Canine hepatozoonosis: two disease syndromes caused by separate *Hepatozoon* spp.

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Hepatozoonosis is caused by apicomplexan haemoparasites of the genus *Hepatozoon*, which are closely related to *Plasmodium* spp. and piroplasms. Recent research revealed that two tick-borne *Hepatozoon* spp. infect dogs and cause distinct syndromes. Comparisons of these related species illustrates that whereas *Hepatozoon canis* appears to be well adapted to its canine host, *Hepatozoon americanum*, an emerging pathogen producing severe and frequently fatal myositis, is highly virulent and might have recently crossed the species barrier from a wild host.

Canine hepatozoonosis is a tick-borne infection caused by apicomplexan protozoa from the family Hepatozoidae. More than 300 species of *Hepatozoon* have been reported to infect animals [1] *Hepatozoon* spp. share a basic life cycle that includes sexual development and sporogony in a haematophagous invertebrate definitive host, and merogony followed by gamontogony in a vertebrate intermediate host. Unlike most tick-borne protozoal and bacterial pathogens that are transmitted via the tick salivary glands, *Hepatozoon* transmission takes place by ingestion of the definitive host, an invertebrate containing *Hepatozoon* oocysts, by the intermediate host. Salivary transfer of this parasite has not been documented. It is presumed that dogs become infected with *Hepatozoon* through grooming ticks from their hair coat or feeding on prey infested with parasitized ticks.

Hepatozoon gamonts from dog isolates originating in different geographical regions are morphologically similar, and therefore it was believed, until 1997, that canine hepatozoonosis was caused by a single species [2]. Recent research has shed light on the pathological and clinical syndromes associated with this infection [3–6], the genetic and antigenic characteristics of *Hepatozoon* isolates [7,8], the vector tick species that transmit canine hepatozoonosis, and the parasite life cycle [9–11]. These studies have led to the recognition that two distinct *Hepatozoon* species infect dogs. Consequently, the parasite that infects dogs in the southern USA was named *Hepatozoon americanum* [4] and separated from the previously described *Hepatozoon canis* prevalent in southern Europe, Africa and Asia. *Hepatozoon americanum* infection is an emerging disease that is spreading north and east from coastal Texas, USA, where it was originally detected in 1978 [2]. It has since been reported also from Louisiana, Alabama, Oklahoma, Georgia, Tennessee and Florida.

Hepatozoon canis

Ingestion of a tick containing mature *H. canis* oocysts is followed by release of sporozoites in the gastrointestinal tract of the dog (Fig. 1). Sporozoites penetrate the intestinal wall and are transported haematogenously to haemolymphatic tissues including the spleen, bone marrow and lymph nodes, where meronts are formed. Merogony can also take place in other visceral organs, and is associated with hepatitis, pneumonia and glomerulonephritis [3]. Upon release from mature meronts, merozoites invade neutrophils in which they develop to gamonts and then circulate in the peripheral blood.

Hepatozoon canis infection (HCI) varies from being asymptomatic in apparently healthy dogs, to a severe and potentially fatal disease that causes extreme lethargy, cachexia and anaemia. A mild disease is the most common presentation of the infection and it is usually associated with a low level of *H. canis* parasitaemia (1–5% of neutrophils are infected). A severe illness is found in dogs with a high parasitaemia, often approaching 100% of the peripheral blood neutrophils [3]. High levels of parasitaemia are frequently accompanied by extreme neutrophilia reaching as high as 150 000 neutrophils per μl of blood. These dogs with leukocytosis and a high parasitaemia could have large numbers of circulating parasites with >50 000 gamonts per μl of blood. This massive parasitaemia reflects the large number of tissue meronts, and takes its toll on the host by demanding nutrients and causing direct injury to affected tissues, leading to extreme weight

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Glossary

Species barrier: crossing the species barrier involves passage of a pathogen from a natural host to a new and different host species. This is sometimes associated with a virulent course of infection in the new non-adapted host.
Subclinical infection: a mild form of infection without apparent clinical manifestations.
Trans-ovarial transmission: transfer of a pathogen from one generation of hosts to the next by invasion of the ovary and infection of the eggs.
Trans-stadial transmission: transfer of a pathogen from one life stage of an arthropod vector to succeeding stages following metamorphosis.

loss and cachexia. However, it appears that most dogs exposed to *H. canis* undergo a SUBCLINICAL INFECTION (see Glossary) with a low parasite burden [12].

Immune suppression induced by an infectious agent or chemotherapy might influence the pathogenesis of new *H. canis* infections or the re-activation of pre-existing ones. Treatment with an immunosuppressive dose of prednisolone was followed by the appearance of parasitaemia in dogs with experimental HCI [10]. In addition, dogs with naturally occurring HCI often have concurrent infections that potentially weaken their immune defences and the capability to resist *H. canis* infection [3].

HCI is mostly diagnosed by microscopic detection of intracellular *H. canis* gamonts within neutrophils in stained blood-smears (Fig. 2a). The gamonts are large (11 μm × 4 μm) and have an ellipsoidal shape. Mature *H. canis* meronts in tissues contain elongated merozoites arranged in a circle around a clear central core, forming a unique shape of a 'wheel spoke' meront (Fig. 2b). HCI is treated with the antiprotozoal imidocarb diproprionate and the tetracycline doxycyline. Elimination of gamonts from the peripheral blood is slow and could require periodic treatment with imidocarb diproprionate for eight weeks.

Hepatozoon americanum

In contrast to the usually mild disease found in HCI, *H. americanum* infection (HAI) is a debilitating and often fatal disease. The most common manifestations of HAI include fever, generalized pain, muscle atrophy, weakness and reluctance to rise [6] caused largely by merogonous cysts in skeletal muscle and bone proliferative lesions induced by *H. americanum*. Gait abnormalities range from stiffness to complete recumbency. A marked leukocytosis ranging between 20 000 and 200 000 leukocytes per μl of blood is typically found with a low parasitaemia, usually not exceeding 0.1% of the circulating leukocytes. Without treatment, chronic wasting commonly leads to death within 12 months [6].

The dissimilarity in the clinical manifestations of HAI and HCI is attributed partly to the different target organs

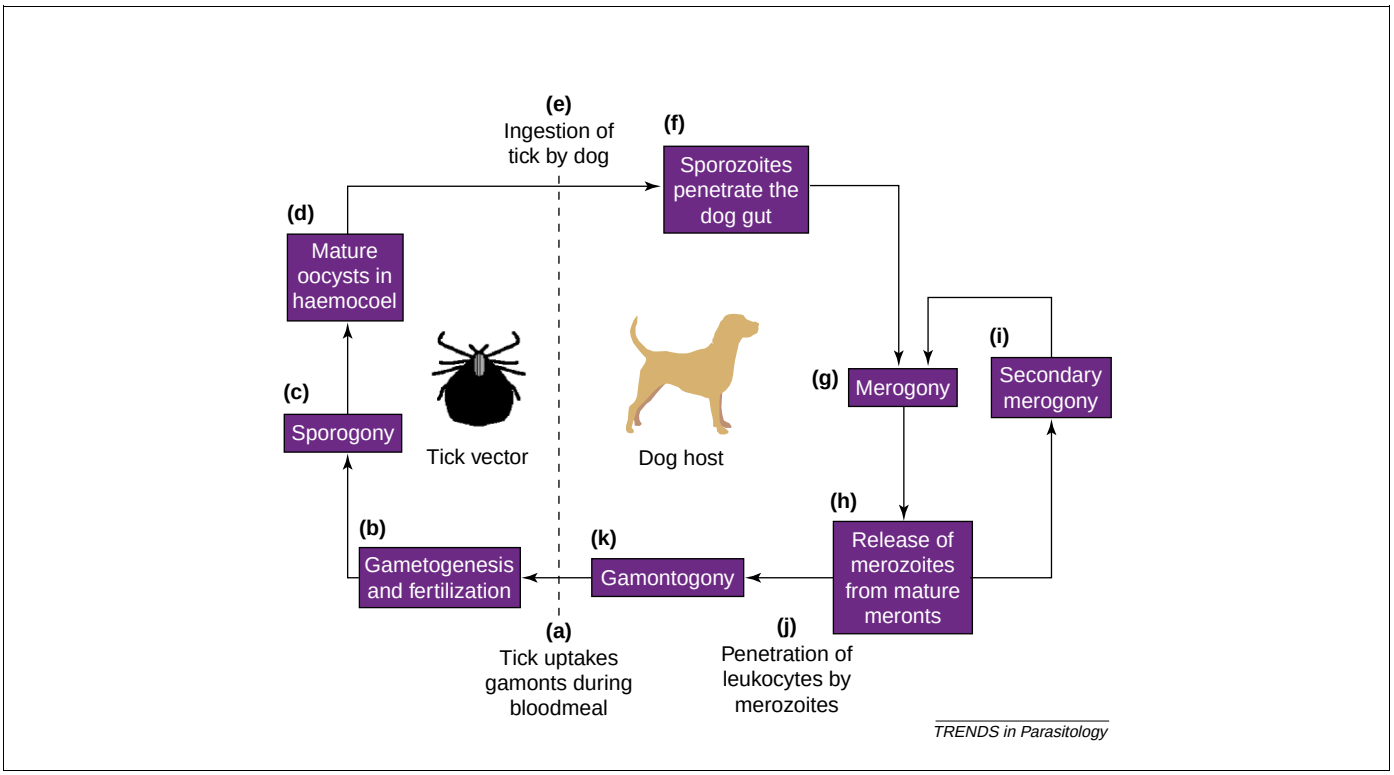

Fig. 1. A general life cycle for *Hepatozoon* spp. of dogs. (a) The leukocytes infected with gamonts, which circulate in the dog's peripheral blood, are taken up by tick during a bloodmeal. (b) The gamonts, which are released from leukocytes in the tick gut, associate in pairs and differentiate to gametes. Fertilization is followed by the creation of a zygote. (c) Meiosis is followed by early sporogony in the tick gut cells. (d) The oocysts formed during sporogony contain numerous sporocysts in which the infective sporozoites develop. Oocysts develop in the gut cells and protrude into the tick's haemocoel as they mature and enlarge. (e) A tick containing *Hepatozoon* oocysts is ingested by a dog or other vertebrate host. (f) *Hepatozoon americanum* sporocysts released from oocysts during ingestion excyst in the dog's gut in the presence of bile. Sporozoites invade the dog gut and are carried (possibly within a phagocytic host cell) to the host target tissues. (g) The formation of meronts in the process of merogony takes place mostly in haemolymphatic organs for *Hepatozoon canis* and in the striated muscles for *Hepatozoon americanum*. Cysts with *H. americanum* parasites surrounded by concentric layers of mucopolysaccharide material are formed in muscular tissues and might, subsequently, undergo merogony. This type of 'onion skin' cyst is not found in *H. canis* infection; however, a distinctive 'wheel spoke' meront comprising merozoites arranged in a circle around a central core is found only in *H. canis* infection. (h) Merozoites are released from mature meronts, which is associated with a painful pyogranulomatous inflammatory reaction in *H. americanum* infection. (i) Merozoites might form secondary tissue meronts. (j) Alternatively, merozoites penetrate leukocytes, and transform to gamonts in the process of gamontogony. (k) Gamonts in the leukocytes reach the blood circulation and could be taken up by tick hosts.

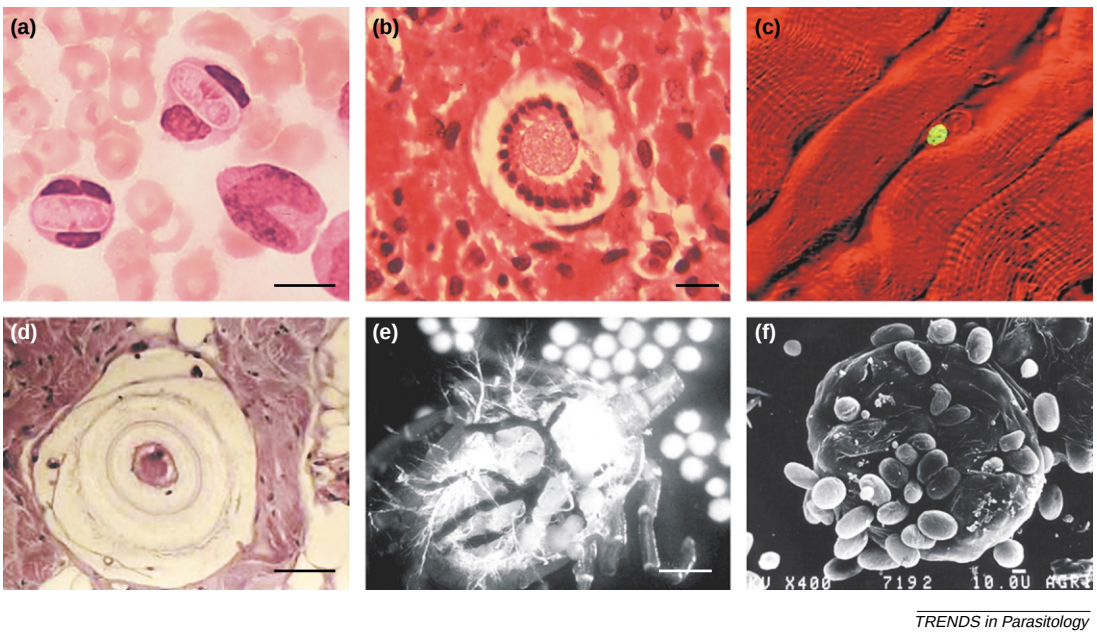

Fig. 2. Stages in the life cycle of *Hepatozoon canis* and *Hepatozoon americanum*. (a) *Hepatozoon canis* gamonts in peripheral blood neutrophils. (b) A 'wheel spoke' *Hepatozoon canis* meront in splenic tissue. (c) A confocal microscopic image of *H. americanum* (green) in a host cell between skeletal muscle fibers. The parasite was labelled with rabbit anti-*H. americanum* antibodies produced against the sporozoite stage. (d) An *H. americanum* 'onion skin' tissue cyst in skeletal muscle. (e) *Amblyomma maculatum* tick with dorsum removed. Note the few, pale, round oocysts attached to the dark gut, and many others spilled from the haemocoel. (f) A scanning electron micrograph of an *H. canis* oocyst surrounded by free sporocysts in the haemocoel of a *Rhipicephalus sanguineus* tick. Scale bars = 10 μm (a,b,f); 50 μm (d); 0.7 mm (e).

in which merogonic development takes place. *Hepatozoon americanum* principally infects skeletal and cardiac muscle, and induces pyogranulomatous myositis. The host cell appears to be a phagocytic cell that is initially located between myocytes (Fig. 2c) [13]. Concentric layers of mucopolysaccharide material are deposited around the host cell and form a large cystic structure (250–500 μm diameter) giving it the appearance of an 'onion skin' cyst, which is not found in HCI [4] (Fig. 2d). Some cysts appear to remain in that state, whereas others undergo merogony. The rupture of mature meronts induces the formation of highly vascularized pyogranulomas, where the released merozoites invade leukocytes and develop to gamonts that circulate in the blood, or possibly give rise to secondary meronts [13,14]. In the absence of an easily detectable parasitaemia, muscle biopsy and recently developed serological testing are the principal means of HAI diagnosis [15].

A therapy for HAI that is capable of eliminating all stages of the organism is not available at present. A combination of trimethoprim–sulfadiazine, clindamycin and pyrimethamine is initially administered every day for 14 days. Although remission of clinical signs usually occurs with this treatment, relapse generally occurs within two to six months. Increased survival times are obtained by long-term follow-up therapy with the anticoccidial decoquinate [16].

Transmission

Similar to all diseases that have arthropod vectors, the distribution of canine hepatozoonosis is tied closely to its acarine definitive hosts. The main vector of *H. canis* is the brown dog tick *Rhipicephalus sanguineus*. It is found in tropical, sub-tropical and temperate regions all over the world, making the potential distribution of *H. canis* worldwide. TRANS-STADIAL TRANSMISSION between the nymph and adult stages of *R. sanguineus* has been shown under experimental conditions, whereas TRANS-OVARIAL TRANSMISSION could not be demonstrated [10]. An experimental model of infecting *R. sanguineus* by percutaneous injection of blood gamonts has been established, thereby enabling transmission of *H. canis* to ticks without having to feed on dogs [10].

Hepatozoon americanum is transmitted by *Amblyomma maculatum*, a tropical tick species commonly called the Gulf Coast tick [9,17]. Older literature regarding distribution of ixodids in the USA indicated that *A. maculatum* was found only along the Gulf of Mexico; however, the tick is now documented to be endemic much further inland. *Amblyomma maculatum* ticks become infected when larvae or nymphs ingest leukocytes containing gamonts that circulate in the peripheral blood of their hosts. Syngamy of *H. americanum* appears to occur in the tick gut lumen, and early stages of sexual development have been observed within gut cells of nymphal and young adult ticks that acquired the parasite as nymphs [11,18]. Following meiosis, sporogony occurs, also within gut cells of the tick, followed by release of mature oocysts into the haemocoel (Fig. 2e). Oocysts contain ~650 sporocysts, each with 10–26 sporozoites. The oocysts of *H. canis* (Fig. 2f) and *H. americanum* are thin shelled and they rupture easily. Although the released sporocysts resist mechanical disruption, they excyst readily in the presence of dog bile to discharge the infective sporozoites [11]. Oocysts from nymphs fed as larvae and also from adult ticks fed as nymphs are infective to dogs [18].

Naturally occurring hepatozoonosis similar or identical to HAI has been reported in coyotes (*Canis latrans*), and a

dog-derived strain of *H. americanum* has produced experimental infection in coyotes [19]. The natural cycle of *H. americanum* might involve this wild canid; however, it is also possible that coyotes and domestic dogs are only secondary intermediate hosts and that a variety of potential hosts on which immature *A. maculatum* feed could be involved in the epidemiology of HAI. Other modes of transmission could exist. Although this has not been documented in canine hepatozoonosis, some *Hepatozoon* spp. are transmitted through predation and ingestion of tissue cysts found in intermediate host tissues [1]. In addition, HCI is often reported in seven to ten-weeks-old puppies and, although the life cycles of *H. canis* and *H. americanum* in the dog are completed within four and five weeks, respectively [10,13], vertical transmission has been reported in HCI [20].

Taxonomic and phylogenetic placement of *Hepatozoon*

The taxonomic placement of the genus *Hepatozoon* has been uncertain for many years largely because of the lack of information surrounding the sporogonic development of these parasites and other haemogregarines. Until recently, the similarity of the intraerythrocytic gamonts of *Haemogregarina* spp., *Hepatozoon* spp., *Karyolysus* spp. and their relatives has provided a rationale for placing all of these parasites in the large family Haemogregarinidae. However, the considerable biological diversity exhibited by parasites within their definitive hosts (vectors) by these adeleorid genera justifies separation into distinct families within the apicomplexan suborder Adeleorina [21]. *Haemogregarina, Cyrilia* and *Desseria* spp. (family Haemogregarinidae) are found in blood cells of cold-blooded vertebrates and are transmitted via the bite of leeches (salivary transmission). *Karyolysus* and *Hemolivia* spp. (Family Karyolysidae) are parasites of cold-blooded vertebrates that are transmitted by ticks or mites. Within their vectors, these parasites undergo complicated sporogonic and merogonic development that can include trans-ovarial transmission in the case of *Karyolysus* spp. Transmission occurs through ingestion of their invertebrate vector containing encysted merozoites. Parasites in the genus *Hepatozoon* are classified within the family Hepatozoidae. Unlike members of the haemogregarines families described previously, *Hepatozoon* spp. form resilient oocysts with numerous sporocysts that are infective for their vertebrate hosts; most such hosts are infected via ingestion of the invertebrate vector. Based on analysis of morphological features and 18S rDNA sequences [8], parasites in the genus *Hepatozoon* are believed to be most closely related to other haemoparasites that use invertebrates as definitive hosts such as *Plasmodium* spp. and piroplasms [22] (Fig. 3).

Conclusions

Despite the morphological and genetic similarities between the two species of *Hepatozoon* infecting dogs, each species causes a distinct disease syndrome. *Hepatozoon canis* appears to be well adapted to its canine host, producing a subclinical to mild disease in most cases. However, *H. americanum* induces a violent and frequently fatal course of disease, and it is suggested that it might have recently crossed the SPECIES BARRIER from a wild host to the domestic dog.

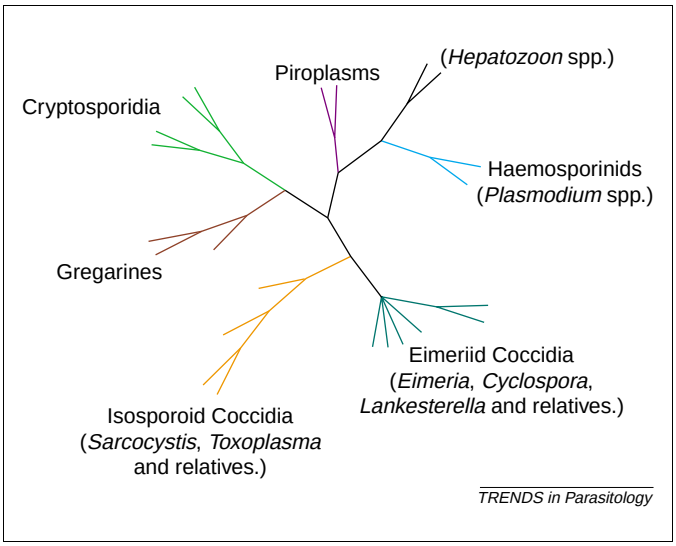

Fig. 3. Unrooted tree based on 18S ribosomal DNA sequences, illustrating the phylogenetic placement of *Hepatozoon* spp. among various major groups of parasites in the phylum Apicomplexa. The tree was generated using maximum parsimony with the following assumptions: (1) equal weighting of all characters; (2) user-defined step matrix to assign double weighting to transversions; and (3) retention of multiple equally parsimonious trees during the heuristic search (mulpars option in effect in PAUP ver. 4.0). Only branching order is shown; branch lengths are not proportional to hypothesized evolutionary change. Monophyly of major widely recognized apicomplexan groups is strongly supported in the cladogram. *Hepatozoon* spp. are found within a monophyletic grouping that includes the piroplasms and malarial organisms. Data from Ref. [22]. Key: blue, haemosporinids; brown, gregarines; pale green, cryptosporidia; orange, isosporoid coccidia; adrk green, eimeriid coccidia; purple, piroplasms.

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