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Canine hepatozoonosis – a summary for the practitioner

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Bayer Parasite Solutions

Introduction

When Bayer HealthCare, Animal Health Division, called for the 1st International CVBD® Symposium in 2006, this was the first step to address the global threat of canine vector-borne diseases (CVBD®). This was based on the belief that vector-borne diseases of the dog should be treated as one topic and dealt with on a global level in an interdisciplinary way. During the past years, four symposia have taken place and CVBD® have become a global issue, even sparking public interest. Many of the parasite-transmitted diseases affect humans as well as animals. The dog as man's best friend plays an important role – both being affected by, and serving as a host for some of the zoonotic pathogens.

At the first symposium, the participants agreed to form the CVBD® World Forum. Besides gathering knowledge, the main task for this group of international experts has been to raise awareness for the specific regional risks of CVBD® and to foster preventive measures. For this reason, the CVBD® World Forum created a website (www.cvbd.org) to provide the veterinary practitioner with cutting-edge and clinically relevant scientific information on CVBD®.

In CVBD® Digest, relevant findings from CVBD® symposia are presented periodically to veterinary practitioners. During the symposia, hepatozoonosis has become a major topic of interest, as it is now regarded as a global problem: meanwhile, *Hepatozoon canis* has been identified in most if not all continents using molecular diagnostics. The recent discovery of a related species in the US, *Hepatozoon americanum*, with severe clinical signs and a poorer prognosis, has accelerated research into the *Hepatozoon* life cycle and associated pathophysiology. Nevertheless, there are still a lot of uncertainties in this research area. One reason might be that these infectious agents have an atypical route of transmission, mostly via ingestion of the transmitting ticks. However, when it comes to prevention, the use of an ectoparasiticide that repels and kills the transmitting ticks, as is recommended to reduce the risk of other CVBD®, is a promising way to minimize the risk of *Hepatozoon* transmission in the dog.

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Canine hepatozoonosis – a summary for the practitioner

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Canine hepatozoonosis is caused by the two apicomplexan parasites *Hepatozoon canis* and *Hepatozoon americanum*. Although phylogenetically related, the two species differ in a variety of aspects, including clinical signs, life cycle, and host spectrum. In contrast to other canine vector-borne diseases (CVBD), the main transmission route for both of these parasites is via ingestion of the infected tick vectors. This article gives an overview of *H. canis* infection with a comparison to *H. americanum* infection. When it comes to prevention, tick control using a repellent ectoparasiticide is beneficial for the dog.

Historical background and taxonomy

The two canine pathogens *Hepatozoon canis* (see Fig. 1) and *H. americanum* are hepatozoid apicomplexan protozoa. They belong to a diverse group of parasites that includes more than 300 *Hepatozoon* spp., of which 46 have been described in mammals.[1] The genus *Hepatozoon* now belongs to the family Hepatozoidae of the suborder Adeleorina.[1,2,3]
Canine hepatozoonosis was first diagnosed in India at the beginning of the last century, and the causative agent was classified as *Leukocytozoon canis.*[4,5] In 1908, Miller[6] described the genus *Hepatozoon*, into which this canine parasite was subsequently transferred.[7] During this time, the Brown Dog tick *Rhipicephalus sanguineus* was established as the main invertebrate host for the protozoan.[8]
Prior to 1978 when the first cases with severe clinical signs were detected in the Gulf Coast region of Texas, USA, canine hepatozoonosis was only known in the Old World.[9,10,11] At first, it was assumed that the pathogen was a virulent strain of *H. canis*, but differences in definitive host, pathology and life cycle[12] led to the description of a separate species *H. americanum* in 1997.[13] Comparative genetic and antigenic studies further substantiated that, although related to *H. canis*, *H. americanum* is a distinct species.[12,14,15] A brief comparison of these pathogens is provided at the end of this text in Tab. 2.

Life cycle

All *Hepatozoon* spp. share a basic life cycle that includes sexual development and sporogony in a hematophagous invertebrate definitive host, and merogony followed by gamontogony in a vertebrate intermediate host. Definitive hosts for *Hepatozoon* spp. are blood-sucking invertebrates, including ticks, lice, reduviid bugs, and leeches. The gamont stage is found in blood cells of the vertebrate host, typically in leukocytes (see Fig. 1).

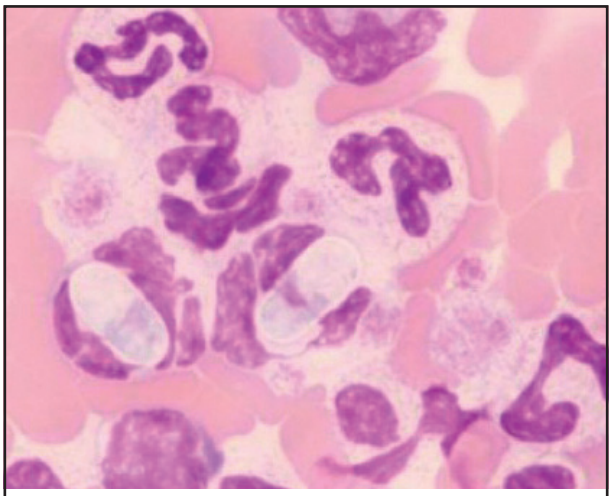

Fig. 1 Blood-smear showing *Hepatozoon canis* gamonts from the blood of a dog. Two protozoa can be observed as oval structures within leukocytes. (With kind permission of Gad Baneth, Rehovot, Israel)

Transmission

Transmission of *Hepatozoon* spp. to vertebrates is by ingestion of all or part of the definitive invertebrate host. In the case of canine hepatozoonosis, a dog may ingest an infected tick, either during grooming or when eating tick infested prey such as small rodents. Direct transmission from infected rodents to dogs is currently being investigated: Ingestion of *H. americanum* sporozoites by cotton rats led to the development of cystozoites in the rats' muscle tissue.[16] Muscle from infected rats was infectious to a dog that subsequently developed the characteristic signs of American canine hepatozoonosis (ACH).[17] However, muscle from *H. americanum* infected dogs did not result in transmission.[13,18] There are no feeding studies evaluating the infectivity of *H. canis* cysts.[19]

Merogony – Development in vertebrates

The exact dispersion route of *H. canis* sporozoites after oral ingestion of *H. canis* oocysts containing sporozoites within sporocysts is unknown. It is not clear whether the sporozoites penetrate the gut and disseminate hematogenously to their target organs or whether they are first engulfed by a phagocytic host cell prior to migration via the lymph or blood to other tissues.[20] Initial merogony may take place in the liver or alternatively the gut lymph tissue or mesenteric lymph nodes.

The bone marrow has also been shown to be a major site for merogony.[7,21,22]

Meronts of *H. canis* are found rarely in muscle and have their own characteristic morphologic "wheel spoke" arrangement of merozoites within the meront (see Fig. 2).[23] It is likely that the invasion of leukocytes by (micro)merozoites and transformation to gamonts takes place in the visceral tissues prior to returning to the circulation[20], where these stages can be taken up again by blood-feeding ixodid ticks.

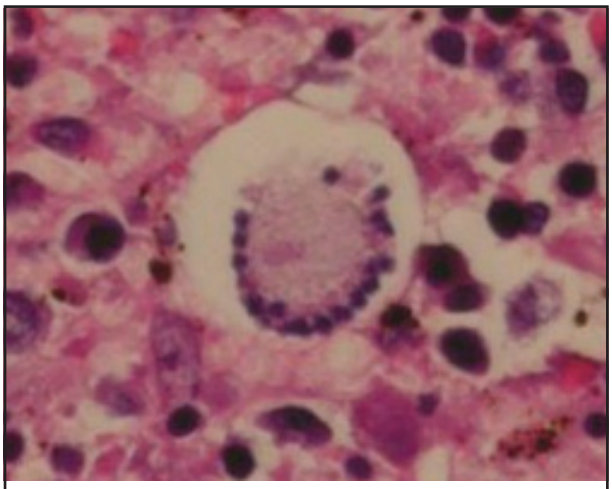

Fig. 2 *Hepatozoon canis* meront in the spleen. Note the "wheel spoke" arrangement of the merozoites within the meront. (With kind permission of Gad Baneth, Rehovot, Israel)

INFO BOX 1

VERTEBRATE LIFE CYCLE OF *H. AMERICANUM*

After the ingestion of oocysts by the vertebrate host, sporocysts are freed, releasing sporozoites. It is assumed that the sporozoites cross the gut wall and are carried via the lymphatic system or the bloodstream to tissues throughout the body.[24] Parasitized host cells have been demonstrated lodged between myofibres in a variety of skeletal muscles soon after experimental exposure to infective oocysts.[25,26] The trophozoite found in macrophage-like cells (mainly in striated muscle) apparently transforms the host cell into a mucopolysaccharide-producing entity, resulting in the so-called "onion skin cysts". This seems to shield the parasite from the dog's immune system until merogony is completed and the cystic structure is ruptured.[24] Mature meronts release merozoites, causing local inflammation with an associated systemic reaction and overt illness. Highly vascular granulomas evolve with parasites in macrophages, where presumably gamogony commences.[27] Parasites enter leukocytes, which subsequently circulate in the bloodstream as gamonts and may be consumed by blood-feeding ixodid ticks.

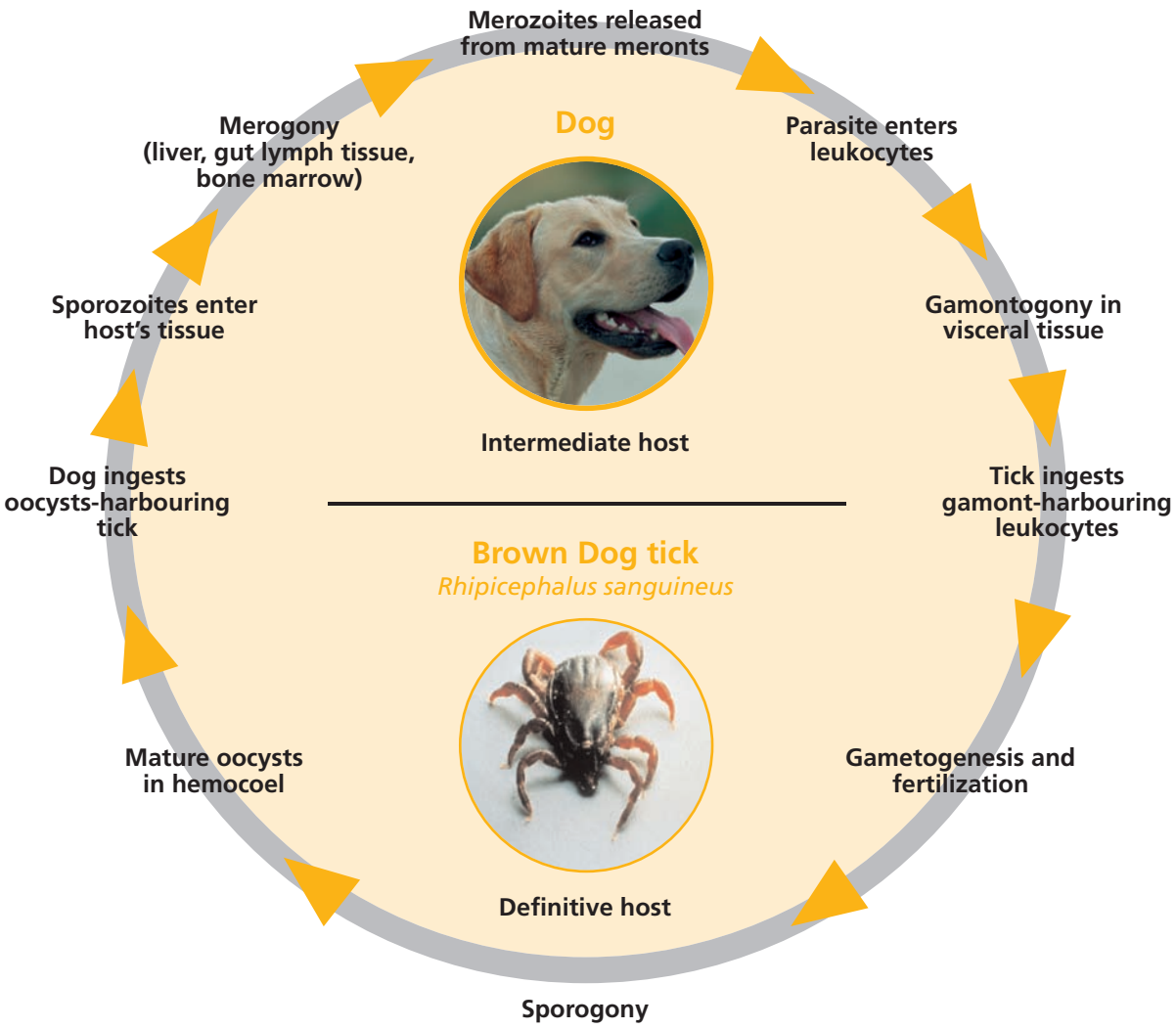

Fig. 3 Life cycle of *Hepatozoon canis* (modified after reference 24). After transmission to the vertebrate host (left side), merogony and gamonotogony both take place in the host's (i.e. dog) tissues. The transmission of gamonts to ticks via blood feeding initiates sporogony inside the tick and development continues until the sporozoites mature and become infective.

Sporogony – Development in invertebrates

After ingestion by the tick, gamonts are released from the canine leukocytes. As shown for *H. americanum*, some time later zygotes can be observed within cells of the tick gut's wall. Sporogony then follows within the tick gut cells, eventually giving rise to oocysts packed with hundreds of sporocysts, each containing sporozoites.[28] The host cell is distorted; some oocysts become dislodged and remain in the tick's body cavity (hemocoel). From there, they are discharged mechanically when the tick's body is ruptured on ingestion by a vertebrate host. A single zygote gives rise to one oocyst containing hundreds of sporocysts and thousands of sporozoites.
For *H. canis*, it has been suggested that sexual development can take place outside the tick gut lumen.[20]

Vertical transmission

As well as ingestion of sporocyst-containing oocysts within ticks and cystozoites in muscle tissue of rats (as in the case of *H. americanum*), transplacental transfer of *H. canis* has been reported in Japanese dogs.[29]

Host range (invertebrate and vertebrate)

Although the invertebrate host for *H. canis* has been known for a century to be the Brown Dog tick, *Rhipicephalus sanguineus*[8], several attempts to transmit *H. americanum* with *R. sanguineus* have not been successful. Other ixodid ticks were also studied (*Dermacentor variabilis, Amblyomma americanum*), until the nymphs of *Amblyomma maculatum*, the Gulf Coast tick, were found to be consistently susceptible to infection with *H. americanum*.[30] The invertebrate host range of *H. americanum* seems to be narrower than that of the vertebrate host.

Reports from South America suggest that the Cayenne tick, *Amblyomma cajennense*, is a possible vector for *Hepatozoon* spp.[24] Japanese scientists have found oocysts in *Haemaphysalis* spp. taken from dogs with hepatozoonosis[31], but it is unclear whether they are those of *H. canis* or a different *Hepatozoon* spp. In dogs, the only *Hepatozoon* species described to date are *H. canis* and *H. americanum*.[19] In other vertebrate hosts, various *Hepatozoon* species have been detected in grey squirrels[32], raccoons[33], bobcats, ocelots[34] and in a crab-eating fox from Brazil.[35]

Distribution

For *H. canis* the distribution of the definitive host, the Brown Dog tick, is decisive. This tick is found in temperate and tropical regions worldwide, and the infection with *H. canis* has been identified in many parts of the Old and some regions in the New World (see Fig. 4). More recently, infections with *H. canis* have also been detected in the USA in dogs (see Tab. 1) either as single or as co-infections with *H. americanum*.[40] This refutes the general assumption that *H. canis* is not present in North America.

The distribution of the definitive host and tick vector of *H. americanum*, the Gulf Coast tick (*A. maculatum*), was historically limited to areas along the Gulf Coast and southern Atlantic coast[41] of North America. Recently, the range of the Gulf Coast tick has expanded northwards[42,43] and the pathogen has also been reported from states outside the recognized range of *A. maculatum*[40], presumably due to relocation of infected dogs from endemic areas.[19]

INFO BOX 2

CURRENT ASSUMPTIONS ON *H. AMERICANUM* EPIDEMIOLOGY

Naturally occurring infection with *Hepatozoon americanum* in wild rodents, rabbits or vertebrates other than canids has not yet been demonstrated, despite testing in endemic areas.[10,19,36] Coyotes (*Canis latrans*) have been reported to be naturally infected with *H. americanum*[10,37,38] and may be an important component of the emerging problem in domestic dogs.[38] It remains to be determined whether both coyotes and domestic dogs are simply being inserted into an already existing enzootic cycle involving Gulf Coast ticks and a vertebrate host such as rodents.

One hypothesis is that *H. americanum* is a newly emerged species and that its appearance in coyotes and dogs is a recent event.[39] Dogs are not among the favored hosts for *A. maculatum*, but all three life stages will feed to repletion on this host under experimental conditions. There is evidence that birds are hosts for larvae and nymphs, and that small mammals such as mice, wood rats, voles, lagomorphs, and shrews are also hosts for these immature stages.[39]

Fig. 4 Geographical distribution of canine hepatozoonosis in different parts of the world. Countries where the endemic occurence has been reported are highlighted in red. The data was gathered by Bayer HealthCare Animal Health from recent scientific publications to provide a comprehensive picture of the endemic situation of several CVBDs including hepatozoonosis in Asia-Pacific (Fig. 4a), Europe (Fig. 4b) and Latin America (Fig. 4c). More specific regional information can be obtained from www.cvbd.org.

State	Total specimens	*H. americanum*	*H. canis*	*H. americanum* and *H. canis*	Total % positive
Alabama	268	83	6	9	36.6%
Georgia	63	18	2	0	31.8%
Mississippi	56	23	3	4	53.6%
Texas	50	12	0	0	24.0%
Louisiana	42	10	0	1	26.2%
Oklahoma	17	9	0	0	52.9%
North Carolina	16	1	0	0	6.3%
Virginia	10	1	1	0	20.0%
Others[a]	92	10	2	0	13.0%
Total	614	167	14	14	31.8%

a) Includes specimens from the remaining 20 states from which less than 10 specimens were submitted, and specimens with unidentifiable sources.

Table 1 Geographical distribution of *Hepatozoon* spp.-positive blood samples in the continental United States. *Hepatozoon* spp. have been amplified from the specimens using 18 S rDNA **F**luorescence **R**esonance **E**nergy **T**ransfer-PCR. In California, Kentucky, Nebraska, North Carolina, Oklahoma, Texas, Vermont, and Washington, *H. americanum*, but not *H. canis*, was detected (after reference 40).

Clinical presentation

H. canis infection ranges from a subclinical state in dogs with low level parasitemia, to a severe life-threatening illness with fever, lethargy, anemia, and emaciation in dogs with high parasitemia[22] or co-infection with other tick-borne pathogens (such as *Ehrlichia*, *Babesia* etc.). Leukocyte counts are usually normal or slightly elevated and numerous gamonts can be observed, infecting up to 100% of neutrophils.[44] Only rarely do *H. canis*-infected dogs display osteoproliferative lesions,[45] in contrast to *H. americanum* infected animals.

INFO BOX 3

CLINICAL PRESENTATION OF AMERICAN CANINE HEPATOZOONOSIS (ACH)

Hepatozoon americanum infected dogs usually present with fever, myalgia, myasthenia, and wasting. They suffer from severe pain and are often reluctant to move. Dogs may also exhibit mucopurulent ocular discharge[46] and usually there is generalized periosteal exostosis of bones on radiograph, and myositis of striated muscles.[47,48] Hematological examination typically reveals leucocytosis (15,000 to more than 200,000/µl), with a mature neutrophilia (greater than 95%)[39], but gamonts are only rarely found in blood smears.

Diagnosis

The following diagnostic tests apply mainly to *H. canis*. For *H. americanum*, some information is provided in the final table. For more information, the corresponding literature should be referred to.[24,40,49]

- **Blood smears:** Parasitemia is usually distinct so that diagnosis of infection can readily be confirmed by examination of blood smears.
- **Serology:** Baneth and colleagues have reported the use of a serological test for the diagnosis of *H. canis* hepatozoonosis in Israel.[50]
- **PCR:** Recently a real-time PCR has been developed using EDTA-whole blood samples and subsequently fluorescence resonance energy transfer (FRET) probes to detect a signature polymorphism in the amplified DNA. This combined test system differentiates between *H. canis* and *H. americanum* and reveals single target nucleic acid copies in a PCR sample derived from an aliquot of ~140 µl canine blood with essentially 100% specificity.[40]

In addition, muscle biopsy might reveal the existence of the characteristic morphologic feature referred to as a "wheel spoke" arrangement of merozoites within the meront.[23] In contrast, *H. americanum* meronts form "onion-skin cysts".

Treatment

There are no substances reported to eliminate all the different developmental stages of *Hepatozoon* sp. making the aim of chemotherapy the alleviation of clinical signs. For *H. canis*, combination therapy of imidocarb diproprionate and tetracyclines or tetracycline hydrochloride has been shown to achieve clinical cure. However, because of very slow elimination of gamonts in the peripheral blood, in certain cases imidocarb diproprionate had to be administered over eight weeks.[51]

Treating dogs with ACH is often frustrating, due to frequent relapses that may result in exacerbating episodes of disease. Additionally with each relapse, the chances of developing complications of glomerulonephropathy, amyloidosis, vasculitis, and cachexia increase.[49] A treatment protocol has been developed that is effective in alleviating overt disease. It consists of a combination of trimethoprim-sulfadiazine, clindamycin, and pyrimethamine (TCP), administered daily for 14 days. This is followed by the long term use of decoquinate, an effective anticoccidial drug. If the protocol is not strictly adhered to, relapse is likely to occur within weeks to months after decoquinate treatment is discontinued.[52]

Apart from this antiprotozoal regimen, supportive care with nonsteroidal anti-inflammatory (NSAIDs) drugs is very important. Some dogs with undiagnosed ACH may recover as a result of good care by owners.[24]

Prevention

As in all canine vector-borne diseases (CVBD), control of the vector is of major importance in prevention of disease by minimizing the risk of disease transmission. This is also the case for *Hepatozoon* spp. infection, even though the way of transmission is not via the tick's saliva, but mainly via ingestion of infected ticks. Since dogs may ingest ticks while grooming their fur, the use of an ectoparasiticide that repels

and kills ticks is advantageous. A broad spectrum ectoparasiticide will also minimise the risk for other CVBDs transmitted by sandflies, fleas or mosquitoes. Additionally, owners should frequently examine their dogs to remove ticks, particularly after hunting or roaming outdoors. With respect to *H. americanum*, dogs in endemic areas should be restricted from eating raw meat or organs from wildlife, that could possibly be infected.

	Hepatozoon canis	*Hepatozoon americanum*
Tick vector	*Rhipicephalus sanguineus* (Brown Dog tick)	*Amblyomma maculatum* (Gulf Coast tick)
Primary clinical signs	frequently asymptomatic; can cause lethargy, fever, weight loss	fever, pain, lameness, mucopurulent ocular discharge; may wax and wane
Common laboratory abnormalities	anemia; extreme leukocytosis is rare but may be seen in dogs with very high parasitemia	extreme leukocytosis (20,000 – 200,000 leukocytes/μm^3), anemia, elevated alkaline phosphatase, low glucose
Concurrent infection or immunosuppression	very common	occasional
Geographic distribution	Africa, Middle East, Asia, Southern Europe, South America recently United States[40]	United States, Central and South America
Tissue stages	"wheel-spoked" meronts found primarily in spleen, bone marrow, lymph nodes	meronts exhibit blastophore formation muscle lesions consisting of "onion-skin" cysts, meronts, pyogranulomas, myositis
Radiographic lesions	none (except one case reported in Japan)	periosteal proliferation
Severity of disease	subclinical to severe, usually mild; good prognosis	severe; guarded prognosis
Frequency of gamonts in peripheral blood	common parasitemia 1–100% (usually ~1%)	rare; parasitemia usually <0.1%
Gamont characteristics	size: 11.0 x 4.3 µm; ultrastructure: fine fibril-like structure surrounding parasitophorous vacuole	size: 8.8 x 3.9 µm; ultrastructure: tail-like appendage; lacks fine fibril-like structure seen in *H. canis*
Antibodies	*H. canis* IFA shows high frequency of antibodies in general dog population (Israel)	*H. americanum* IFA shows good correlation with muscle biopsy and low cross-reactivity on *H. canis* IFA
PCR	real-time PCR on EDTA-whole blood samples, with subsequent use of FRET probes, differentiating between *H. canis* and *H. americanum*; 100% specifity[40]	real-time PCR on EDTA-whole blood samples, with subsequent use of FRET probes, differentiating between *H. canis* and *H. americanum*; 100% specifity[40]
Treatment	imidocarb diproprionate, doxycycline	trimethoprim/sulfadiazine, pyrimethamine, clindamycin, decoquinate

Table 2 Comparison of *Hepatozoon americanum* and *Hepatozoon canis* (modified after reference 49)

IFA: immunofluorescent antibody; FRET: fluorescence resonance energy transfer

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