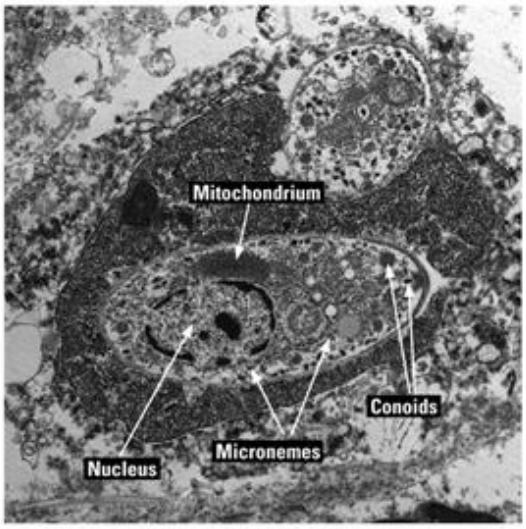

FIG. 2.11 Transmission electron microscopy (TEM) of an intracellular merozoite of *Sarcocystis neurona*. The organism is located directly in the host cell cytoplasm, without any apparent parasitophorous vacuole. Note the presence of conoids (C), micronemes (Mi) distributed throughout the parasite, a nucleus (N), a mitochondrium (Mc), and absence of rhoptries. The latter feature distinguishes *Sarcocystis* merozoites from other Apicomplexa tachyzoites. *(Reproduced with permission from Roberta di Terlizzi.)*

CRYPTOSPORIDIUM CANIS

- Recently reclassified as a gregarine instead of coccidian.
- Infection orally from oocysts; the infection dose is very low.
- Clinically, diarrhea is the primary sign.
- Diagnosis from fecal sample with Ziehl-Nielsen stain or immunological tests.
- No specific treatment available.

Identification

The *Cryptosporidium* genus has been recently classified as gregarine (phylum Apicomplexa, class Gregarinomorphea, subclass Cryptogregaria) instead of belonging to Coccidia, as it was previously classified. Gregarines are capable of completing their lifecycle even without a host. The oocyst of *Cryptosporidium* is small, only about 5 µm in diameter (about the size of a red blood cell). The oocyst contains four sporozoites but no sporocysts. Oocysts are secreted in feces in a sporulated form with an immediate infective effect.

Low Zoonosis Risk Associated With Canine Cryptosporidiosis

Cryptosporidium canis has been isolated in human samples in rare cases involving immunocompromised patients or young children. Even in at-risk groups (young children, organ transfer patients, cancer patients, or people receiving immunosuppressive glucocorticoid medication or having other immunosuppressive conditions), the transfer of cryptosporidia from dog to human is very rare, considering the close coexistence of these two species. Human cryptosporidiosis is often transferred via water, and this method of contagion cannot be ruled out in canine infections either.

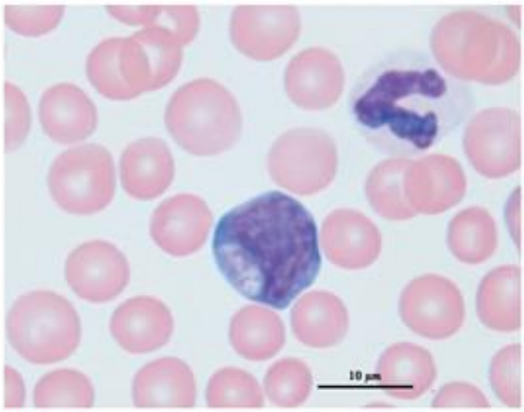

FIG. 2.12 Peripheral blood of a dog, Wright's stain. A reactive lymphocyte contains a *Sarcocystis neurona* merozoite. *(Reproduced with permission from Roberta di Terlizzi.)*

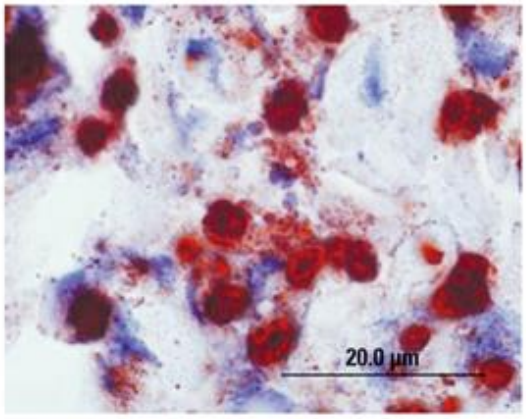

FIG. 2.13 Immunohistochemical staining of a tissue sample from a dog. Strong positive reaction for *Sarcocystis neurona* can be observed with anti *S. neurona* polyclonal antibodies. *(Reproduced with permission from Roberta di Terlizzi.)*

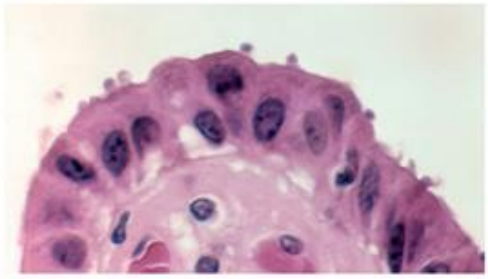

FIG. 2.14 *Cryptosporidium* protozoa seen as spherical structures within microvillus surface of epithelium. *(Courtesy of Dr. Edwin P. Ewing, Jr./ CDC.)*

Life Cycle

The dog receives the infection orally from *Cryptosporidium* oocysts. The infectious dose is very small and the prepatent period varies from days to weeks. Sporozoites that are released from the oocyst penetrate the gut epithelial cells and multiply through asexual (merogony/schizogony) (Figs. 2.14 and 2.15) and sexual cycles (gametogony), resulting in oocysts that sporulate inside the host and are readily infectious when they reach the environment. This early sporulation also enables autoinfection. An infected dog secretes large amounts of oocysts in the feces. An illustrated life cycle of *Cryptosporidium* is presented in Fig. 2.16.

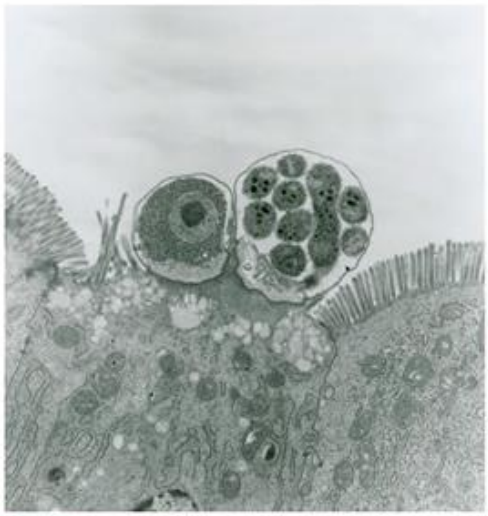

FIG. 2.15 Transmission electron microscopy showing *Cryptosporidium* protozoa in the intestinal microvillus surface of epithelium. Two developmental stages can be seen: a trophozoite (left) and meront (right). *(Reproduced with permission from Science Source Images. Photo by Biophoto Associates.)*

Distribution

Cryptosporidium infections are ubiquitous in humans as well as in animals. Many host species have their specific *Cryptosporidium* species. *C. canis* is specific to dogs. It is common especially in kennels with high dog density. It is also known that dogs frequently visiting dog parks are at greater risk of infection than other dogs.

Importance to Canine Health

The pathogenesis of canine cryptosporidiosis is poorly known, but it is assumed that the canine cryptosporidia behave in the same way as those infecting mice, cattle, and humans, which are better known and studied.

The penetration of cryptosporidia into the intestinal epithelium is limited. Yet they do affect the surface membrane of epithelial cells, which facilitates their access inside the cells. Clinical signs may be caused either by cryptosporidia themselves or by the dog's immune response activated by the infection. Signs may be triggered, for instance, by the inflammation mediators, the shortening of the microvilli covering the epithelial cells of the intestine, or the increased permeability of the gut epithelial lining. The cryptosporidia may loosen the intercellular adhesion between the epithelial cells, which may in turn weaken the epithelial defense barrier. This leads to increased epithelial cell death and the activation of mucosal associated immune-defense. There is little published information on the clinical importance of cryptosporidia to dogs. It appears that *C. canis* is well adjusted to coexistence with dogs, and consequently subclinical infections are common. The infection may manifest in especially young dogs as diarrhea of small-intestine origin, and immunocompromised dogs or dogs with concomitant diseases may have chronic signs, such as weight loss and signs associated with nutrient malabsorption.

Diagnosis

Oocysts in fecal sample are colored bright red in modified Ziehl-Nielsen stain (Fig. 2.17). Several commercial ELISA or immunofluorescence assays have been developed for Crytosporidium diagnosis (Fig. 2.17). The species is defined with PCR.

Therapy and Prevention

There is no specific medical therapy for canine cryptosporidiosis. The treatment is symptomatic and supportive. Sick animals should be isolated from the healthy, if possible, and the environment should be cleaned and good hygiene maintained. *Cryptosporidium* oocysts are resistant to disinfectants but sensitive to heat and drought.

Thorough mechanical cleaning of surfaces is important. Exposure to 3% hydrogen peroxide for 20 min reduces the number of infective oocysts. The cleaned surfaces must be totally dry before dogs are allowed in the space.

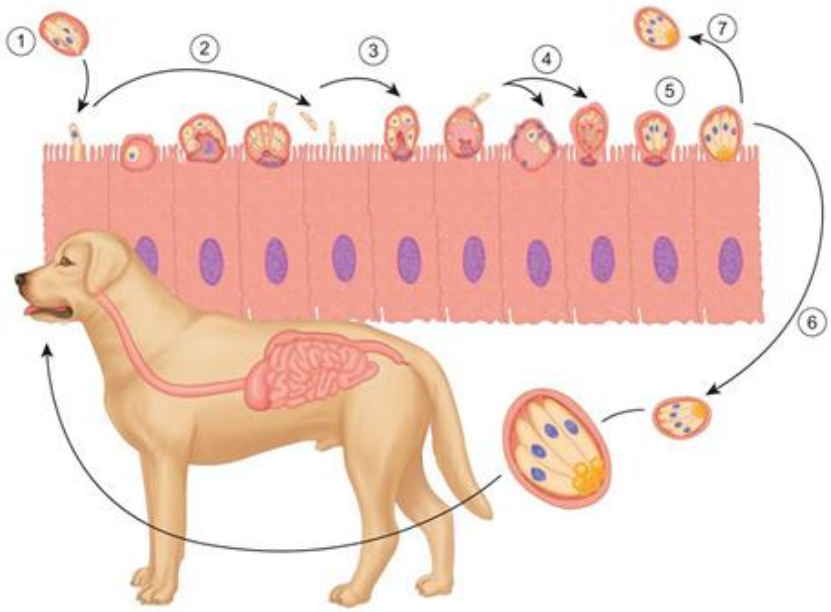

FIG. 2.16 Life cycle of *Cryptosporidium* spp.: (1) the dog gets the infection orally from *Cryptosporidium* oocysts. The infectious dose required is very low and the prepatent period varies from days to weeks. Sporozoites released from the oocyst penetrate the gut epithelial cells; (2) cryptosporia multiply first by merogonia resulting in type I meront, followed by another asexual cycle, type II meront as an end-result (3); (4) type II meronts, or rather the gamonts inside them, serve as a source for sexual cycle called gametogony, producing microgamonts (male) and macrogamonts (female); (5) microgametes fertilize the macrogamont, resulting in the oocyst, which sporulates in the infected host. Oocysts are infective upon excretion, thus permitting direct and immediate fecal-oral transmission as well as auto-infection. There are thick-walled oocysts, which are commonly excreted from the host (6); and thin-walled oocysts, which enable the autoinfection (7). An infected dog secretes large amounts of oocysts in feces.

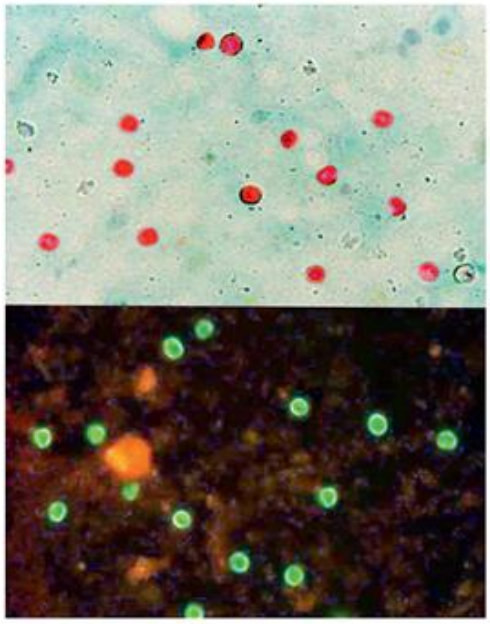

FIG. 2.17 Cryptosporidia in a fecal sample. The small-sized cryptosporidia have been made to stand out and stained red with modified Ziehl-Nielsen staining. A strong specific immunofluorescence reaction can be seen in the immunofluorescence assay (micrograph below). *(Reproduced with permission from Heidi Enemark.)*

BABESIA SPECIES

- Protozoans within the red blood cells.
- Tick-borne parasitic infection.
- Clinical signs resulting from hemolysis.
- Diagnosis from blood smear or immunological tests.
- Treatment depends on *Babesia* species; tick control is an important preventive measure.

Identification

Babesia are protozoans found within red blood cells and belong to the order Piroplasmida. The so-called large canine piroplasms, *B. canis*, *B. rossi*, and *B. vogeli*, are drop-shaped, about 4–5 µm in diameter, and commonly found in pairs (Fig. 2.18). Small canine piroplasms, *B. gibsoni*, *B. annae* (also known as *B. microti*-like and *Theileria annae*), and *B. conradae*, are round or oval, less than 2 µm in diameter, and are usually found singly (Fig. 2.19).

Life Cycle

Babesiosis is a tick-borne disease. When the tick sucks blood, infective sporozoites of *Babesia* are transferred to the dog's blood circulation and penetrate red blood cells. During the repeated asexual cycles (merogony), erythrocytes are broken down and merozoites are liberated to infect yet more red blood cells. Another tick ingests *Babesia* during the blood meal. In the tick, *Babesia* reproduces through sexual cycles and invades the organs of the tick including ovaries, thus enabling also the infection of the future tick generation. In dogs, the prepatent time varies according to the species. Usually it is 2–3 weeks. For instance, the prepatent time of *B. canis* infection is 10–21 days and that of *B. gibsoni* 14–28 days. The life cycle of *Babesia canis* is presented in Fig. 2.20.

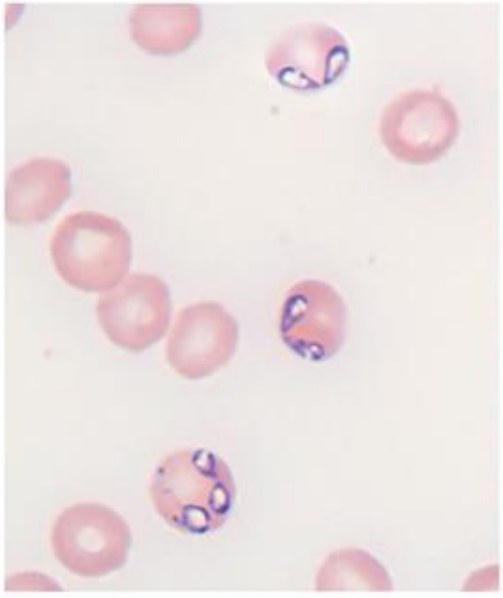

FIG. 2.18 Merozoite stages of *Babesia canis* in erythrocytes. Blood smear stained with Giemsa.

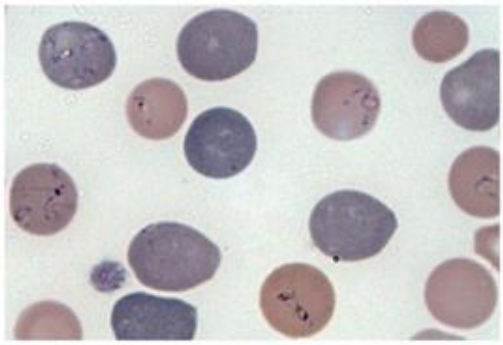

FIG. 2.19 *Babesia gibsoni* is classified as so-called small *Babesia*. Typical forms are seen in several erythrocytes. Blood smear stained with Giemsa.

Distribution

The distribution of *Babesia* species depends on which tick species are endemic in the area. *B. canis* is transmitted by *Dermacentor reticulatus*, which is endemic in southern and central Europe. The vector of *B. rossi* is *Haemaphysalis leachi*, endemic in South Africa. *Babesia vogeli* is carried by the brown dog tick *Rhipicephalus sanguineus*, which is found in Asia, Africa, the Americas, Australia, and Europe. *Babesia gibsoni* is endemic in Asia, North Africa, and occasionally North America and Europe. Its vectors are *Haemaphysalis bispinosa*, *Haemaphysalis longicornis*, and, according to some sources, *R. sanguineus*. Evidently, *B. gibsoni* could be spread by dog bite or intraplacentally without vector involvement. This is supported by the relatively high prevalence in fighting dogs compared to other dogs. It has been suspected that the vector of *B. annae*, endemic in southern Europe, is *Ixodes hexagonus*. Infections caused by canine *Babesia* have not been diagnosed in humans. Traveling dogs also import *Babesia* cases into nonendemic areas, but the emergence of these cases depends on tick species present in new areas.

Importance to Canine Health

The clinical signs of babesiosis may vary and involve many organs. The seriousness depends on the *Babesia* species and

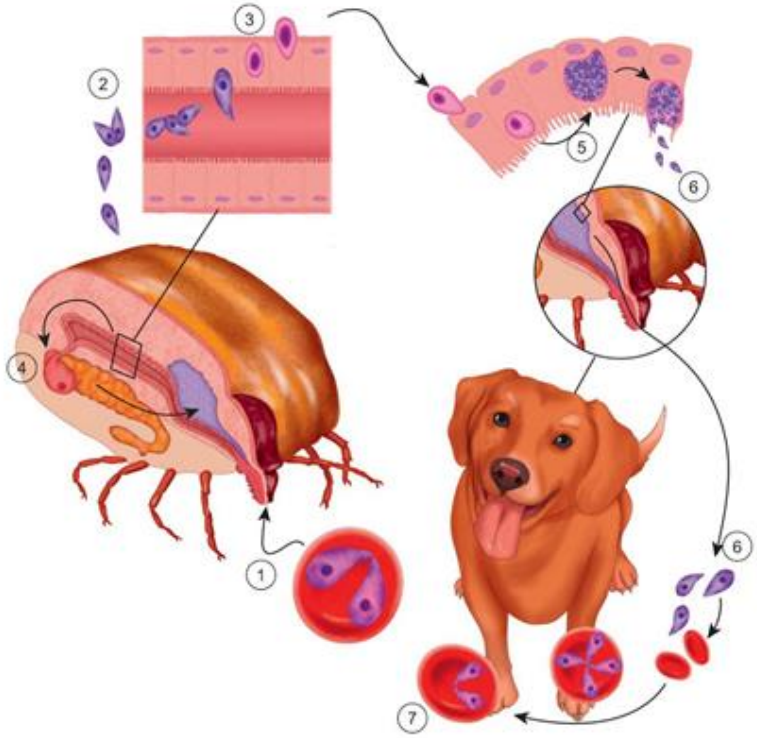

FIG. 2.20 Life cycle of *Babesia canis*: (1) the tick ingests *Babesia* merozoites while sucking blood. Merozoites liberated from disintegrated erythrocytes change their form, developing spiky and filamentous appendages; (2) two *Babesia* with appendages, or so-called ray bodies, join forming a zygote and followed by a stage called kinete; (3) kinetes migrate from the intestine to other parts of the tick and infect different cell types, including muscle cells, cells of Malpighian tubules, ovary cells, and gonads; (4) *Babesia* may pass into the offspring of the female tick in the gametes. Intracellular kinetes change their form and go through several division cycles. The result is the development of new kinetes. These also penetrate the salivary gland epithelium; (5) when the tick sucks blood from a dog in its next developmental stage, the parasites in the salivary gland activate and start dividing, resulting in a large number of sporozoites; (6) sporozoites invade though the salivary ducts into the dog when blood is being sucked. The transfer usually takes place about two days after the attachment of the tick; and (7) in the dog, the sporozoites infect erythrocytes and multiply in them asexually. These new *Babesia* infect more red blood cells.

strain, other infections, the dog's age, and immune defense. Babesiosis cases may be divided into uncomplicated and complicated forms on the basis of the clinical signs. In an uncomplicated case of babesiosis, the signs are usually limited to anemia, while the complicated form affects several organ systems. Both types manifest as a result of the host's inflammatory response. When the erythrocytes undergo lysis, hemolytic anemia develops, causing lethargy, weakness, enlargement of the spleen and liver, and possibly disturbances in the function of several organs. Fever, poor appetite, and pale mucous membranes or jaundice may be the initial observed signs. Tachycardia, hemoglobinuria, uremia, mucous membrane petechiae, low blood pressure, ischemia, coma, and ultimately death may follow. Central nervous system signs are possible. The signs associated with especially *B. rossi* infection can be peracute. The infection caused by *B. vogeli* is the least severe in adult dogs, but can cause severe signs in puppies, whereas *B. gibsoni* infection is characterized by chronic signs and transient fever. Because the elimination of red blood cells damaged by protozoa takes place in the spleen, splenectomized dogs tend to have more severe clinical signs. Dogs with compromised immunity or those on immunosuppressive medication may become seriously ill. In these dogs, chronic babesiosis may become active and start showing clinical signs.

Diagnosis

Babesia organisms may be seen in a stained blood smear. The sensitivity of the smear may be enhanced by puncturing a peripheral capillary vein (e.g., the tip of pinna or tail), in order to yield a maximum number of protozoa-carrying erythrocytes. Infected red blood cells are often found in the edges

of the smear. In a Giemsa-stained blood smear, the parasites are visible inside erythrocytes and the morphological evaluation may reveal whether the *Babesia* is of a small or large type (Figs. 2.18 and 2.19). Several commercial immunological assays are available. The *Babesia* species is determined with PCR methods and also with ELISA tests. It is therapeutically important to distinguish large and small *Babesia*, as they are sensitive to different drugs.

Other hematological findings in babesiosis reflect the hemolytic anemia caused by the infection. Thrombocytopenia is a common finding in babesiosis regardless of the protozoa species, and it often precedes anemia and circulatory parasitemia.

Treatment and Prevention

In the endemic areas of vector ticks, dogs should be protected with continuous preventative medication for the duration of the tick season. Attached ticks must be removed without delay, because the transmission of the tick-borne disease takes place for a few days, and in the case of *B. canis* at least 48 h. *Babesia* vaccines are available in some countries. They do not stop the infection, but do prevent the development of severe signs. The efficacy of the vaccine depends on the *Babesia* species.

Whenever dogs are given a blood transfusion, the *Babesia* status of the donor should be checked to prevent iatrogenic infection.

The disease caused by large *Babesia* is treated with imidocarb given twice at a 2-week interval. Small *Babesia* are more difficult to manage. There are varying therapy recommendations, including treatment with atovaquone combined with azitromycin daily for 10 days. Symptomatic treatment is also necessary. The prognosis of a treated dog is good, but naturally depends on the therapy and the *Babesia* species. It is important to remember that an infected dog is considered a carrier of *Babesia* even after it has been cured of the disease.

HEPATOZOON CANIS

- A tick-borne protozoan, but the infection comes from eating the tick, not from the bite.
- Usually subclinical, but unspecific signs of lethargy, fever, anemia, etc. may appear.
- Parasitic gamonts can be seen inside the neutrophilic granylocytes in a blood smear.
- Treatment with imidocarb; prevention by acarisidic substances.

Identification

Of the currently recognized 300 *Hepatozoon* protozoa, two, *H. canis* and *H. americanum*, are known to infect dogs. Of the life cycle stages of Hepatozoon, gamonts are the most likely to be detected in the laboratory. In a stained blood smear, they are visible in the cytoplasm of white blood cells (neutrophilic granylocytes, rarely also in monocytes) Gamonts are elongated, ellipsoidal, and surrounded by a membrane, with an eccentrically located nucleus (Fig. 2.21). The nucleus of *H. canis* is elongated and sometimes shaped like a horseshoe. Its gamont is about 8–12 µm long and 3–6 µm wide.

Another form found in the dog is the meront (also known as the schizont). It can be seen in histological sections or cytological samples taken from lymph nodes, spleen, or bone marrow. Meront is a round or oval tissue cyst and is capsulated in the tissue. At an early stage, the meront contains only amorphic material, but with the development of the cyst, a nucleus develops, which divides into elongated merozoites. The size of a typical meront of *H. canis* is about 30×30 µm. Within the meront, there are two or four macromerozoites, or over 20 micromerozoites. Micromerozoites are often in a cartwheel pattern around the central core of the meront (Fig. 2.22).

Life Cycle

Hepatozoonosis is a tick-borne disease. Unlike in many other tick-borne infections, the dog is infected orally and not during the tick's blood meal. Infection takes place when the dog ingests a hepatozoon-carrying tick from its fur or from a prey animal that has ticks on its skin. The scene of sexual reproduction and hence the definitive host in the life cycle of *H. canis* is the brown dog tick, *R. sanguineus*. The infection is passed transtadially from one stage of the tick development to another. The dog is usually infected by an adult tick, which has been infected during the larval or nymphal stage. Both male and female ticks can infect the dog.

After the dog has eaten the tick carrying *H. canis*, the sporozoites penetrate the gut wall. They infect circulatory monocytes and macrophages, which transfer the sporozoites primarily to lymph nodes, bone marrow, and spleen.

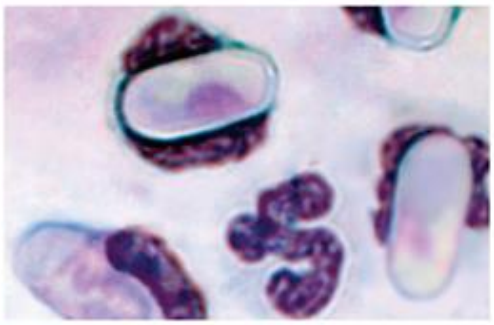

FIG. 2.21 Gamont forms of *Hepatozoon canis* inside granylocytes in a Giemsa-stained blood smear. Since they struggle to fit into the cytoplasm of the neutrophil, they distort its morphology.