses). Another very common source of infection are aquatic mammals (muskrats, beavers, otters and nutria). Consumption of fresh vegetables, fruits or shellfish has also been reported as infection sources.

Cysts of *Giardia* can survive in the water or soil for several months. Freezing during winter destroys the cysts. Cysts are sensitive to UV light. Exposure to chlorine and chloramines during the water disinfection is ineffective against *Giardia* cysts. Cysts are sensitive to ozone treatment.

**Pathogenesis.** The pathogenicity depends on the strain (assemblage, genotypes etc.) and on the host. Most of the cases are asymptomatic.

Several mechanisms have been incriminated in the pathogenesis of giardiosis in humans and animals. These include: production of toxins, intestinal dysmicrobism, inhibition of normal enzymatic activity of the enterocytes, intestinal motility disorders. The permanent attachment of trophozoites to the enterocytes induces mucus hypersecretion and destruction of microvilli.

The anaerobic metabolism is responsible for gas production resulting in flatulence and intestinal distension with pain. The results of all these is diarrhea and malabsorption. Impaired absorption of fats leads to fatty stools, while the malabsorption of other nutrients together with dehydration induces weight loss.

**Immunology.** Most of the knowledge on the immune response in giardiosis is either from humans or laboratory animal models. Infection with *Giardia* induces a strong humoral immune response. The most important antibodies are IgA, which are secreted in the intestinal lumen. They have good in vitro effect, but the antigenic variation of *Giardia* reduces their efficacy in vivo. The role of the immune system in the defense against *Giardia* is evident mainly in the light of several clinical signs in immunodeficient hosts. The role of cellular immunity is not fully understood, but it seems it plays a less important role in protection.

**Clinical signs.** Most of the infections in animals and humans are inapparent. In domestic animals, the clinical disease is reported occasionally in dogs, cats, ruminants and pigs. Symptoms are more common in young animals.

In dogs and cats, the symptoms vary from subclinical to moderate or severe abdominal discomfort and pain. Diarrhea is not constant, but when it is present it is soft, watery and coated with mucus and often with steatorrhea and strong odor. The diarrhea is self-limiting in dogs and cats with normal immune status.

As a result of long-term malabsorption and diarrhea, chronic, persistent infections are accompanied by dehydration and weigh loss. Diarrhea can be continuous or intermittent. Flatulence may be present, mainly in humans. Typically, the blood is not present in feces

of animals suffering from giardiosis. If blood is present it might be the results of concurrent bacterial or coccidian infections. Some dogs and cats may vomit. Fever is usually absent. Differential diagnosis in dogs and cats include pancreatic insufficiency or other malabsorption syndromes.

In calves and other livestock, the clinical picture is more or less similar to the symptoms described above for dogs and cats. Diarrhea which is not responsive to antibiotic or anticoccidial treatment in young animals (1-6 months) might be an indication of giardiosis or crypto-sporidiosis (see Chapter 2.4.2.1).

In such cases, confirmation must be using laboratory methods. In livestock, the economic impact of the disease is also important, as infection results in a decreased feed efficiency and weight gain.

Hematology and biochemistry laboratory values are usually within physiological limits. They can reflect only dehydration with loss of electrolytes if diarrhea is severe.

**Pathology.** Gross intestinal lesions are rarely evident. Microscopic lesions consist in the villous atrophy of the enterocytes.

**Diagnosis.** The preferred method for the diagnosis of *Giardia* cysts in feces is ELISA, employed for detection of coproantigens. Direct detection of cysts (**figure 2.33**) or even trophozoites (**figure 2.34**) by microscopic examination is possible. Various methods are used, including flotation and staining methods (iodine).

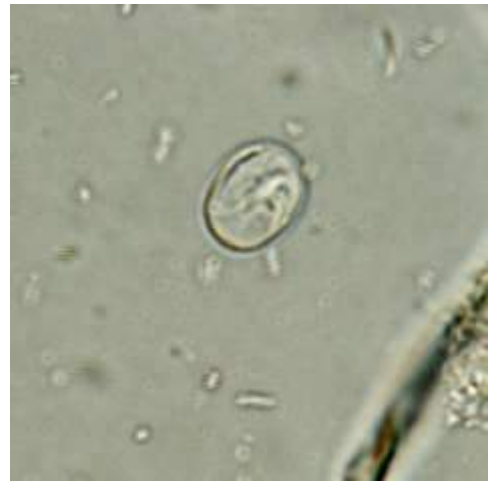

**Figure 2.33** Cyst of *Giardia duodenalis* from the feces of a dog. *(Photo Viorica Mircean)*

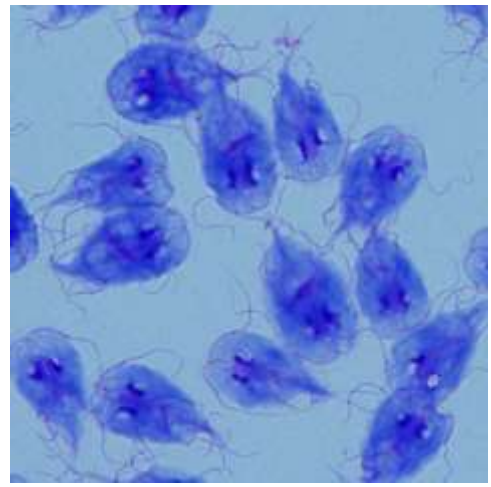

**Figure 2.34** Trophozoites of *Giardia duodenalis* from culture. *(Photo Andrei D. Mihalca)*

Other diagnosis methods include immunofluorescence and phase contrast microscopy. Identification of assemblages and genotypes is done by molecular techniques (e.g. PCR).

**Treatment.** Various drugs have been used for the treatment of giardiosis in animals. Most effective group of drugs are benzimidazoles.

There are no licensed drugs to be used in farm animals. In pets (dogs and cats), several protocols are approved and licensed. The following protocols can be used:

- Fenbendazole, 50 mg/kg body weight/day, orally for three consecutive days. It is recommended for treatment of dogs, including pregnant and lactating females. It is not approved for cats. In ruminants, the dose is 5-20 mg/kg body weight/day, orally for three consecutive days. Fenbendazole has also been used in birds.
- Oxfendazole, 11.3 mg/kg body weight/day, orally for three consecutive days is used also in dogs. It is not approved, but it is effective.
- Albendazole, 25 mg/kg body weight/day, orally for 4-5 consecutive days in dogs and cats. It is not approved but it is effective. Bone marrow toxicity of albendazole has been reported. In calves, the same dose given for fenbendazole is recommended.
- Pyrantel and febantel combination (each at 30 mg/kg body weight/day, orally, single dose, are effective in canine giardiosis.
- Metronidazole, 25 mg/kg body weight/twice a day, orally, for 5-7 consecutive days is 65% effective in dogs and cats. Side effects include anorexia and vomiting.
- Furazolidone, 4 mg/kg body weight/day, orally, for one week is effective in dogs and cats.
- Paromomycin, 50–75 mg/kg body weight/day, orally, for 5 consecutive days is used in calves.

These drugs may not eliminate completely the infection, but they reduce significantly the oocyst shed and severity of symptoms.

**Control.** The most effective prevention is by avoiding ingestion of cysts. In humans, drinking pure water (boiled, filtered) is the key. In animals, these general preventive measures are more difficult to be applied. However, a good waste management in farms greatly reduce the environmental pollution.

Specific prevention is available for dogs and cats as vaccination. The commercial product is a killed vaccine, efficient for both, prevention and treatment.

## 2.4 Apicomplexa

Phylum Apicomplexa includes exclusively parasitic organisms, some of them with huge medical importance in both human and veterinary medicine. Prominent examples of medically important apicomplexans are the agents of human malaria (genus *Plasmodium*) which are responsible for more than one million deaths annually. In animals, genus *Eimeria* has a huge economic impact on

poultry farming. Other species have major zoonotic impact (e.g. *Toxoplasma*, *Cryptosporidium*).

The name Apicomplexa is derived from the presence of a series of ultrastructural cell organelles, known as the ***apical complex*** (**figure 2.35**). The apical complex is present only in "zoite"-like stage (e.g. sporozoites, merozoites).

The apical complex includes several structures:

- one or two ***polar rings***, which surround the apical tip of the cell; from the polar rings, two subpellicular microtubules radiate posteriorly, parallel to the body axis.
- the ***conoid***, consists of a conic spiral of fibrillar structures. It is not present in all apicomplexans. Based on its presence or absence, Phylum Apicomplexa is divided in two classes: Conoidasida (with conoid) and Aconoidasida (without conoid).
- two or more ***rhoptries***, under the shape of elongated bodies.
- ***micronemes***, like smaller elongated bodies.

**Table 2.8** Taxa of veterinary importance in phylum Apicomplexa

| Class | Order | Family | Most important genera | Disease (Chapter) |
|---|---|---|---|---|
| Coccidia | Eimeriorina | Eimeriidae | *Eimeria* | Intestinal coccidiosis in mammals (2.4.1.1)<br>Intestinal eimeriosis in birds (2.4.1.2)<br>Hepatic eimeriosis in rabbits (2.4.1.3)<br>Renal eimeriosis in geese (2.4.1.4) |
| | | | *Isospora* | Isosporosis in pigs (2.4.1.5)<br>Isosporosis in carnivores (2.4.1.6) |
| | | Sarcocystidae | *Sarcocystis* | Sarcocystosis in domestic animals (2.4.3.1) |
| | | | *Toxoplasma* | Toxoplasmosis in domestic animals (2.4.3.2) |
| | | | *Neospora* | Neosporosis (2.4.3.3) |
| | | | *Hammondia* | Hammondiosis (2.4.3.4) |
| | | | *Besnoitia* | Besnoitiosis (2.4.3.5) |
| | Adeleorina | Hepatozoidae | *Hepatozoon* | Hepatozoonosis (2.4.4.1) |
| Cryptosporidea | | Cryptosporidiidae | *Cryptosporidium* | Cryptosporidiosis in domestic animals (2.4.2.1) |
| Haematozoea | Piroplasmida | Babesiidae | *Babesia* | Babesiosis in domestic animals (2.4.6.1) |
| | | Theileriidae | *Theileria* | Theileriosis in domestic animals (2.4.7.1) |

***Micropores*** located along the body margins of the "zoites" have role in the ingestion of food by the parasitic organism. Movement cell organelles (flagella, cilia, pseudopodia) are absent in apicomplexans, with the exception of very few developmental stages (like some gametes).

Additionally, the apicomplexan cell contains various cell organelles and structures typical to eukaryote cells: nucleus, mitochondria, Golgi body etc. Apicomplexans have a very complex life cycle (**figure 2.36**), with three main types of pathways.

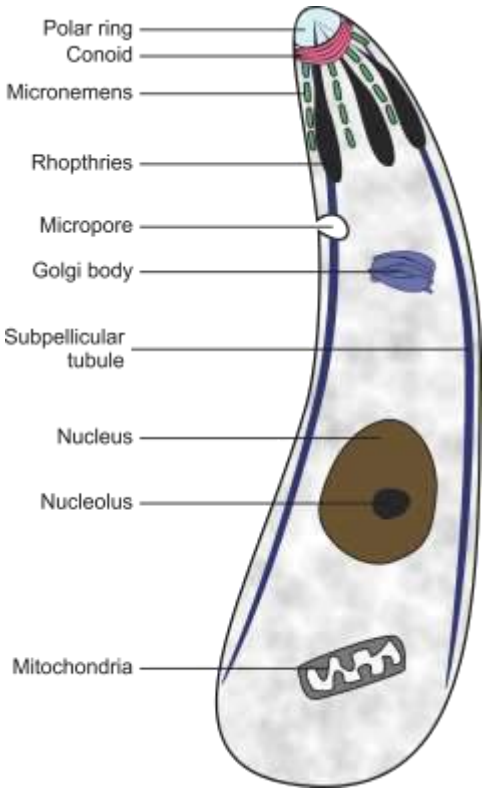


**Figure 2.35** General structure of the cell in Apicomplexa.

- Some groups (Eimeriidae, Cryptosporidiidae) have homoxenous life cycle (black pathway in **figure 2.36**), with endogenous and exogenous stages. Infection of the host is made with infectious stages from the environment.
- Some others (*Babesia, Theileria, Hepatozoon*) are heteroxenous, with no stages in the external environment (red pathway in **figure 2.36**). These include vector-borne species which are transmitted to the vector by blood meal and to the host by blood meal or ingestion of vector.
- The third group includes members of Sarcocystidae family (*Sarcocystis*, *Toxoplasma*, *Neospora*) with heteroxenous development (blue pathway in **figure 2.36**). Transmission from the definitive host (which is usually a carnivorous species) to the intermediate host is through the environment, and from the intermediate host to the definitive host by predatorism.

The multiplication in Apicomplexa takes place in both ways: asexually (binary fission, multiple fission or endopolyogeny) and sexually (by male and female gametes). The sexual reproduction takes place always in the definitive host. The vast majority of species are intracellular parasites. All domestic species are affected by diseases produced by apicomplexans.

The apicomplexan species of veterinary importance are included in three classes

A more detailed list of genera and associated diseases is shown in **table 2.8**.

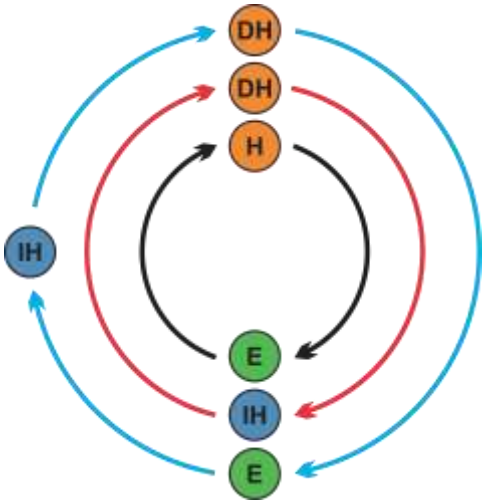


**Figure 2.36** General life cycle paths in Apicomplexa of domestic animals. H = Host; DH = Definitive Host; IH = Intermediate Host; E = Environment. For detailed explanations please refer to the text.

Coccidia and Cryptosporidea inhabit the epithelium of digestive tube, the liver, kidney, blood cells and various other tissues of vertebrates and invertebrates.

The typical life cycle of coccidia has ***three phases: merogony, gametogony and sporogony***. The infective stages are called sporozoites. These sausage-shaped (or banana-shaped) cells infect the host via various routes and after entering the host cell they become trophozoites and start the merogonic development. By asexual multiplication, during merogony numerous merozoites are produce which escape from the host cell and infect other susceptible cells, re-starting the merogony once again. Merogony is repeated several times, depending on several factors. The last generation of merozoites will transform into gamonts, initiating the gametogony. There are two types of gamonts (gametocytes): microgametocytes (corresponding to male cells) and macrogametocytes (female). Each macrogametocyte develops into one macrogamete. Each microgametocyte divides by multiple fission producing numerous biflagellated microgametes. The macrogamete is fertilized by a microgamete and this produces the zygote. Zygotes develop into oocysts. By sporogony, inside each oocyst the sporozoites will be formed.

### 2.4.1 Eimeriidae

**Introduction.** Family Eimeriidae include thousands of species parasitic in vertebrates and invertebrates. There are three genera of veterinary importance: *Eimeria*, *Isospora* and *Tyzzeria*.

**General morphology.** Each developmental stage has a typical morphology. Nevertheless, the most important stage from diagnostic point of view and for specific identification is the oocyst. The internal structure of the sporulated oocysts is different in the various genera of the family (**figure 2.37**).

In genus *Eimeria* the sporulated oocysts contains 4 sporocysts, each with two sporozoites. In genus *Isospora*, there are two sporocysts, each with four sporozoites. In genus *Tyzzeria*, there are eight sporozoites, free in the oocyst and

not within a sporocyst as mentioned for the previous two genera. The fine structure will be detailed, as an example for genus *Eimeria* (**figure 2.38**).

The oocyst wall has two layers. In many species there is a small opening in the oocysts wall called micropyle. This opening is used for the emergence of sporozoites when the oocyst reaches the digestive tube of the host. The micropyle, if present is covered by a cap. A refractile structure called polar granule may be also present. The internal structure of typical sporulated oocysts comprises sporocysts in different numbers.

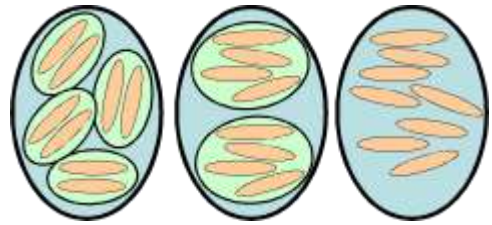

**Figure 2.37** General morphology of the most representative species of Eimeriidae: left - *Eimeria* (four sporocysts, each with two sporozoites); center - *Isospora* (two sporocysts, each with four sporozoites); right - *Tyzzeria* (asporocystic oocyst, eight free sporozoites).

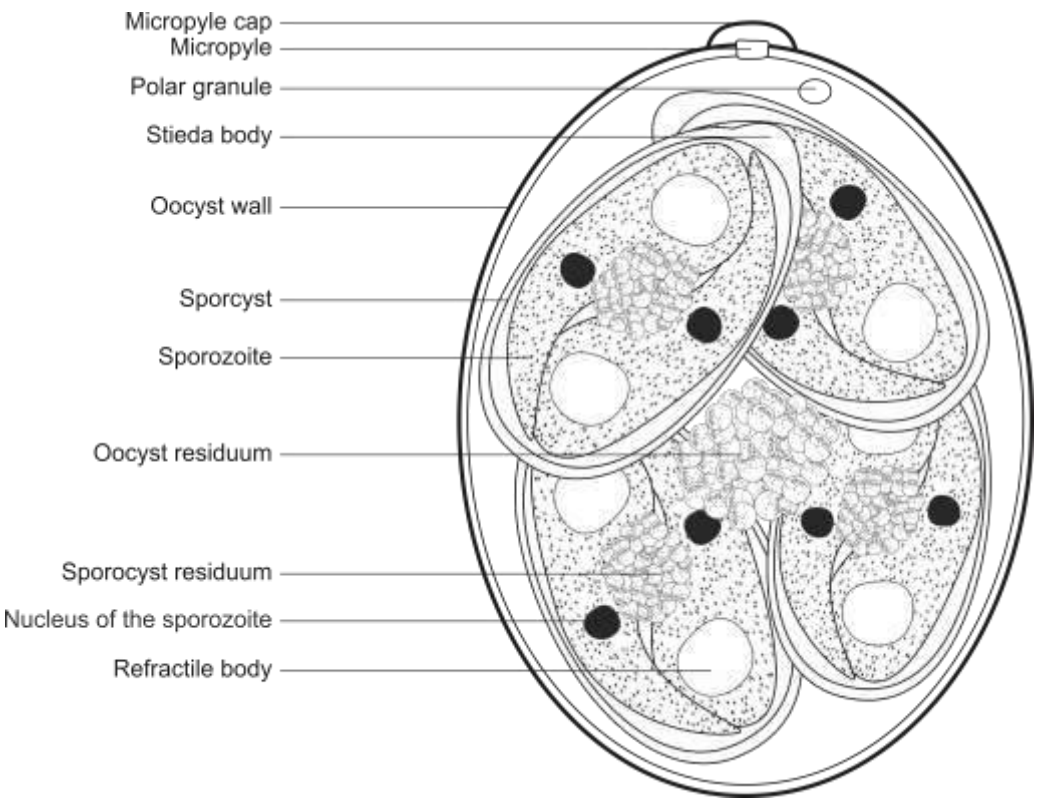


**Figure 2.38** Diagrammatic representation of a typical sporulated oocyst of *Eimeria*.

Between sporocysts, an oocyst residuum may be present, as a result of unincorporated cytoplasmic material during sporogony.

Similarly, inside each sporocyst, as sporocyst residuum is evident. Each sporocyst is delimited by a sporocyst wall. At the anterior end of the sporocysts a small plug-like structure known as Stieda body may be visible. Less typical is the general morphology of the unsporulated oocyst. Unsporulated oocysts (**figure 2.39**) are present in fresh fecal material and they have similar morphology in all Eimeriidae genera. It is impossible to identify the species based on the morphology of unsporulated oocysts. They contain one single round central body called sporont, which has a fine granular structure.

**Ecology and transmission.** All species inhabit the intracellular environment of epithelial cells, usually enterocytes. They can be parasitic intracytoplasmatically or intranuclearly. As a rule, all species are homoxenous, although exceptions are documented. Merogony and gametogony take place within the host, while sporogony occurs in the external environment. The infective stage is the sporulated oocyst. Transmission is by ingestion of infective oocysts. Usually they are highly specific parasites, and interspecific transmission is very limited. Eimeriidae are not zoonotic. Moreover, in certain host species (like chicken for instance) there is also a very pronounced organ specificity.

**Medical importance.** Members of Eimeriidae are responsible for severe intestinal infections in poultry but also in other livestock, affecting mainly the young animals. Their economic impact is huge and losses are due to mortalities or expenses for prophylaxis and treatment.

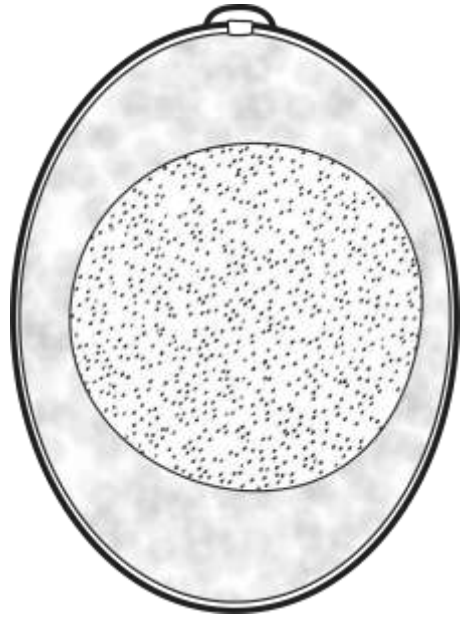

**Figure 2.39** Diagrammatic representation of an unsporulated oocyst of Eimeriidae.

### 2.4.1.1 Intestinal coccidiosis in mammals

**Introduction.** Intestinal coccidiosis in domestic mammals are globally distributed in infections responsible for hemorrhagic diarrhea in young individuals, with possible mortality and with important economic losses. Under the name of "eimeriosis" we include diseases caused by species of genera *Eimeria* and *Isospora*.

**Historical notes.** The first oocysts of *Eimeria* were seen in the liver of an

infected rabbit, by Antonie van Leeuwenhoek in 1674 but it was not described as a new species during those times. The first description of a species in genus *Eimeria* came in 1870, when Eimer, a German zoologist, named a small organism found in house mice as *Gregarina falciformis*. Five years later Johann Gottlob Schneider, another German naturalist erected a new genus in the memory of Eimer and named it *Eimeria*. In the following years many other species parasitic in domestic and wild animals have been described. Genus *Isospora* was proposed by the same Schneider, in 1881.

**Etiology.** The diversity of the species in genus *Eimeria* parasitic in domestic mammals is huge. The quasi-complete list of them is shown below for ruminants (**table 2.9**), equids and camelids (**table 2.10**), pigs (**table 2.11**), rabbits (**table 2.12**) and laboratory rodents (**table 2.13**).

In domestic animals, the species of genus *Isospora* are parasitic only in pigs and carnivores (**table 2.14**).

The taxonomic value of genus *Isospora* is being under debate for some years. In 1977, a new genus was proposed (*Cystoisospora*) to include species parasitic in carnivores with facultative heteroxenous life cycle. For educational purposes, these taxonomic debates are more confusing, hence we will use in this textbook the name *Isospora* for all species.

One should note that in ruminants, horses and rabbits the only parasitic genus of Eimeriidae is *Eimeria* while carnivores harbor only genus *Isospora*. Pigs are the only domestic species to be infected with both genera. Previous reports of genus *Eimeria* in carnivores are probably pseudoparasitic species from their prey which are passed passively through the feces.

**Table 2.9** Species of genus *Eimeria* parasitic in domestic ruminants

| Species | Host |
|---|---|
| *E. alabamensis* | Cattle, Buffalo |
| *E. auburnensis* | Cattle, Buffalo |
| *E. bovis* | Cattle |
| *E. brasiliensis* | Cattle |
| *E. bukidnonensis* | Cattle |
| *E. canadensis* | Cattle |
| *E. cylindrica* | Cattle |
| *E. ellipsoidalis* | Cattle |
| *E. illinoisensis* | Cattle |
| *E. pellita* | Cattle |
| *E. subspherica* | Cattle |
| *E. thianethi* | Cattle, Buffalo |
| *E. wyomingensis* | Cattle |
| *E. zuernii* | Cattle, Buffalo |
| *E. ankarensis* | Buffalo |
| *E. bareillyi* | Buffalo |
| *E. gokaki* | Buffalo |
| *E. ovoidalis* | Buffalo |
| *E. ahsata* | Sheep |
| *E. crandallis* | Sheep |
| *E. danielle* | Sheep |
| *E. faurei* | Sheep |
| *E. gilruthi* | Sheep |
| *E. gonzalezi* | Sheep |
| *E. granulosa* | Sheep |
| *E. intricata* | Sheep |
| *E. marsica* | Sheep |
| *E. ovina* | Sheep |
| *E. ovinoidalis* | Sheep |
| *E. pallida* | Sheep, Goat |
| *E. parva* | Sheep |
| *E. punctata* | Sheep, Goat |
| *E. absheronae* | Goat |
| *E. africiensis* | Goat |
| *E. alijevi* | Goat |
| *E. arloingi* | Goat |
| *E. caprovina* | Goat |
| *E. christenseni* | Goat |
| *E. gilruthi* | Goat |
| *E. hirci* | Goat |
| *E. jolchijevi* | Goat |
| *E. kocharii* | Goat |
| *E. ninakohlyakimovae* | Goat |

**Morphology.** The general morphology for Eimeriidae was described previously.

**Table 2.10** Species of genus *Eimeria* parasitic in domestic equids and camelids

| Species | Host |
|---|---|
| *E. leuckarti* | Horse |
| *E. solipedum* | Horse |
| *E. uniungulati* | Horse |
| *E. bactriani* | Camels |
| *E. cameli* | Camels |
| *E. dromedari* | Camels |
| *E. pellerdyi* | Camels |
| *E. rajasthani* | Camels |
| *E. alpacae* | Llama, Alpaca |
| *E. lamae* | Llama, Alpaca |
| *E. macusaniensis* | Llama, Alpaca |
| *E. peruviana* | Llama |
| *E. punoensis* | Llama, Alpaca |

**Table 2.11** Species of genus *Eimeria* parasitic in domestic pigs

| Species | Host |
|---|---|
| *E. almaataensis* | Swine |
| *E. betica* | Swine |
| *E. debliecki* | Swine |
| *E. guevarai* | Swine |
| *E. ibrahimovae* | Swine |
| *E. neodebliecki* | Swine |
| *E. perminuta* | Swine |
| *E. polita* | Swine |
| *E. porci* | Swine |
| *E. residualis* | Swine |
| *E. scabra* | Swine |
| *E. spinosa* | Swine |
| *E. suis* | Swine |

**Table 2.12** Species of genus *Eimeria* parasitic in domestic rabbits

| Species | Host |
|---|---|
| *E. coecicola* | Rabbit |
| *E. elongata* | Rabbit |
| *E. exigua* | Rabbit |
| *E. flavescens* | Rabbit |
| *E. intestinalis* | Rabbit |
| *E. irresidua* | Rabbit |
| *E. magna* | Rabbit |
| *E. matsubayashii* | Rabbit |
| *E. media* | Rabbit |
| *E. neoleporis* | Rabbit |
| *E. perforans* | Rabbit |
| *E. piriformis* | Rabbit |

**Table 2.13** Species of genus *Eimeria* parasitic in laboratory rodents

| Species | Host |
|---|---|
| *E. caviae* | Guinea pig |
| *E. amburdariana* | Golden hamster |
| *E. aurata* | Golden hamster |
| *E. razgovica* | Golden hamster |
| *E. arasinaensis* | House mouse |
| *E. baghdadensis* | House mouse |
| *E. falciformis* | House mouse |
| *E. ferrisi* | House mouse |
| *E. hansonorum* | House mouse |
| *E. hindlei* | House mouse |
| *E. keilini* | House mouse |
| *E. krijgsmanni* | House mouse |
| *E. musculi* | House mouse |
| *E. musculoidei* | House mouse |
| *E. papillata* | House mouse |
| *E. paragachaica* | House mouse |
| *E. schueffneri* | House mouse |
| *E. vermiformis* | House mouse |

**Table 2.14** Species of genus *Isospora* parasitic in domestic mammals

| Species | Host |
|---|---|
| *I. suis* | Swine |
| *I. burrowsi* | Dog |
| *I. canis* | Dog |
| *I. neorivolta* | Dog |
| *I. ohioensis* | Dog |
| *I. felis* | Cat |
| *I. rivolta* | Cat |

Specific morphology for sporulated oocysts is given for selected species in **tables 2.15**, **2.16**, **2.17**, **2.18** and **2.19**. Identification of species can be done solely if the oocysts are sporulated. Measurements are necessary for such a purpose not only for the oocyst but also for internal structures (sporocyst, sporozoites). Other specific characteristics must be also observed: presence or absence of the micropyle, Stieda body, polar granule, aspect of oocyst and sporocyst residuum etc. In fresh feces, the oocysts are not

sporulated and their specific identification is not achievable.

**Life cycle.** The common aspects of the life cycle were described under general considerations about Eimeriidae (Chapter 2.4.1). Intestinal *Eimeria* species develop intracellularly in epithelial cells of the intestine (enterocytes) (**figure 2.40**). Each species has a specific habitat. Some species inhabit the small intestine, some other the large intestine. Other species are very limited (only duodenum) others are more generalist and can infect portion of the intestine. Hosts acquire the infection when ingesting sporulated oocysts (figure 2.40 - 1). Sporozoites escape (figure 2.40 - 2) through the opened micropyle. In the species which lack micropyle, the oocyst wall ruptures in order to release the sporozoites. Sporozoites will enter a host cell (figure 2.40 - 3) and start the merogony. They soon become enlarged trophozoites (figure 2.40 - 4), divide their nucleus and become multinucleated cells (called meronts (figure 2.40 - 5). Each nucleus will be incorporated in the structures of the merozoites (figure 2.40 - 6). Merozoites than rupture the host cell (figure 2.40 - 7) and actively search for a new cell which they infect, repeating the merogony (figure 2.40 - 8). The last generation of merozoites (figure 2.40 - 9) will enter enterocytes (figure 2.40 - 10 and 11) and become macrogametes (figure 2.40 - 12) and biflagellated microgametes (figure 2.40 - 13). The microgametes will fertilize the macrogamete (figure 2.40 - 14) forming the zygote (figure 2.40 - 15) which eventually becomes an oocyst (figure 2.40 - 16).

**Table 2.15** Basic morphology of *Eimeria* parasitic in domestic ruminants

| Species | Oocyst shape | Size (µm) |
|---|---|---|
| *E. alabamensis* | subellipsoidal | 13-24 x 11-16 |
| *E. auburnensis* | elongated-ovoidal | 32-46 x 20-25 |
| *E. bovis* | ovoidal | 23-34 x 17-23 |
| *E. brasiliensis* | ovoidal | 34-42 x 24-29 |
| *E. bukidnonensis* | piriform | 33-41 x 24-28 |
| *E. canadensis* | ellipsoidal | 28-37 x 20-27 |
| *E. cylindrica* | cylindrical | 16-27 x 12-15 |
| *E. ellipsoidalis* | ellipsoidal | 12-27 x 10-18 |
| *E. pellita* | ovoidal | 32-42 x 22-27 |
| *E. subspherica* | subspherical | 9-13 x 8-12 |
| *E. wyomingensis* | ovoidal | 37-45 x 26-30 |
| *E. zuernii* | spherical | 15-22 x 13-18 |
| *E. ankarensis* | elongated-ovoidal | 32-43 x 25-29 |
| *E. bareillyi* | piriform | 23-29 x 16-22 |
| *E. gokaki* | ovoidal | 22-32 x 18-25 |
| *E. ovoidalis* | ovoidal | 32-40 x 20-28 |
| *E. ahsata* | ellipsoidal | 29-37 x 17-28 |
| *E. crandallis* | ellipsoidal | 17-23 x 17-22 |
| *E. faurei* | ovoidal | 25-36 x 19-28 |
| *E. gonzalezi* | ellipsoidal | 26-38 x 20-26 |
| *E. granulosa* | ellipsoidal | 22-35 x 17-25 |
| *E. intricata* | ellipsoidal | 39-53 x 27-34 |
| *E. ovina* | ellipsoidal | 23-36 x 16-24 |
| *E. parva* | subspherical | 12-23 x 10-19 |
| *E. punctata* | ellipsoidal | 18-25 x 16-21 |
| *E. arloingi* | ellipsoidal | 17-42 x 13-27 |
| *E. christenseni* | ovoidal | 34-41 x 23-28 |
| *E. hirci* | round | 18-23 x 14-19 |

**Table 2.16 Basic morphology of Eimeria parasitic in domestic equids and camelids**

| Species | Oocyst shape | Size (µm) |
|---|---|---|
| *E. leuckarti* | piriform | 75-88 x 50-59 |
| *E. solipedum* | spherical | 15-28 x 15-28 |
| *E. uniungulati* | oval-ellipsoidal | 15-24 x 12-17 |
| *E. bactriani* | subspherical | 32 x 25-27 |
| *E. cameli* | oval | 81-99 x 63-94 |
| *E. dromedari* | ovoidal | 23-33 x 20-25 |
| *E. pellerdyi* | ellipsoidal | 22-24 x 12-13 |
| *E. rajasthani* | ellipsoidal | 34-39 x 25-27 |
| *E. alpacae* | ellipsoidal | 22-26 x 18-21 |
| *E. lamae* | ovoidal | 30-40 x 21-30 |
| *E. macusaniensis* | ovoidal | 81-99 x 61-80 |
| *E. peruviana* | ovoidal | 28-37 x 18-22 |
| *E. punoensis* | ellipsoidal | 17-22 x 14-18 |

**Table 2.17** Basic morphology of *Eimeria* parasitic in domestic pigs

| Species | Oocyst shape | Size (µm) |
|---|---|---|
| *E. debliecki* | ellipsoidal | 18-24 x 15-20 |
| *E. guevarai* | piriform | 26-32 x 15-19 |
| *E. neodebliecki* | ellipsoidal | 17-26 x 13-20 |
| *E. perminuta* | ovoidal | 11-16 x 10-13 |
| *E. polita* | ellipsoidal | 22-31 x 17-22 |
| *E. porci* | ovoidal | 18-27 x 13-18 |
| *E. scabra* | ovoidal | 22-36 x 16-26 |
| *E. spinosa* | ellipsoidal | 16-22 x 13-16 |

Oocysts are eliminated into the environment through the feces of the host (figure 2.40 - 17) where they sporulate (figure 2.40 - 18).

**Table 2.18** Basic morphology of *Eimeria* parasitic in domestic rabbits

| Species | Oocyst shape | Size (µm) |
|---|---|---|
| *E. exigua* | subspherical | 10-18 x 9-16 |
| *E. intestinalis* | piriform | 27-32 x 17-20 |
| *E. irresidua* | ovoidal | 31-43 x 22-27 |
| *E. magna* | ovoidal | 31-40 x 22-26 |
| *E. matsubayashii* | ovoidal | 22-29 x 16-22 |
| *E. media* | ovoidal | 27-36 x 15-22 |
| *E. neoleporis* | subcylindrical | 33-44 x 16-23 |
| *E. perforans* | ellipsoidal | 15-29 x 11-17 |
| *E. piriformis* | piriform | 26-32 x 17-21 |

**Table 2.19** Basic morphology of *Isospora* parasitic in domestic mammals

| Species | Oocyst shape | Size (µm) |
|---|---|---|
| *I. suis* | subspherical | 20-24 x 18-21 |
| *I. burrowsi* | ellipsoidal | 17-22 x 16-19 |
| *I. canis* | ellipsoidal | 34-42 x 27-33 |
| *I. neorivolta* | oval | 15-19 x 13-16 |
| *I. ohioensis* | oval | 23-25 x 19-20 |
| *I. felis* | oval | 39-48 x 26-37 |
| *I. rivolta* | oval | 20-25 x 15-20 |

The time needed for sporulation is variable, from species to species and it depends a lot on various environmental factors like temperature and humidity. The average sporulation times for selected species of *Eimeria* and *Isospora* are given in tables **2.20**, **2.21**, **2.22**, **2.23** and **2.24**.

In genus *Isospora*, the general life cycle is similar with the one described for

*Eimeria*. However, in several species infecting carnivores, the presence of paratenic hosts (facultative heteroxenous life cycle) has been reported. In this case, the final hosts (dogs, cats) become infected after preying on infected hosts. Moreover, in species of genus *Isospora* parasitic in carnivores, extraintestinal replication can take place in mesenteric lymph nodes, spleen or liver.

**Table 2.20** Sporulation times of *Eimeria* parasitic in domestic ruminants

| Species | Sporulation time (days) |
|---|---|
| *E. alabamensis* | 4-5 |
| *E. auburnensis* | 2-3 |
| *E. bovis* | 2-3 |
| *E. brasiliensis* | 6 |
| *E. bukidnonensis* | 4-7 |
| *E. canadensis* | 3-4 |
| *E. cylindrica* | 2 |
| *E. ellipsoidalis* | 2-3 |
| *E. pellita* | 10-12 |
| *E. subspherica* | 4-5 |
| *E. wyomingensis* | 5-7 |
| *E. zuernii* | 2-3 |
| *E. ankarensis* | 3-4 |
| *E. bareillyi* | 3-4 |
| *E. gokaki* | 7 |
| *E. ovoidalis* | 4-5 |
| *E. ahsata* | 2-3 |
| *E. crandallis* | 1-3 |
| *E. faurei* | 1-2 |
| *E. gonzalezi* | 5-6 |
| *E. granulosa* | 3-4 |
| *E. intricata* | 3-5 |
| *E. ovina* | 2-4 |
| *E. parva* | 2 |
| *E. punctata* | 1-2 |
| *E. arloingi* | 1-2 |
| *E. christenseni* | 6 |
| *E. hirci* | 2-3 |
| *E. ninakohlyakimovae* | 1-4 |

**Epidemiology.** In general, intestinal eimeriosis in mammals is distributed worldwide. However, some species are known only from certain parts of the world.

**Table 2.21** Sporulation times of *Eimeria* parasitic in domestic equids and camelids

| Species | Sporulation time (days) |
|---|---|
| *E. leuckarti* | 14-21 |
| *E. bactriani* | 10 |
| *E. cameli* | 10-15 |
| *E. dromedari* | 15-17 |
| *E. pellerdyi* | 5 |
| *E. rajasthani* | 7 |

**Table 2.22** Sporulation times of *Eimeria* parasitic in domestic pigs

| Species | Sporulation time (days) |
|---|---|
| *E. debliecki* | 7 |
| *E. guevarai* | 10 |
| *E. neodebliecki* | 13 |
| *E. perminuta* | 9-11 |
| *E. polita* | 8-9 |
| *E. porci* | 9 |
| *E. scabra* | 9-12 |
| *E. spinosa* | 10-12 |

**Table 2.23** Sporulation times of *Eimeria* parasitic in domestic rabbits

| Species | Sporulation time (days) |
|---|---|
| *E. intestinalis* | 1-2 |
| *E. irresidua* | 2 |
| *E. magna* | 2-3 |
| *E. matsubayashii* | 1-2 |
| *E. media* | 2 |
| *E. neoleporis* | 2-3 |
| *E. perforans* | 3-5 |
| *E. piriformis* | 1-2 |

**Table 2.24** Sporulation times of *Isospora* parasitic in domestic animals

| Species | Sporulation time (days) |
|---|---|
| *I. suis* | 3-5 |
| *I. canis* | 2 |
| *I. neorivolta* | 2 |
| *I. ohioensis* | 4 |
| *I. felis* | 2 |
| *I. rivolta* | 4 |

It is beyond the scope of this textbook to provide detailed information on the

distribution of each mammalian *Eimeria* or *Isospora* species. Generally, they are more prevalent in areas with warmer and humid climate (i.e tropics, subtropics). Usually, infection is present in all age groups, at lower intensities in adult animals which are asymptomatic carriers and a permanent source of infection for young animals. Severity of the disease is exacerbated by various stress factors like overcrowding, poor nutrition, post-weaning stress, transportation etc. The disease is hence more common and more severe in farmed animals than in backyard ones.

In ***cattle*** the disease occurs all over the world. It causes huge economic losses. Mortality can reach up to 50%. Infection is rare in suckling calves. The most affected age group is 9-12 months.

In ***sheep and goats***, the disease is very common, with moderate to high mortality in young animals. It is distributed worldwide and responsible for important economic losses. The most susceptible age is 6-12 weeks, usually in the first few weeks after the first contact with the pasture. Outbreaks are acute, and they usually affect a substantial part of the flock.

In ***horses***, the infection with *Eimeria* is sporadic, and it has been reported so far only Europe and parts of Asia. All species can infect horses, donkeys and their hybrids.

The infection in ***pigs*** is very common, although rarely symptomatic. Suckling piglets acquire the infection from the skin around the mammary glands.

Intestinal *Eimeria* of ***domestic rabbits*** is extremely common. Virtually all rabbits are infected. Coprophagia has been incriminated as a cause for such a high prevalence. The rabbits are susceptible from the age of 16 days.

In ***dogs and cats***, younger individuals are more commonly affected. Cats under four years are at increased risk. Purebred animals are more susceptible.

The resistance of oocysts in the environment is normally high, with specific limits for each species. In general, Oocysts survive up to one year at temperatures between -30°C and +40°C. Prolonged freezing and direct sun light kill most oocysts on the pastures.

**Pathogenesis.** It is believed that the severity of symptoms is correlated with the relative length of the intestine and the potential number of host cells. This is why species affecting the large intestine or specific portions of the small intestine (which have rather low lengths) are usually more pathogenic than those with less selective histotropism. Moreover, the large intestine has a much lower turn-over rate of the epithelial cell population and as consequence, a lower regenerative potential.

The pathogenic effect is mostly caused by the direct damage of the enterocytes during the merogonic phase of the life cycle. Hence, the acute form of the disease is during parasitic merogony. This has a practical impact on the diagnosis, as during the acute diarrhea the typical oocysts are absent from the feces.

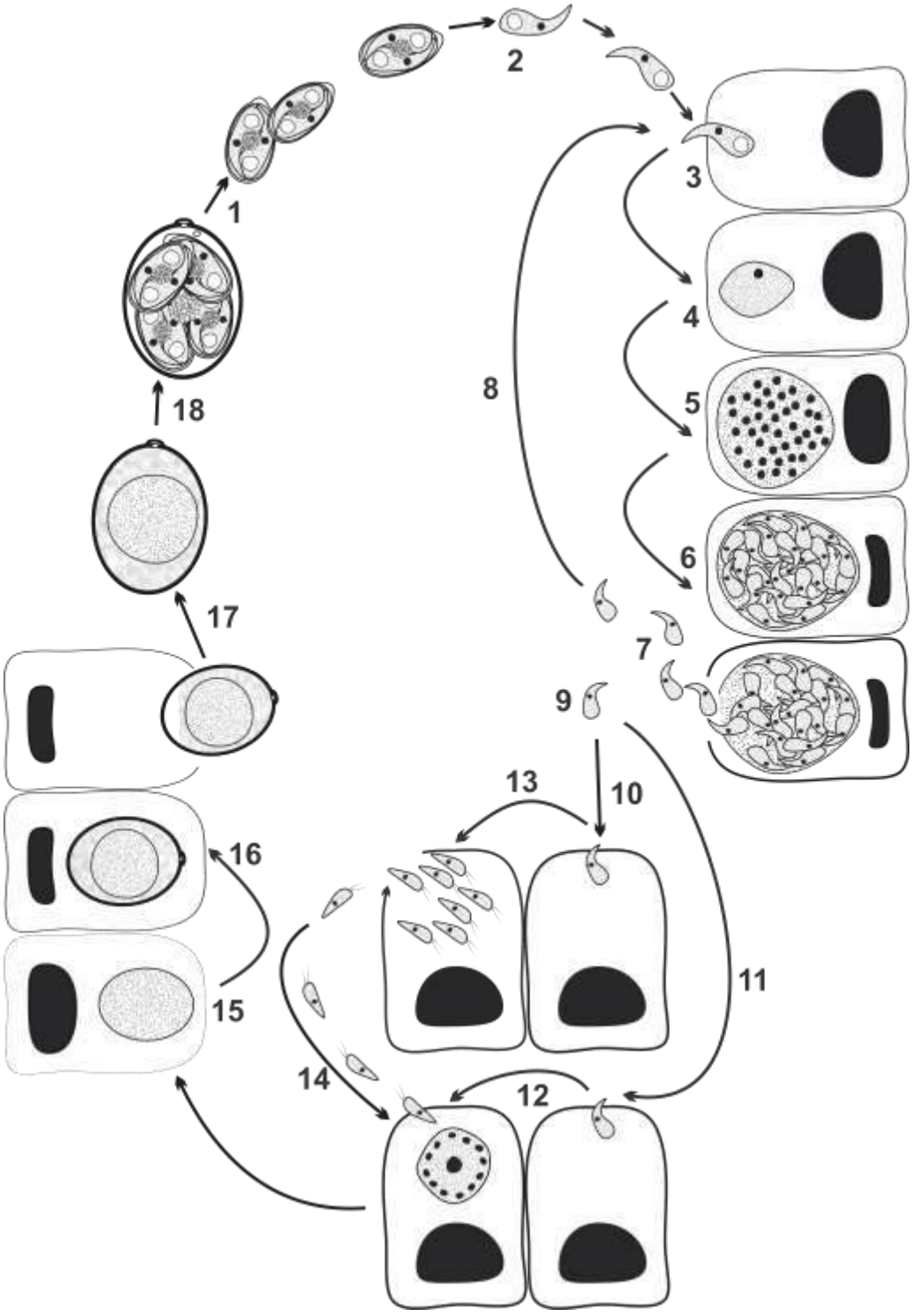


**Figure 2.40** Life cycle of genus *Eimeria*. For the meaning of numbers, please refer to the text.

Denudation of the intestine with the destruction of the epithelial lining results in intestinal hemorrhage, reduced water resorption with consecutive diarrhea. Chronic infections with catarrhal inflammation are responsible for malabsorption and various consequent nutritional deficiencies.

**Immunology.** As only young animals are susceptible and stress is a favoring factor it is evident that the immune system has a well-established role in the active protection against coccidial infection. The main problem with post-infective immune protection is the lack of cross-immunity between the different species of *Eimeria* infecting certain hosts.

There are no extensive studies on all species of *Eimeria*. However, some aspects can be concluded based on several experimental studies. It is thought that certain species are highly immunogenic and the infection with very few oocysts (less than 10) can induce a strong immunity.

Both components of the immune system are involved in the anti-eimerial protection. IgG antibodies and lymphocytes are responsible for protection. A strong immune response can be detected at around 14 days after the initial infection.

**Clinical signs.** Despite the diversity of etiological agents in the various domestic hosts, the clinical signs of coccidiosis are rather uniform. In adult animals the infection is usually asymptomatic. Clinical signs in young animals are predominantly digestive, with diarrhea, dehydration, anemia and weight loss. The diarrhea is catarrhal in the beginning and hemorrhagic during the merogonic development of the coccidia.

In ***bovines*** the most common species involved in clinical cases are *E. zuernii* and *E. bovis*. The first two days of clinical infection are characterized by catarrhal diarrhea, followed by hemorrhagic discharges. Other symptoms reported in cattle include: anorexia, accelerated respiration, convulsions, emaciation and tremors. Fever is rarely present. In dairy cows, milk production might be affected. Cows with chronic infection display intermittent/recurrent diarrhea.

Eimeriosis of ***sheep*** is a very severe parasitic problem, with significant mortality in lambs and less severe in ***goats*** (kids). Lambs with acute infections show a profuse diarrhea, often hemorrhagic, with almost liquid feces, which lasts several days. Debilitated lambs become weak, they cease eating and some die.

In ***horses***, the infection is usually asymptomatic. If clinical cases occur in foal, they are generally mild, with moderate and self-limiting diarrhea.

Coccidiosis in ***pigs*** is rarely a clinical problem. Diarrhea is not usually hemorrhagic. More severe cases show loss of appetite, emaciation and retarded growth. Mortality is very rare.

The main symptoms in ***domestic rabbits*** are diarrhea and bloating. Mortality is high, sometimes even superacute, without any prodromal signs.

In ***dogs and cats***, diarrhea is uncommon, unless associated with

immunosuppressive condition or concurrent infection. In kennels, outbreaks can be enzootic.

**Pathology.** The main lesions are located in the intestine. Generally, they consist in an inflammation of the intestinal epithelium (enteritis). Their severity and location depends on the age of the animal and the species of coccidia involved. Some species are located deeply, under the lamina propria of the enterocyte lining and are responsible for hemorrhagic lesions. Some other are more superficial and the lesions are catarrhal. Often, whitish spots are easily visible on the intestinal surface. They represent the merogonic stages of the eimerids.

In ***cattle***, the body of animals which died of acute eimeriosis is weak, with the posterior parts (perianal area, ventral surface of the tail) soiled with red-colored feces. In the abdominal cavity there are small amounts of reddish exudate. The body is generally anemic, with pale colored organs. Mesenteric blood vessels are hyperemic. The most characteristic lesions are at the level on intestinal mucosa. The luminal surface of the intestines is coated with reddish mucus, congestion is present and the mucosa is congested, mainly in the terminal part of the ileum and in the large intestine (cecum and colon). The intestinal content is liquid and malodorous. The hemorrhages on the intestinal mucosa can be punctiform or confluent.

In ***sheep and goats***, the lesions are different depending on the causative species. In general they consist of petechial hemorrhages and necrotic enteritis with edema of the intestinal wall.

The lesions ***in horses*** were described after experimental infection. They consist in catarrhal enteritis, with whitish foci easily visible at gross examination.

In ***pigs***, the gross lesions consist in catarrhal enteritis mainly in the large intestine.

***Domestic rabbits*** show catarrhal enteritis, with whitish deposits.

In ***dogs and cats***, lesions are usually catarrhal, rarely hemorrhagic.

**Diagnosis.** Based on clinical signs, diagnosis must be confirmed microscopically. The presence in the samples of any of the developmental stages of *Eimeria* or *Isospora* has diagnostic value. During the acute stage of infection, when the life cycle has barely reached its merogonic or gametogonic phases, the oocysts (which appear after the gametogony) are absent from the feces. During this clinical stage, other developmental forms can be detected in the feces (e.g. meronts). Examination of large number of samples from the same flock can be helpful. For the detection of most *Eimeria* and *Isospora* oocysts, flotation methods are recommended. In certain *Eimeria* species (i.e. *E. leuckarti* from horses) better results are obtain using sedimentation.

The oocysts from feces are normally not sporulated (**figure 2.41**), thus their specific identification is impossible at this stage. For detailed morphological studies and specific diagnosis, they can be

sporulated in the laboratory at room temperature, using a 2.5% solution of potassium dichromate in the presence of oxygen. In older fecal samples, the oocyst can begin the sporulation process and they are found in various stages (**figure 2.42**).

The presence of oocysts in the feces of ruminants, horses, and rabbits normally means genus *Eimeria*. In swine, both genera can be present (*Eimeria* and *Isospora*). The most controversial coproscopic diagnosis is in dogs and cats, as domestic carnivores pass in feces oocysts of several species of intestinal coccidia: *Isospora*, *Toxoplasma*, *Neospora*, *Hammondia* and *Besnoitia*. Usually, the differentiation should be made on the basis of size. In dogs, "*Isospora*"-like oocysts which are larger than 15 µm are with high probability members of genus *Isospora*. If they are smaller, they belong to *Neospora caninum* (10-12 µm) or *Hammondia heydorni* (11-12 µm).

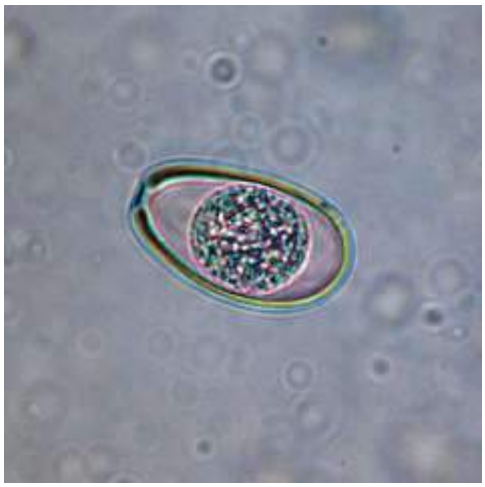

**Figure 2.41** Unsporulated oocyst of *Eimeria* sp. from the feces of a domestic rabbit. *(Photo Andrei D. Mihalca)*

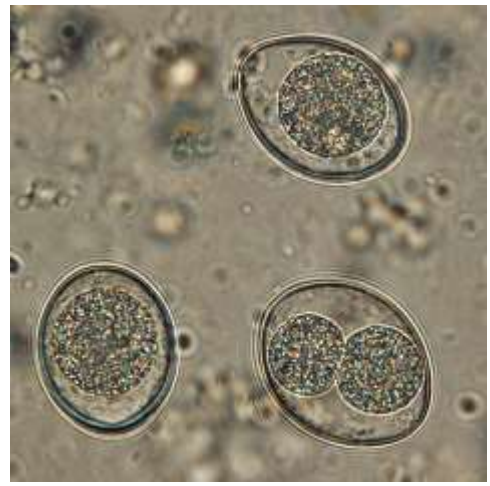

**Figure 2.42** Unsporulated oocyst of *Isospora* sp. from the feces of a cat. Note the oocyst beginning the sporulation process (lower right). *(Photo Andrei D. Mihalca)*

In cats, oocysts larger than 20 µm are likely to be *Isospora*, while those smaller than 12 µm are one of the heteroxenous species (*Toxoplasma gondii*, *Hammondia hammondi*, *Besnoitia darlingi* or *Besnoitia oryctofelisi*).

However, *Besnoitia wallacei* also reported in cats has slightly larger oocysts than its congeners (17 x 12 µm).

Post-mortem diagnosis is achieved by direct examination of the intestinal content from the parts with lesions or by histopathology, when all developmental stages can be identified. For dogs and cats, differentiation of small oocysts can be achieved also by PCR.

**Treatment.** The number of available drug used for the treatment of coccidial infections in mammals is huge. They are known generically as anticoccidial drugs.

**Table 2.25** Drugs used for the treatment of coccidiosis in domestic mammals

| Drug | Species | Dose (mg/kg) | Route | Duration (days) |
|---|---|---|---|---|
| Sulfadimetoxine | cats, dogs | 50-60 | PO | 5-20 |
| | sheep | 50-100 | PO | 5 |
| Sulfadimidine | cattle | 140 | PO | 3 |
| | sheep | 25-50 | PO | 3 |
| Sulfaguanidine | cats, dogs | 100-200 | PO | 5 |
| | sheep | 250 | PO | 7 |
| | swine | 200 | PO | 3-4 |
| Sulfamethazine | cattle | 110 | PO | 5 |
| | sheep | 60 | PO | 3-5 |
| Sulfaquinoxaline | cattle | 6 | PO | 3-5 |
| | sheep | 60 | PO | 3-5 |
| Trimethoprim-sulfonamide | dogs | 30-60 | PO, SC | 5 |
| | cats, dogs | 15-30 | PO, SC | 5 |
| Ormetoprim-sulfadimethoxide | dogs | 66 | PO | 7-23 |
| Furazolidone | cats, dogs | 8-20 | PO | 5 |
| Amprolium | cats, dogs | 300-400 | PO | 5 |
| | cats, dogs | 110-200 | PO | 7-12 |
| | cats | 60-100 | PO | 7 |
| | cattle | 10 | PO | 5 |
| | cattle | 65 | PO | 1 |
| | sheep | 50-65 | PO | 4-7 |
| | swine | 25-65 | PO | 3-4 |
| | rabbits | 25 | PO | 4-5 |
| Quinacrine | cats, dogs | 10 | PO | 5 |
| Clindamycin | cats | 10 | PO, SC, IM | 7-28 |
| Toltrazuril | dogs | 15-30 | PO | 1-6 |
| | cattle | 15 | PO | 1 |
| | sheep | 20-40 | PO | 1 |
| | rabbits | 20 | PO | 1-3 |
| Diclazuril | cats | 25 | PO | 1 |
| Ponazuril | dogs | 30-50 | PO | 1-7 |
| | cats | 15 | PO | 7 |
| Monensin | cattle | 1 | PO | 10 |
| | sheep | 1.5 | PO | 21 |

Treatment must be implemented for treating animals with clinical symptoms or to eliminate asymptomatic carriers, as a control measure. Drugs can be used as chemoprophylaxis.

A check-list of available drugs and their dosage for various domestic mammals is given in **table 2.25**.

**Control.** The most efficient way for controlling coccidiosis in mammals is prevention. This can be achieved by using general measures and in the animals exposed to risk, chemoprophylaxis. The most important factor for avoiding the onset of clinical disease is to maintain a good overall health status in young animals. Neonates should be given colostrum. Proper environmental conditions, microclimate, a balanced nutrition and avoidance of overcrowding are essential factors. Moreover, sanitation, disinfections, use of clean water and watering devices are also important.

Unlike in birds, the use of chemoprophylaxis is not compulsory only when outbreaks are imminent (i.e. previous history of disease, introduction of new animals). In principle, the same molecules which are used for treatment can be used for chemoprophylaxis, but in lower doses and long-term administration. In ruminants, the use of decoquinate and ionophores was shown to be effective. During the first month in calves exposed to infection, lasalocid, monensin or amprolium are efficient. In pigs, sulfonamides and amprolium were successfully used.

### 2.4.1.2 Intestinal eimeriosis in birds

**Introduction.** *Eimeria* infections in poultry, mainly in chicken, are among the most important diseases in industrial farms. They have a huge economical dimension, mainly by expenses with the prophylaxis. The estimated annual cost related to poultry eimeriosis is situated around 2 billion euros.

**Historical notes.** The first description of a coccidian life cycle from birds came in 1910, when Fantham published his work on red grouse. In 1929, Tyzzer described the life cycle for several species of *Eimeria* parasitic in chicken (*E. acervulina*, *E. mitis, E. maxima*, *E. tenella*). The first anticoccidial drugs have been introduced in 1948, when FDA approved in USA the use of sulphaquinoxaline and nitrofurazone. The first commercial vaccine was introduced in 1952.

**Etiology.** There are ten species of coccidia parasitic in chicken (**table 2.26**). Not only they are host specific, but they have different tropism for various segments of the intestine (**figure 2.43**). Other species of *Eimeria* parasitic in birds are shown in **tables 2.27**, **2.28** and **2.29**.

**Morphology.** General morphology of *Eimeria* parasitic in poultry is concordant with genus characteristics, discussed above. Specific morphology for selected species parasitic in chicken is given in **table 2.30**. No data will be provided for species parasitic in other domestic bird species.

**Table 2.26** Species of genus *Eimeria* parasitic in chicken

| Species | Intestinal segment |
|---|---|
| *E. acervulina* | Duodenum |
| *E. brunetti* | Large intestine |
| *E. dispersa* | Small intestine |
| *E. hagani* | Duodenum |
| *E. maxima* | Small intestine |
| *E. mitis* | Duodenum |
| *E. mivati* | Duodenum |
| *E. necatrix* | Small intestine |
| *E. praecox* | Duodenum |
| *E. tenella* | Cecum |

**Life cycle.** The life cycle follows the same phases and developmental stages as for species parasitic in mammals. In birds, the life cycle is somehow faster that in species parasitic in mammals. For some species (i.e. *E. tenella*) the entire development takes 4-6 days.

**Epidemiology.** The disease is present everywhere where the hosts are presents. Their distribution is hence global. As the parasitism is strictly host-specific, the only sources of infection are conspecific birds.

**Table 2.27** Species of genus *Eimeria* parasitic in other galliformes

| Species | Host |
|---|---|
| *E. adenoeides* | Turkey |
| *E. dispersa* | Turkey |
| *E. gallopavonis* | Turkey |
| *E. innocua* | Turkey |
| *E. meleagridis* | Turkey |
| *E. meleagrimitis* | Turkey |
| *E. subrotunda* | Turkey |
| *E. gorakhpuri* | Guinea fowl |
| *E. grenieri* | Guinea fowl |
| *E. numidae* | Guinea fowl |
| *E. colchici* | Pheasant |
| *E. dispersa* | Pheasant |
| *E. duodenalis* | Pheasant |
| *E. langeroni* | Pheasant |
| *E. megalostomata* | Pheasant |
| *E. pacifica* | Pheasant |
| *E. phasiani* | Pheasant |
| *E. mandali* | Peacock |
| *E. mayurai* | Peacock |
| *E. patnaiki* | Peacock |
| *E. pavonina* | Peacock |
| *E. pavonis* | Peacock |

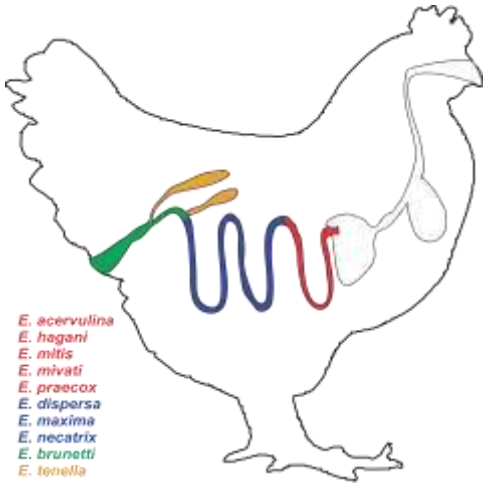


**Figure 2.43** Location of *Eimeria* species in chicken.

As a consequence, unlike in other bacterial or viral disease of poultry, wild birds are not infection sources. The most important way for spreading the coccidia in poultry farms is mechanical, by personnel.

**Table 2.28** Species of genus *Eimeria* parasitic in ducks and geese

| Species | Host |
|---|---|
| *E. abramovi* | Duck |
| *E. anatis* | Duck |
| *E. battakhi* | Duck |
| *E. boschadis* | Duck |
| *E. danailovi* | Duck |
| *E. mulardi* | Duck |
| *E. nottion* | Duck |
| *E. saitamae* | Duck |
| *E. schachdagica* | Duck |
| *E. anseris* | Goose |
| *E. fulva* | Goose |
| *E. hermani* | Goose |
| *E. kotlani* | Goose |
| *E. magnalabia* | Goose |
| *E. nocens* | Goose |
| *E. stigmosa* | Goose |
| *E. striata* | Goose |

**Table 2.29** Species of genus *Eimeria* parasitic in pigeons

| Species | Host |
|---|---|
| *E. columbae* | Pigeon |
| *E. columbarum* | Pigeon |
| *E. kapotei* | Pigeon |
| *E. labbeana* | Pigeon |

**Table 2.30** Basic morphology of *Eimeria* parasitic in chicken

| Species | Oocyst shape | Size (µm) |
|---|---|---|
| *E. acervulina* | ovoidal | 17.7-20.2 x 13.7-16.3 |
| *E. brunetti* | ovoidal | 20.7-30.3 x 18.1-24.2 |
| *E. maxima* | ovoidal | 21.5-42.5 x 16.5-29.8 |
| *E. mitis* | subspherical | 11.7-18.7 x 11.0-18.0 |
| *E. mivati* | ellipsoidal | 11.1-19.9 x 10.5-16.2 |
| *E. necatrix* | ovoidal | 13.2-22.7 x 11.3-18.3 |
| *E. praecox* | ovoidal | 19.8-34.7 x 15.7-19.8 |
| *E. tenella* | ovoidal | 19.5-26.0 x 16.5-22.8 |

There is a clear age predisposition. The most sensitive age is variable in each *Eimeria* species. For instance in the case of the most commonly occurring species ***in chicken***, *E. tenella* (cecal eimeriosis), the most affected age is 5-7 days in birds are exposed to high infective doses immediately after hatching. Normally, cecal eimeriosis appears at 21-25 days. Cecal coccidiosis is also among the most severe forms of disease, with an acute onset and high mortalities (50-100%) in few days, if not treated. Infection with *E. necatrix* produces clinical infection in 5-7 weeks old chicken, and the onset and evolution is slower. Mortality is much lower. Infections with *E. brunetti* are rare but the onset is very fast. *Eimeria acervulina* affects mainly older chicken and even adults, while *E. maxima* is responsible for coccidiosis in laying hens.

***Turkeys*** are less sensitive to clinical eimeriosis, and outbreaks are less severe than in chicken. The most susceptible age is 6-8 weeks. ***In ducks and geese***, the infection is sporadic. ***In pigeons***, the infection is common and mortality is high (15-70%) at the age of 3-4 months.

Resistance of oocysts in the environment is relatively high. Alternation of freezing-defreezing or direct sunlight exposure kills them rapidly. However, the most important aspect regarding oocyst resistance is within the microenvironment of the farm. Common disinfectants have limited efficacy on the oocysts. Most of them inhibit sporulation; hence unsporulated oocysts are more sensitive.

**Pathogenesis.** Some species are more pathogenic than others (see symptoms and lesions below). The pathogenicity is also host-dependent (age, immune status, species) and is influenced by the presence of concurrent infections. Although the lesions are generally local, they affect the whole organism.

The most important pathogenic effect is due to destruction of enterocytes and other associated tissues (e.g. lamina propria, submucosal layers, blood vessels) by the merogonic development. Massive destructions are responsible for intestinal hemorrhages. Intestinal tissue damage results in motility disorders, altered absorption of nutrients, decreased water resorption, malnutrition etc. Their severity is variable and depends on the surface of damaged epithelium. Epithelial destruction allows undisturbed access of other pathogens (mainly bacteria) to the blood stream and tissues. Chronic infections, although not life-threatening produce long-term debilities (i.e. rickets).

In order to assess the severity of infection, mostly in experimental trials for testing anticoccidial drugs or vaccines, a scoring system has been developed (please refer for details to Conway and McKenzie (2007).

**Immunology.** The knowledge on the immunology of eimeriosis in chicken is very extensive, as the quest for safer and more ecological preventive measures (i.e. vaccination) is permanent. There is an evident acquired immunity, as adult birds are non-receptive to clinical infection and the development in their intestine is self-limiting. Local intestinal cellular immunity from the associated lymphoid tissues are responsible for the post-

infective resistance. B cells produce anti-*Eimeria* antibodies shortly after the debut of infection. However, their protective role is limited. The most important components seem to be intraepithelial and lamina propria cytotoxic T lymphocytes.

**Clinical signs**. Clinical signs are mainly digestive and certainly not characteristic.

Clinical signs ***in chicken*** range from asymptomatic infections in adult birds to mild, moderate or severe disease in young birds. Clinical eimeriosis in chicken varies from chronic forms with decreased growth rate to severe diarrhea, often with high and fast mortality. There are some differences in the clinical signs between *Eimeria* species involved. In the case of cecal eimeriosis (caused by *E. tenella*), the diarrhea is watery and contains often hemorrhagic droppings. As a consequence, chicken are anemic and may exhibit even nervous signs. Hemorrhagic feces can be present also in infections caused by other species of *Eimeria*, but they are not as severe as in the case of *E. tenella*. In the case of *E. brunetti*, *E. necatrix*, *E. acervulina*, *E. maxima* or *E. mivati*, the hemorrhage in the feces is weaker and it appears as discrete streaking on the droppings. Moreover, in these later species, the feces are rarely watery, or even with normal consistence and soaked with slightly hemorrhagic mucus.

Sometimes the infection with *Eimeria* in chicken does not result in changes in the consistence of feces, nor other evident symptoms. However, even low levels of infection can induce low weight gain or decreased feed conversion rate.

The most pathogenic species ***in turkeys*** is *E. adenoides*. The feces are liquid and may be coated with hemorrhagic mucus. Mortality can be present if infective doses are high. Chronic infections are responsible for significant weight loss.

***In ducks and geese***, the signs of infection with *E. anseris* are: anorexia, weight loss, general weakness, distress, diarrhea and even mortality.

***In pigeons***, the common signs of infection are greenish diarrhea, anorexia, dehydration and extreme weight loss. The feces may be hemorrhagic. Chronic eimeriosis can cause mineral deficiencies (**figure 2.44**).

**Figure 2.44** Typical "S" shaped keel as a result of secondary calcium deficiency in a pigeon suffering from chronic eimeriosis. *(Photo Andrei D. Mihalca)*

**Pathology.** The lesions in poultry coccidiosis are different for the various species of *Eimeria* involved. Their location and gross aspect are of great

diagnostic value, some almost pathognomonic. In cecal coccidiosis (*E. tenella*) the most prominent finding is a severe hemorrhagic typhlitis (**figure 2.45**), with a dilated cecum (**figure 2.46**) containing liquid or partially clotted blood.

The extent of the lesion might be reduced to some patches which in severe forms are confluent and affect both ceca, totally. Sometimes the lesions consist in hemorrhagic-necrotic typhlitis or even with the presence of fibrinous material.

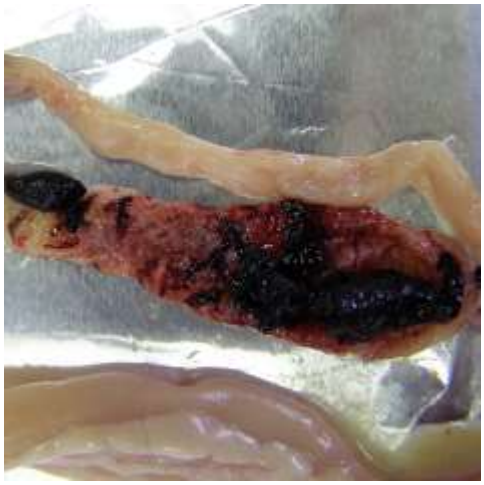

**Figure 2.45** Hemorrhagic typhlitis caused by *Eimeria tenella* in chicken. *(Photo Adriana Györke)*

The infection with *E. necatrix* is responsible for intestinal congestion, with pinhead-sized hemorrhagic spots (**figure 2.47**). In severe cases, these spots become confluent and the middle part of the small intestine becomes entirely hemorrhagic (**figure 2.48**). Sometimes the lesions are fibrinous or necrotic (**figure 2.49**).

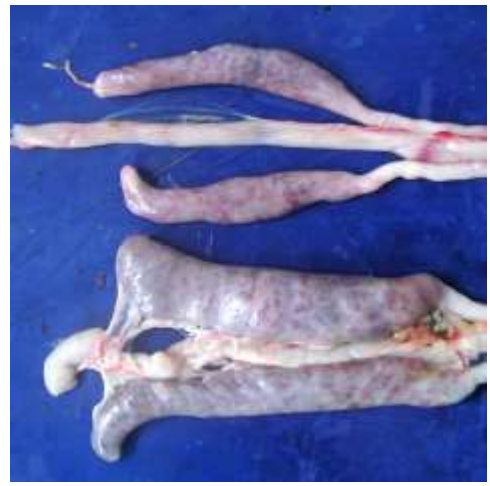

**Figure 2.46** Dilated ceca with focal hemorrhagic typhlitis caused by *Eimeria tenella* in chicken. *(Photo Adriana Györke)*

The same situation is encountered in the infection with *E. brunetti*, but the punctiform hemorrhages are located in the rectum and cloaca. Necrosis of the rectal wall may be present.

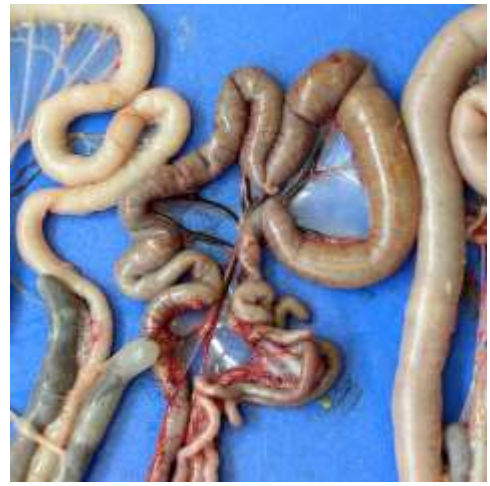

**Figure 2.47** Small intestine enteritis caused by the infection with *E. necatrix*. *(Photo Adriana Györke)*

**Figure 2.48** Severe hemorrhagic enteritis caused by the infection with *E. necatrix*. *(Photo Adriana Györke)*

*Eimeria maxima* is responsible for hemorrhagic enteritis in the anterior and medial segment of the small intestine, very clearly delimited from the healthy contiguous intestinal segments.

*Eimeria acervulina* and *E. mivati* produce more or less similar lesions, with focal (petechial) hemorrhagic enteritis in the small intestine with the presence of whitish colonies.

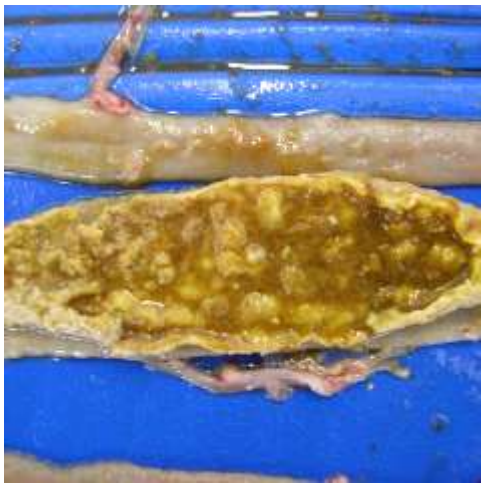

**Figure 2.49** Fibrinous-necrotic enteritis caused by the infection with *E. necatrix*. *(Photo Adriana Györke)*

The lesions in other bird species vary from catarrhal enteritis to various degrees of hemorrhagic enteritis (focal or generalized) or fibrinous-necrotic inflammations.

**Diagnosis.** Based on clinical signs and necropsy, the etiology must be confirmed in the laboratory. This is done by direct identification of various stages of *Eimeria* in the feces of living birds or in the lesions in the case of dead birds (**figures 2.50, 2.51, 2.52, 2.53**).

Please refer to the diagnosis section from the previous Chapter (2.4.1.1) on mammal coccidiosis.

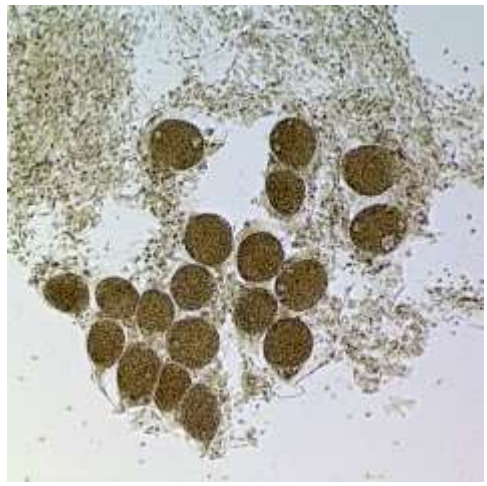

**Figure 2.50** Meronts of *Eimeria* sp. in wet mount from intestinal lesions of chicken. *(Photo Adriana Györke)*

**Treatment.** The disease in chicken is very severe. Treatment in large bird communities (farms) is most often impossible, mainly because of logistic reasons.

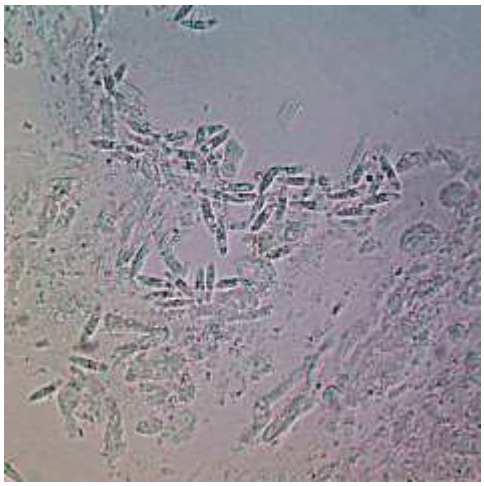

**Figure 2.51** Merozoites of *Eimeria* sp. in wet mount from intestinal lesions of chicken. *(Photo Adriana Györke)*

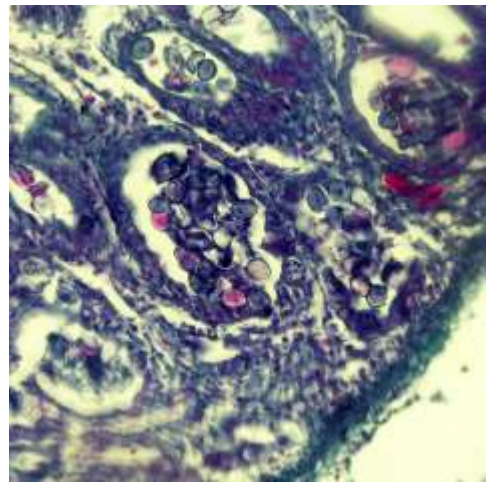

**Figure 2.53** Various developmental stages of *Eimeria* sp. in histological section from intestinal lesions of chicken. *(Photo Andrei D. Mihalca)*

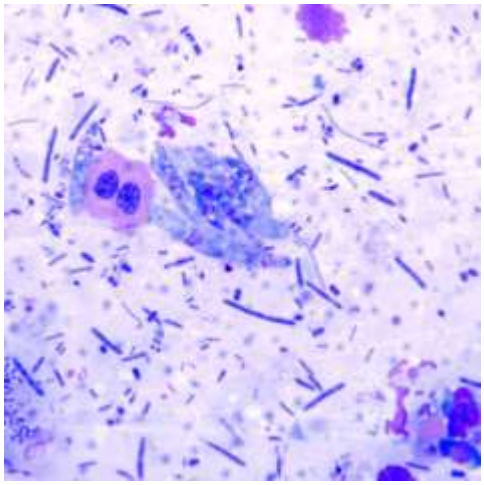

**Figure 2.52** Merozoites of *Eimeria* sp. in stained smear from intestinal lesions of chicken. *(Photo Adriana Györke)*

Even if the parasites are successfully eliminated using specific anticoccidial medication, the lesions do not heal fast enough in order to allow full recovery.

Several drugs have been used in chicken in traditional (backyard), free-range systems. Sulfaquinoxaline (125 mg/kg) or Sulfadimidine (150-200 mg/kg) for 3-5 days are effective against *E. tenella*. In pigeons, sulfonamides and clazuril are used in the drinking water.

**Control.** Prevention of eimeriosis in chicken is crucial in the poultry industry. It represents the only viable solution for controlling outbreaks. Otherwise, any clinical outbreaks are equivalent with economic disaster.

General hygiene methods and specific methods are used for controlling eimeriosis in birds.

***General measures*** include: maintaining the general health status of birds at optimal levels, decreasing the level of stress, use of proper and balanced diet, optimal environment, avoiding of overcrowding, good lighting and

ventilation and general biosecurity measures (strict access control, cleaning, disinfections).

Specific measures include ***chemoprophylaxis*** (with low and long term/continuous administration of anticoccidial drugs) and ***immune prophylaxis*** (using vaccines) or an alternation of them.

Various ***anticoccidial drugs*** are currently being used in the chemoprophylaxis of eimeriosis in chicken. They are grouped in two categories, based on their chemical structure (classification based on Peek and Landman, 2011).

***(1) Synthetic compounds***

- amprolium (competes for the absorption of vitamin $B_1$ by the parasites);
- aprinocid (interferes with DNA synthesis);
- clopidol (inhibits development of sporozoites);
- decoquinate (inhibits development of sporozoites);
- diclazuril (blocks the excretion of oocysts);
- dinitolmide (inhibits development of meronts);
- halofuginone (inhibits maturation of merozoites);
- nequinate;
- nicarbazin (inhibits development of meronts)
- robenidine (inhibits development of meronts).

***(2) Polyether antibiotics or ionophores***

Their mechanism of action is by interfering with the metabolism of sodium and potassium. Three types are known:

- monovalent ionophores (monensin, narasin, salinomycin);
- monovalent glycosidic ionophores (maduramicin, semduramycin);
- bivalent ionophores (lasalocid).

Ionophores cannot be associated with certain other antibiotics (e.g. tiamulin, chloramphenicol, erythromycin, oleandromycin, sulphonamides).

Additionally, there are various commercial products where a combination of the aforementioned products is used. The poultry category and the concentration to be given to chicken in food for each drug is listed in **table 2.31**.

The main problem when using chemoprophylaxis is the emergence of resistant strains. In the case of chicken eimeriosis, resistance is known for all compounds and it has been reported worldwide. Despite this, severe outbreaks are occasional, as the insidious presence of *Eimeria* due to resistance stimulates immunity.

To reduce the impact and occurrence of resistance, rotation of anticoccidial drugs is recommended. Each drug should be used for maximum 2 months, or in broilers, for maximum two fattening cycles.

**Table 2.31** Anticoccidials used in the prophylactic treatment of eimeriosis in chicken (after Peek and Landman, 2011)

| Drug | Poultry category | Concentration in fodder (ppm) |
|---|---|---|
| Amprolium | Broiler, rearing | 125-250 |
| Amprolium+ ethopabate | Broiler, rearing | 125-250+ 4 |
| Aprinocid | Broiler | 60 |
| Clopidol | Broiler, rearing | 125 |
| Decoquinate | Broiler | 30 |
| Diclazuril | Broiler, rearing | 1 |
| Dinitolmide | Broiler, rearing | 125 |
| Halofuginone | Broiler, rearing | 3 |
| Nequinate | Broiler, rearing | 20 |
| Nicarbazin | Broiler | 125 |
| Robenidine | Broiler | 33 |
| Lasalocid | Broiler | 75-125 |
| Maduramicin | Broiler | 5-6 |
| Monensin | Broiler, rearing | 100-120 |
| Narasin | Broiler | 60-80 |
| Salinomycin | Broiler, rearing | 44-66 |
| Semduramycin | Broiler | 25 |

Shuttle programs (simultaneous use of two or more drugs with different mechanism of action during the same technologic cycle) are also recommended.

Before implementing chemoprophylaxis programs, it is highly recommended to perform in vivo Anticoccidial Sensitivity Tests, using local strains.

New approaches to the management of anticoccidial drug resistance include the rotation of chemoprophylaxis with immune-prophylaxis (vaccination).

There are several types of ***vaccines*** available today.

***(1) Subunit vaccines*** contain antigens (either native or recombinant proteins expressed from DNA) of different developmental stages (sporozoites, merozoites, gametogonic stages). There is a single subunit commercial vaccine available (CoxAbic). It is used for vaccination of mother hens and immunity is transmitted to broiler chicken via trans-vitelline route.

***(2) Live vaccines*** contain non-attenuated or attenuated sporulated oocysts of various species of *Eimeria*. Two vaccination strategies are employed: single shot (high dose) or multiples shot (low dose). The vaccinal strains might be sensitive to anticoccidial drugs. Thus, discontinuation of these is essential in vaccination strategies are to be implemented.

Non-attenuated vaccines have been used for long time (CocciVac, Inovocox, Advent, Imunocox), but their administration is risky, as they can induce clinical infection if protocols are not followed strictly.

Attenuated vaccines (with decreased virulence obtained by repeated in vitro passages on chicken embryos) are safer but they lose immunogenicity over time and their production is more costly. Examples of attenuated vaccines include Livacox or Paracox.

However, antigenic variability between various *Eimeria* species restrict the commercial value of live vaccines. Some of the vaccines are also available for turkeys (e.g. Coccivac, Immunocox).

### 2.4.1.3 Hepatic eimeriosis in rabbits

**Introduction**. Hepatic eimeriosis is a severe condition of rabbits caused by *E. stiedai*, with high mortality in young animals.

**Historical notes.** *E. stiedai* is probably the first ever unicellular "animal"-like organism ever observed (by Antonie van Leeuwenhoek in 1674). The species was described as *Monocystis stiedae* by Lindemann, in 1865. The first study on its life cycle date from 1903 (Metzner). The first reports of disease date back from the mid-19th century.

**Etiology.** The causative agent of hepatic eimeriosis in rabbits is *Eimeria stiedai*. Except domestic rabbits, *E. stiedai* is parasitic in various wild rabbit and hare species (Lagomorpha): European rabbit (*Oryctolagus cuniculus*), European brown hare (*Lepus europaeus*), Snowshoe hare (*Lepus americanus*), mountain hare (*Lepus timidus*) and Eastern cottontail (*Sylvilagus floridanus*).

**Morphology.** The oocysts are oval or narrow oval, sometimes asymmetrical and they have a micropyle. The size of oocysts is 28-40 x16-25 µm.

**Life cycle.** *Eimeria stiedai* follows the typical life cycle of genus *Eimeria*, as described in Chapter 2.4.1.1. However, there are few particular aspects which require some attention. First of all, their typical habitat is the epithelial lining of biliary ducts. After sporulated oocysts are ingested by rabbits they typically excyst in the duodenum. Sporozoites leave the intestine by penetrating the mucosa and reach the liver via lymphatic system or via blood through the portal, free or in macrophages. The sporozoites reach the sinusoid veins between liver cells 1-6 days after the infection. They subsequently migrate to the biliary ducts and penetrate the epithelial cells where they start the merogony. Merogony takes around 12 days. Gametogony lasts for 4 more days. Unsporulated oocysts appear in feces as early as 16 days after the infection. In the environment, the sporulation takes 2-3 days.

**Epidemiology.** *Eimeria stiedai* is present all over the world, affecting various species of rabbits and hares. Adult rabbits are the source of infection for young rabbits. Wild rabbits can also harbor the infection and contaminate the environment. Contaminated grass fed to rabbits can bring the infection. After elimination through the feces, they become infected in 2-3 days. Rabbits up to two weeks old are resistant to infection. The rabbits are normally receptive to the infection with *E. stiedai* after 16-18 days of life up to 4 months. After this age, they become completely resistant, and clinical cases are normally absent.

**Pathogenesis.** As parasites are invading the biliary ducts and merogonic stages are developing, the epithelial cells are being destroyed. The biliary ducts become distended and filled with cellular debris and nodules appear in the liver parenchyma. These changes are most prominent by day 16, when merogonic gametogonic phases are already complete (**figure 2.54**). If rabbits survive and no massive reinfection takes place, all these lesions heal completely. These lesions are

able to produce significant functional disorders. The main alteration seems to be in the metabolism of vitamin E.

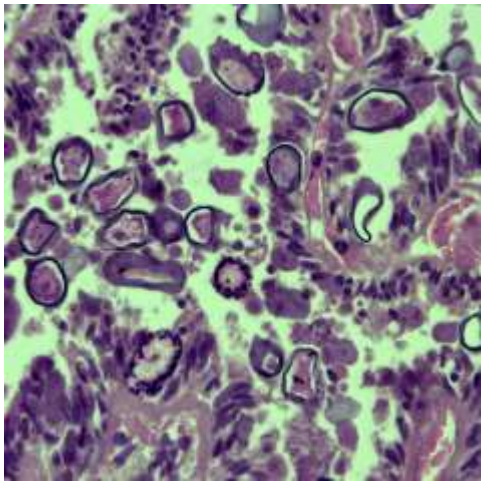

**Figure 2.54** Various developmental stages of *Eimeria stiedai* in histological section rabbit liver. *(Photo Andrei D. Mihalca)*

**Immunology.** All infections, regardless they produce clinical disease or not are ultimately inducing a strong immunity. Experimental data suggest that development of protective immunity requires around 21-30 days. It seems the most important component of this post-infective resistance is humoral immunity. Cross immunity with intestinal *Eimeria* have been suspected (i.e. with *E. magna*).

**Clinical signs.** Infection with *E. stiedai* may be asymptomatic, may cause various clinical signs or may even result in sudden death without any prodromal signs. Symptoms can vary from only moderate weight loss to more evident signs like bloating, loss of appetite, diarrhea or constipation. If function of the liver is severely affected, jaundice, ascites and fever may be present. Mortality can be present.

**Pathology.** The gross lesions in rabbits which died of coccidiosis consist in hepatomegaly with the presence of numerous, disseminated white or grey-white nodules (**figure 2.55**) even in the more profound regions of the liver (**figure 2.56**).

There are various degrees of lesion severity. In mild forms, the nodules are few and small, while in the most severe cases nodules are confluent and normal liver tissue is almost absent. The bile is full of debris. Bile ducts are fibrous and enlarged.

Liver lesions must be differentiated from the white and fibrous migratory routes or the vesicle-like cysts of the larval stages of *Taenia pisiformis* (i.e. "Cysticercus pisiformis").

**Figure 2.55** Typical gross lesions in the liver of rabbits suffering of hepatic eimeriosis: hepatomegaly with the presence of multiple whitish nodules. *(Photo Andrei D. Mihalca)*

**Figure 2.56** More detailed view of the lesion pictured previously. *(Photo Andrei D. Mihalca)*

**Figure 2.57** Wet mounts from the hepatic lesions reveal usually high number of oocysts. *(Photo Andrei D. Mihalca)*

**Diagnosis.** In living rabbits, the positive diagnosis is based on the demonstration of oocysts in the feces. By the time clinical signs are present, the oocysts are already present in stools. They must be differentiated from intestinal *Eimeria*.

In dead animals, the lesions are almost pathognomonic. Fresh preparations (wet mounts) done directly from the whitish nodules from the liver or from the bile reveal the presence of various developmental stages but mainly of unsporulated oocysts in impressive numbers (**figure 2.57**).

**Treatment.** Suffering rabbits are treated using oral medication. The anticoccidial drugs recommended for treating clinical cases in rabbits is toltrazuril (25 ppm in drinking water for two days).

Other anticoccidials may be used.

**Control.** Asymptomatic carriers can be treated to eliminate the infections sources for the young susceptible rabbits. Sulfaquinoxaline (0.04% in drinking water for 30 days or 0.025% in food for 20 days), sulfadimetoxine (0.06% in drinking water), sulfadimerazine (0.2% in drinking water) or amprolium 9.6% (in drinking water) are all effective for chemoprophylaxis. Withdrawal time for sulfaquinoxaline is 10 days.

General hygiene measures (frequent removal of feces from pens and disinfection with 10% ammonia help in reducing the infective preasure.

#### 2.4.1.4 Renal eimeriosis in geese

**Introduction.** It is a locally relatively common protozoal infection of domestic

geese which is responsible for acute symptoms and high mortality in goslings.

**Historical notes.** The etiological agent, *Eimeria truncata* was described as *Coccidium truncatum* by Railliet and Lucet in 1891 from domestic geese.

**Etiology.** The only known species able to infect the epithelial layer of the renal tubules in domestic geese is *Eimeria truncata*. The species was reported subsequently from various other wild avian hosts.

**Morphology.** The oocysts of *E. truncata* are oval but with a truncation of the narrow end. The size is 20-22 x 13-16 μm. A micropyle is present.

**Life cycle.** All stages develop in the tubular epithelial cells of the kidneys. As experimental infection is difficult, it is not yet fully understood how the infective sporozoites reach from the intestine to the kidneys. The contamination route is oral, although some authors have questioned this. Oocysts can be recovered from the feces 5-6 days after the infection. Sporulation takes 1-2 days.

**Epidemiology.** The disease seems to be spread worldwide. Outbreaks are sporadic, but when they occur, they are able to affect a significant number of animals. The most susceptible age groups are goslings between 3 weeks and 3 months old, in which mortality can reach high values. The prepatent period is 5-6 days.

**Pathogenesis.** Development of the various stages in the epithelial cell of the renal tubules is responsible for desquamation followed by the presence of debris and possible micro-obstructions of the tubules. This results in significant increase of the kidneys' size and deposition of urates.

**Immunity.** No studies are available, but birds which survive the infection become refractory to new infections.

**Clinical signs.** The usual form of the disease is acute. Renal coccidiosis may be a very serious disease of goslings, with severe depression, general weakness, whitish diarrhea and anorexia. During more chronic forms, birds have polydipsia and nervous signs may be present (gait). Neurologic sequelae include vertigo and torticollis.

**Pathology.** The most characteristic lesion is the enlarged aspect of kidneys which have a greyish-yellow to yellowish red colored surface (normal color is reddish brown). Kidneys often protrude from their sacral bed. The surface is covered with small pinhead-sized grayish-white foci. Hemorrhagic lesions can be occasionally present.

**Diagnosis** is based on clinical sign and lesions, followed by demonstration of oocysts in the droppings or in the kidney lesions.

**Treatment and control.** No reliable experimental studies are available. Anecdotal reports suggest sulfonamides as being effective. Keeping goslings separated from the adults reduces the infective pressure.

## 2.4.2 Cryptosporidiidae

**Introduction.** The family includes a single genus, genus *Cryptosporidium*, with 20 species parasitic in all vertebrate classes.

The name (Greek: *kryptos* = hidden; *spora* = seed) suggests its very small size and the difficulty in detecting it.

**General morphology.** The morphology is discussed in more detail in the corresponding section from Chapter 2.4.2.1.

**Ecology and transmission.** All species are primarily parasitic in the digestive tract of all vertebrates groups, but some may inhabit also other organ systems (e.g. respiratory).

They have a homoxenous life cycle and are transmitted from host to host via fecal-oral contamination.

**Medical importance.** Species of genus *Cryptosporidium* are distributed worldwide and are able to produce clinical infection in a large variety of hosts. Some species are host specific, some others not.

In general, there is a relative host class specificity. This means that species parasitic in reptiles are not able for instance to infect birds or mammals.

On the other hand, some species seem to lack host specificity and infect a wide range of species within a class. One prominent example is *Cryptosporidium parvum* which has been reported from more than 150 mammal species.

### 2.4.2.1 Cryptosporidiosis

**Introduction.** Cryptosporidiosis is a widespread zoonotic parasitic infection of all vertebrate groups, produced by small apicomplexan parasites from genus *Cryptosporidium*. It affects mainly very young or immunosuppressed individuals, producing severe diarrhea and dehydration.

**Historical notes.** The first observations of these parasites came in 1907 by Tyzzer, when he described *Cryptosporidium muris* from mice.

The first association of *Cryptosporidium* with clinical cases of diarrhea in turkeys has been reported in 1955 and in cattle came only more than half century after its discovery, in 1971. The first human cases were reported in 1976 and two years later, *Cryptosporidium parvum* was designated as a zoonotic species. In 2004, the complete genome of a couple of *Cryptosporidium* species was published.

**Etiology.** Although the taxonomy of genus *Cryptosporidium* is still debated, generally 20 named species are currently recognized (valid) (**table 2.32**). Additionally, there are several other genotypes which do not have assigned a specific epithet.

A new genus (*Piscicrytposporidium*) was erected to designated two species parasitic in fish: *P. cichlidis* and *P. reichenbachklinkei*.

**Morphology.** The number of studies on the morphology of various species of *Cryptosporidium* produced huge amount of information.

**Table 2.32** The species of genus *Cryptosporidium*

| Species | Hosts |
|---|---|
| *C. molnari* | fish |
| *C. scophthalmi* | fish |
| *C. fragile* | amphibians |
| *C. serpentis* | reptiles |
| *C. varanii* | reptiles |
| *C. meleagridis* | birds, mammals |
| *C. bailey* | birds |
| *C. galli* | birds |
| *C. andersoni* | cattle, camels, humans |
| *C. bovis* | cattle |
| *C. canis* | canids, humans |
| *C. fayeri* | marsupials, sheep |
| *C. felis* | cats, humans |
| *C. hominis* | humans, cattle, goats |
| *C. macropodum* | marsupials |
| *C. muris* | rodents, camels, goats, primates, pigs, dogs |
| *C. parvum* | various mammals |
| *C. ryanae* | cattle |
| *C. suis* | pigs, cattle, humans |
| *C. wrairii* | guinea pigs |

Nevertheless, the identification of species is more routinely based nowadays on molecular data.

The most important stage from diagnostic point of view is the oocyst. In general, oocysts are very small (2-6 µm in diameter). The shape is usually spherical. Under direct light, they are highly refractile. Each sporulated oocyst contains 4 free, slender or fusiform sporozoites. Unlike most other coccidian parasites (*Eimeria*, *Isospora*, *Sarcocystis*, *Toxoplasma* etc.) oocysts of *Cryptosporidium* lack a sporocyst.

**Life cycle** is homoxenous (**figure 2.58**), typically with three phases: merogony, gametogony and sporogony. The only exogenous stages known are sporulated oocysts, which are eliminated to the environment by infected hosts (figure 2.58 - 1). Infection of the host takes place by ingestion of these sporulated oocysts (figure 2.58 - 2). Immediately after ingestion by a suitable host, the oocysts will start excystation (figure 2.58 - 3) and the free motile sporozoites approach the apical end of the potential host-cells (figure 2.58 - 4) and invade them (figure 2.58 - 5). After attachment, the sporozoites become oval or spherical and vacuoles form inside them (figure 2.58 - 6). This stage is called trophozoite. The newly formed parasitophorous vacuoles are located intracellularly in the host cell, but as they are not in direct contact with its cytoplasm their location is called epicellular. The next stage is the merogony, which is the phase of intense asexual multiplication. This phase is different in the various species of *Cryptosporidium*. In general, the nucleus of the trophozoite will divide several times (figure 2.58 - 7), resulting in multinucleated structures called meronts. Each nucleus will eventually be incorporated in the structure of the forming merozoites (figure 2.58 - 8).

The mature merozoites will leave the surface of the infected host cells (figure 2.58 - 9) and will re-infect other cells (figure 2.58 - 10). The number of merozoites per meronts is different from species to species, but it is usually eight. These meronts are called type I meronts. The last generations of meronts (type II) always contain only four merozoites and these will be responsible for the initiation of the next phase of the life cycle, the gametogony (figure 2.58 - 11).

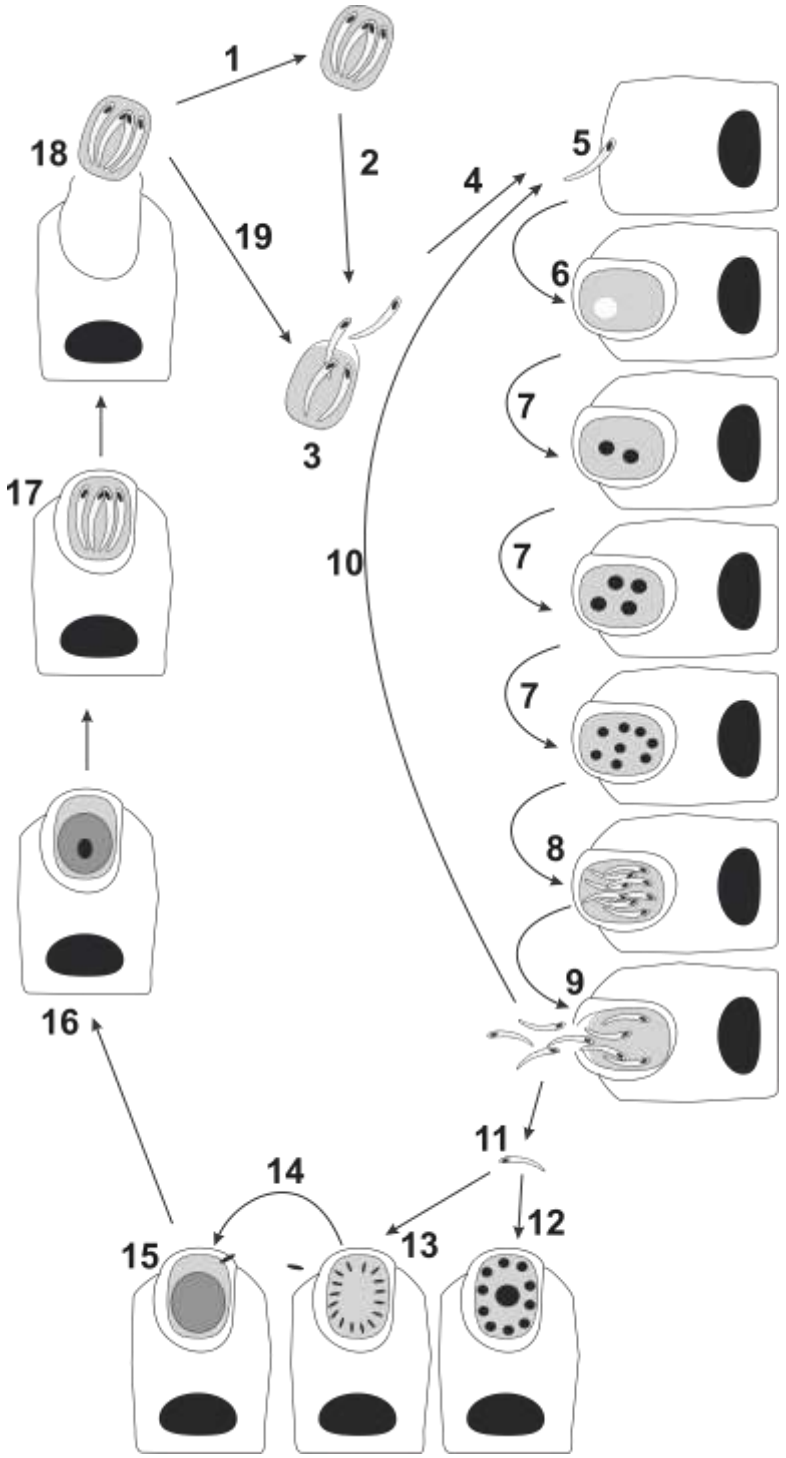


**Figure 2.58** Life cycle of *Cryptosporidium*. For the meaning of numbers, please refer to the text.

Hence, the last generation merozoites will differentiate into female macrogamonts (syn. macrogametocytes) (figure 2.58 - 12) and male microgamonts (syn. microgametocytes) (figure 2.58 - 13). Microgamonts will develop into microgametes and will penetrate the female gametes (figure 2.58 - 14) in order to produce fertilization with formation of the egg cell (zygote) (figure 2.58 - 15) and ultimately unsporulated oocysts (figure 2.58 - 16).

Oocyst will sporulate in situ (figure 2.58 - 17), hence in *Cryptosporidium* the sporulation is typically endogenous. This important from epidemiological point of view, as the oocysts from the feces of infected animals will be immediately infectious to new hosts. Sporulated oocysts are released from the host-cell (figure 2.58 - 18) and are eliminated in the feces (figure 2.58 - 1).

Some sporulated oocysts might excyst before being eliminated in the feces, and the released sporozoites are responsible for autoinfection (figure 2.58 - 19).

*Cryptosporidium* species are responsible for the infection of various parts of the digestive system. In mammals, some species inhabit the distal small intestine, cecum and colon. *Cryptosporidium andersoni* is found in the digestive glands of the abomasum. In birds, some species are able to infect also the respiratory system, conjunctival mucosa or the bursa of Fabricius.

**Epidemiology.** The distribution of *Cryptosporidium* in domestic animals is global. Prepatent periods are 2-7 days in calves infected with *C. parvum*, 10-12 days for calves infected with *C. bovis*, 2-9 days in pigs infected with *C. suis* and 5-6 days for cats infected with *C. felis*.

The sources of infection are oocysts from the environment and the route of infections is ingestion. Oocysts of *Cryptosporidium* are sporulated when they are eliminated so they are immediately infective. The sources of environmental pollution are infected animals (wild or domestic) or humans.

Farm husbandry practices which enhance the transmission cycles include shared feeding of neonates with older animals not necessarily conspecific. Improper disposal of manure and other fecal wastes is contaminating water the sources. Raw sea food (i.e. oysters, clams) have been incriminated is several human outbreaks.

Susceptibility is higher in very young animals and decreases with the age. Immunodeficient individuals are particularly sensitive. Calves are sensitive between 1 and 4 weeks of age for the infection with intestinal species.

*Cryptosporidium andersoni* infects only cattle older than 5 months. In sheep and goats, young animals can be infected and seriously affected from the first days of life. In pigs, the highest susceptibility is between weaning time up to the age of 2 months. In horses, the susceptible age group is 5-8 weeks.

*Cryptosporidium* oocysts are relatively resistant in the environment. At optimal temperatures (5-15°C) and humidity they remain infective for 6 months. Deep freezing destroys them in 24 hours.

**Pathogenesis.** Unlike other apicomplexans, *Cryptosporidium* is not an intracellular parasite. They are typically located on the epithelial surface (**figure 2.59**), and their pathogenicity is related to the villous atrophy (loss and shortening of microvilli) or even detachment of enterocytes. This results in diarrhea, intestinal malabsorption and hypersecretion of chloride and water. After attachment to the cell, *C. parvum* is able to use the host cell's membrane transport systems for its own metabolic processes. Abomasal infection with *C. andersoni* causes inhibition of proteolytic enzymes. Moreover, the epicellular location (between the cell membrane and cell cytoplasm) makes them very refractory to chemotherapy. Significant part of the pathogenetic process is dependent on the host's immune system and will be detailed in the section on immunity.

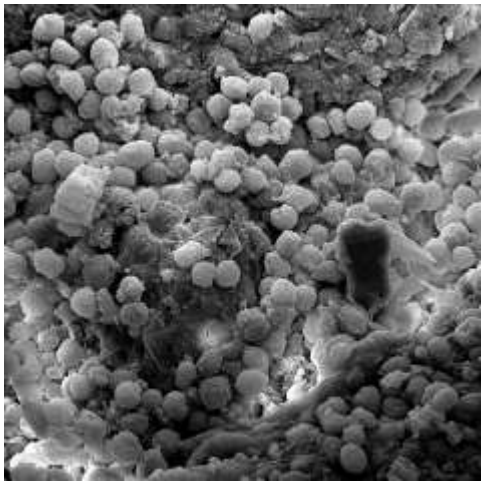

**Figure 2.59** Oocysts of *Cryptosporidium parvum* covering the intestinal epithelium in a naturally-infected goat kid. *(Photo Alexandru Bejan)*

**Immunology.** The role of immunity is very important in the case of cryptosporidiosis. It is very evident that immunocompromised hosts are severely affected by the infection. In humans with AIDS, cryptosporidiosis can be a potentially lethal complication. The first line of defense is the innate immunity. Toll-like receptors on the intestinal epithelial cells are enabling the pathogen recognition. In the case of *C. parvum*, the recognition activates the nuclear factor kappa-light-chain-enhancer of activated B cells pathway and expression of pro-inflammatory cytokines. Interferon-γ and natural killer (NK) cells have a significant effect against *C. parvum*. The phagocytic system also plays an important role in cryptosporidiosis. The production of the free radicals of the nitric oxide by neutrophils and macrophages is increased during the infection with *C. parvum*. The complement system is activated by *C. parvum* on the classical and lectin pathways.

The acquired immunity plays crucial role in the defense against the parasite but also in the pathogenesis. T lymphocytes are mediating the microvillus atrophy.

**Clinical signs.** The most important symptom in clinical cryptosporidiosis is diarrhea in unweaned animals, mainly livestock (calves, lambs, goat kids). In adult animals the infection is regularly asymptomatic, while in very young ones the diarrhea is severe (scouring). Mortality is uncommon and it if is present, it is usually because of coinfections with other pathogens. Infected calves may cause various associated symptoms: dehydration,

anorexia, dullness, weight loss and even fever.

Cryptosporidiosis of pigs is a rare condition. The clinical signs described after experimental infections are: inappetence, depression, vomiting, diarrhea. Clinical cryptosporidiosis has been reported also in other livestock, but rarely.

In dogs and cats the clinical infection is rare, but even inapparent infections pose an increased zoonotic risk. Clinical symptoms in pets include: diarrhea, anorexia, weight loss, tenesmus, etc. Cats infected with FeLV or FIV are more prone to develop clinical cryptosporidiosis. In horses the infection is relatively common but rarely causes serious problems.

In birds, the infection with *C. baileyi* is rarely responsible for digestive signs. More commonly it produces respiratory symptoms: sneezing, coughing, orthopneic position. The respiratory signs can last up to 4 weeks.

*Cryptosporidium meleagridis* infects the ileum of turkeys and other birds producing severe diarrhea. *Cryptosporidium galli* infects the proventriculus and the clinical infection in chicken (even adults) results in puffed plumage and decreased growth.

In quails, unnamed *Cryptosporidium* species produce similar digestive and respiratory signs.

**Pathology.** Lesions are located at the site of infection. Usually no other systemic lesions are found, except situations where bacterial or viral pathogens complicate the diseases.

The main lesion in calves infected with *C. parvum* is enteritis in the small intestine, atrophy of villi with the presence of various developmental stages on the surface of the epithelial cells. Histological lesions consist of cellular infiltrates in lamina propria and hyperplasia of epithelial cells of the intestinal crypts. The infection with *C. andersoni* invades the peptic and pyloric glands in the stomach, producing their dilatation with hypertrophy of the gastric mucosa. Similar intestinal lesions are found in small ruminants and pigs. In dogs and cats, lesions can be found also in the large intestine.

**Diagnosis.** Clinical signs of diarrhea in young animals is an indication for cryptosporidiosis, mainly if the usual treatments are not efficient. Diagnosis should be based on the identification of oocysts in the feces of animals. As oocysts are very small, their detection in feces is not always an easy task. Regular coproscopic methods (i.e. salt flotation) are not very sensitive. Moreover, when the number of oocysts in the feces is very low their detection is even harder.

There are three main method of choice for the detection of *Cryptosporidium* in the feces, or other sample types.

***(1) Direct detection (visualization) of oocysts*** in the feces is based either on their concentration from fecal material, or special staining methods. The most commonly used concentration methods are: sucrose flotation or formalin-ether method. For stools which have large fat contents, the formalin-ether method is recommended. As oocysts are very small and conventional light microscopy does

not allow accurate identification, staining methods have been developed. The most commonly used staining method for fecal smears is the Ziehl-Neelsen modified by Henriksen and Pohlenz. Using this method, the oocysts appear bright red on a green background (**figure 2.60**).

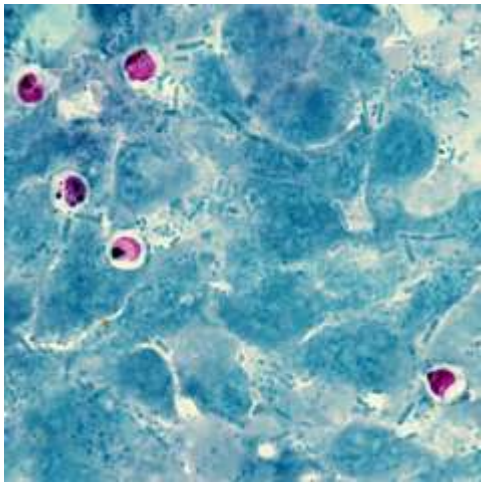

**Figure 2.60** Oocysts of *Cryptosporidium sp.* in a fecal smear as they appear at Ziehl Neelsen modified by Henriksen and Pohlenz method. *(Photo Andrei D. Mihalca)*

***(2) Immunological methods*** include direct immunofluorescence, ELISA or other immunochromatographic methods, all used to identify antigens in fecal material. ELISA tests are available also for detection of serum antibodies.

***(3) Detection of DNA*** are highly sensitive and they are used mainly for genotyping.

**Treatment.** Various chemical compounds are used for the treatment of cryptosporidiosis in animals (**table 2.33**) but their efficacy is often moderate. Usually animals recover if only given symptomatic treatment (i.e. preventing dehydration and correcting electrolyte balance). The minimum duration of chemical treatment should be at least 5 days.

**Table 2.33** Drugs used in the treatment of cryptosporidiosis in domestic animals (after Stockdale et al., 2008; Scorza and Lappin, 2012)

| Drug | Animal | Dose (/kg) |
|---|---|---|
| Paromomycin | calf | 25-100 mg |
| | goat kid | 100 mg |
| | dogs, cats | 125-165 mg |
| Halofuginone lactate | calf | 30-500 µm |
| Lasalocide | calf | 6-15 mg |
| Sulfadimidine | calf | 200 mg |
| Sulfaquinoxaline | goat kid | 100 mg |
| β-Cyclodextrin | lamb | 500 mg |
| Nitazoxanide | dogs, cats | 100 mg |
| Azithromycin | dogs | 5-10 mg |
| | cats | 7-15 mg |

In birds, anticoccidial drugs can be used, but with low moderate success.

**Control.** Controlling the disease is not easy. Although there are some chemoprophylaxis protocols, they are rarely used. General hygiene methods are recommended. As infection is potentially dangerous during the first days of life, the maternity area of the farms should be kept as clean as possible. Colostrum must be fed to newborns, mainly in calves. In farms where imminent risk exists (i.e. history of recent cryptosporidiosis) newborn calves must be kept separated, with no calf-to-calf contact during the first two weeks of life. Sick animals with diarrhea must be isolated from the healthy ones. All in-all out system with thorough disinfection between animal series must be strictly followed. As host

barrier for some species is low, the farm must be kept free of free ranging dogs, cats and rodents. Water sources must be clean.

As zoonotic risk is high, rules and regulations for waste management must be strictly followed.

### 2.4.3 Sarcocystidae

**Introduction.** Family Sarcocystidae includes several genera of great veterinary and public health importance. The family currently includes two subfamilies: Sarcocystinae (with genera *Sarcocystis*, *Frenkelia*) and Toxoplasmatinae (with genera *Toxoplasma*, *Neospora*, *Hammondia*, *Besnoitia* and others).

**General morphology.** All species have *Isospora*-like oocysts. This means, that sporulated oocysts contain two sporocysts, each with four sporozoites. Members of genera *Toxoplasma*, *Neospora*, *Besnoitia* and *Hammondia* have relatively small oocysts. Their differentiation by morphological criteria is virtually unachievable. Except oocysts, the morphology of the other developmental stages is also important for diagnosis purposes, as some of them are commonly found in various tissues of their intermediate hosts.

**Ecology and transmission.** All species are intracellular parasite in various tissues and organs of animal and human hosts. They are parasitic in a great variety of vertebrate species, including amphibians, reptiles, birds and mammals. Life cycle is heteroxenous. Definitive hosts are usually carnivorous species which acquire the infection after ingesting infected intermediate hosts. In the definitive host, the gametogony typically takes place in the intestinal epithelial cells.

The differentiation between the two subfamilies is based on the nature of stages found in the intestine of their definitive hosts and the type of cysts from the tissues of intermediate hosts. In subfamily Sarcocystinae gametogony and sporogony are both endogenous while in Toxoplasmatinae the sporogony is exogenous.

The infective stage for the intermediate host is the sporulated oocyst. After it is ingestion, the sporozoites infect various tissues and rapidly produce the first generation of merozoites known as tachyzoites (Greek: *tachy-* = swift, fast, speed). They subsequently infect other tissues of the intermediate hosts and continue merogony by a slow asexual multiplication resulting in the production of later generations of merozoites known as bradyzoites (Greek: *brady-* = slow).

They typically remain in this stage (known as tissue cysts) until a new suitable definitive host preys on the infected intermediate host. In the intestine of the definitive host, the "zoites" (tachyzoites or bradyzoites) are released and invade the enterocytes where they finish their merogonic development and undergo gametogony with the formation of unsporulated oocysts. In genera *Toxoplasma*, *Neospora*, *Besnoitia* and *Hammondia*, the unsporulated oocysts are shed with the feces and they undergo sporulation in the

environment, becoming infective for a new intermediate host.

In genus *Sarcocystis*, gametogony is followed by endogenous (intraintestinal) sporogony with the formation of typical sporulate *Isospora*-like oocysts. The oocyst wall of *Sarcocystis* is very thin and fragile and it ruptures releasing the sporocysts. The definitive hosts in this case shed through their feces the infective sporocysts (each of them with four sporozoites).

For some genera (i.e. *Toxoplasma*), other complex transmission mechanisms are described, like for instance the transmission from an intermediate host to another intermediate host, without the presence of definitive hosts. The life cycle for each genus will be detailed in the following chapters.

**Medical importance.** The members of Sarcocystidae are important human and animal pathogens. Some of them are responsible for systemic disease (*Toxoplasma*, *Sarcocystis neurona*), others produce mild intestinal infections (*Hammondia*, *Sarcocystis*) or reproductive and congenital disorders (*Toxoplasma*, *Neospora*). *Toxoplasma gondii* and certain species of genus *Sarcocystis* are of zoonotic importance.

### 2.4.3.1 Sarcocystoses

**Introduction.** Sarcocystoses are extremely common parasitic infections produced by member of genus *Sarcocystis* (Greek: *sarx*- = meat, flesh; *kystis* = bladder, pouch). They generally infect the skeletal muscle of their intermediate hosts and the intestinal epithelium of the definitive host with little clinical impact. However, their zoonotic transmission confers them a public health importance.

**Historical notes.** In 1843, Miescher found some white thread-like structure in the skeletal muscle of house mice. He did not realize they are parasites, and for more than 20 years, they were known as the "Meischer's tubules". Similar structures were found in the muscle of domestic pigs in 1865 and described as *Synchytrium miescheriana* by Kühn. In 1892, Lankester erected the genus *Sarcocystis* and in 1899, Labbé transferred the species earlier described in pigs to this new genus and named it *Sarcocystis miescheriana*. In the beginning they were considered to be fungi. Their correct taxonomic position was established in 1967. The heteroxenous life cycle was discovered only in 1970. First clinical cases of equine protozoal myeloencephalitis were described in 1970, but its etiology was clarified only in 1974, when Dubey and his team concluded the agent was a *Sarcocystis* species. The name *S. neurona* was proposed in 1991 by the same American author, Dubey. Its life cycle was elucidated only at the end of the 1990s.

**Etiology.** The diversity of species parasitic in domestic animals is shown in **tables 2.34** (dogs as definitive hosts), **2.35** (cats as definitive hosts), **2.36** (humans as definitive hosts) and **2.37** (wild or unknown definitive hosts).

**Morphology.** Two developmental stages are important from morphologic point of view.

**Table 2.34** Species of genus *Sarcocystis* with domestic dogs as definitive host

| Species | Intermediate host |
|---|---|
| *S. bertrami, S. fayeri* | Equids |
| *S. cruzi, S. levinei* | Cattle |
| *S. arieticanis, S. micros, S. mihoensis, S. tenella* | Sheep |
| *S. capracanis, S. hircicanis* | Goats |
| *S. miescheriana* | Swine |
| *S. alceslatrans, S. capreolicanis, S. gracilis, S. cervicanis, S. sybillensis, S. wapiti, S. grueneri, S. hemionilatrantis, S. odocoileocanis* | Cervids |
| *S. aucheniae* | Llamas, Alpaca |
| *S. cameli* | Camels |
| *S. poephagicanis* | Yaks |
| *S. baibacinacanis* | Squirrels |
| *S. erdmanae* | Skunks |
| *S. wenzeli* | Chicken |
| *S. peckai* | Pheasants |

**Table 2.35** Species of genus *Sarcocystis* with domestic cats as definitive host

| Species | Intermediate host |
|---|---|
| *S. buffalonis, S. fusiformis, S. hirsuta* | Cattle |
| *S. gigantea, S. medusiformis* | Sheep |
| *S. moulei* | Goats |
| *S. porcifelis* | Swine |
| *S. cuniculorum, S. leporum* | Rabbits |
| *S. muris, S. rodentifelis, S. neotomafelis, S. cymruensis* | Rodents |
| *S. odoi* | Cervids |
| *S. wenzeli* | Chicken |

**Table 2.36** Species of genus *Sarcocystis* with humans as definitive host and domestic animals as intermediate hosts

| Species | Intermediate host |
|---|---|
| *S. hominis* | Cattle |
| *S. suihominis* | Swine |

These are the stages in the striated muscles of intermediate hosts and the stage in the feces of the definitive hosts.

**Table 2.37** Species of genus *Sarcocystis* with domestic animals as intermediate hosts and wild or unknown definitive hosts

| Species | Intermediate host |
|---|---|
| *S. neurona* | Horse |
| *S. novaki* | Cattle |
| *S. ippeni* | Dromedary |
| *S. asinus* | Donkeys |
| *S. canis* | Dog |
| *S. felis* | Cats, Dogs |

In the intestine and feces of the definitive hosts (carnivorous mammals) two developmental stages can be found: oocysts and sporocysts.

The sporulated oocyst contains two sporocysts, each of them with four sporozoites ("*Isospora*"-like). The oocysts are thin-walled and they normally rupture while still inside the intestine and release the two sporocysts. Sporocysts (**figure 2.61**) found in feces are small stages, and their morphology is relatively similar, regardless the species. They contain four sporozoites and well-visible sporocyst residuum.

The size of sporocyst found in the feces is variable from species to species (**table 2.38**).

The stages found in the muscle of the intermediate hosts are called muscular cysts or sarcocysts. Biologically they are the last generation of meronts. Their size, shape and location is variable, but as a rule all are whitish, cyst-like structures located in the skeletal muscles of various animals (**figure 2.62**). A practical classification based on their size classifies them in macrocysts (visible with the naked eye) and microcysts (visible only under the microscope) (**figure 2.63**).

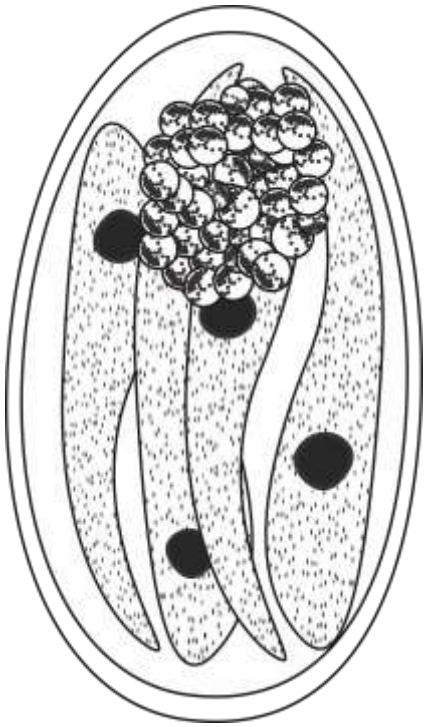

**Figure 2.61** Sporocyst of *Sarcocystis* sp.

**Table 2.38** Sporocyst size of selected species of *Sarcocystis* found in feces of dogs, cats and humans

| Species | Size (µm) |
|---|---|
| *S. cruzi* | 10.8 x 16.3 |
| *S. hominis* | 9.3-14.7 |
| *S. tenella* | 9.9 x 14.8 |
| *S. fusiformis* | 7.8 x 12.5 |
| *S. gigantea* | 8.1 x 12.4 |
| *S. miescheriana* | 9.6 x 12.6 |
| *S. suihominis* | 10.5-13.5 |
| *S. bertrami* | 10.0 x 15.2 |
| *S. neurona* | 10 x 8 |

Sometimes, sarcocysts are very long and thin, like for instance certain species found in rodents (**figure 2.64**).Internally, muscular cysts contain variable number of bradyzoites (**figure 2.65**).

The sarcocysts are usually located longitudinally, along the length and in between of the muscle fibers.

**Figure 2.62** Muscular cysts of *Sarcocystis* sp. from a naturally infected pig are visible during meat inspection. *(Photo Andrei D. Mihalca)*

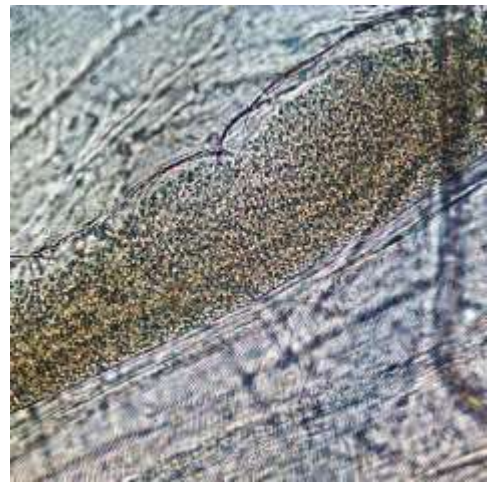

**Figure 2.63** Microcysts of *Sarcocystis* sp. are visible only under the microscope. *(Photo Andrei D. Mihalca)*

Meronts and *S. cruzi* (dog-cattle cycle) from the endothelial cells are very small, 2-8 µm. The cyst in the muscle is fairly large (0.5-5 mm) and often visible with the naked eye.

**Figure 2.64** In some hosts, the muscular cysts of *Sarcocystis* are very thin and long, like in this laboratory mouse. *(Photo Andrei D. Mihalca)*

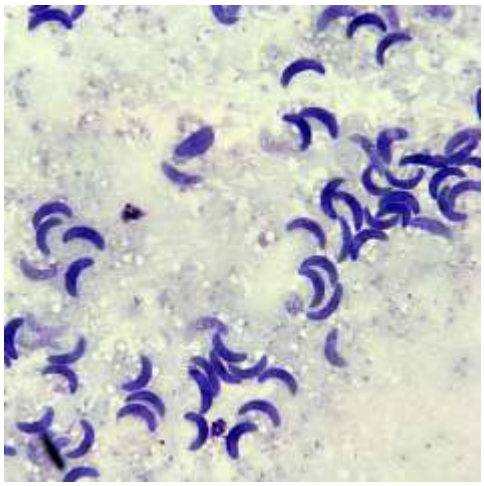

**Figure 2.65** Content of a squashed muscular cyst of *Sarcocystis* reveals the presence of the sausage-like bradyzoites *(Photo Andrei D. Mihalca)*

The cyst wall is thin and smooth. Muscular cysts of *S. hirsuta* (cat-cattle cycle) are relatively big (8 mm) and have a striated wall. Endothelial meronts are bigger (37 x 22 µm with 100 tachyzoites first generation meronts; 14 x 6.5 µm with 35 tachyzoites the second generation meronts). Muscular cysts of *Sarcocystis hominis* (human-cattle cycle) are microscopic, with a thin wall (6 µm) with radial striations.

First generation meronts of *S. tenella* (dog-sheep cycle) are 19-29 x 7.5-24 µm, with 120-280 tachyzoites. The muscular cysts are relatively small (0.1-0.6 mm), have a thick and radially striated wall. Endothelial meronts of *S. gigantea* (cat-sheep cycle) are small (2-8 µm) but their muscular cyst is very large (hence the name) and measure up to 1.5 x 0.5 cm.

*Sarcocystis capracanis* (dog-goat cycle) develops small muscular cysts (130-800 x 50-70 µm) with a thick (2.5 µm) and radially striated wall. On the other hand, the second species with dog-goat cycle, *S. hircicanis* produces larger muscular cysts, of around 2.5 µm but with thinner and smooth wall. The muscular cysts of *S. moulei* (cat-goat cycle) are even bigger (12 mm) with a thick and striated wall.

Muscular cysts of *S. miescheriana* (dog-pig cycle) are large, 0.5-2.2 mm x 160-260 µm. In *S. suihominis* the muscular cysts are thin-walled (4-9 µm) and visible with the naked eye (1.5 mm).

In *S. bertrami* (dog-horse cycle), the muscular cysts are large, up to 10 mm in size, with a smooth and very thin wall. *Sarcocystis fayeri* (dog-horse cycle) has smaller muscular cysts (0.9 mm x 70 µm) and a thin, striated cyst wall. *Sarcocystis neurona* develops small meronts (5-20 x 4-40 µm) in various tissues of naturally or experimentally infected hosts

**Life cycle.** The life cycle of *Sarcocystis* is heteroxenous (**figure 2.66**). Each species is relatively host specific.

The definitive hosts (dogs, cats, humans, wild carnivores) eliminate through their feces the already infective sporocysts. If they are ingested by a suitable intermediate hosts (figure 2.66 - 1), in their intestine the sporocyst wall ruptures and the free sporozoites migrate through the epithelium of the gut and invade the endothelial cell of blood vessels in various internal organs (figure 2.66 - 2)

*Sarcocystis miescheriana* will be used as example in this section. Inside the endothelial cells, they undergo usually two merogonic developments.

The first merogony (figure 2.66 - 3) takes place at 5-6 days. The first generation of merozoites (tachyzoites) invade other endothelial cells (figure 2.66 - 4) and undergo the second merogony at 12-17 days after infection.

The second generation of merozoites (tachyzoites) travels via the blood stream and when they reach the striated muscles they undergo the final merogony and become tissue (muscular) cysts with bradyzoites (figure 2.66 - 5). They remain in this stage for a long time, until the hosts or its flesh (raw meat) are consumed by a suitable carnivorous definitive host (figure 2.66 - 6).

After being ingested by the definitive host, muscular cysts are broken, the bradyzoites invade intestinal epithelial cell and start the gametogonic development with the formation of micro- and macrogametes as early as 14 hours after the infection. They fuse (figure 2.66 - 7) resulting in the sexual formation of the zygote (figure 2.66 - 8). Each zygote will become on oocysts (figure 2.66 - 9).

The oocysts will sporulate in the intestine of the host (figure 2.66 - 10) with the formation of two sporocysts each with four sporozoites. The very fine oocyst wall ruptures (figure 2.66 - 11) and the two sporocysts are freed in the intestinal lumen. They are subsequently eliminated in the environment together with the host's feces (figure 2.66 - 12).

*Sarcocystis neurona* is one of the most interesting species of the genus. Its natural life cycle includes two species of opossums (*Didelphis virginiana* and *D. albiventris*) in North and South America. The natural intermediate host is not known, but the infection was found in a great variety of mammal and bird species. Among domestic animals, the natural infection was reported in horses, cats, and dogs. All are however considered aberrant (accidental) intermediate hosts.

The life cycle is not yet fully understood. Asexual merogonic stages develop in the central nervous system (brain and spinal cord) of horses and other intermediate hosts. A single neuron can harbor as many as 13 meronts with altogether several hundred merozoites. In experimental infection in cats, cysts developed also in the skeletal muscles.

**Epidemiology.** All species parasitic with domestic life cycles (both, the definitive host and the intermediate host are domestic animals) have a global distribution.

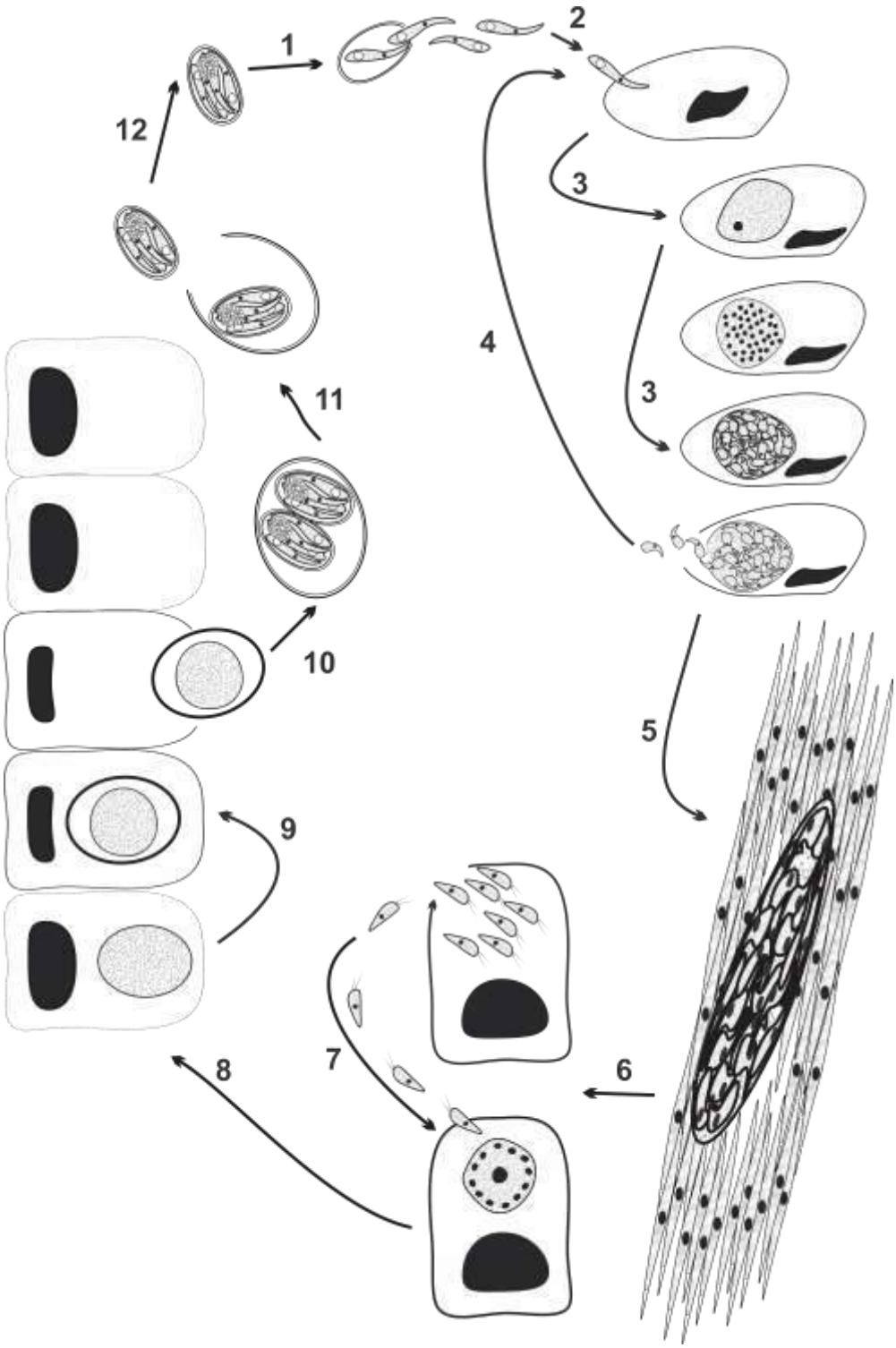


**Figure 2.66** Life cycle of genus *Sarcocystis*. For the meaning of numbers, please refer to the text.

*Sarcocystis neurona* has been reported only in the Americas, its distribution being determined by its strict specificity to the definitive hosts.

The data regarding prevalence of infection in intermediate hosts is very abundant in the literature. However, most studies did not attempt to identify the species; hence their zoonotic potential is not fully known. The prevalence of muscular cysts in slaughtered cattle is very high in certain areas, but the most common species is *S. cruzi* which is infective for dogs. Intestinal sarcocystosis in humans is the most common in Europe (between 7.3 and 10.4%). The main source of infection for humans is raw meat from cattle and pigs.

In the case of *S. neurona*, the disease is most commonly reported in racing animals aged between 3 and 6 years. In USA, the seroprevalence of anti-*S. neurona* is between 30 and 50%.

Resistance of sporocysts is high. Sporocysts of *S. cruzi* remain infective if kept at -22°C for 10 days or at 56°C for 10 minutes. Sporocysts of *S. miescheriana* are destroyed in the meat after 20 minutes at 60°C, after 15 minutes at 70°C and in 5 minutes at 100°C. Freezing does not inactivate sarcocysts.

**Pathogenesis.** In the intermediate hosts the most important pathogenic effect is caused by the first and the second merogonic development which takes place in the vascular endothelial cells. In certain species (i.e. *S. tenella* in sheep), merogonic development can be also responsible for severe myositis encephalomyelitis. In pregnant females, heavy infections with certain species (*S. cruzi*, *S. tenella*) are able to induce abortions. *Sarcocystis suihominis* develops in the endothelial cells of blood vessels from the liver. Sever muscular destruction is responsible for gait and myalgia.

*Sarcocystis neurona* was found in neurons, mononuclear cells and glial cells but not peripheral nerves. Although not too many information are available on the migration route, experimental infection in ponies by Elitsur et al. (2007) revealed the presence of *S. neurona* in lymph nodes, liver and lungs. At 9-21 days after the infection, parasites reach the central nervous system and infect various parts: cerebellum (with depression, seizures, behavioral changes), brainstem and spinal cord (abnormal gait) or damage of the cranial nerve nuclei (various paralysis).

The intestinal development of gametogonic and sporogonic stages of *Sarcocystis* in the definitive host is usually non-pathogenic. The main difference from the severe impact of *Eimeria* and *Isospora* on the intestinal mucosa consists in the different phase of the life cycle taking place here. In *Eimeria* and *Isospora*, the repeated merogony is responsible for the epithelial destruction, while in *Sarcocystis* only the gametogony and sporogony take place here, both of them being non-repetitive.

**Immunology.** In intermediate hosts, the immune reaction during the muscular phase of the life cycle is predominantly cellular. In time, the organism is able to eliminate the infection. Definitive hosts

are easily susceptible to reinfections, demonstrating a decreased immune response.

**Clinical signs.** With the exception of *S. neurona*, sarcocystoses are benign muscular infections in intermediate hosts and asymptomatic or light intestinal infections in their definitive host. Clinical, signs are very rare, and they are reported mostly after very high infective doses. The pathogenicity and symptoms in such cases are variable from species to species.

In ***cattle*** heavily infected with *S. cruzi* anorexia, fever, weight loss, anemia and difficulty in movements have been reported. Loss of hair on the tip of the tail, submandibular edema, exophthalmia, lymphadenopathy and abortion are also possible. This particular condition has been known also as the Dalmeny disease. Infection of cattle with the species of cat-origin (*S. hirsuta*) is usually asymptomatic, but heavy infective disease may induce anorexia, weight loss, anemia, fever and even diarrhea. *Sarcocystis hominis* infection is asymptomatic.

Massive infection with *S. tenella* in ***sheep*** can be responsible for anorexia, loss of weight, fever, anemia, recumbency and abortions in pregnant ewes. The cat-origin species parasitic in sheep (*S. gigantea*, *S. medusiformis*) are rarely responsible for clinical infections. In goats, *S. capracanis* and *S. hircicanis* can produce similar clinical signs, including abortions. Infection with *S. hircifelis* is asymptomatic.

From the three species parasitic in ***pigs***, only two seem to be pathogenic. *Sarcocystis miescheriana* produces enteritis with diarrhea, myositis with impaired movement and lameness, fever, anorexia and weight loss. Infections wirh *S. suihominis* can be acute, with two fever peaks (days 5-9 and 11-15), apathy, dyspnea, anemia, cyanosis, muscles spasms and tremors, hyperexcitability, prostration and abortion.

In ***horses***, two forms of sarcocystoses have been described. As in all other domestic animals, horses usually ***develop asymptomatic muscular*** sarcocystosis (with *S. bertrami* or *S. fayeri*, both from dogs). However, *S. fayeri* may be responsible for myalgia (associated with myositis).

The second type of sarcocystosis in horse is the very severe infection with *S. neurona* causing a distinct parasitic disease known as ***equine protozoal myeloencephalitis***. This is a debilitating disease, with progressive clinical evolution, responsible for a variety of nervous signs. The disease may be acute or chronic. In general, the first observed symptoms are lameness which gradually increases in intensity leading to ataxia. One of the most typical clinical signs are the asymmetrical gait disorders with focal muscular atrophy. Some horses have abnormal upper respiratory function, difficulties in standing or walking, dysphagia. Neurologic examination reveals asymmetric signs of weakness, spasticity, hypoalgesia or complete loss of sensitivity. If the function of cranial nerves is affected horses have tilted head and facial nerve paralysis. During all the course of the disease, the animals are bright and alert.

Affected horses usually die during the acute stage of the disease. Dogs and cats can be also infected with *S. neurona* and they develop similar signs of fatal myeloencephalitis.

In ***dogs*** infected with *S. canis* the parasite produces a systemic disease involving the central nervous system and liver necrosis.

In the ***definitive hosts***, heavy infections are responsible for mild diarrhea. ***Humans*** infected with *S. miescheriana* after eating raw infected pig meat can develop bloat, diarrhea, stomach ache, nausea, vomiting or loss of appetite. In ***dogs*** and ***cats*** with intestinal sarcocystosis, the infection is normally asymptomatic. However, the author of this book has found sporocysts in dogs with diarrhea.

**Pathology.** In the intermediate hosts, the merogonic stages produce lesions of different degrees, dependent mainly on the infective dose. The most striking aspect during the gross necropsy is the disseminated presence of white nodules in the skeletal muscles, in the striated muscle of the esophagus in ruminants and even in the cardiac muscle. Histology reveals the presence of intramuscular parasitic cysts (**figure 2.67**) associated with myositis, myodystrophy or myocarditis. Cellular inflammatory infiltration around the cyst can be present.

In ***bovines***, meronts of *S. cruzi* are responsible for destructions of the endothelial lining of capillaries. The muscular stage produces myositis, with the parasitic cysts surrounded by inflammatory cells, mainly macrophages and lymphocytes. Older cysts are surrounded by thick capsules and may degenerate. Muscular cysts of *S. hirsuta* are easily visible and are located mainly in the striated muscle fibers from the esophagus.

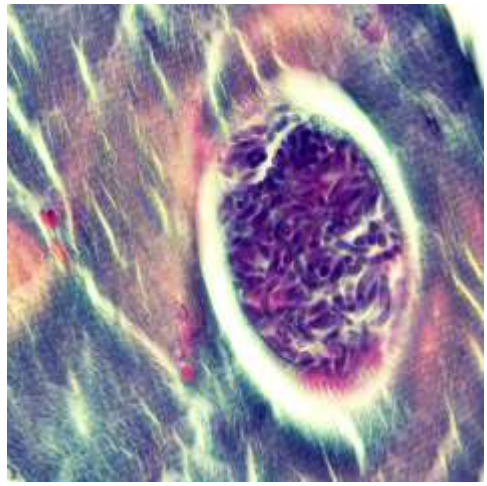

**Figure 2.67** Histological aspect of a muscular cyst of *Sarcocystis*. Bradyzoites are clearly visible. *(Photo Andrei D. Mihalca)*

In ***sheep, goats*** and ***pigs***, the lesions are similar to those described in cattle. Cysts of some species (*S. tenella*, *S. capracanis*, *S. hircicanis*) can be found also in the cardiac muscles.

**Diagnosis.** The diagnosis in the definitive host is based on the detection of sporocysts in the feces (**figure 2.68**) by flotation methods. Differentiation of species based on sporocyst morphology is not easy.

Diagnosis of muscular cysticercosis in intermediate hosts is usually post-

mortem, during the compulsory meat inspection in slaughterhouses. The detection of large white cysts in the striated or cardiac muscles has diagnostic value.

They have to be differentiated from other cystic structure like for instance from the bladder-like cysts of "Cysticercus bovis" (the larval stage of *Taenia saginata*) in cattle or "Cysticercus cellulosae" (the larval stage of *Taenia solium*) in swine. However, the detection of microcysts is impossible when examining the meat by naked eye. In pigs microcysts can be accidentally detected during the microscopical examination of meat for *Trichinella*.

In vivo, the infection (asymptomatic or not) can be detected using serological tools (immuno-fluorescence, ELISA) or by correlating the clinical signs (if present) with increased serum levels of bilirubin, creatine phosphokinase and lactic acid.

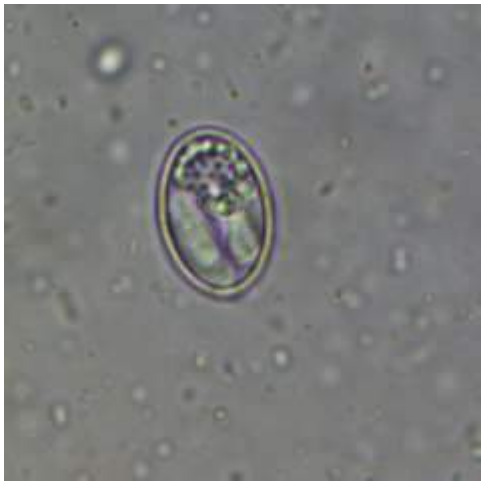

**Figure 2.68** Sporocyst of *Sarcocystis* sp. from the feces of a grey wolf. *(Photo Călin M. Gherman)*

Diagnostic of equine protozoal myeloencephalitis caused by *S. neurona* is done by serological confirmation (immunoblotting) of cases clinically corresponding to the disease. The presence of the parasite can be demonstrated also in the cerebrospinal fluid by western blot.

**Treatment**. Animals infected with muscular sarcocysts are not usually treated, as the diagnosis is most commonly done post-mortem. There are several therapeutic studies done in experimental infections. In acute sarcocystosis of lambs, amprolium (50-100 mg/kg) decreased the intensity of clinical signs. Salinomycin (1-2 mg/kg) and halofuginone (0.67 mg/kg) had the same protective value in sheep and goats. Monensin was found to be effective for the treatment of acute bovine muscular sarcocystosis.

The treatment of equine protozoal myeloencephalitis is an emergency, and if it is applied in time and correctly, the success rate is up to 75%. The most common therapeutic protocol for horses is the use of sulfadiazine (20 mg/kg, PO) once or twice per day combined with pyrimethamine (1 mg/kg, PO) once per day. The duration of treatment is long (84-120 days) and it must be discontinued only when the cerebrospinal fluid is negative by western blot. In the case that this treatment fails to yield good results, the drugs of choice are diclazuril, toltrazuril or nitazoxanide.

The treatment of intestinal sarcocystosis in the definitive host is similar to the treatment of other intestinal coccidiosis (see Chapter 2.4.1.1).

**Control.** The most important means for prevention are the general hygiene measures which interrupt the life cycle of the parasite. Free-ranging dogs and cats must be excluded from farms. The infected meat should not be given to carnivores. There is no specific prophylactic method yet, but vaccines are being developed for immune-prophylaxis of the infection with *S. neurona* in horses.

### 2.4.3.2 Toxoplasmosis

**Introduction.** Toxoplasmosis is one of the most widely distributed parasitic infections on Earth, affecting humans and animals as well. Its highly zoonotic potential and the severity of infection in certain host categories make it one of the most intensively studies parasites. The most important aspect of toxoplasmosis is probably its congenital transmission, the subsequent clinical problems in children and the resulting social impact.

**Historical notes.** The description of the parasite came in 1908, when Nicolle and Manceaux, two researchers from Pasteur Institute in Tunis have noticed protozoan stages in the tissues of a laboratory kept rodent known as the common gundi, *Ctenodactylus gundi*. Initially, they misidentified the parasite as *Leishmania* but subsequently, in 1909, they described it as a new species, *Toxoplasma gondii*. The generic name was given according to the morphology of the stages they found (Lat. *toxo* = arc or bow; *plasma* = life) and the specific epithet is an erroneous spelling of the host's name. The first case of correctly diagnosed congenital toxoplasmosis in humans was reported in 1938 in an infant girl who died at the age of one month in New York. However, previous reports of human congenital chorioretinitis and encephalomyelitis, initially attributed to some other agents, were later shown to have been caused by *Toxoplasma*. The first case of human acquired toxoplasmosis has been identified in 1940. Nevertheless, first reports of animal toxoplasmosis date back to 1910, when Mello described an acute case in a dog from Italy. Although recognized as a widespread and sometimes severe zoonotic infection, the full life-history and the role of the cats in the biology and transmission of *T. gondii* was not fully understood until the 1970s, when Dubey and Frenkel described the entire developmental cycle.

**Etiology.** The only known species is *Toxoplasma gondii*. It has a worldwide distribution and an immense host spectrum. Virtually it can undergo its asexual development in any warm-blooded host, mammal or bird.

The genetic analysis of various strains of *Toxoplasma gondii* from Europe and North America revealed the presence of three major genotypes (I, II and III). Subsequently, new genetic variants were identified worldwide and they were classified into 12 haplogroups. All these genetic variants differ in their pathogenicity on various hosts.

**Morphology.** *Toxoplasma gondii* is a very common parasite of humans and animals and its diagnosis in the definitive and intermediate hosts is based on the detection of various developmental stages.

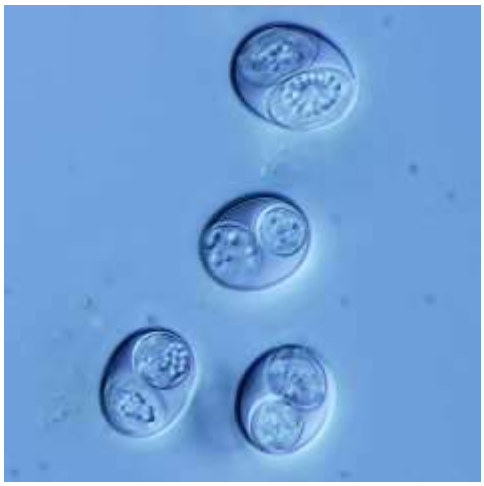

**Figure 2.69** Sporulated oocysts of *Toxoplasma gondii* from the feces of a cat. *(Photo Jana Juránková)*

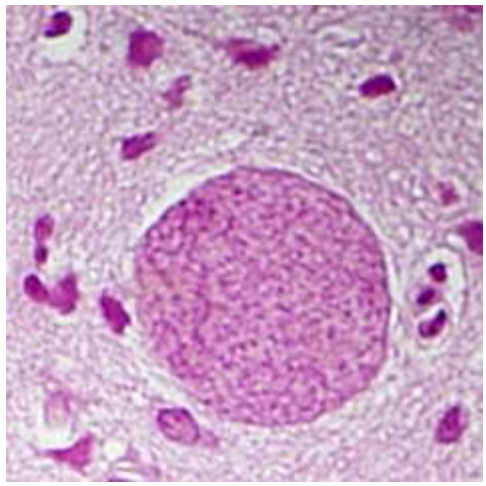

**Figure 2.70** Tissue cyst of *Toxoplasma gondii* in the nervous system of an infected mouse. *(Photo Jana Juránková)*

The typical stage found in the intestine of cats as definitive hosts is the unsporulated ***oocysts***. In older feces, the oocysts are sporulated and they are "*Isospora*"-like (two sporocysts, each with four sporozoites) (**figure 2.69**).

The ***tachyzoites*** are crescent-shaped (Greek: *toxon* = arc or bow), 2-6 µm with pointed anterior and rounded posterior end. The nucleus is in central position. They are found intracellularly in almost any cell type, except red blood cells. The group of tachyzoites resulting after repeated multiplication by endodyogeny inside a cell is also known as ***pseudocyst***.

***Bradyzoites*** (5-8.5 x 1-3 µm, posterior nucleus, more slender than tachyzoites) are found in ***tissue cysts***. Tissue cysts are also intracellular. They multiply by endodyogeny within the tissue cysts (**figure 2.70**). The size of the tissue cysts is variable and it depends on the number of contained bradyzoites.

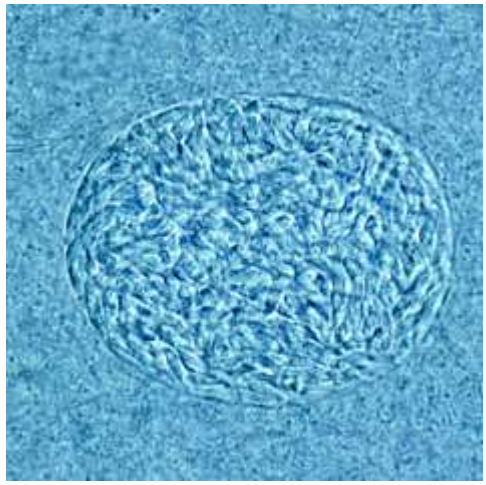

**Figure 2.71** Tissue cyst of *Toxoplasma gondii* freed from its host tissue. Numerous bradyzoites are visible inside *(Photo Břetislav Koudela)*

Small tissue cysts (~5 µm) contain two bradyzoites while larger cysts (70-100 µm) contain thousands of them (**figure 2.71**). The shape and size of the tissue cysts depend also on the host tissue.

Tissues in the brain are usually smaller (~70 μm) and round while those in the muscles for instance are larger (~100 μm) and elongated. The tissue cyst wall is elastic and thin (<0.5 μm). In older tissue cysts, the wall may be hardly visible.

**Life cycle.** The biology of *Toxoplasma gondii* typically includes two obligatory host, the cats and other felids as definitive hosts and rodents as natural intermediate hosts (**figure 2.72**). In many aspects, this typical life cycle is similar to *Sarcocystis* spp. However, several particular biology aspects make *Toxoplasma gondii* a unique parasite:

(1) Extremely ***broad host specificity*** for its asexual stage (merogony). *Toxoplasma gondii* can use virtually any mammal and any bird for its merogonic development.

(2) Natural transmission can be done directly from an ***intermediate host to another intermediate host***, without the presence of the definitive host. For instance, humans (which are intermediate host) can get the infection after eating infected tissues from livestock (also intermediate hosts).

(3) ***All "zoite" stages are infective*** to any susceptible host. Sporozoites (from the feces of cat definitive hosts), and tachyzoites or bradyzoites (from the tissues of intermediate hosts) are infective to any other intermediate host. Cats are also susceptible to infection with any of these stages, but depending on which stage is ingested, they act as intermediate or definitive hosts.

(4) ***Diversity of infected tissues*** is great, and as the parasite has systemic distribution, virtually any organ can be infected and infective. However, they are more common in the brain, eyes, skeletal muscles and myocardium.

(5) Infected pregnant females (including women) are able to pass the infection to the fetus via ***transplacentary route***.

*Toxoplasma gondii* is an obligatory intracellular parasite, with tropism mainly for nervous and muscular tissues. However, it can be found occasionally in any other organs. It is usually located in the cytoplasm of the infected cells, but sometimes it can invade also the nucleus.

Typically, intermediate hosts are infected after ingesting sporulated oocysts from the cat's feces (figure 2.72 - 1). After ingestion, the oocyst wall ruptures releasing the sporozoites which, after passing through the intestinal wall will penetrate into various types of cells (macrophages, endothelial cells, fibroblasts etc.) (figure 2.72 - 2) where they multiply by endodyogeny (figure 2.72 - 3) producing the tachyzoites. Tachyzoites may rupture the host cell and invade other cells (figure 2.72 - 4) or migrate through the host's body, reaching various tissues (including the fetus) where they continue their multiplication in a slower rate. The result is the formation of tissue cysts with bradyzoites (figure 2.72 - 5). If cats or other felids feed on tissue cysts originating from an infected intermediate host (figure 2.72 - B2), they become infected (2.72-6).

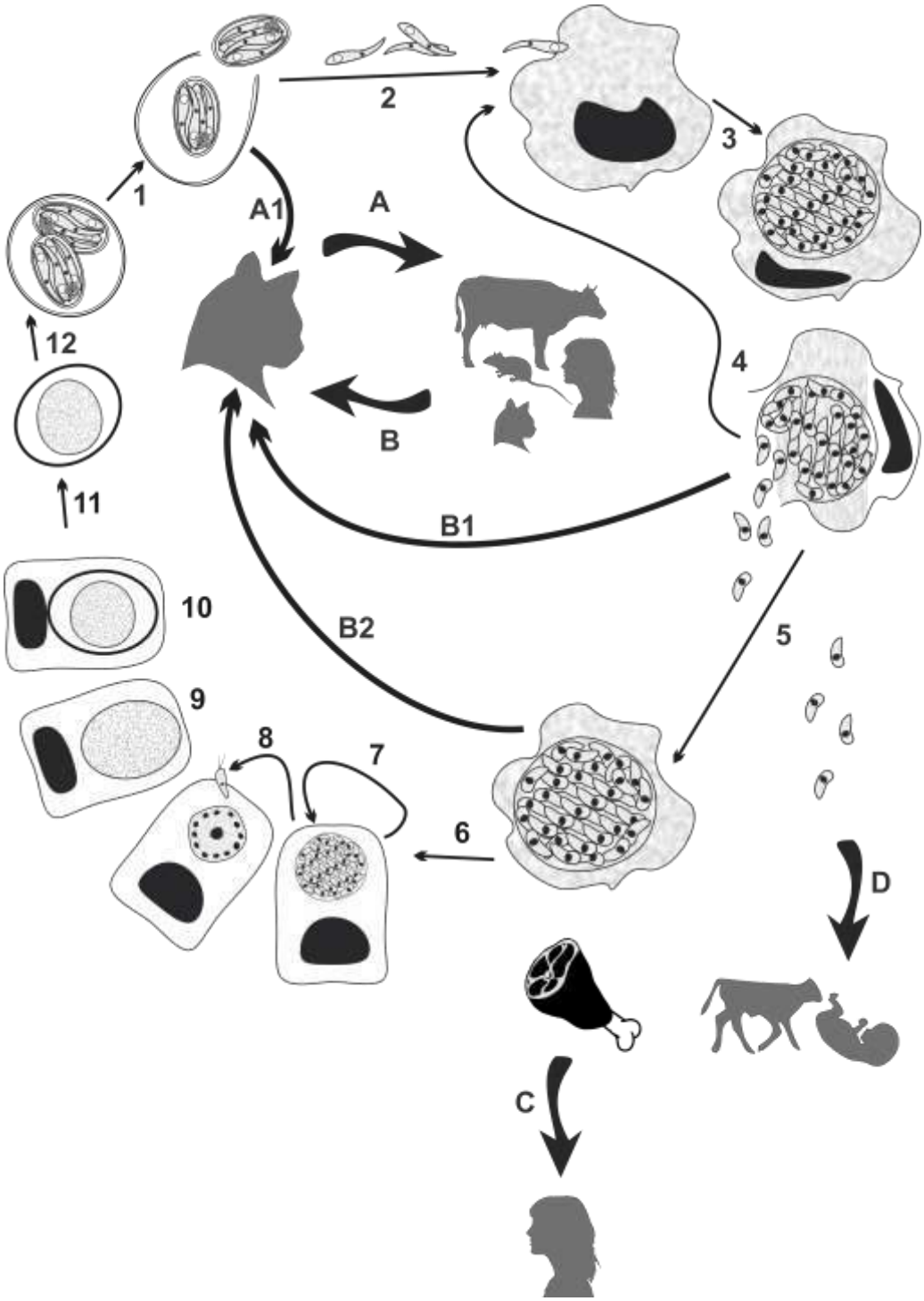


**Figure 2.72** Life cycle of *Toxoplasma gondii*. For the meaning of numbers and letters, please refer to the text.

The tissue cyst wall is dissolved by proteolytic enzymes from the feline stomach and intestine, realizing the bradyzoites. The bradyzoites invade the epithelial cells of the small intestine of cats and undergo repeated asexual multiplication (figure 2.72 - 7), passing through multiple generations of meronts, classified in five types (type A to E). After this supplementary asexual merogony takes place in the intestine, the sexual gametogonic development starts (figure 2.72 - 8) with the formation of the zygotes (figure 2.72 - 9) and ultimately the unsporulated oocysts (figure 2.72 - 10). Unsporulated oocysts are eliminated through the feline feces (figure 2.72 - 11) intro the environment where they sporulate in 1-5 days (figure 2.72 - 12), becoming infective.

Except this typical heteroxenous life cycle (figure 2.72 - A and B), there are several other pathways which can be followed by *Toxoplasma gondii*. If cats are infected following ingestion of tachyzoites (figure 2.72 - B1) the life cycle in the cat is slower. It is considered that tachyzoites are less acid-resistant than bradyzoites but they can be still infective.

If cats ingest oocysts (figure 2.72 - A1) the course of infection is different. In this case, in the first stage the cat acts as an intermediate host with the formation of tachyzoites and bradyzoites in the tissue. In some of the cats infected with oocysts, after almost three weeks, oocysts can be found again in the feces. The hypothesized scenario is that some bradyzoites reach back the intestine and start gametogony in the enterocytes. However, not all cats infected with oocysts will ultimately shed oocysts in their feces. The prepatent period in cats is different according to the infection source. After ingesting tissue cysts (bradyzoites), cats shed oocysts after 3-10 days. After being infected with oocysts or tachyzoites, the prepatent period is longer (18 days).

One of the most important life cycle pathways is the possibility of transmission form an intermediate host to another intermediate host by carnivorism (figure 2.72 - C). According to numerous opinions, this is the most common way of infection for humans (i.e. following eating raw or undercooked meat from infected animals). This bradyzoites-induced cycle in the intermediate host is following similar patterns to that of the oocyst-induced infection. However, the infectivity of bradyzoites to intermediate hosts is lower than the infectivity of sporozoites. This is why oocyst and their hosts (cats) are an absolutely essential link in the complex transmission chains of *Toxoplasma gondii*.

From clinical point of view, the most important contamination route is the vertical transmission of tachyzoites (figure 2.72 - D), mainly in humans. Various studies have shown that the transplacental infection occurs only if the mother is primo-infected during the pregnancy. The chances of transplacental transmission are low if the women acquire the infection just before the pregnancy or during older, pre-natal chronic infections. Nevertheless, in immunosuppressed pregnant women with chronic infection, transplacental

infection is possible. Transplacental infection was also reported in domestic animals. The risk of congenital infection is lowest when maternal infection is in the first trimester (10–15%) and highest when infection occurs during the third trimester (60–90%). If maternal infection occurs early in pregnancy, it results in fewer infected babies, but they are more severely affected than the greater number of infected babies born when infection is acquired later in pregnancy. The highest risk to the fetus is when infection is acquired between the 10th and 24th week of gestation. Fortunately about 60–70% of babies born of infected mothers escape infection.

In certain species (i.e. laboratory rodents) transplacental transmission occurs even if the infection is chronic and took place before the pregnancy. In domestic animals (i.e. sheep), various observation suggest that the situation is similar to humans, and only tachyzoites can pass transplacentally (i.e. infection must be during pregnancy). Transplacental transmission was not documented in all domestic species. There are no confirmed reports from dogs, horses or cattle.

**Epidemiology.** As stated before, *Toxoplasma gondii* is probably the most common parasite on Earth. It is present wherever cats are present which means everywhere where humans are present. Despite its global ***distribution***, *Toxoplasma* is more common in warmer climates than in colder ones and in lowland compared to highlands. This is most probably related to the lower survival of oocysts and their lower sporulation success at lower temperatures.

The ***infections sources*** are various. The direct sources of infective oocysts are always cats. However, cat owners are not more exposed to infective oocysts compared to people who never had a cat, as oocysts from cat feces can contaminate fruits and vegetables. Dogs can also shed *T. gondii* oocysts following coprophagia of cat feces. Dogs which rolled over infected cat feces pose also a risk for humans. Defecation sites in public parks are important and oocysts may be mechanically carried on shoes.

Another source of infective oocysts is represented by wild felids which acquire the infection from their wild prey. Hence, the wildlife reservoirs can be locally important also.

The main sources of infection with *Toxoplasma* for humans are not the same everywhere. In the areas where cats are abundant and live in close contact with people the oocysts are probably the principal infection sources. In developed countries, where cats are mostly indoors and they feed on commercial food, the most probable source for human infection is raw or undercooked meat. Raw goat milk was reported as a source of infection for humans. Raw cow milk or uncooked chicken eggs are not considered dangerous for the transmission of *Toxoplasma*.

Other sources of infection are uncommon. Tachyzoites accidentally reaching the cornea during laboratory manipulation can induce the infection. Transfusion of whole blood is not

infective as the number of circulating tachyzoites is very low and the period of parasitemia is low. However, transfusion of packed leukocytes can be a risk. Organ transplants are also incriminated. The presence of tachyzoites has been demonstrated in semen and saliva but no venereal or salivary transmission were reported.

The ***prevalence in humans*** is not necessarily related to the prevalence in cats, but rather to cultural habits. In nations where eating raw or undercooked meat is a common practice (i.e. France) the seroprevalence of toxoplasmosis in humans is higher. Eating raw meat seems to be a more common habit in more developed countries. In third-world countries, meat is usually well cooked because of other health risks. This explains the lower prevalence in humans from Africa and Asia. The prevalence in humans is also influenced by the species they most commonly eat. In countries where mutton is a common dish, the prevalence of human toxoplasmosis is higher. Viable *Toxoplasma* cysts are commonly found in pigs and sheep; in cattle, viable cysts are very rare. Reported values for seroprevalence in humans are very heterogeneous and they depend on various factors. The higher values have been reported in South America, and the lowest in Eastern Asia.

The ***prevalence of infection in cats*** is dependent on various factors, but the most important factor seems to be the presence and availability of infected rodents. Interestingly, mice infected with *T. gondii* are less neophobic and show less aversion to cat odor. This host-manipulation makes infected mice easier preys to cats.

The values for the infection prevalence in cats are variable. If we assess this epidemiologic parameter on the basis of the presence of oocysts in the feces the prevalence is very low (average less than 1%). However, if we assess the prevalence of anti-*Toxoplasma* antibodies (seroprevalence) it can reach an astonishing 100% in certain cat population. This can be explained if we consider the biology of *T. gondii* in its definitive host, as the time for oocysts elimination in cat's feces is very short (1-2 weeks) and the number of oocysts can be low, under the coproscopic detection threshold (<1000 oocysts per gram). Nevertheless, PCR detection of *Toxoplasma* DNA in feline feces can yield higher prevalences (up to 11%) and is able to differentiate between other small intestinal coccidia like *Hammondia*.

Another very important epidemiological question is if cats shed oocysts more than once in their lifetime or they acquire kind of immunity. Data from experimental studies are controversial. Even though, probably the number of oocysts eliminated by cats during their first infection is much higher than during subsequent infection. More details are given in the immunity section. Quantitative assessments found impressive number of oocysts in cats with primary infection (up to 13 million oocysts per gram of feces). The average values are of course lower, but still impressive (around 10 million oocysts per cat).

The seropositivity in cats increases with the age and it is higher in feral cats than in indoor cats. Prevalence of cats with tissue cysts is between 5 and 70% in various countries.

In ***sheep*** the infection was also found worldwide. Except cats, seroprevalence in sheep is among the highest from all domestic animals. In certain adult populations, it can easily reach 95%. In intensive farming system the seroprevalence is lower than in semi-intensive systems. Risk factors include mostly the age, presence of free-ranging cats in the farm and history of abortions. It is estimated that between 10 and 23% of abortions in sheep are caused by *T. gondii*. Toxoplasmosis in ***goats*** is also globally distributed, with variable seroprevalence which is generally lower than in sheep. In ***cattle*** the global seroprevalence is generally lower than in sheep.

The same worldwide distribution was reported in ***pigs***. The seroprevalence is much higher in backyard and free-range pigs and almost absent in pigs from intensive farm system. There is also a clear age related distribution of seropositivity values. Adult saws show higher prevalences than market-age pigs.

In ***dogs*** the distribution is global. Seropositivity is variable and higher in older dogs and in rural dogs. In general, the seroprevalence in dog is high (up to almost 90%). In ***horses*** the seroprevalence is generally low.

Multiple seroprevalence studies are available for ***chicken***, as their meat forms a significant part of human diet. Seropositivity greatly depends on the farming system. The infection is virtually absent from intensive farms and more common in free-ranging birds.

Surprisingly, the ***resistance*** of *T. gondii* oocysts in the environment is not too well known under natural conditions. Under experimental conditions they proved to be relatively resistant. At -21°C in water they were not killed. They also resisted 4.5 years in water at 4°C and more than 1 year in water at 22°C. Warmer aquatic medium kills them faster (1 month at 40°C; 2 minutes at 55°C; 1 minute at 60°C). If kept in cat feces, they resist several years at temperature between 15 and 35°C. In dry condition they die faster (11 days in air at 11% relative humidity and 2 days at 0% relative humidity). Unsporulated oocysts are generally more sensitive to environmental factors than sporulated oocysts. Oocysts are also very resistant to disinfectants. Kept in 10% formalin they resist 48 hours. The 5% ammonium hydroxide kills them in 30 minutes but not in 10 minutes. Iodine tincture (2%) kills oocysts in 3 hours but not in 10 minutes. Oocysts survive standard water chlorination for at least 24 hours.

Tissue cyst resistance is a key epidemiologic factor. Treatment and injection with salt of meat-based products kills bradyzoites from tissue cysts. Tissue cysts are sensitive to conventional cooking. They are killed as the internal temperature is over 60°C. Freezing also kills tissue cysts (at -12°C in 1 day). In decomposing carcasses bradyzoites survive for several days. Resistance of tachyzoites is very low

compared to the resistance of bradyzoites or oocysts.

**Pathogenesis.** Despite the huge attention given to *T. gondii*, clinical infections are rare. After the infection with "zoites" (bradyzoites from tissue cysts, tachyzoites from pseudocysts, sporozoites from oocysts in cat feces), they penetrate the intestinal wall and multiply locally in adjacent tissues including mesenteric lymph nodes. They cause here local necrosis. Similar necrotic lesions are produced in various other organs by the asexual development of tachyzoites. Necrosis is the result of intracellular development cellular death and not the result of toxins. No toxin has been so far detected in *T. gondii*. The initial necrosis may kill the host if it is extensive enough and affects vital organs. Otherwise, the necrosis is gradually replaced by local chronic inflammation and the host develops immunity. Tachyzoites usually disappear from the internal organs in three weeks after the infection and afterwards, bradyzoites are located in cysts mainly in the muscular tissue (striated and cardiac) and central nervous system.

Tissue cysts remain arrested awaiting a carnivorous predator to prey on the host. However, in certain situations (mainly related to a sudden decrease in immunity) the tissue cysts can rupture and the released bradyzoites invade other tissues forming new cysts.

Pathogenicity is dependent on various factors. Certain host species (New World monkeys, Australian marsupials) are more susceptible to develop clinical signs than others (Old World cattle, horses, cattle). Other determinant factors for the pathogenicity are the route of infection, the source of infection and the parasite strain. Oocyst-induced infections are more severe that bradyzoites-induced infections. Type I genotype is pathogenic for mice, but not type II and III. In humans, the most pathogenic genotypes are I and III, but in France, severe cases were reported also with type II. Certainly the immune system of the host plays also a great role (see next section).

Parasitemia with tachyzoites during pregnancy is a key factor for transplacental transmission. After invading the placenta, they produce placental necrosis associated with embryonic death, fetal resorption, mummification, abortion or stillbirth. If the fetus survives, tachyzoites invading the fetal tissue are able to cause necrotic lesions. As some cases of severe placental necrosis did not result in abortions, also hormonal imbalances have been incriminated in the pathogenesis of toxoplasmic abortion.

Behavioral alterations in laboratory rodents and humans are linked to perturbations in dopamine production.

**Immunology.** Immunity against *Toxoplasma* is very complex. It involves both types of immunity, innate and acquired. The intracellular location protects the parasite from the direct contact with the host's immune effectors. The fact that clinical cases are rare suggests that the immune system work effectively against *Toxoplasma* in most of the cases. This is supported by the extremely high and widespread seroprevalence values reported in

various hosts worldwide. However, the ubiquity of this parasite and also high prevalence values for the presence of infective viable tissue cysts suggest that despite not being able to produce clinical infection, *Toxoplasma* is capable of surviving in the host and remaining infective.

Cellular response is also very strong. CD4+ and CD8+ T-cells are crucial in the recovery form the primary infection. The protective role in subsequent infection is probably held by antibodies. This theory of cellular-mediate immunity is also sustained by the increased susceptibility of human patients suffering from AIDS, a condition caused by the HIV virus which causes depletion of CD4+ cells.

In general, the humoral response is strong. In cats infected with tissue cysts, the seroconversion appears after 10 days and is very persistent. Antibodies can be detected even years after the infection. Seropositive mother cats transfer protective antibodies to kitten. This situation is probably valid also for other animal species but it has not been investigated in detail. This is why post-natal infection in newborns is rare, and most animal become susceptible to oral infection after several weeks of life. In cats for instance, the passive maternally - acquired immunity disappears by the age of 3 month.

Various experimental trials showed that cats shed massive number of oocysts only during the first (primary) infection. All subsequent infection of already immune cats result in no or very low levels of fecal oocyst elimination. The immune protection responsible for inhibition of oocysts formation is lost after several years if no reinfections take place. An interesting immunologic interaction with other feline coccidia was reported in cats with latent toxoplasmosis. When infected with *Isospora felis*, the bradyzoites from the cat's tissue cyst become active again and start to shed *Toxoplasma* oocysts in feces. On the other hand, another coccidian parasite of cats, *I. rivolta* is not able to induce the relapse in oocyst shedding.

**Clinical signs.** Despite most of the infections are asymptomatic, toxoplasmosis still remains a major disease.

Symptoms are not characteristic and this may account for false negative diagnosis and subsequent limitation of its clinical importance. Moreover, certain signs of infection in humans are not considered to be real symptoms of a disease (decreased reaction times, tendency for accidents, personality changes, lower guilt proneness, higher chance for more promiscuous lifestyle) and their correlation with toxoplasmosis is quasi-impossible in practice. In animals, these "hidden" signs are even more difficult (if not impossible) to trace. In mice infected to *T. gondii*, various behavioral changes which enhance the chance of being predated by cats have been recorded: decreased learning capacity, higher activity levels, lower ability to differentiate familiar and novel surroundings or reduced predator avoidance.

Clinical signs and severity of disease vary with the host species, age and immune status.

In ***cats*** with tissular infections, the clinical signs consist in one or more of the following symptoms: fever, anorexia, respiratory abnormalities (dyspnea, polypnea), abdominal pain (due to hepatitis or pancreatitis), icterus, neurologic signs (blindness, anisocoria, slow pupillary light reflex, ear twitch, circling, torticollis, seizures, incoordination, increased affection, stupor, atypical cry, central hypothermia), cutaneous signs (nodules, ulcerations), locomotory problems (lameness, articular pain), and ocular signs (iritis, mydriasis, hyphema, retinal hemorrhages). A statistical analysis on 100 feline cases showed that 36% had systemic infection, 26% showed pulmonary involvement, 16% abdominal lesions, 12% hepatic involvement, 7% neurologic involvement, 4% ocular involvement while other location (cutaneous, pancreatic, cardiac) were less common. Clinical tissue infections in cats might result in sporadic cases of mortality. Cats infected with FIV might have aggravated symptoms and chronic asymptomatic cases may become acute. Congenital toxoplasmosis results in significant mortalities in kitten.

No clinical signs were described in cats with intestinal infection with oocysts. Lesions of severe enteritis were described in cats but they were caused by tissue cysts.

In ***sheep***, the most important clinical sign is abortion. *Toxoplasma gondii* is one of the main causes of infective abortion worldwide. Toxoplasmic abortions in sheep are in the mid or last term of gestation. Mummified fetuses or fetal resorption are also reported in infected sheep. Sterility is also possible in *Toxoplasma*-infected ewes. In sheep flocks in which toxoplasmic abortions are present, a great number of lambs born-alive can suffer of subclinical congenital toxoplasmosis. Except abortion, other symptoms associated with post-natal infection in sheep are fever and diarrhea.

In ***goats***, clinical toxoplasmosis is similar with the situation described for sheep. Abortions and neonatal mortality are not uncommon. Goats are more susceptible to clinical toxoplasmosis than sheep. Mortality following natural infection was reported even in adult goats.

Clinical toxoplasmosis in ***pigs*** is a rare condition. Clinical signs of the acute form (postnatal) include fever, anorexia, dyspnea, weakness of the limbs, neurologic signs and abortions. Generally, pigs recover after 3 weeks. Mortality is possible, but rare. Congenital (neonatal) toxoplasmosis is associated with gait abnormalities, dyspnea, diarrhea and mortality up to the second week of life.

In ***dogs*** clinical cases are usually associated with lower immunologic status, mainly after surviving canine distemper. Reported symptoms include: orchitis, respiratory signs, nervous signs and death. Toxoplasmosis usually is diagnosed post-mortem and it seems to complicate canine distemper cases in puppies. Rare cases of acute toxoplasmosis in adult dogs included ocular and hepatic involvement. No congenital cases are known.

Except very few cases of abortion, in ***cattle*** clinical toxoplasmosis is a rare

condition. Clinical toxoplasmosis in ***horses*** is virtually absent. However, in both these cattle and horses, any reported case of toxoplasmosis must be regarded suspicious. Instead, the other related conditions are definitely more prevalent: neosporosis in cattle (see Chapter 2.4.3.3) and equine protozoal myeloencephalitis (see Chapter 2.4.3.1).

In ***chicken***, there are very few known cases of clinical toxoplasmosis. These scarce reports include sudden death and nervous signs (torticollis, lateral recumbency).

**Pathology.** The lesions associated with *Toxoplasma* infection are located in various organs and tissues. During acute toxoplasmosis, tachyzoites are responsible for producing necrotic lesions in various tissues (mesenteric lymph nodes, liver, intestinal lamina propria, spleen, pancreas, lungs, adrenal glands, kidneys). Same tachyzoites-induced lesions are present in internal organs of early aborted or stillborn fetuses or in newborns suffering of congenital toxoplasmosis.

Additionally, necrotic or degenerative encephalitis, endocarditis and retinitis have been described in congenital toxoplasmosis in goat kids, lambs, kittens, puppies and piglets. Histologically, in all the affected organs tachyzoites are visible.

Tachyzoite infection in pregnant females results in degenerative lesions of the placenta. The main lesion is necrotizing placentitis with the presence of large amounts of tachyzoites in the trophoblastic layer.

Focal myocarditis and encephalitis with the presence of bradyzoites in tissue cysts are the main lesions in chronic toxoplasmosis.

**Diagnosis.** Diagnosis has different approach in the feces of the definitive host or in the samples from intermediate hosts.

Detection of oocysts in the feces of cats is done by classical flotation methods. However, finding non-sporulated or even sporulated oocysts with *Toxoplasma*-like morphology is not enough to say it is *Toxoplasma*. As shown in other sections of this textbook (see Chapters 2.4.1.1 and 2.4.3.4), various coccidia are parasitic in the small intestine of cats. Their differentiation cannot be done relying strictly on morphological features (**table 2.39**).

**Table 2.39** Oocyst size of coccidia found in feces of cats (after Dubey and Greene, 2012)

| Species | Average size (µm) |
|---|---|
| *Isospora felis* | 40 x 30 |
| *Isospora rivolta* | 22 x 20 |
| *Toxoplasma gondii* | 12 x 10 |
| *Hammondia hammondi* | 12 x 11 |
| *Besnoitia wallacei* | 17 x 12 |
| *Besnoitia darlingi* | 12 x 11 |
| *Besnoitia oryctofelisi* | 12 x 11 |
| *Sarcocystis* spp.* | 11 x 9 |

*sporocysts

For safety reasons, all small oocysts found in the feces of cats must be regarded as *Toxoplasma*. Specific identification can be done only by molecular biology (copro-PCR) or after sporulation by ultrastructural studies or xenodiagnosis.

The diagnosis in systemic infections of intermediate hosts is more complex. As clinical signs are not characteristic, diagnosis must rely on detection of the organism or its DNA in various tissues or the detection of antibodies. The most difficult task is the differentiation of subclinical from clinical toxoplasmosis and to correlate the presence of *Toxoplasma* with the symptoms.

For diagnosing clinical toxoplasmosis, various symptoms must be correlated with imagery and clinical laboratory followed by identification of the organism from various samples, including biopsies by cytology, histology, PCR, xenodiagnosis (artificial infection in rodents) or serology.

In vivo diagnosis of ***clinical toxoplasmosis*** is important for acute post-natal cases and congenital infections. It is mainly of interest in pets. Fever in cats, correlated with unresponsiveness to antibiotic treatment is one of the most common findings of acute toxoplasmosis. The procedure in this case is to continue with further clinical procedures. Fundic ocular examination reveals multifocal iridocyclochoroiditis. Cytology during the acute disease can reveal the presence of free tachyzoites in various tissue and body fluids. Tachyzoites are more common in thoracic and peritoneal pathologic effusions but they can be rarely detected also in other samples (blood, cerebrospinal fluid, tracheal washings). These samples should be further analyzed by PCR for confirmation. Thoracic radiology can also help, showing a diffuse interstitial to alveolar pattern and peribronchial markings. Abdominal radiology shows the enlarged mesenteric lymph-nodes. Other imagery techniques used mainly for detection of lesions in the central nervous system include myelography, computer tomography (CT) and magnetic resonance imaging (MRI). Clinical laboratory findings are not specific but they may help in the diagnosis. They include: anemia, leukocytosis with neutrophilia, eosinophilia, lymphocytosis and monocytosis. Terminally ill cats present leukopenia (with absolute lympho-cytopenia and neutropenia). Usually the leukocytosis is indicative of recovery and a better prognosis.

Clinical biochemistry shows hypoproteinemia, hypoalbumin-emia, increase of alanine amino-transferase, aspartate aminotransferase and alkaline phosphatase associated with hepatic and muscular necrosis. The serum level of creatine kinase is increased when muscular necrosis is present. In animals with acute hepatic necrosis, the levels of serum bilirubin are also increased. Increased serum amylase and lipase activities are indication of pancreatic involvement. All these clinical laboratory findings are not enough for a positive diagnosis of toxoplasmosis.

***Isolation*** of the agent from aborted fetuses and fetal membranes is routinely done by inoculation to laboratory mice. For this scope, the fetal brain and placental cotyledons yield optimal results.

Identification in ***tissue section*** is done by histology and more specifically by immunohistochemistry. Histopathology

is essential for concluding on the cause-effect association.

***Serologic diagnosis*** include detection of humoral antibodies: Dye test known also as Sabin-Feldman test, indirect hemagglutination test, complement fixation test, modified agglutination test, latex agglutination test, indirect fluorescent antibody test, ELISA, immunoglobulin M immunosorbent agglutination assay test and Western blotting.

An ELISA method has been developed for detection of *Toxoplasma* antigens in cats.

***Molecular techniques*** are employed for the detection of parasite DNA. PCR is widely used on various samples mainly for diagnosing abortions and for molecular epidemiology surveys.

For details on the technique of isolation, cultivation and serologic procedures refer to Dubey (2010) and to OIE (2012).

Differential diagnosis is variable in each species. Because of the great variety of clinical signs in cats, the list of diseases to be considered for the differential diagnosis is too long to be given here. Readers should refer to a more general feline medicine book. In the case of toxoplasmic abortion, differential diagnosis must include all the other causes (infectious and non-infectious). In dogs the differential diagnosis must be done against neosporosis.

**Treatment.** Treatment has two major indications: to treat the clinical systemic cases and two treat the cats with intestinal infection to stop the oocyst elimination.

In ***cats*** with systemic infection the treatment is done by combining two drugs. Sulfonamides (20-30 mg/kg, orally, every 6-24 hours, for 2-4 weeks) in combination with pyrimethamine (0.5-1 mg/kg, orally, every 24 hours, for 2-4 weeks) are the drugs of choice. Pyrimethamine can be given parenterally, after dilution. Sulfonamides can be also used in combination with trimethoprim. The use of these drugs may induce thrombocytopenia and/or leukopenia. In such a case, treatment should not be discontinued but the cats should receive folinic acid and yeast supplement. Clindamycin (8-17 mg/kg, orally or intramuscularly, every 8-12 hours, four 4 weeks) is also highly effective in feline toxoplasmosis.

The treatment of choice in ***dogs*** is similar with the one in cats, but doses are slightly different: clindamycin (3-13 mg/kg, orally or intramuscularly, every 8 hours or 10-20 mg/kg, same routes, every 12 hours) for 4 weeks.

As in the other domestic animal species acute toxoplasmosis is rare, no therapeutic protocols are recommended. In the case of toxoplasmic abortion, chemoprophylaxis must be performed in pregnant animals.

Treatment of ***cats which shed oocysts*** is done by: clindamycin (25-50 mg/kg, orally or intramuscularly, every 12-24 hours, for 1-2 weeks); a combination of sulfonamides (100 mg/kg, orally, every 24 hours, for 1-2 weeks) with pyrimethamine (1 mg/kg, orally, every 24 hours, for 1-2 weeks); toltrazuril (5-10 mg/kg, orally, every 24 hours, for 2 weeks). Monensin given in food for one

two weeks is also effective and stops oocysts shedding.

**Control.** Preventing infection with *Toxoplasma* is essential in all host categories (humans, cats, livestock). Prevention in cats is easy if they are kept indoors and never receive uncooked meat, bones or organs from animals, even if these are bought from grocery stores. In the case cats do not eat dry or canned food or cooked food, the option of choice is to give them raw meat which was deep-frozen before or beef which is usually free of infective cysts. Raw liver is an essential part of feline diet, as it is a perfect dietary supplement of vitamin A. As infective *Toxoplasma* cysts are very common in the liver, in such cases the organs must be deep frozen before fed to cats. Owners must avoid letting cats to hunt outside or to scavenge in the trash can. In farms, cat populations must be controlled. In farms where abortions occur, fetuses and fetal membranes must be eliminated promptly to avoid placentophagia by cats or other animals. Outdoor cats must be under permanent surveillance and treated if they are shedding small coccidian oocysts.

Prevention of infection in humans include: washing the hands after handling meat or after petting cats and dogs. Fruits and vegetables must be washed thoroughly. Any consumed meat must be properly cooked. Tasting of food during cooking is not recommended. Microwave cooking does not kill the bradyzoites. Freezing meat at -12°C kills the tissue cysts in few days. Pregnant women should avoid contact with cats, raw meat and unwashed fruits or vegetables.

Chemical prevention is done mainly in humans in certain cases when patients are exposed to risk of clinical infection or the risk of congenital toxoplasmosis is high. If a human patient is serological negative, meaning there is no protection against *Toxoplasma*, and an immunosuppressive treatment must be applied (i.e. before transplants), the prophylactic therapy is recommended. Prophylactic treatment has been used also in sheep, in flocks with history of toxoplasmic abortion. The drug of choice in this case is monensin (17-28 mg/kg, daily, for five days), given in the last half of gestation. Other drugs (sulfamethazine, pyrimethamine, decoquinate) have been shown to have similar effects in sheep.

One commercial vaccine (Toxovax) is available for prevention of toxoplasmic abortions in sheep.

### 2.4.3.3 Neosporosis

**Introduction.** Neosporosis is a disease of cattle and dogs with huge economic impact in dairy and beef cow farms. It is considered one of the most important infectious causes of abortion in cattle, worldwide. It has been estimated that annual global losses due to neosporosis are between 1.2 and 2.3 billion US dollars. Additionally, another *Neospora* species is responsible for a neurologic disease in horses.

**Historical notes.** The history of neosporosis is very recent. Until 1988 when the etiological agent was discovered, it was confused with *Toxoplasma gondii*. The first case of

neosporosis was described in 1984, in dogs from Norway by Bjerkas et al. Three years later, in 1987, O'Toole and Jeffrey described a clinical case in a newborn calf. Both reports were designated to be caused by an unidentified protozoan, similar to *Toxoplasma* and *Sarcocystis*. The agent was described only in 1988 by Dubey and named *Neospora caninum*. This does not mean the disease is new, but that until 1988, all cases (except the two listed above) were considered to be toxoplasmosis.

More recently, in 1998, a new species, *Neospora hughesi* was described from the central nervous system of a horse in California by Marsh et al.

**Etiology.** Two species are known in genus *Neospora*. *Neospora caninum* is typically parasitic in dogs (which act as definitive hosts) and cattle (intermediate hosts). Several other species have been found naturally infected with viable *N. caninum* (sheep, buffaloes, horses, bison, deer). The second species, *Neospora hughesi* is responsible for a form of equine protozoal myeloencephalitis.

**Morphology.** Oocysts are small (10.6-12.4 x 10.6-12.0 µm) and morphologically not differentiable from other small oocysts found in canine feces (see **table 2.40**). Oocyst wall is colorless. Sporulated oocysts contain two sporocysts (7.4-9.4 x 5.6-6.4 µm), each with four sporozoites (5.8-7.0 x 1.8-2.2 µm). Oocysts ("*Isospora*"-like) can be found in the feces of dogs and other canids.

Tachyzoites (3-7 x 1-5 µm) are found in intracellular parasitophorous vacuoles in the cytoplasm of various cells in the intermediate hosts (see life-cycle). Their typical shape is crescent-like (**figure 2.73**). Bradyzoites (8 x 2 µm) are found in tissue cysts surrounded by a thick-wall (4 µm), located in muscular and nervous tissues.

**Table 2.40** Oocyst size of coccidia found in feces of dogs (modified after Dubey and Greene, 2012)

| Species | Average size (µm) |
|---|---|
| *Isospora canis* | 38 x 30 |
| *Isospora ohioensis* | 24 x 20 |
| *Isospora neorivolta* | 17 x 15 |
| *Isospora burrowsi* | 20 x 17 |
| *Neospora caninum* | 12 x 10 |
| *Hammondia heydorni* | 12 x 11 |
| *Sarcocystis* spp.* | 11 x 9 |

*sporocysts

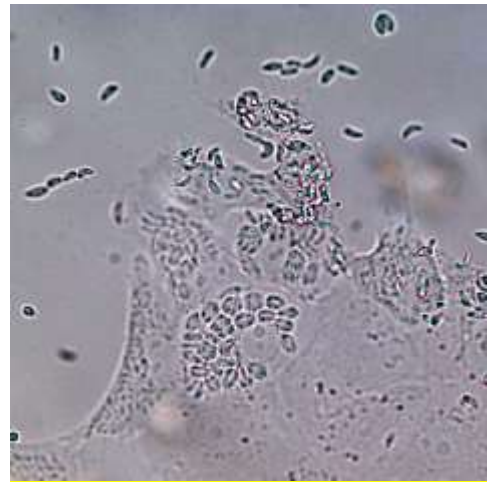

**Figure 2.73** Tachyzoites of *Neospora caninum* in cell culture. *(Photo Ovidiu Şuteu)*

Although many genetic strains of *N. caninum* have been described, little is known on their variation in virulence. In the case of *N. hughesi*, only the asexual

stages in the intermediate host (horses) are known. Tachyzoites are located in a parasitophorous vacuole inside host cell cytoplasm. Bradyzoites (2-3 x 4-7 µm) are within a tissue cyst (7-16 x 10-19 µm), surrounded by a wall (0.1-1 µm thick).

**Life cycle.** In many aspects, the life cycle of *Neospora caninum* is similar to that of *Toxoplasma gondii*. The life cycle of *N. caninum* is heteroxenous (**figure 2.74**). Domestic dogs and other canids (coyotes, grey wolves, dingoes) are definitive hosts. The typical intermediate hosts are cattle, but various other warm-blooded vertebrates can serve as hosts for the asexual stages. However, the spectrum of recorded intermediate hosts is not as diverse as in the case of *T. gondii*. One very important aspect is the lack of human infections reported. So far, neosporosis is not considered a zoonotic disease.

Dogs shed in their feces unsporulated oocysts which eventually undergo exogenous sporogony (24-72 hours) and become infectious (sporulated) oocysts. If sporulated oocysts are ingested by a suitable intermediate hosts (e.g. cattle), they excyst and the freed sporozoites will penetrate the intestinal wall and invade various cell types: macrophages, neural cells, fibroblasts, endothelial cells, muscle cells and hepatocytes where the multiply by endodyogeny and producing tachyzoites. Tachyzoites divide around twenty times before becoming bradyzoites in tissue cysts (in cca. three weeks after the infection). Tissue cysts with bradyzoites are found typically in the central nervous system and striated muscle cells where they can remain for all the life of the animal.

The typical life cycle continues when tissue cysts with bradyzoites are ingested by a canine definitive host. What happens here is still largely unknown, but it is presumed that part of the bradyzoites reconvert into tachyzoites and produce systemic infection which ultimately result in the formation of tissue cysts.

Systemic infection with tachyzoites can result in the transplacental transmission to puppies. Other bradyzoites will invade the epithelial cells of the dog's intestine and they probably follow a similar development as *Toxoplasma gondii* does in cats, continuing merogony, and then gametogony with oocysts formation. Infected dogs shed through their feces unsporulated oocysts 5 days after ingestion of tissue cysts.

The oocyst elimination by dogs is at a much lower rate than in cats infected with *T. gondii*. It has been estimated that dogs shed around 500,000 oocysts (compared to 1 billion oocysts excreted by one infected cat). In dogs, the time they shed oocysts extends up to 4 months (unlike in cats which shed *T. gondii* only for 1-2 weeks). Considering the lower overall number of eliminated oocysts and the longer period, the chance of finding *Neospora caninum* oocysts in dog feces is very low. Another difference from *Toxoplasma gondii* is that dogs are not susceptible for the infection with *N. caninum* oocysts or tachyzoites (like cats are for *T. gondii*). Dogs can be infected only with tissue cysts with bradyzoites from intermediate hosts.

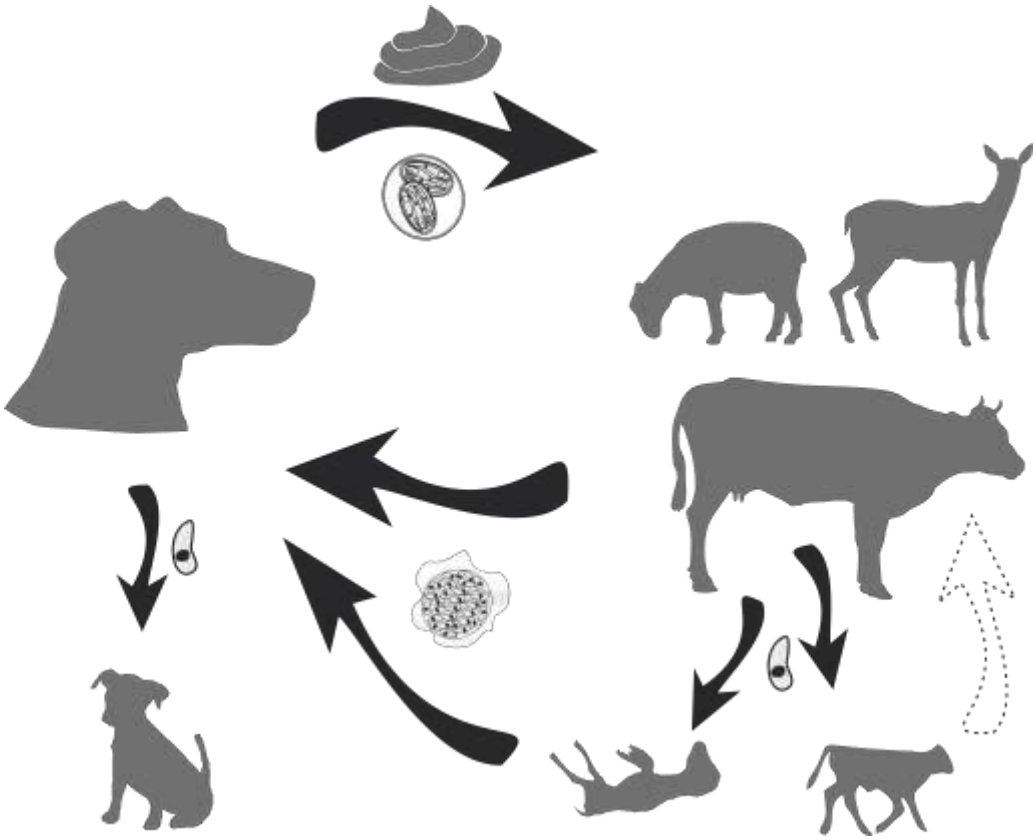

**Figure 2.74** Life cycle of *Neospora caninum*. Contamination of dogs as definitive hosts takes place after ingestion of tissue cysts from meat or organs of infected intermediate hosts (including aborted fetuses and fetal membranes). Intermediate hosts acquire the infection after eating sporulated oocysts passed by dogs in the feces. Infected intermediate host females are able to pass the infection to their offspring which will develop lifelong infection. Subsequently they are able to transmit the infection transplacentally again and again (dotted contour arrow). After ingesting tissues cysts, dogs develop both enteroepithelial and systemic infection and are able to pass tachyzoites transplacentally to puppies.

Additionally, the only known way of horizontal transmission to intermediate hosts is by ingestion of oocysts.

Although placentophagia in cows was suggested as a mode of transmission (horizontal, intermediate to intermediate host), no experimental trials support this theory yet.

Except this typical life cycle involving alternation of the definitive and intermediate hosts, *Neospora caninum* can be transmitted vertically, from infected pregnant females to the fetus. There are two types of transplacental transmission described for *N. caninum* in cattle: (1) The exogenous transplacental

transmission occurs when non-infected pregnant cows ingest infective oocysts. In this case, the tachyzoites will invade the fetus, transported probably by the mononuclear phagocytes via the blood stream. (2) The endogenous transplacental transmission happens in cows which are already infected before pregnancy. In such a case, bradyzoites from tissue cyst are reactivated and they differentiate into tachyzoites which ultimately infect the fetus. The endogenous transplacental transmission explains why female calves with congenital neosporosis are able to pass the infection to their offspring when they become reproductive females.

It is not known if congenital transmission follows the same rule in dogs or other hosts. Infected female dogs can give birth to infected puppies during several pregnancies.

Transmission is not possible through milk or venereal route.

The life cycle of *N. hughesi* is not known, as only the asexual stages in the intermediate hosts were described. Probably the definitive host is a carnivorous mammal.

**Epidemiology.** Neosporosis is distributed worldwide in cows and dogs, as numerous seroprevalence and molecular epidemiology studies show. Antibodies were found also in small ruminants, but the clinical importance in ovine abortion is still debated. The prevalence of neosporosis is highly variable between countries, between regions of the same country and between beef and dairy cows and between cattle breeds. The highest seroprevalence in cattle has been found in those farms where dogs were more abundant.

Seroprevalence data in dogs is reported worldwide. In random populations it is usually less than 20%. Purebred dogs are more likely to develop seropositivity than mixed breeds. Stray dogs with access to raw organs and meat have significantly higher prevalence than indoor dogs fed exclusively with commercial diet. Serologic prevalence is higher in cattle farm dogs than in dogs living in cities.

The most important epidemiological factor from clinical point of view is the prevalence of abortions caused by *N. caninum* in cows. Two epidemiologic patterns of *Neospora*-induced abortions are known: endemic abortion and epidemic abortions. The endemic pattern consists of persistent abortion rate (around 5-10%, all year around). The most dramatic situation (epidemic pattern) is the so called "***abortion storm***", when more than 10% of the pregnant cows from a single farm abort within a time frame on 12 weeks. Abortions can persist from several months to several years.

As dogs shed a very low number of oocysts, the main source of infection for cows is the transplacental transmission from their mothers. However, not all infected cows transmit the infection to their offspring. The transplacental transmission rate varies roughly from 30 to 60%. It is not known if the transmission rate is different between endogenous and exogenous transplacental transmission routes. Some authors suggest that post-natal infection

is equally important, as seropositivity may increase dramatically in certain flocks at a given time. In the recent years, wildlife reservoirs increased in their epidemiologic importance. Abortion storms are likely caused by exogenous transplacental transmission following exposure to oocysts.

Resistance of oocysts of *N. caninum* in the environment is considered to be more or less similar with *T. gondii*. High temperatures (100°C) inactivate them in one minute and treatment with 10% sodium hypochlorite in 1 hour. Bradyzoites in tissue cysts remain infective in tissues for 7-10 days.

**Pathogenesis.** It is not fully understood why some infected ***cows*** are able to give birth normally while others abort. Various factors have been incriminated, including the infective oocyst dose. It is also not known if there are any correlations between the clinical outcome and the mode of transplacental infection (endogenous or exogenous). Other hypothesized factors include the existence of *N. caninum* strain with different virulence or the susceptibility of cows under metabolic stress (e.g. dairy cows). The factors affecting the risk of abortion were summarized by Goodswen et al. (2013).

For the exogenous transmission cycle, the risk factors are the number of oocysts ingested and the gestation stage. In the case of endogenous transmission cycle, cows with higher antibody titers are more likely to abort.

Reactivation of bradyzoites from tissue cysts and their reconversion to tachyzoites is thought to be related to hormonal and immune factors of the pregnant cow.

The pathogenesis of abortion is related to the damage of the placenta by rapidly multiplying tachyzoites. They initially invade the maternal caruncular septum and subsequently the fetal placental villi. The abortion occurs in two situations. When the placenta is severely damaged, the fetus receives insufficient oxygen supply and nutrition. Other situation is when tachyzoites destroy directly the fetal tissues. Immune-mediated fetal expulsion has been also suggested, associated with maternal pro-inflammatory cytokines (IL-10, gamma interferon).

Pathogenesis in ***dogs*** is caused by the rapid multiplication of tachyzoites in various tissues. This can occur mainly during primary infections but also if bradyzoites are reconverted to tachyzoites during persistent infection (e.g. stress, pregnancy, immuno-suppressive diseases). Development in the brain of dogs causes monocyte-mediated lesions with altered function of the nervous centers involved. Lower motor neuron damage and severe myositis are the causes of gradual hind limb paralysis in puppies with congenital neosporosis. Destruction of the muscular layers of the esophagus can result in megaesophagus with dysphagia.

**Immunology.** Immunity plays a crucial role in the development of neosporal infection. Weather the infected cows abort or they transmit the infection transplacentally is greatly dependent on the immune response. Moreover, some

offspring which are born alive display signs of disease, some others not. The immune response in cows infected with *Neospora caninum* is associated with high levels of IFN-γ, IgG-2 antibodies and Th1 cells. IFN-γ inhibits the growth of tachyzoites. IFN-γ activates the response of macrophages which play a major role in the differentiation of tachyzoites to bradyzoites and vice-versa (during reactivation of infection).

The immunological maturity of the fetus when it is infected determines probably its survival chance. As most organs involved in fetal immunity (thymus spleen, lymph nodes) mature in the last period of gestation, the risk of fetal death and abortion is higher during the early gestation. The immunity can be protective as seropositive cows are less likely to abort. Based on this, vaccination has been developed.

**Clinical signs.** Most infections are asymptomatic. In ***cows***, the characteristic clinical sign is abortion (**figure 2.75**). The outcome of gestation resulting from infected cow can be: fetal resorption, fetal mummification, fetal autolysis, stillbirth, born alive with clinical signs, born with no clinical signs but with persistent life-long infection and born without infection. About 80-90% of the infected cows produce apparently normal calves, part of them infected.

Abortion can be present in cows of any age, from the age of gestation of three month to term. However, most abortions are reported to be between 4 and 6 months of gestation. Repeated abortion is uncommon, and usually it is encountered in less than 5% of the aborting cows. Post-abortion sterility is not considered a problem.

**Figure 2.75** Six months old bovine aborted fetus caused by *Neospora caninum*. *(Photo Ovidiu Şuteu)*

***Congenital neosporosis in calves*** (<2 months old) include variable clinical signs: inability to rise, ataxia, flexed or hyperextended for or hind limbs, decreased patellar reflex, loss of proprioception, exophthalmia and decreased weight. Calves can be born alive, but with severe congenital deformities (like hydrocephalus) which are incompatible with life. Other calves are born with neonatal encephalomyelitis and are paralyzed.

The infection in ***dogs*** is usually asymptomatic. It can affect dogs of all ages (post natal infection) but also neonates (congenital neosporosis). Though, most cases are congenital. Puppies born from infected mothers do not necessarily develop clinical signs. If

they do, anyhow they are usually born with no evident sign of infection.

First clinical signs of ***congenital neosporosis in puppies*** appear at 3-9 weeks of age. The most common symptom is paralysis of the hind legs, often with spastic character (arthrogryposis) and gradual muscle atrophy and stiffness. Usually the paralysis is gradual and ascending. Forelimbs may be also affected but less severely.

Other clinical symptoms in puppies include: joint deformations, cervical weakness, dysphagia (caused by megaesophagus). During all this time dogs are fully conscious and alert. They can survive in this state for several months until finally death occur. Not all puppies from the same litter show clinical signs.

In ***dogs older than six month***, clinical neosporosis can be caused by the primary infection or by reactivation of a chronic infection due to various factors (see pathogenesis).

The clinical signs are related to the multifocal nervous lesions or to polymyositis. Other clinical signs recorded in adult dogs are: coughing, progressive ataxia, dysphagia, cutaneous ulcers. Dogs with severe multifocal central nervous involvement usually die.

Clinical biochemistry findings in dogs include increased levels of creatine kinase and aspartate aminotransferase (due to severe myositis and hepatitis). The cerebrospinal fluid has an increased level of proteins and pleocytosis. Tachyzoites may be present in the cerebrospinal fluid. There are no recorded clinical cases of canine intestinal neosporosis caused by the enteroepithelial cycle.

Clinical signs caused by *N. caninum* in other domestic hosts are rare and they include abortions in sheep, goats and South American camelids. The infection with *N. hughesi* in horses is responsible for a similar syndrome with the equine protozoal myeloencephalitis produced by *S. neurona*. *Neospora hughesi* is not pathogenic for dogs.

**Pathology.** Lesions caused by *N. caninum* are very important mainly from diagnostic point of view, as the presence of histopathological changes can represent a reliable cause-effect correlation. They are different in each host type.

***Lesions in aborted fetuses*** include serosanguinolent fluid accumulation in the body cavities (**figure 2.76**). Sometimes, the fetal tissues are in incipient or moderate autolysis (**figure 2.77**). Other gross lesions are more difficult to be detected (pale white foci in the muscles). Histopathology from fetal tissues reveals generalized non-suppurative infiltrates. The brain is the site of somehow more characteristic lesions. They consist of scattered foci of non-suppurative cellular infiltrates, sometimes necrotic. *Neospora caninum* developmental stages can be visible or not in these histological sections. Other lesions in aborted bovine fetus include: epicarditis, myocarditis, myositis, hepatitis, all focal, with non-suppurative cellular infiltrates and even focal necrosis.

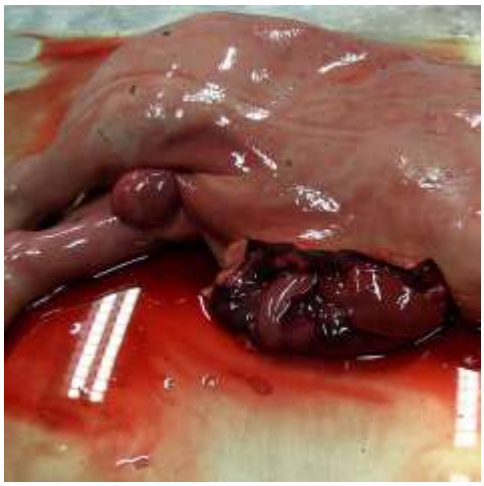

**Figure 2.76** Serosanguinolent fluid accumulation in the abdominal cavity of an aborted bovine fetus caused by *Neospora caninum*. *(Photo Ovidiu Şuteu)*

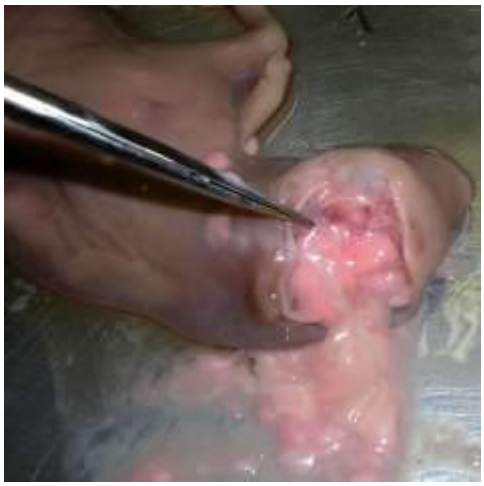

**Figure 2.77** Autolysis of the central nervous system of an aborted bovine fetus caused by *Neospora caninum*. *(Photo Ovidiu Şuteu)*

***Lesions in congenital neosporosis*** of calves are more or less similar with lesions from aborted fetuses. They include mainly non-suppurative encephalomyelitis.

Gross lesions in ***dogs*** include: multifocal areas of necrosis and/or fibrosis with or without mineralization in the striated muscles (mainly diaphragm), hepatitis with hepatomegaly, pneumonia and discoloration of certain areas of the central nervous system (visible on gross tissue sections). Histological sections in puppies which died of congenital neosporosis reveal the presence of lesions and parasitic stages in the following organs: muscles, myocardium, retina, nerve roots, thymus, kidney, liver, adrenal gland, brain, spinal cord, stomach wall or dermis. The characteristic lesions are non-suppurative myeloencephalitis, myositis, polyradiculoneuritis (mainly in puppies), lymphonoditis, myositis, cerebral cortical necrosis etc.

**Diagnosis.** The most difficult task in aborting cows is to ***correlate the presence of Neospora caninum with the abortion***. Finding antibodies in a cow which aborted or in the cow population from a farm with endemic or epidemic abortions is not enough. Moreover, finding the parasite in the tissues of an aborted fetus (by PCR or immunohistochemistry) is not always enough.

The etiologic confirmation of abortions must be done in specialized laboratories. All of them have to be correlated with the presence of *Neospora caninum* in histological lesions from the fetus or from the placenta and with other epidemiological data, including the age of fetus and the immune status of the cow. The lesions in fetuses aborted because of

*Neospora* usually have disseminated inflammatory lesions in most of their internal organs (brain, heart, kidneys, liver, lungs, muscles). Additionally, other abortion causes must be excluded.

Various serologic tests are available for the detection of anti-*Neospora* antibodies (IFAT, ELISA, direct agglutination test, Western blot). They are widely used for seroepidemiologic surveys. However, their use in clinical diagnosis has many limitations. Most of them are not able to discern between chronic and acute infection. Moreover, the antibody titers in seropositive cows are fluctuant.

Some seropositive cows may become seronegative (antibody titers below the cut-off value). Serologic testing can be used also for fetal samples. Serologic testing in calves must be done after the age of 6 months, to eliminate the positivity due to maternally transferred antibodies.

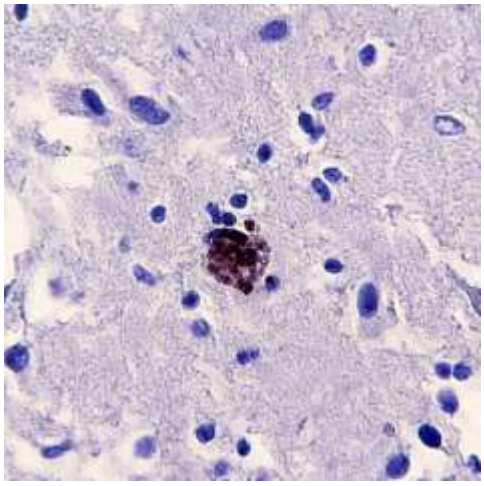

**Figure 2.78** Cyst of *N. caninum* in the brain (IHC) *(Photo Ovidiu Şuteu)*

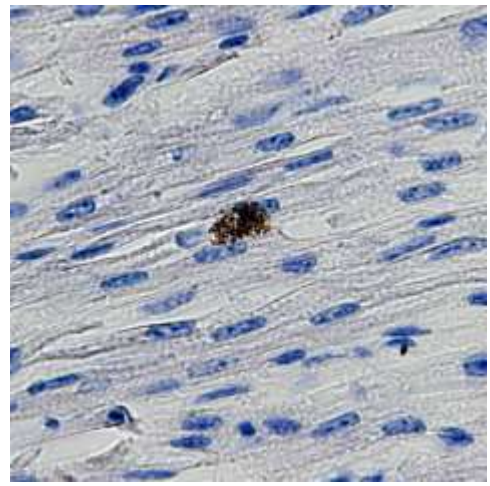

**Figure 2.79** Cyst of *N. caninum* in the cardiac muscles (IHC) *(Photo Ovidiu Şuteu)*

Immunohistochemistry (IHC) is a very useful tool for differentiating *Neospora* tissue cysts from other protozoal cysts (**figures 2.78** and **2.79**). PCR is also highly sensitive and specific.

Serologic diagnosis using recombinant proteins from tachyzoites (NcGRA7) and bradyzoites (NcSAG4) allows differentiating between chronic and acute infections. If both proteins yield positive results, this might be indicative of a reactivated infection during pregnancy.

The same diagnostic principles as for the abortion apply for diagnosis of congenital neosporosis in calves.

Diagnosis in ***dogs*** relies greatly on the correlation of symptoms, clinical laboratory (blood and CSF) and results of various serological assays (IF, ELISA, immunoprecipitation). The definitive diagnosis can be based on the demonstration of the presence of *Neospora caninum* in the CSF. However,

this is a difficult task, as the number of tachyzoites is very low. In dead animals, the presence of the parasite can be made also in histological sections from brain, heart or muscles.

Differentiation from other cyst-forming coccidia is based on PCR or immunohistochemistry. Differential diagnosis in dogs must be done from many diseases which produce similar symptoms and lesions: infection with *Hepatozoon canis* and *Hepatozoon americanum*, leishmaniosis, sarcocystosis, clostridial myositis, leptospirosis, ehrlichiosis, trichinellosis, trypanosomosis etc.

Serological tests in horses and possibly also in other animals can be difficult to interpret because of suspected cross-reaction between antibodies against *N. caninum* and *N. hughesi*.

For the isolation of the *Neospora caninum* in the lab and for experimental trials, laboratory animals such as mice, gerbils and fat-tailed dunnarts are widely used.

**Treatment** is not routinely done in bovine neosporosis. Experimental administration of toltrazuril to calves can delay the tachyzoite multiplication and spread. There is no data on the effect of toltrazuril on bradyzoites.

The treatment of ***canine*** neosporosis must be approached in all animals with clinical signs and with a positive diagnosis of neosporosis. Otherwise, the condition is often fatal. The treatments of choice in dogs are:

- a combination of trimethoprim-sulfadiazine (15 mg/kg, orally, every 12 hours, for at least 4 weeks) with pyrimethamine (1 mg/kg, orally, every 24 hours, for at least 4 weeks);
- clindamycin (10 mg/kg, orally, every 8 hours, for 4 weeks) in adult dogs;
- clindamycin (75-150 mg/dog, orally, every 12 hour, for 6 months) for 13 months old puppies with congenital neosporosis;
- sequential treatment with clindamycin hydrochloride, trimethoprim-sulfadiazine and pyrimethamine

Treatment of dogs does not always result in the complete recovery or in the full elimination of the parasite. However, clinical signs may improve. Sometimes the treatment must be done for very long time (up to 18 months) until improvement is seen.

**Control.** It is essential mainly in farms. In uninfected bovine herds, preventing the introduction of neosporosis is the main assignment. Newly introduced cows must be purchased from *Neospora*-free farms and they should be serologically tested before.

Prevention of horizontal transmission is achieved by restricting the access of freely moving dogs in cattle farms. If they are present, their direct access to the animals or their food must be avoided. This is more difficult (or virtually impossible) if cattle are grazed on pastures where dogs or wild canids have access. Monitoring programs must be introduced (serological testing of cows, laboratory examination of each aborted fetus).

In infected herds the primary goals are to prevent abortions, to prevent spreading the disease (both horizontally and vertically), to avoid introduction of new infected animals and ultimately the elimination of infection.

Permanent serologic monitoring of the herd is recommended, and negative animals must be selected for breeding on long term. Animals with high antibody titers or history of repeated neosporal abortions must be considered for culling. Embryo transfer from seropositive donors implanted to seronegative recipients produces non-infected offspring.

Proper disposal of fetal membranes (placenta) and aborted fetuses must be strictly followed.

A killed anti-*Neospora caninum* vaccine is commercially available in United States. The vaccine must be used twice in early gestation, mainly in farms where the infection is present. Vaccination was shown to reduce the rate of abortions. The main disadvantage of the vaccine is the production of seropositive cows; post-vaccinal seropositivity is not differentiable from the post-infective seropositivity.

To prevent infection of pet dogs, administration of raw meat or organs, mainly of bovine origin must be avoided. In bitches known to be infected with the parasite, birth control programs (including spaying) are to be considered for prevention of vertical transmission. There is no vaccine available against canine neosporosis.

#### 2.4.3.4 Other heteroxenous coccidia parasitic in domestic animals

Except the three major heteroxenous coccidia presented before (*Sarcocystis*, *Toxoplasma*, *Neospora*), several other genera have been identified in domestic animals. Their importance is rather for the differential diagnosis than for their clinical importance.

**Genus *Hammondia*** is represented by two species. Both are heteroxenous. *Hammondia hammondi* has felids as definitive hosts and goats and mice as natural intermediate hosts. Many others mammal species serve as experimental intermediate hosts. Cats are infected only after eating tissue cysts and develop only intestinal infection.

Cats eliminate unsporulated oocysts, morphologically similar to those of *Toxoplasma*. Oocysts of *Hammondia* sporulate in the environment. Intermediate hosts are infected after ingesting sporulated oocysts. Sporozoites invade the mesenteric lymph nodes and other abdominal organs where tachyzoites develop. Ultimately, they encyst as bradyzoites in the skeletal muscles. The second species, *H. heydorni* uses dogs as definitive hosts and various domestic and wild mammals as intermediate hosts. The life cycle is similar to *H. hammondi*. The reports of clinical signs associated with *Hammondia* infection are scarce. Mild diarrhea has been occasionally reported in dogs. In intermediate hosts the symptoms are also normally absent. In experimentally infected goats, fever has been reported.

**Genus *Besnoitia*** includes nine species parasitic in a great variety of hosts. The life cycle is known, although not in great details, only for few species. It seems the life cycle is more similar to *Toxoplasma gondii* than to *Hammondia*, as extraintestinal development takes place also in the definitive host.

Four of the species have cats as definitive hosts. These species and the respective intermediate hosts for each are: *B. wallacei* (rodents), *B. darlingi* (opossums, lizards), *B. oryctofelisi* (rabbits) and *B. neotomofelis* (woodrats). After cats ingest tissue cysts from the connective tissues of intermediate hosts, the bradyzoites follow enteroepithelial merogony and gametogony, followed by extraction of unsporulated oocysts. Part of the bradyzoites will invade also extraintestinal sites in the cat host, as *Toxoplasma* does.

Dogs are not known to harbor any species of *Besnoitia* as definitive hosts.

The life cycles for the other 5 species of the genus are not known only from few studies on the asexual development in the intermediate hosts. Among these five species, three are parasitic in domestic animals: *Besnoitia besnoiti* (bovines), *B. bennetti* (equids) and *B. tarandi* (raindeers).

The most important species of veterinary importance is *Besnoitia besnoiti*, responsible for the ***bovine besnoitiosis***. Its life cycle is not fully understood. Bovines are intermediate hosts; the definitive hosts are not known. Not all infections in cattle become clinically evident. If they do, the principal clinical signs include initial fever, anorexia, tachycardia and tachypnea, corresponding to the rapid multiplication of tachyzoites in various tissues and internal organs. The next stage of the disease consists in cutaneous signs: skin congestions with increased sensitivity in various body areas and anasarca (generalized edema). In the next stage of the disease, the skin becomes sclerodermic, with complete loss of hair, severe lichenification and hyperpigmentation. Sexual organs are also affected. In this chronic stage, the affected areas are full of tissue cysts, which are large enough to be seen at gross necropsy. There is no treatment for besnoitiosis.

### 2.4.4 Hepatozoidae

**Introduction.** The family has a single genus, *Hepatozoon*. The genus includes around 300 species parasitic in all groups of tetrapod vertebrates.

**Ecology and transmission.** The variety of life cycles is very high within genus *Hepatozoon*. The full development and complete life cycle is not known only for few species. The life cycle is heteroxenous, at least for those species where it is known, but probably for all. One of the hosts is always a vertebrate (amphibian, reptile, bird or mammal) and the other is always an invertebrate. The merogonic and gametogonic developments take place in various tissues of the vertebrate host. The invertebrate is hosting the sporogonic development with the oocyst formation.

More details are given in the section of canine hepatozoonosis.

**Medical importance.** Most species are parasitic in cold-blooded vertebrates (amphibians and reptiles) where they usually cause asymptomatic infection. The only two species of veterinary importance are *H. canis* and *H. americanum*.

### 2.4.4.1 Canine hepatozoonosis

**Definition.** Hepatozoonosis is a tick-borne disease of dogs, occurring worldwide with usually sub-clinical infection. The data in this chapter is mostly based on the excellent review of Baneth (2011).

**Etiology**. Genus *Hepatozoon* contains more than 300 species, parasitic in a variety of vertebrate hosts. Two species are responsible for canine hepatozoonosis: *H. canis* and *H. americanum*.

**Morphology.** The complexity of the life cycle results in a great diversity of developmental stages. However, two stages are practically important for the diagnosis of the infection in dogs: the gamonts within the neutrophils (**figure 2.80**) and the meronts within the tissues. Gamonts of *H. canis* are typically located within the cytoplasm of the circulating neutrophils. They have an ellipsoidal shape and the average size is 4 x 11 µm. The meronts of *H. canis* found in infected tissues contain elongated micromerozoites arranged in a circle around a clear central centre forming the typical "wheel spoke" aspect. The gamonts of *H. americanum* are located in various leukocytes. The meronts of *H. americanum* are described as multi-layered "onion skin" cysts, located between the muscular fibers, having 250–500 micrometer in diameter and containing a central nucleus surrounded by concentric rings of membranes.

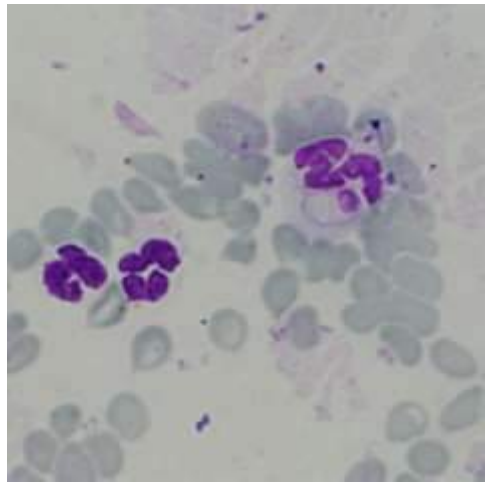

**Figure 2.80** Gamont of *Hepatozoon canis* in a neutrophil of an infected dog. *(Photo Barbora Mitková)*

**Life cycle**. *Hepatozoon canis* has a heteroxenous life cycle (**figure 2.81**). Dogs are the intermediate hosts and certain tick species are the definitive hosts. Although hepatozoonosis is a tick-borne disease, its transmission from ticks to dogs is not via the tick bite. Though, the dogs have to ingest infected ticks to acquire the infection with *H. canis* (figure 2.81 - 1). After an infected tick or parts of it are ingested by a dog, the sporozoites of *H. canis* leave the body of the tick, penetrate the epithelial lining of the intestine and invade mononuclear cells (figure 2.81 - 2). They are transported by

the blood and lymph to the target tissues and organs: bone marrow, spleen, lymph nodes, liver, kidneys and lungs. Immediately after they reach these organs, the merogonic development starts, resulting in asexual multiplication with the formation of merozoites within tissue meronts. Two types of meronts are formed: type 1, containing up to four larger merozoites (macromerozoites, figure 2.81 - 3) and type 2, containing 20 to 30 small merozoites (micromerozoites, figure 2.81 - 4). It is believed that macromerozoites are released and are responsible for the production of secondary meronts in the same target tissues. Eventually, the micromerozoites will invade monocytes and neutrophils (figure 2.81 - 5) and become gamonts (figure 2.81 - 6). The formation of gamonts corresponds to the beginning of the next stage of the life cycle, the gametogony.

If blood of parasitemic dogs is ingested by ticks (figure 2.81 - 7), the gamonts will be released in the tick's intestine. If the tick is a suitable host for *H. canis*, male and female gamonts will develop into male and female gametes and will associate (figure 2.81 - 8) for the formation of the zygote within the tick's gut. The zygote begins the last phase of the life cycle, the sporogony with the ultimate formation of the oocysts in the tick's hemocoel (figure 2.81 - 9). Each oocyst contains hundreds of sporozoites which are infective for dogs if ingested. The overall duration of the life cycle of *H. canis* is almost 3 month.

The life cycle of *H. americanum* is more or less similar with the one described for *H. canis*. The main difference consists in the target tissues, which in the case of *H. americanum* are skeletal and cardiac muscles. The parasitic stages are transported to the muscle fibers by macrophages. The merogonic stage produces a meront, which finally becomes a cyst surrounded by mucopolysaccharide layers. When the cyst ruptures, the free merozoites invade the surrounding tissues. Merozoites enter into leukocytes where they may undergo an additional merogonic multiplication. Within the leukocytes, the last generations of merozoites become gamonts. Ticks feeding on the blood of infected dogs take the leukocytes infected with gamonts. In the body of the tick, gamonts continue to develop into gametocytes. Gametogony is followed by sexual reproduction with the formation of the zygote and sporogony with the formation of polysporocystic oocysts in the tick's hemocoel.

Transmission of *H. americanum* to dogs is similar to *H. canis*, via the ingestion of infected vector ticks.

Several tick species have been shown to act as suitable definitive hosts for *H. canis*. The most widely distributed vector is *Rhipicephalus sanguineus*, but other ticks species were also shown to transmit the infection under experimental or natural conditions: *Amblyomma ovale* in Brazil and *Haemaphysalis longicornis* and *H. flava* in Japan. The only know tick-vector for *H. americanum* is *Amblyomma maculatum*. Transstadial transmission in ticks occurs in both *Hepatozoon* species. However, there is no evidence for transovarial transmission in ticks.

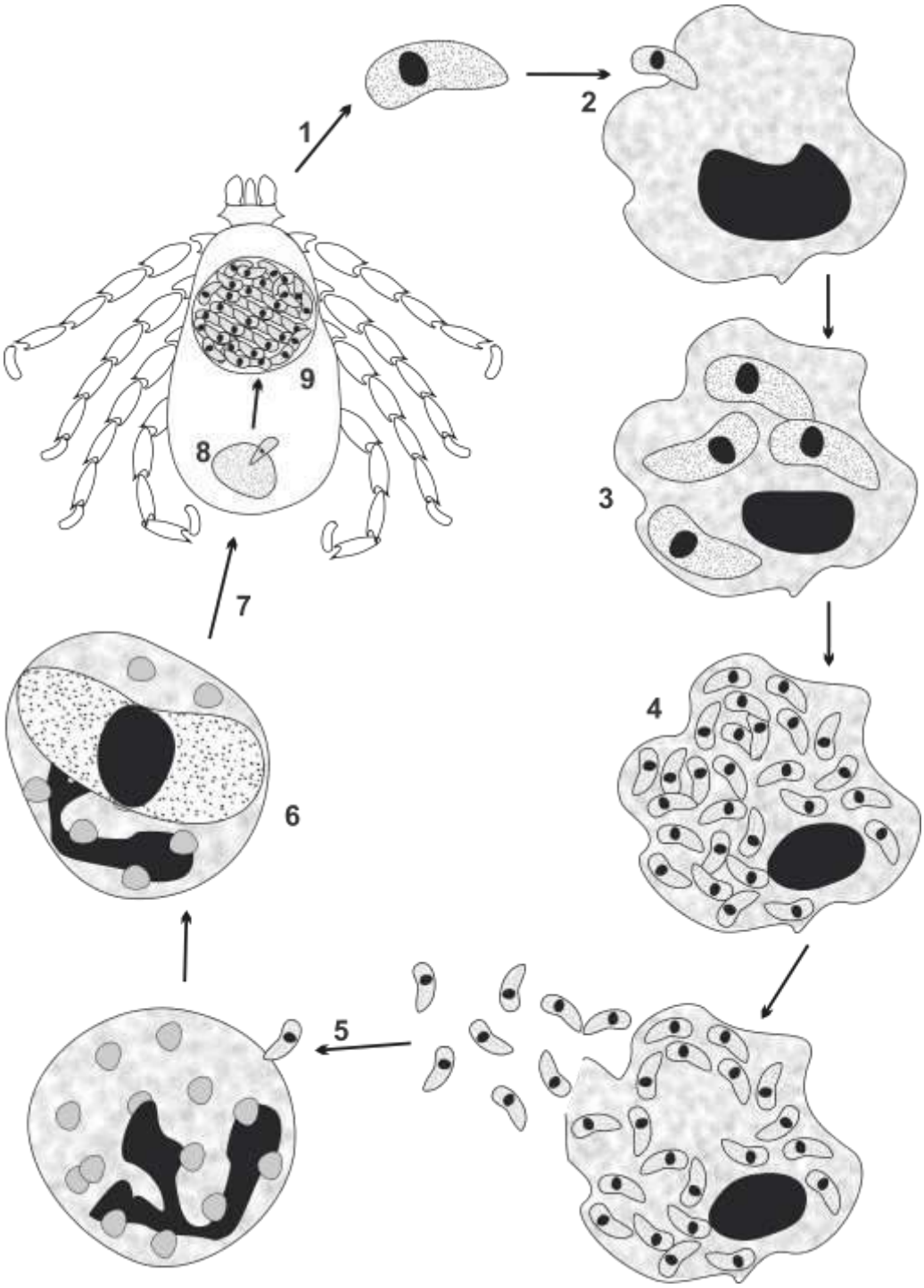


**Figure 2.81** Life cycle of *Hepatozoon canis*. For the meaning of numbers, please refer to the text.

Transplacental transmission has also been demonstrated for *H. canis*. Naturally infected pregnant bitches gave birth to infected puppies. Although not proven, another suspected route of infection for dogs is via carnivorism by predation on other infected intermediate hosts.

**Epidemiology.** Natural (autochthonous) infections with *H. canis* generally overlap with the distribution of its main vector, the tick *R. sanguineus*. Thus, canine hepatozoonosis with *H. canis* occurs in most tropical and subtropical (including Mediterranean climate) regions, but cases are known also from countries with warmer temperate climate. Nevertheless, imported cases have been reported in many other countries. Although the main vertebrate host is probably the domestic dog, several other wild canids are suspected to act as natural reservoirs. The infection with *H. canis* has been detected in red foxes (*Vulpes vulpes*), crab-eating fox (*Cerocyon thous*), jackals (*Canis aureus*, *C. mesomelas*), African wild dogs (*Lycaon pictus*) and spotted hyenas (*Crocuta crocuta*).

*Amblyomma maculatum* is found along the US Gulf Coast and Southern Atlantic, hence the distribution of *H. americanum* infection in dogs is limited to this area.

**Pathogenesis.** There is an evident difference in pathogenicity between *H. canis* and *H. americanum*. The dogs infected with *H. canis* are usually asymptomatic. Severe symptoms are more common in young dogs or are frequently associated with concurrent infections. It seems that the immune suppression induced by other infectious agents or the immature immune system in young animals influence the pathogenesis of new *H. canis* infections or favors the reactivation of preexisting ones. The role of the immune system in the development of clinical infections has been also demonstrated by the appearance of parasitemia in infected dogs following immunosuppressive doses of prednisolone.

In the infection with *H. americanum*, the main pathogenesis is caused by the merogonic development in the muscular tissues. The release of merozoites from the muscular cysts induces an intense local inflammatory response associated with severe musculoskeletal pain.

**Symptoms.** Several studies suggest that the severity of clinical signs in dogs infected with *H. canis* is positively correlated with the level of parasitemia. The most common appearance of *H. canis*-hepatozoonosis is subclinical or mild diseases (moderate fever, lethargy, muscle pains). These cases are associated with low levels of the parasitemia (1-5% neutrophils infected with gamonts). The severe cases can evolve with life-threatening symptoms (extreme lethargy, cachexia and anemia) and are usually associated with high parasitemia levels (even 100% of the neutrophils infected with gamonts). The hematology of these cases reveals anemia, extreme neutrophilia (as high as 150,000 leukocytes/µl blood). Blood biochemistry findings include hyperproteinemia with polyclonal hyperglobulinaemia and hypoalbuminaemia, and elevated creatine kinase and alkaline phosphatase activities. Often, symptoms of hepatozoonosis are aggravated by other

overlapping vector-borne disease: ehrlichiosis (*Ehrlichia canis*), anaplasmosis (*Anaplasma phagocytophilum*), leishmaniosis (*Leishmania infantum*) and babesiosis (*Babesia canis*, *B. vogeli*). Concurrent infections with parvovirus or *Toxoplasma gondii* have also been reported.

The dogs infected with *H. americanum* usually show fever, abnormal gait (limb stiffness, inability to rise), muscular pain and generalized muscular atrophy. Often, a mucopurulent ocular discharge is present, as a result of decreased tear production caused by with inflammation of the external ocular muscles. American canine hepatozoonosis evolves as an acute infection but can also become chronic. In either situation, the muscular pain is present and this can be generalized or localized, usually at the level of lumbar and cervical spine. The chronic infections can be complicated by immune-mediated glomerulonephritis and uveitis. Clinical hematology and blood biochemistry are similar to those described for the infection with *H. canis*.

**Lesions.** Following death subsequent to severe hepatozoonosis, the meronts of *H. canis* can be found in various tissues: liver, lungs, kidneys, spleen, bone marrow or lymph nodes. Associated lesions include hepatitis, pneumonia and glomerulonephritis. In the case of *H. americanum*, the most important lesion is the myositis.

**Diagnosis.** Direct detection of *H. canis* gamonts in the neutrophils of infected dogs is dependent on the level of parasitemia. As symptomatic dogs usually have significant parasitemia, examination of blood smears from dogs with clinical infection might reveal the presence of gamonts. They are present in the cytoplasm of the infected neutrophils. Histopathology from target tissues (liver, lymph nodes, spleen etc.) reveals the presents of typical meronts with the "wheel spoke" aspect.

In the case of *H. americanum*, the parasitemia is generally lower than in dogs infected with *H. canis*. Therefore, the usual direct confirmation of the disease is by showing the presence of parasites in muscle biopsies. Additionally, radiography of long bones showing periostitis is another indication for *H. americanum*-hepatozoonosis.

Several serological methods (IFAT, ELISA) are available for the diagnosis of canine hepatozoonosis. The most sensitive method for the detection of *Hepatozoon* spp. in the blood of dogs is PCR and quantitative evaluation is possible by real-time PCR.

**Treatment.** The treatment of the dogs with clinical infection with *H. canis* is done using imidocarb dipropionate, 5-6 mg/kg every 14 days until gamonts disappear from the blood smears. However, the absence of gamonts from the blood smears is not equal to zero parasitemia. Studies using more sensitive detection methods (i.e. PCR), showed that no treatment can completely eliminate the infection. As dogs with low parasitemia usually show no clinical signs, the prognosis of such cases is generally good.

The treatment of dogs infected with *H. americanum* must follow a combination

of oral therapy with trimethoprim-sulfadiazine (15 mg/kg every 12 h), pyrimethamine (0.25 mg/kg every 24 h) and clindamycin (10 mg/kg every 8 h) for 14 days.

In order to avoid clinical relapses, it is recommended that after relief of acute forms, an anti-coccidial drug to be given orally for long term. The treatment suggested is decoquinate at 15 mg/kg mixed in food every 12 h for two years. Supportive therapy might be considered for pain relief.

**Control.** Although canine hepatozoonosis is a vector-borne disease, its unusual transmission route makes its prevention different than for the other arthropod-transmitted disease. It is recommended to avoid the oral ingestion of ticks by dogs, both from the environment and while grooming. Hence, the use of topical and environmental acaricides is encouraged. No vaccine is currently available.

#### 2.4.4.2 Feline hepatozoonosis

Feline hepatozoonosis is by far less common than the canine infection. It has been reported from several countries in Asia, Africa, North America and Europe. The species of *Hepatozoon* responsible for the feline infection are not well defined, nor its life cycle known. The development of *Hepatozoon* meronts in cats takes place in the skeletal muscles and the myocardium causing elevated levels of creatine kinase.

Decreased immunity caused by FIV and FeLV favor the development of clinical disease. The symptoms are not specific and highly diverse: weakness, lethargy, anorexia, weight loss, fever, hypersalivation, mucosal ulcers, enlarged lymph nodes, anemia. The disease can be successfully treated using doxycycline (5 mg/kg, orally, every 12 hours), oxytetracycline (50 mg/kg, every 12 hours) until recovery signs or with a single dose of primaquine (2 mg/kg, orally).

### 2.4.5 Babesiidae

**Introduction.** Includes several genera parasitic mostly in warm-blooded vertebrates.

**Ecology and transmission.** All the members of the family undergo the merogonic development only in erythrocytes with no preliminary development in white leukocytes or other types of cells. The transmission of the species for which the life cycle is known is by the bite of ticks.

**Medical importance.** Species of genus *Babesia* are very important veterinary parasites, causing severe clinical diseases, mortality and economic losses. Some species are zoonotic, but clinical signs in humans are present with few exceptions only in splenectomized people.

The diseases caused by Babesiidae and Theileriidae are generically known as piroplasmosis. As the life cycle and pathogenesis is different, more accurate designation babesiosis and theileriosis or their plural forms (babesioses, theilerioses) will be used herein.

### 2.4.5.1 Babesioses

**Introduction.** Babesioses are tick-borne diseases found in all species of domestic mammals and in many wild mammals, with great economic impact but also medical and veterinary importance. The disease is clinically severe in non-immune hosts, causing anemia, fever, jaundice, hemoglobinuria and sometimes death.

**Historical notes.** Two distinct geographical areas (Eastern Europe and North America) are particularly important from historical point of view. At the end of the 19th century (1888), Victor Babeş, a Romanian microbiologist, discovered the agent of the disease in cattle. One year later, Theobald Smith, a medical doctor from New York, described the agent of Texas fever in USA and was the first to elucidate the tick-borne nature. It was the first time ever when arthropods were shown to transmit a disease. Babeş originally named the species *Hematococcus bovis*. Smith and Killborne named the Texas fever agent *Pyrosoma bigeminum*. In 1893, Starcovici erects genus *Babesia* and includes both species there. In the years to follow, many new species have been described from various domestic and wildlife hosts.

**Etiology.** The taxonomy and species status of genus *Babesia* is highly dynamic. As most species descriptions were based mostly on morphology of the erythrocytic stages and host specificity, the validity of some still needs molecular confirmation. The list of valid *Babesia* species parasitic in domestic animals is shown in **table 2.41**.

**Table 2.41** Named species of *Babesia* parasitic in domestic animals (modified and updated after Schnittger et al. 2012)

| Host | Species | Distribution |
|---|---|---|
| Cattle | *B. bovis* | Worldwide |
| | *B. bigemina* | Worldwide |
| | *B. divergens* | Europe |
| | *B. major* | Asia, Europe |
| | *B. occultans* | Africa |
| | *B. ovata* | Asia |
| Buffalo | *B. bovis* | America, Asia |
| | *B. bigemina* | America, Asia |
| | *B. orientalis* | Asia |
| Horse, donkey | *B. caballi* | Africa, America, Asia, Europe |
| Pig | *B. trautmanni* | Africa, Europe |
| Sheep, goat | *B. crassa* | Asia |
| | *B. motasi* | Africa, Asia, Europe |
| | *B. ovis* | Africa, Asia, Europe |
| Dog | *B. canis* | Europe |
| | *B. conradae* | North America |
| | *B. gibsoni* | Asia, Africa, America, Europe |
| | *B. rossi* | Africa |
| | *B. vogeli* | Worldwide |
| Cat | *B. felis* | Africa |
| | *B. presentii* | Asia |

Certain species of *Babesia* have been recorded in several mammal species, raising the question if they are really host specific. For instance, *Babesia caballi* is commonly found in dogs and *Babesia canis* is also present in cats. Molecular surveys have shown also other such cross-infections, but their clinical and epidemiological importance is not yet fully understood.

**Morphology.** Among the various developmental forms of *Babesia* in the definitive (ticks) and intermediate (vertebrate) hosts, the most important stages from morphologic (diagnostic) point of view are those found in the erythrocytes of the domestic mammals. The most common intraerythrocytic

stages are the merozoites. They are usually found in pairs, forming a typical "V" shape (**figure 2.82**). In some of the species of *Babesia*, multiple divisions result in the formation of groups of 4 or 8 intraerythrocytic merozoites.

Based on the size of the intraerythrocytic stages, the species of *Babesia* are divided in two types: small *Babesia* (1.0-2.5 µm) and large *Babesia* (2.5-5.0 µm). Merozoites of small species usually form obtuse angles, while the larger ones form acute angles to each other. Sometimes, atypical forms (amoeboid, ring-shapes, round) can be found in the erythrocytes (**figure 2.83**).

In other species of *Babesia*, 4 or 10 merozoites can be seen inside a single erythrocyte. Detection of free stages in the plasma is possible but very rare event.

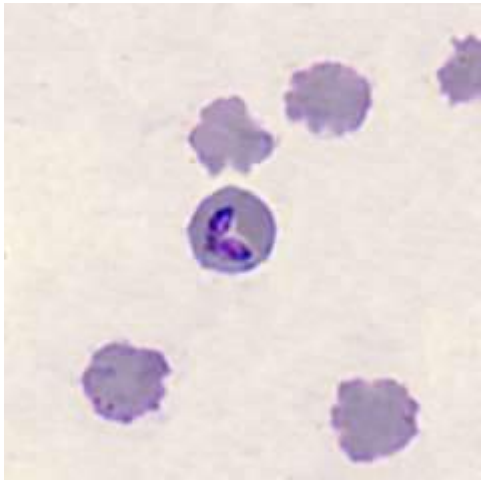

**Figure 2.82** "V"-shaped disposition of merozoites of *Babesia canis* in an erythrocyte from infected dog. (*Photo Andrei D. Mihalca)*

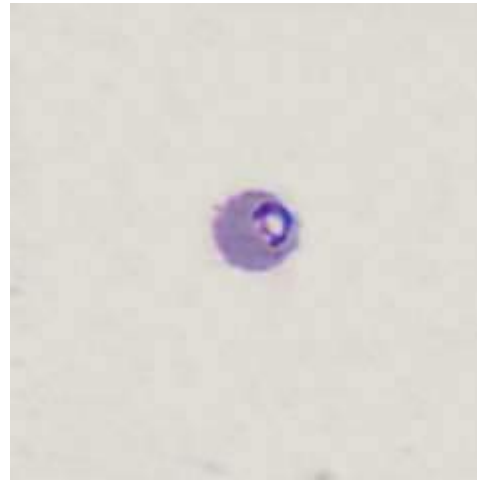

**Figure 2.83** Ring-shaped aspect of *Babesia canis* in an erythrocyte from infected dog. (*Photo Andrei D. Mihalca)*

**Life cycle.** The life cycle of all species is heteroxenous (**figure 2.84**). As the sexual development takes place in ticks, they are considered definitive hosts. Mammals are intermediate hosts as they are harboring the asexual stages.

Mammals acquire the infection after a bite of an infected tick. Through the saliva, ticks inject into the blood stream of the vertebrate the infective sporozoites (figure 2.84 - 1).

They attach to erythrocytes (figure 2.84 - 2) and by endocytosis they enter inside them (figure 2.84 - 3). Inside the red blood cells, sporozoites start to feed, becoming trophozoites (figure 2.84 - 4) which subsequently divide by binary fission resulting in the formation of two merozoites in each erythrocyte (figure 2.84 - 5).

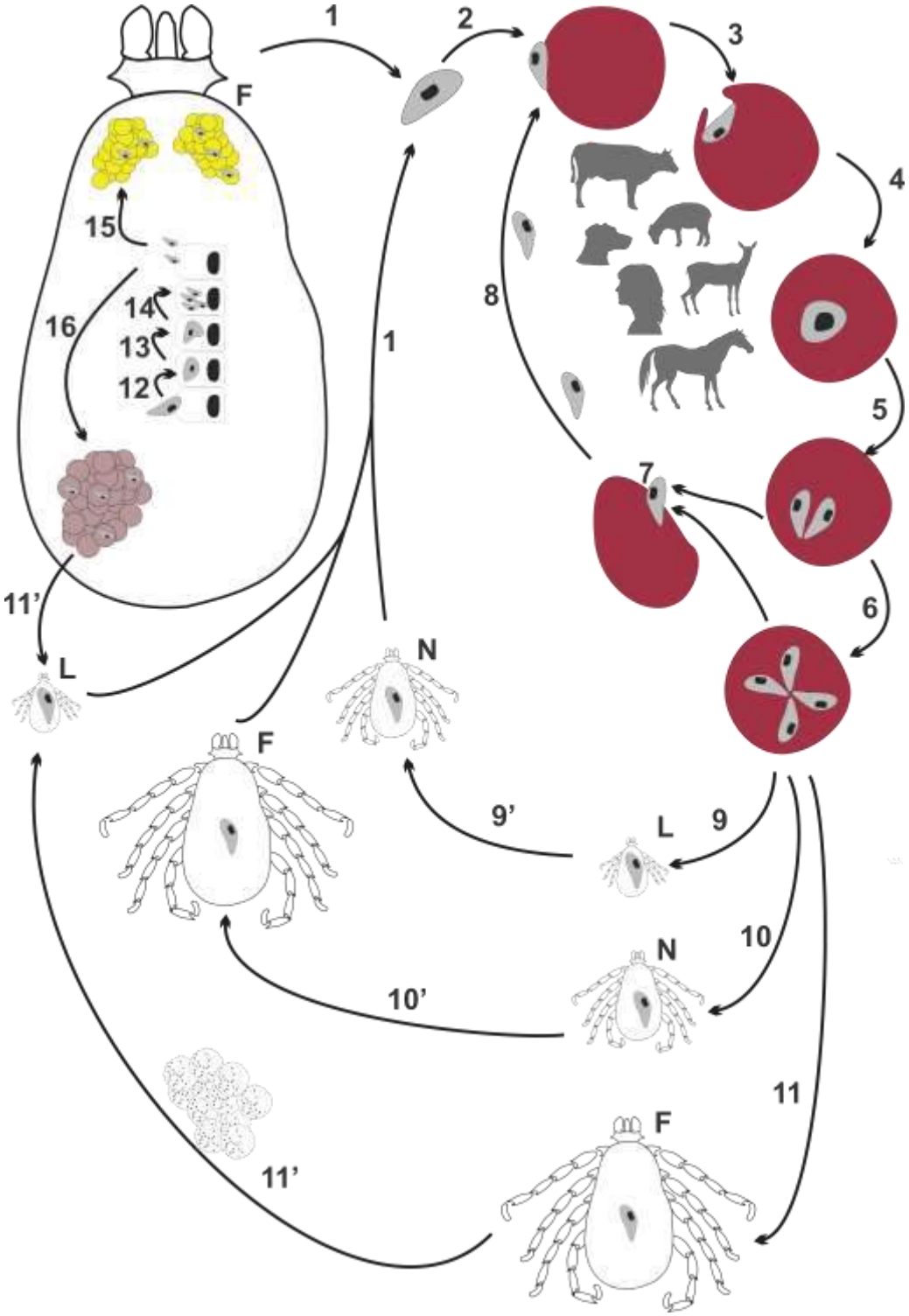


**Figure 2.84** Life cycle of *Babesia* spp. For the meaning of numbers and letters, please refer to the text.

Sometimes, one additional cell division results in the formation of four merozoites in each erythrocyte (figure 2.84 - 6). Merozoites rupture the infected erythrocytes (figure 2.84 - 7) and invade new ones (figure 2.84 - 8), repeating the merogony several times until many red blood cells are destroyed. During the course of infection, some merozoites are transformed into gamonts while still in the erythrocytes of the intermediate hosts. When a new tick (figure 2.84 - 9, 10, 11) feeds on the infected blood of the intermediate host, the erythrocytes with gamonts reach its intestine. They will invade the epithelial cells (figure 2.84 - 12) and start the gametogony process. It seems that the other stages (i.e. merozoites, sporozoites, trophozoites) ingested by the vector are not able to produce the intestinal infection in the tick. After gamonts sexually join, the zygote is formed. Each zygote differentiates into a mobile oocyst-like structure called kinete (figure 2.84 - 13). Through the hemolymph, the kinetes will invade all the tick's tissue, including ovaries (figure 2.84 - 15) and salivary glands (figure 2.84 - 14). When reaching the salivary glands, the kinetes start the sporogony with the formation of sporozoites. Kinetes from the ovaries will be responsible for the transovarial transmission to the eggs produced by the female tick and eventually part of the next generation larvae will be already infected when hatch. The sporozoites from the salivary gland will infect a new host when the tick feeds again.

In order to understand the ecological rationale behind the transmission of pathogens by ticks, it is essential to present some brief insights into the tick biology and mechanism of vectorial transmission. Ticks have three feeding developmental stages: larvae, nymphs and adults (male and females). Most ticks follow a so-called three host life cycle, meaning that each stage feeds on a different host. After larvae hatch from the eggs, they attach to a host, feed with blood and detach. After detachment, larvae fall-off in the environment where they undergo the first molting and become nymphs. Nymphs search for a new host, they attach to it and feed again. After fully engorged they detach, fall into the environment and molt for the second time, becoming adults. Part of the nymphs become males, the others become females. Adult ticks attach to the third host, the feed and reproduce sexually. Fully engorged and fertilized females fall-off the host. After some time spent in the environment, they lay thousands of eggs and die. Males usually do not feed, and they are attached to the host only for finding the females. Considering all these events, it is evident that each stage of the tick feeds only once and only on one host. The next time it will feed it will be already as another developmental stage. If a larvae (figure 2.84 - L) feeds on a host infected with *Babesia*, it will acquire the infection (figure 2.84 - 9). The next time it will feed, it will be as a nymph, on another host. Hence, the maintenance of the infection from a stage to another (figure 2.84 - 9') is a sine qua non prerequisite for the existence and transmission for any tick-borne disease. This essential event is known as ***transstadial passage*** (or less accurately, transstadial

transmission). The same situation happens if a nymph (figure 2.84 - N) feeds on an infected host (figure 2.84 - 10). It will pass the infection to adults (figure 2.84 - 10') which will eventually infect a new host. In the case the stage which is infected is an adult female (figure 2.84 - F) (figure 2.84 - 11), for the infection to be ecologically continuous, the female must be able to pass the acquired pathogen to the offspring (figure 2.84 - 11'). This is possible in certain tick-borne pathogens (but not in all) because of the presence of anatomical structures which connect the digestive tube of the tick with the ovaries. This feature is known as ***transovarial transmission***. Not all species of *Babesia* are able to pass by this transovarial route. It seems that *B. felis* from cats or *B. microti* from rodents are such species.

Given the relatively strict host specificity of *Babesia*, one more extremely important barrier is not only the alternation of ecological hosts but also the host species. Not all the three hosts from a complete life cycle of a tick are belonging to the same species. For instance in the case of *Dermacentor reticulatus* the larvae and nymphs feed usually on micromammals and only adult ticks use dogs. It is known that *D. reticulatus* is the vector of *Babesia canis*. If *Babesia canis* infects only dogs, it is evident that the only stages which acquire the infection from dogs are adults. It logically means that infected adults females pass the infection to the eggs. Infected larvae which hatch will feed on small mammals, possibly not infecting them. However, the larvae maintain the infection in their body, pass it to nymphs and then to adults. So the next adult generation will be ultimately responsible for infecting a new dog. This means sometimes even years.

An interesting mechanism of transmission for those species vectored by one-host ticks (i.e. all stages feed on the same host individual) is by males which move from an animal to the other.

One more essential factor in the transmission mechanism of tick-borne disease is the infectivity of the tick saliva in the first hours or days after attachment. It was said before that *Babesia* moves from the intestine of the tick to the salivary glands where sporozoites will be formed. This in-tick migration takes place for most of the tick-borne pathogens only after the ticks attaches to a host which is suitable for the pathogen's development. Factors from the vertebrate's blood will activate the migration of the pathogens to the salivary gland and eventually its transmission to the host. This key aspect is essential from practical point of view. If a tick dies because of an antiparasitic treatment or it is mechanically removed from the host before the salivary migration occurred, the risk for pathogen transmission is limited.

The vectors for *Babesia* species of domestic animals are always hard-ticks (Ixodidae). Not all tick species transmit all *Babesia* species. The spectrum of vectors for each *Babesia* species is shown in **table 2.42**.

A possible but rather unusual route of transmission for *Babesia* is the vertical transmission in vertebrates, from an

infected mother to the fetus. Transplacental transmission has been reported in humans, cattle, horses and dogs. Transmission through infected needles or blood transfusion is also possible.

**Table 2.42** Tick vectors for selected *Babesia* species of domestic animals

| *Babesia* species | Tick vector |
|---|---|
| *B. bigemina* | *Rhipicephalus microplus*, *R. annulatus*, *R. decoloratus*, *R. geigyi*, *R. evertsi*, *Haemaphysalis punctata* |
| *B. bovis* | *Rhipicephalus microplus*, *R. annulatus*, *R. geigyi* |
| *B. caballi* | *Rhipicephalus evertsi*, *R. bursa*, *R. sanguineus*, *Dermacentor albipictus*, *D. variabilis*, *D. nitens*, *D. marginatus*, *D. reticulatus*, *D. silvarum*, *Hyalomma anatolicum*, *H. dromedary*, *H. marginatum*, *H. scupense*, *H. truncatum* |
| *B. canis* | *Dermacentor reticulatus*, *Rhipicephalus sanguineus* (?) |
| *B. conradae* | *Rhipicephalus sanguineus* (?) |
| *B. crassa* | Unknown |
| *B. divergens* | *Ixodes ricinus*, *I. persulcatus* |
| *B. felis* | Unknown |
| *B. gibsoni* | *Haemaphysalis longicornis* |
| *B. major* | *Haemaphysalis punctata*, *H. longicornis* |
| *B. motasi* | *Haemaphysalis punctata*, *Rhipicephalus bursa*, *H. qinghaiensis* (?), *Amblyomma variegatum* (?) |
| *B. occultans* | *Hyalomma marginatum*, *H. rufipes*, *H. anatolicum*, *H. truncatum* |
| *B. orientalis* | *Rhipicephalus haemaphysaloides* |
| *B. ovata* | *Haemaphysalis longicornis* |
| *B. ovis* | *Rhipicephalus bursa*, *R. sanguineus* (?), *R. turanicus* (?) |
| *B. presentii* | Unknown |
| *B. rossi* | *Haemaphysalis elliptica* |
| *B. trautmanni* | *Rhipicephalus turanicus* |
| *B. vogeli* | *Rhipicephalus sanguineus* |

**Epidemiology.** The geographical distribution of various *Babesia* species is shown in **table 2.41**. Certain species are distributed worldwide (of course except the polar regions), some others are more or less limited. As domestic animals are everywhere where people are present, the spatial distribution of *Babesia* is largely influenced by the degree of specificity to the tick-host and the distribution of those ticks.

As with most other vector-borne parasites, the occurrence of *Babesia* is influenced by the ecology of the vectors. Transmission to the vertebrate host is seasonal and correlated with the ticks' dynamics. The outbreaks of acute babesiosis typically take place during the warm seasons.

The newly introduced animals are more susceptible to acute infection. Long-term parasite-host associations result in acquired resistance, but with the presence of parasites in the blood of clinically healthy animals. This results in a permanent infection source for ticks and through them to *Babesia*-free individuals. The local breeds are more resistant than imported breeds. *Bos indicus* breeds are more unlikely to develop clinical babesiosis.

The concept of endemic stability is very important in the epidemiology of babesiosis, mainly in large animal communities as livestock (cattle, small ruminants). The endemic stability means that the pathogen is present in the host population but the clinical disease occurs rarely.

Although the disease can be transmitted by a single infected tick, the prevalence of

infection in the tick population is usually very low.

The susceptibility to develop clinical babesiosis is higher in adult animals than in young ones. This rather unusual situation is possibly related to immune factors, most probably to the acquired transplacental or colostral passive immunity.

*Babesia*, as most vector-borne parasites spends all their life inside a host. No exogenous stages exist. Therefore, we cannot discuss about the environmental resistance of *Babesia*. *Babesia* can survive in ticks for several years, until the life cycle of that individual tick ends.

**Pathogenesis and immunology.** Most of the pathogenesis of babesiosis is related to the altered immune response of the hosts. This is the reason why the sections are discussed together.

The most important pathogenetic mechanism is related to the destruction of erythrocytes. Two mechanisms are involved in this process: immune-mediated erythrolysis and non-immune erythrolysis.

After the erythrocytes are infected they display on their outer surface modified antigenic structures. Therefore, they target the synthesis of opsonizing antibodies and as a consequence, the infected erythrocytes are destroyed by the mononuclear-phagocyte system. Even uninfected erythrocytes can be the target of autoimmunity, as soluble parasitic antigens adhere to their surface.

Other mechanisms are also involved in the destruction of infected erythrocytes. They become osmotically fragile. Moreover, during penetration by sporozoites or merozoites, direct injury of the cellular membrane can result in mechanical destruction.

The altered erythrocyte metabolisms and membranary processes slow down their circulation speed in capillaries and favors sludging. The most intense erythrocyte sludging occurs in the central nervous system and in the lungs.

The excessive production of cytokines and other pharmacologically active compounds cause vasodilatation, hypotension, increased capillary permeability and endothelial damage. These are ultimately responsible for the disseminated intravascular coagulation, which is usually lethal. The increased capillary permeability together with the resulting edema are responsible for the severe respiratory distress in certain species.

The erythrocyte depletion causes tissular hypoxia and subsequent failure of various internal organs, including kidneys. As a result of hypoxia, there is an over production of lactic acid, resulting in metabolic acidosis. Hyperventilation, as a result of anemia is also considered to be a compensatory attempt to fight acidosis. Overall, certain species of *Babesia* are responsible for the systemic inflammatory response syndrome which results in multiple organ failure: acute renal failure (typically in dogs), hepatopathy or pulmonary edema.

Massive destruction of erythrocytes is followed by hemoglobinuria and jaundice. However, these seem to be the feature of moderately pathogenic species.

Interestingly, the level of parasitemia is not correlated with the severity of clinical signs. In cattle infected with *B. bovis* and with severe acute signs, the parasitemia is normally less than 1%, while subclinical or moderate cases with *B. bigemina* are associated with 10-30% infected erythrocytes. This could be explained also by the higher rate of erythrocytic destruction in the case of more pathogenic species, resulting in false "low" intensity, as most of the infected erythrocytes could have been already destroyed.

The low infective doses in immune animals are responsible for life-long resistance. This non-sterile immunity, also known as premunition is the most prominent immunologic feature in persistent, subclinical *Babesia* infection. Both components of the immune system are important in the anti-*Babesia* resistance. The innate immunity is the first line defining the host specificity of *Babesia* species. Experimental splenectomy allows the infection of otherwise resistant host species. The involvement of innate immunity has been suspected to be responsible for the resistance of young animals to infection. One key circumstantial proof for such a hypothesis is the resistance to infection of calves born from non-immune mothers. However, the colostrally passed antibodies also play a role. The most important components of the innate immunity active against *Babesia* are monocytes, macrophages and neutrophils.

The role of the specific immunity is related to the production of antibodies. They will mediate the cytotoxic killing of parasites during re-infections.

**Clinical signs.** Most infections are at a very low level and they are clinically asymptomatic. Clinical signs usually occur in individuals which have never been in contact with the pathogen (see immunology). The clinical infection has been documented in most domestic species, in humans as well as in wildlife. Because of the associated mortality, babesioses of livestock are economically important. Although the clinical course and symptoms vary in each host species and also between *Babesia* species, there is a general picture which can be considered.

In all species, the infection with *Babesia* can be acute, sub-acute or chronic.

Common features of acute babesiosis include fever, anemia, jaundice and hemoglobinuria. The general status of the animals with acute signs can be severely altered, with malaise, lethargy, tachypnea, muscle tremors, anorexia and weight loss. An alternation of diarrhea with constipation can be present. The duration of the acute form is usually around one week.

Sub-acute and chronic babesioses are similar but less severe that acute forms. Hyperacute forms are reported, but rarely. They usually consist in sudden death, within few hours from the onset of symptoms.

As there are certain specific features for various *Babesia* species-host species associations, the most common of them will be discussed.

In ***bovines*** the most pathogenic species is *B. bovis*. Other species (*B. bigemina*, *B. divergens*) are moderately pathogenic while others (*B. occultans*, *B. major*) are most often non-pathogenic. Babesiosis produced by *B. bovis* (incubation period 10-12 days) is characterized by high fever, ataxia, incoordination, anorexia, hemoglobinuria, nervous signs, circulatory shock and death. The infections with *B. divergens* or *B. bigemina* (incubation period 4-5 days) produce fever, hemoglobinuria and anemia, but nervous signs are absent.

In ***sheep*** and ***goats***, the most pathogenic species are *B. motasi* and *B. ovis*. It is responsible for fever, anorexia, listlessness, anemia, jaundice. Hemoglobinuria is not constant.

Acute cases caused by *B. caballi* in ***horses*** (incubation period 10-30 days) are associated with high fever, dyspnea, mucosal congestion, edemas, and anemia. The icterus and hemoglobinuria may be present, but are less severe that in the infection with *Theileria equi*.

The clinical course in ***dogs*** is often very severe, but symptoms greatly vary. They depend on various factors, one of them being the species. Certain *Babesia* species (i.e. *B. vogeli*, *B. gibsoni*) have lower pathogenicity and they typically produce mild or subclinical infection. Others (*B. canis*, *B. rossi*, *B. conradae*) are responsible for acute forms, with more severe outcome. The acute form in dogs is associated with a great diversity of clinical signs: anorexia, lethargy, fever, weakness, weight loss, hemolytic anemia, icterus, splenomegaly, lymphadenopathy, vomiting, ascites, diarrhea, buccal ulcers, seizures, ataxia, paresis etc.

In ***cats*** the published reports are few. The main sign of the infection with *B. felis* is fever associated with depression, loss of appetite, weight loss, weakness, tachycardia, tachypnea, vomiting, and diarrhea. Jaundice is uncommon.

**Pathology.** Gross pathology of babesiosis is as diverse as the clinical picture is, both reflecting its complex pathophysiology.

In ***cattle*** infected with *B. bovis*, the acute lesions include: generalized congestion of abdominal organs (soft and pulpy spleen, hepatomegaly, congested kidneys), generalized anemia and jaundice, presence of dark-red urine in the urinary bladder, distended gall-bladder with thick and granular content or pulmonary edema.

The lesions in ***dogs*** which died of acute babesiosis (usually caused by small *Babesia*) consist of staining of tissues with hemoglobin, hepatomegaly, splenomegaly, lymphadenopathy, nephrosis, signs of disseminated intravascular coagulation. Histologic findings are consistent with the pathophysiology: capillary congestion, thrombosis in various organs, erythroid hyperplasia in the bone marrow and vasculitis.

The lesions in other host species are similar with those described in cattle and dogs.

**Diagnosis.** The clinical signs and lesions in babesiosis are characteristic but not pathognomonic. Hence, the diagnosis must always be confirmed in the laboratory. The most reliable methods are the microscopic detection methods when the stages of the parasite are seen directly by the observer.

Detection of the parasite is possible in blood smears, when the characteristic parasitic forms are seen inside the erythrocytes. From dead animals, other cytological examination can be performed if blood is not available anymore (i.e. brain smears, blood from internal organs). Although the method is highly specific, its sensitivity is reduced, as usually during the chronic phase the parasitemia is relatively low, under the detection threshold. Another disadvantage is the relatively long time necessary for the thorough examination of a blood smear. Specific identification is not always possible, mainly in hosts where more *Babesia* species are known.

Various immunological methods are available for the detection of anti-*Babesia* antibodies in the sera of infected animals: the Indirect Fluorescent Antibody Test (IFAT), ELISA, Immunochromatography Test (ICT). The disadvantage of serological methods consist in failure to detect acute infection (the antibodies are not yet produced) and difficulty in interpreting the seropositivity (post-vaccinal versus post-infective).

The most sensitive and specific methods are those targeting the parasite's DNA. The most widely used is the PCR, but also Reverse Line Blot Hybridization (RLB), Real Time PCR or Loop Mediated Isothermal Amplification (LAMP).

**Treatment.** The efficacy of various drugs is different against the various species of *Babesia*. Usually, small *Babesia* species are more resistant than large *Babesia* species. Two drugs are currently widely used for the treatment of babesiosis in animals: imidocarb and diminazene aceturate. Treatment does not always eliminate the infection but it can significantly reduce the clinical impact. In severe cases, supportive therapy is required. It is forbidden to use the treatment together with anti-*Babesia* vaccination, as the drugs kill the vaccinal strain and interfere with a proper installation of immunity.

In ***cattle***, diminazene aceturate is effective at 3.5 mg/kg, intramuscularly, 1-2 administrations. The injection protects the cows against reinfection with *B. bovis* and *B. bigemina* for 2-4 weeks. Imidocarb at 1.2 mg/kg, single dose, given subcutaneously is also effective. Higher doses (3 mg/kg) have even protective effect for 1-2 months.

In ***dogs*** infected with large piroplasms (i.e. *Babesia canis*), the treatment of choice is imidocarb (5-6 mg/kg, intramuscularly, single dose and then repeated in two to three weeks). Imidocarb is not effective against the infection with small *Babesia* (i.e. *B. gibsoni*).

Diminazene aceturate has been used in the past for the treatment of *B. gibsoni* infection, but currently, the most effective treatment is considered to be the

combination between atovaquone and azithromycin.

In ***cats*** infected with small *Babesia* the only effective treatment is with primaquine phosphate (0.5-1.0 mg/kg intramuscularly, orally or intravenously, single dose or daily for 3 consecutive days). Diminazene aceturate (3.5 mg/kg, intramuscularly, single dose) is effective against large *Babesia* of cats. There are no reliable clinical studies to show the efficacy of imidocarb in cats.

Diminazene aceturate is recommended also for sheep, goats, swine and equines. The dose is between 3.5-5 mg/kg, intramuscularly, twice at 24 hours interval.

**Control.** There are several approaches in preventing and controlling babesiosis in domestic animals. One of the most successful approaches is the control of vectors. Long-term strategic programs to reduce the tick parasitism in livestock result in great improvements.

This can be achieved by the use of acaricide drugs. They are expensive and very toxic to the environment so their large scale use is often limited. Selecting resistant breeds is another option. *Bos indicus* breeds are more resistant than *Bos taurus* breeds. The areas with endemic babesiosis foci should be populated with breeds known to have natural resistance.

The most modern approach for controlling babesiosis is the use of immunoprophylaxis. Commercial vaccines are available for bovines and dogs.

### 2.4.6 Theileriidae

**Introduction.** All Theileriidae are tick-borne intracellular parasites of wild and domestic animals with significant economic importance. Two genera are particularly important in domestic animals: *Theileria* and *Cytauxzoon*.

**Morphology.** The morphological appearance of *Theileria* is more heterogeneous than *Babesia*. Intracellular stages in the mammalian host includes round, ovoid, rod-like or irregular forms.

**Ecology and transmission.** The biology of Theileriidae is comparable to Babesiidae, but with two main differences. ***In the vertebrate host***, species of *Theileria* undergo the first multiplication in white blood cells and only later in the course of infection they are able to infect the erythrocytes. ***In the tick host***, there is no transovarial transmission.

**Medical importance.** Theilerioses are important diseases of cattle, sheep, goats and horses in regions with tropical climate, mainly in Africa but also in warmer regions of Asia and Europe. They are not zoonotic. Recently, the infection with Theileriidae gained importance also in dogs and cats.

#### 2.4.6.1 Theilerioses

**Introduction.** Species of genus *Theileria* are responsible for several nominal diseases mainly in cattle, small ruminants, horses and dogs from tropical countries (**table 2.43**).

**Historical notes.** The first to observe *Theileria* parasites in the blood of cattle was Robert Koch. In 1897, he found rod-like parasites inside the red blood cells of cows from the current Tanzania. He considered them to be morphs of *Babesia bigemina*. In 1901, when Australian cows were introduced to Zimbabwe, many died of babesiosis. The surviving cows were moved to another location. Three weeks later they died of another, more acute and unknown diseases. Again, Koch realized it is a different disease than babesiosis, Theiler described in 1904 the new agent as *Piroplasma parvum*. In the same year, Dschunkowsky and Luhs described the second species, *Piroplasma annulatum* in Transcaucasia. Genus *Theileria* was erected in 1907 by Bettencourt, Franca & Borges.

**Etiology.** The species of *Theileria* parasitic in domestic animals are shown in **table 2.44**. Certain species of *Theileria* have been divided into subspecies, but their taxonomic status is still debated. Moreover, these subspecies are serologically and morphologically indistinguishable.

The main difference between them is in the epidemiological features and geographical distribution. However, as they are commonly used in various textbooks, they will be briefly mentioned herein. *Theileria parva* has been divided into three subspecies: *T. parva parva* (the typical agent of East Coast Fever), *T. parva lawrencei* (causing the Corridor disease) and *T. parva bovis* (only mildly pathogenic).

**Table 2.43** Species of *Theileria* and *Cytauxzoon* parasitic in domestic animals

| Host | Species | Vector genus | Disease | Distribution |
|---|---|---|---|---|
| Cattle | *T. annulata* | *Hyalomma* | Mediterranean Coast Fever | Europe, Africa, Asia |
| | *T. buffeli* | *Haemaphysalis* | Benign theileriosis | Worldwide |
| | *T. mutans* | *Amblyomma* | Benign theileriosis | Africa |
| | *T. parva* | *Rhipicephalus* | East Coast Fever, Corridor Disease | Africa |
| | *T. sergenti* | *Haemaphysalis* | Oriental theileriosis | Asia, Europe, Australia, Africa |
| | *T. sinensis* | *Haemaphysalis* | Benign theileriosis | Asia |
| | *T. taurotragi* | *Rhipicephalus* | Benign theileriosis | Africa |
| | *T. velifera* | *Amblyomma* | Benign theileriosis | Africa |
| Sheep, Goats | *T. lestoquardi* (syn. *T. hirci*) | *Hyalomma* | Ovine and caprine theileriosis | Europe, Asia, Africa |
| | *T. luwenshuni* | *Haemaphysalis* | Ovine theileriosis | Asia |
| | *T. ovis* | *Rhipicephalus* *Hyalomma* | Benign theileriosis | Africa, Europe |
| | *T. separata* | *Rhipicephalus* | Benign theileriosis | Africa |
| | *T. uilenbergi* | *Haemaphysalis* | Ovine theileriosis | Asia |
| Horses | *T. equi* | *Dermacentor, Hyalomma, Rhipicephalus* | Equine theileriosis | Worldwide |
| Dogs | *T. annae* | *Ixodes* | Canine theileriosis | Europe, America |
| Cats | *C. felis* | *Dermacentor, Amblyomma* | Cytauxzoonosis | America |

**Morphology.** From diagnostic point of view, the stages in the mammalian host are important. In the white blood cells, the schizonts of *T. parva* appear as circular or irregularly shaped structures, also known as Koch's blue bodies. They measure between 2 and 12 µm (average 8 µm). The macroschizonts have around 8 nuclei and the microschizonts have 50-120 nuclei.

In the red blood cells, the merozoites are usually single (there is no intraerythrocytic division), they have rod-shape and measure 1.5 x 0.5-1.0 µm. Sometimes, other forms can be seen in the erythrocytes: round, oval, ring etc. The morphology of other species is more or less similar. The main differences consist in morphometric values.

**Life cycle.** As a model of life cycle for *Theileria*, the development of *T. parva* will be described in the following paragraphs. In other species, there are small differences in the intensity and ration of multiplication in erythrocytes and lymphocytes. For instance, if in *T. parva* there is no intraerythrocytic division, in *T. mutans*, the predominant multiplication takes place in the red blood cells.

Transmission of the infection to the mammalian host is by the bite of nymph and adult ticks. As the infection is not transmitted by vertical (transovarial) route, larvae are not infective. After the sporozoites are injected into the blood stream, they infect the lymphocytes where they start the merogonic development producing merozoites. Two types of schizonts (meronts) are known: macroschizonts, containing around 90 macromerozoites (2-2.5 µm) and microschizonts with 80-90 micromerozoites (0.7-1 µm). After the merozoites rupture the lymphocytes, they can follow two ways. They can either infect other lymphocytes and maintain the lymphocytic infection or penetrate into erythrocytes, where they usually remain until engorgement by vector ticks. In the tick's gut, they undergo gametogony and sexual reproduction, resulting in the formation of the zygote which ultimately becomes motile and bears the name of kinete. They remain in this stage in the intestinal cells of the ticks until they molt. After the subsequent stages (nymphs or adults) start to feed on the next host, kinetes invade the salivary glands of the ticks and produce thousands of sporozoites. Sporozoites from the salivary gland will infect a new host. There is no transovarial transmission in *Theileria*.

The life cycle in genus *Cytauxzoon* is similar to genus *Theileria*.

Transplacental transmission from infected mother to offspring is suspected for certain species, including *T. annae* in dogs.

**Epidemiology.** The spread and distribution of the vector is responsible for the distribution of the diseases. Although some species of *Theileria* have a more broad distribution, with different vectors in each area they occur, they are causing endemic diseases only on few regions. For instance, *T. parva* causing the East Coast Fever is distributed in South, East and Central Africa. The group of *T. parva* causing the corridor disease is endemic to East and Central Africa, as the

main reservoir is the African buffalo. *Theileria annulata* is responsible for the Mediterranean Coast Fever and its distribution ranges from Northern Africa (Morocco) to Middle East and Southern Russia.

The host specificity of *Theileria* is an important epidemiological feature. As for instance species parasitic in cattle infect also buffaloes or various wild ruminants, the diversity of reservoir hosts creates permanent risks of outbreaks. Often, the pathogenicity in wild ruminants is almost absent, but when the infection occurs in domestic cattle it has devastating impact.

The mortality is also variable. *Theileria lestoquardi* causes between 50 and 100% mortality in sheep and goats. When East Coast Fever infects previously non-exposed groups, the mortality can reach 90% or more of the animals.

Young animals are more resistant than adults. Local breeds from endemic areas are usually resistant to the infection.

**Pathogenesis.** The pathogenicity is different from *Theileria* species to *Theileria* species and even within the same species, between hosts.

In cattle, the infection of lymphocytes with *T. parva* induces blastogenesis resulting in an increased production of lymphoblasts which invade various tissues ("leukemia"-like infection). The pathogenetic effect is dominated by the massive alteration of the structure and functions of the lymphoid tissue and by the subsequent anemia caused by erythrolysis. The oxidative stress has been incriminated as the main mechanism of erythrolysis. These induce changes in the structure of the membranary proteins of the erythrocyte.

Like in the case of babesiosis, the severity of disease is not correlated with the intensity of parasitemia.

**Immunology.** The primo-infection is always severe. If animals survive they are usually resistant to clinical infection. The infection with one species does not confer cross immunity against the infection with another species.

The main immunological mechanism involves cellular immunity. In the infection with *T. parva*, the bovine major histocompatibility complex is mediating the immune response through cytotoxic T lymphocytes. The infection with *T. annulata* activates the release of cytokines by CD4+ T cells. They produce IFN-γ which triggers the production of α tumor necrosis factor and nitric oxide by non-infected macrophages. These active compounds will destroy the infected cells. The extracellular merozoites are killed by antibodies produced by B lymphocytes.

Like in babesiosis, the altered immune response is ultimately responsible for the pathogenesis of the diseases. The increased production of cytokines results in increased vascular fragility and permeability.

**Clinical signs.** Despite the great species diversity, the disease has several common clinical features. Usually the adult animals are more susceptible and signs of disease are more severe. The presence of fever and generalized lymphadenopathy are characteristic.

The ***East Coast Fever*** is characterized by very high mortality in susceptible cattle populations. Incubation is 1-4 weeks. The main clinical signs include: high fever, lymphadenopathy, severe dyspnea, tachycardia, and extreme weight loss. The fever is high persists until recovery or death. Infected animals have aural and palpebral edema and hyperlacrimation. The severe respiratory distress and the excessive frothy nasal discharge are indicative of the severe pulmonary edema which usually precedes death. Some animals show hemorrhagic diarrhea. The clinical hematology findings include leucopenia,

The clinical signs and course of the ***Corridor Disease*** are similar to the East Coast Fever but outbreaks are associated with the presence of wild buffaloes.

The ***Mediterranean Coast Fever*** has a much lower mortality rate and occurs after 1-4 weeks of incubation with fever, depression, hyperlacrimation, superficial lymph node swelling, nasal discharge and weight loss. Except these signs which are similar but slightly less severe than in the case of African theileriosis, the infection with *T. annulata* produces also anemia with hemoglobinuria.

***Benign bovine theilerioses*** (produced by *T. buffeli*, *T. mutans*, *T. sinensis*, *T. taurotragi* and *T. velifera*) and ***Oriental theileriosis*** (*T. sergenti*) are either subacute or chronic and if symptoms are present they are typically mild. Death is uncommon.

***Ovine and caprine theileriosis*** produced by *T. lestoquardi* is a severe disease, clinically similar to East Coast Fever of cattle. Symptoms include high fever, listlessness, frothy nasal discharge, jaundice and enlargement of superficial lymph nodes.

***Equine theileriosis*** produced by *T. equi* is a severe disease. Infected horses have high fever, listlessness, hyperlacrimation, palpebral edema, severe anemia, hemoglobinuria and icterus (signs typical to babesiosis).

***Canine theileriosis*** produced by *T. annae* is a severe disease causing acute fever, weakness, lethargy, tachypnea, tachycardia, anemia and hemoglobinuria. Clinical hematology reveals regenerative anemia, reticulocytosis, and thrombocytopenia.

***Feline cytauxzoonosis*** is characterized by fever, acute onset of anorexia and lethargy. Following these acute signs, increased the cats show increased vocalization, weakness, jaundice, dyspnea, seizures, delayed capillary refill time, lymphadenopathy. The outcome of the disease is often death, preceded by deep coma.

**Pathology.** The lesions of animals which died of theilerioses consist in severe pulmonary edema, generalized lymphadenopathy with enlarged lymph nodes and multifocal lymphoid hyperplasia in various parenchymatous organs (i.e. kidneys, brain). The spleen is enlarged and congested. In species producing marked anemia, the tissues are icteric and the urinary bladder may contain hemoglobinuric urine.

**Diagnosis.** Acute clinical signs insusceptible animals correlated with the lesions and the history of the local

endemicity are good diagnosis indicators. The confirmation of the diagnosis is based on demonstration of the *Theileria* in various samples. The presence of schizonts (meronts) in the infected white blood cells or merozoites in the erythrocytes can be observed under the microscope in blood smears or lymph node biopsies.

The most widely used serological test for screening of animals, mainly in the international trade is indirect fluorescent antibody test.

Other serological methods (i.e. ELISA) are also used. Molecular methods are highly sensitive but not implemented in the routine surveys.

**Treatment.** Most of the drugs are improving the clinical presentation but are not eliminating the infection.

The treatment of infected ***cattle*** is recommended only during the very early phase of the clinical diseases. Two molecules are widely used: parvaquone and its derivative buparvaquone.

Parvaquone is used as single dose (20 mg/kg, intramuscularly) in cattle infected with *T. annulata* or at 10 mg/kg, intramuscularly, twice at 48 hours interval against the infection with *T. parva* and *T. mutans*. Buparvaquone (2.5 mg/kg, single dose, intramuscularly) is effective against bovine theilerioses.

In cattle but also other species, various other drugs are used with variable efficacy. In ***equids*** infected with *T. equi*, chlortetracycline hydrochloride and oxytetracycline hydrochloride are effective when given intravenously (5.5 mg/kg, daily for 2-5 days). Imidocarb or diminazene diaceturate (see babesiosis) is also effective in horses.

***Dogs*** infected with *T. annae* can be treated with limited success with babesicidal drugs. ***Feline cytauxzoonosis*** is treated with a combination of atovaquone (15 mg/kg, orally, every 8 hours) and azithromycin (10 mg/kg, orally, every 24 hours) for 10 days.

**Control.** The most efficient and widely used method employed for controlling theileriosis is the control of ticks using acaricides. Factors contributing to an unsuccessful control include acaricide resistance of ticks, improper use of acaricide drugs (cheap, poor quality products, underdosing) or illegal animal movements. Chemoprophylaxis with theilericidal drugs is an option for animals newly introduced to an endemic area.

Prevention by immune prophylaxis is possible in cattle. Live vaccines have been developed for *T. parva* and *T. annulata*.

## 2.5 Ciliophora

Phylum Ciliophora includes very diverse groups of highly mobile protozoans known generically as ciliates. Their defining feature is the presence of hair-like movement organelles, known as cilia (singular cilium). Most species are free-living, and they usually inhabit aquatic environments, both marine and freshwater. The majority of them are predators of smaller microorganisms (i.e. bacteria). Other species are symbiotic and they are associated with a huge

variety of hosts within most animal phyla, both invertebrates and vertebrates. Certain groups inhabit the digestive system of their host where they are mutualists, commensals and few species parasitic. Others are living on the tegumentary surface of aquatic animals, mostly fish as commensals or parasites. Their medical importance is mainly known in aquaculture, as they produce outbreaks in farmed fish (e.g. *Ichthyophthirius, Trichodina, Chilodonella*). In domestic animals very few species are known to be potentially pathogenic, and the most prominent example is *Neobalantidium coli*.

All species are unicellular and they bear on their surface variable number of cilia. Their arrangement and patterns are important in classification. The ingestion of nutrients is through a small opening in the cell, known as cytopharynx, surrounded by a group of cilia. Waste material is eliminated from the cell through another opening, known as cytoproct (or "cellular anus"). The internal structure of ciliates is also very characteristic. They have two nuclei, one larger (macronucleus) and one smaller (micronucleus). The macronucleus is the true-nucleus and it is controlling the metabolic activity of the cell. The role of micronucleus is in the sexual reproduction. Like all eukaryotic cell, ciliates have all the typical cell organelles (mitochondria, Golgi body etc.).

The life cycle of all parasitic ciliates is homoxenous, and transmission is directly by contact (in species parasitic on the skin of fish) or via ingestion of cysts (in species parasitic in the digestive tube). There are two types of reproduction in ciliates. The asexual reproduction is by binary fission. The sexual reproduction is a very interesting process and it takes place by conjugation. During conjugation, two cells which are of complementary mating type, are fertilizing each other.

Two genera are of veterinary importance, both included in Class Litostomatea: *Neobalantidium* and *Buxtonella*.

### 2.5.1 Ciliates of domestic animals

Ciliates are the least represented protozoans in the veterinary parasitology. They are usually opportunistic pathogens, and the clinical infections are sporadic. Two diseases will be discussed: balantidiosis of swine and buxtonellosis of cattle.

#### 2.5.1.1 Balantidiosis

**Introduction.** Balantidiosis is a worldwide distributed parasitic disease of the lower intestinal tract of pigs and humans, with usual subclinical course but problematic in immunocompromised patients.

**Historical notes.** In 1857, Malmstein described a new species of ciliates from the feces of dysenteric patients. He named it *Paramecium coli*. One year later, Claparède and Lachmann erected the genus *Balantidium* for a newly described species, *B. entozoon* from frogs. In 1858, Stein moved *Paramecium coli* into genus *Balantidium*. However, due to evident phylogenetic differences, in 2013, Pomajbíková et al., erected the new genus

*Neobalantidium* and placed within it *Parameciu coli*.

**Etiology.** The species responsible for the infection of pigs is *Neobalantidium coli*. It is not clear yet if all *Balantidium*-like ciliates described from pigs, primates and camels are conspecific with *N. coli*. However, until new data is available, we list *N. coli* as a zoonotic agent.

**Morphology.** According to the description by Pomajbíková et al. (2013), trophozoite is elongated, rounded at the posterior end and narrow at the anterior end. The size of the trophozoite is 30-300 x 30-100 µm. The surface is covered by cilia, arranged in longitudinal rows. The cytostome-cytopharyngeal complex is located at the apical end and surrounded by longer cilia. The cysts (40-60 µm) are spherical or ovoidal s one hypobiotic trophozoite.

**Life cycle.** *Neobalantidium coli* inhabits the cecum and colon of its hosts. It is not clear if interspecific transmission is possible. The life cycle is direct (homoxenous) (**figure 2.85**)

The organisms are passed in feces as cysts. Cysts are ingested via contaminated food or water by a new host (figure 2.87 - 1). In the intestine of the new host, trophozoites excyst and grow. They inhabit the surface of the mucosa where they feed through their cytopharynx with various particles. They multiply asexually by binary fission (figure 2.87 - 2) or sexually, by conjugation (figure 2.87 - 3). When the intestinal content begins dehydration prior to fecal transmission, *N. coli* starts the encystment process (figure 2.87 - 4). The newly formed cysts are passed via the feces.

**Epidemiology.** The disease is virtually present wherever pigs are present. *Neobalantidium coli* is found in more than half of the adult pigs. In young pigs the prevalence is lower. In some pig populations the prevalence can be 100%.

In humans, the disease is sporadic and the geographic areas with high prevalences include Latin America, Philippines, Indonesia, Papua New Guinea and Middle East. Pigs-to-humans and humans-to-humans cycle are described. The occurrence of the disease in humans is influenced by several factors. The close contact between humans and pigs together with improper waste management seem to be such factors.

Survival of cysts is longer in warm and humid climate. This is why the prevalence of human disease is higher in humid tropical areas.

**Pathogenesis.** *Neobalantidium coli* is typically a commensal of the large intestine. It typically feeds through the cytopharynx with debris and other particles from the intestinal content without any pathogenic effect. Under certain condition (decrease of immunity, intestinal dysmicrobism) it becomes pathogenic by producing proteolytic enzymes which destroy the intestinal epithelium resulting in acute diarrhea. Persistent mucosal damage may result in humans in the production of ulcers. Deep ulcers can be complicated by potential fatal intestinal perforations.

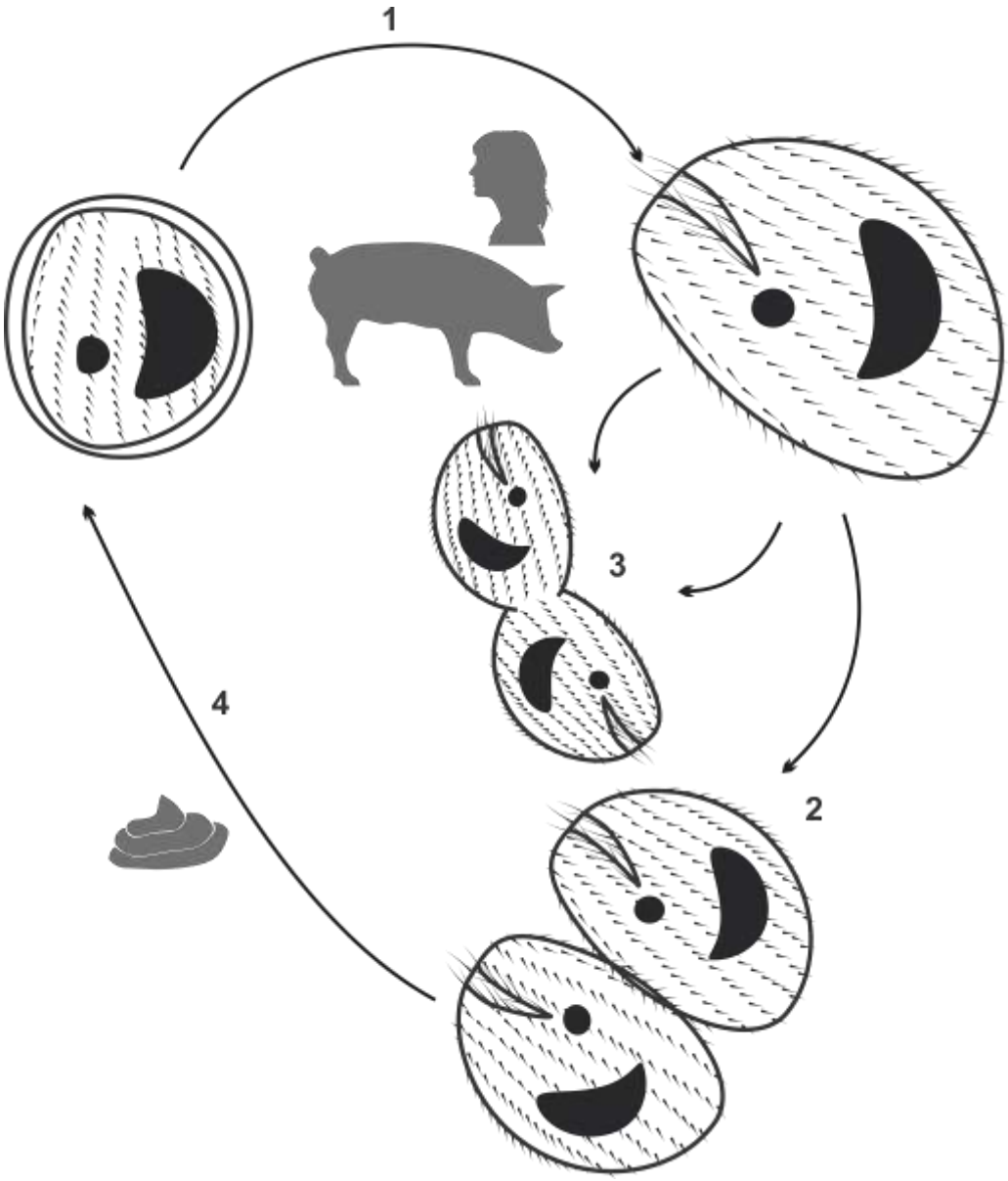


**Figure 2.85** Life cycle of *Neobalantidium coli*. For the meaning of numbers, please refer to the text.

In pigs, they can invade lesions produced by other pathogenic organisms. Production of hyaluronidase by *N. coli* aggravates the ulcers.

**Immunology.** The role of immunity is not clear. Indirect evidences however suggest the role of the immune system in maintaining the infection asymptomatic. In immunocompromised patients, *Neobalantidium coli* can produce a

systemic disease, infecting the lungs, lymph nodes.

**Clinical signs.** The vast majority of infected pigs have no clinical signs. Under certain conditions, some pigs will show moderate to severe acute diarrhea.

**Pathology.** In non-clinical infections, even large numbers of organisms are not associated with lesions. In clinical cases, the lesions consist in focal colitis.

**Diagnosis.** The cysts can be detected in fecal samples by sedimentation methods. Occasionally on direct examination,. mobile trophozoites can be seen.

**Treatment.** Usually not necessary. Metronidazole is effective but forbidden in animals used for meat production. Tetracycline antibiotics are also effective.

**Control.** General hygiene rules, proper feeding, avoiding stress and maintaining an overall health status of the farmed pigs is enough for prevention of clinical cases.

### 2.5.1.2 Buxtonellosis

Buxtonellosis is a common infection of the lower digestive tract of cows produced by the ciliate *Buxtonella sulcata*. The morphology of the trophozoite resembles *Neobalantidium coli*. The body is ovoidal, 100 x 72 µm and cilia are present all over the body surface. The cysts are indistinguishable from *N. coli*. Life cycle is identical with the

The infection is usually asymptomatic. Rarely, cows show signs of disease which can be associated with the presence of *B. sulcata*. The signs may include mild diarrhea.

Identification of "*Balantidium*"-like cysts in the feces of cows is indication of *Buxtonella sulcata*.

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