



*Mini-review*

**HEPATOZOON CANIS AND HEPATOZOONOSIS IN THE DOG**

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**ABSTRACT**

This review summarizes data from the numerous investigations from the beginning of the last century to the present. The studies concerned the main issues of the morphology, the life cycle, hosts and localization of *Hepatozoon canis* (phylum *Apicomplexa*, suborder *Adeleorina*, family *Hepatozoidae*). The characteristic features of hepatozoonosis, caused by *Hepatozoon canis* in the dog, are evaluated. A survey of clinical signs, gross pathological changes, epidemiology, diagnosis and treatment of the disease was made. The measures for prevention of *Hepatozoon canis* infection in animals are listed. The importance of hepatozoonosis with regard to public health was evaluated. The studies on the subject, performed in Bulgaria, are discussed.

**Key words:** hepatozoonosis, dog, *Hepatozoon canis*, *Rhipicephalus sanguineus*

**INTRODUCTION**

*Hepatozoon canis* is a protozoan from the phylum *Apicomplexa*, detected for the first time in the blood of dogs in India and determined as *Leukocytozoon canis* (1, 2). *Hepatozoon canis* belongs to the family *Hepatozoidae* (*Hemogregarinidae*) of suborder *Adeleorina*. This family consists of more than 300 species, described in reptiles, birds and mammals (3). All *Hepatozoon* species have the same life cycle: gametogony and sporogony in the definitive host (a blood-sucking invertebrate) and schizogony followed by formation of gametes in the intermediate host (a vertebrate). The definitive host of *Hepatozoon canis* is the brown dog tick *Rhipicephalus sanguineus* (2), and the intermediate hosts are dogs and wild canids.

Hepatozoon infection in the dog, caused by *Hepatozoon canis* is widely spread in South Europe (4, 5), Africa (6), Asia (7, 8), and South America (9). The infection could be asymptomatic in dogs with low level of parasitaemia or could be manifested as a severe life-threatening disease with fever, lethargy, anaemia, cachexia in dogs with high parasitaemia (10).

The reviews of some authors

summarize the information about the biology, epidemiology, pathogenesis, clinical manifestation, diagnosis and prevention of canine hepatozoonosis. However, the studies on the range of possible hosts, the routes of transmission, the distribution and methods for prevention and control are still continuing (11, 12, 13).

The lack of relevant investigations in Bulgaria and the necessity for more comprehensive information on this problem motivated the present detailed review of available literature.

**LIFE CYCLE**

The studies providing data upon the life cycle of *Hepatozoon canis* could be classified in several directions:

- investigations on the development of *Hepatozoon canis* in naturally infected dogs and ticks (1, 2, 14, 15);
- performance of experimental *Hepatozoon canis* infections of ticks and dogs with follow-up of the different stages of development (16, 17, 18);
- investigations on the analogous development of *Hepatozoon canis* in ticks and canids, based on information obtained from other *Hepatozoon* species, parasitising different mammalian hosts (15, 19).

The vector tick *Rhipicephalus sanguineus* becomes infected in the nymph stage by

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ingesting blood of a dog, possessing *Hepatozoon canis* gamonts located within leukocytes (11, 12, 20, 21). In the gut of ticks, gamonts are dividing and by the 24<sup>th</sup> hour after the infection, macro- and microgametes are formed, and after the 8<sup>th</sup> day – zygotes and young oocysts could be detected. The time of sporogony probably corresponds to the nymph moulting stage and ends approximately by the 50<sup>th</sup> day after ingestion of infected blood (20, 21).

There is not a study demonstrating the complete development of *Hepatozoon canis* in ticks, but investigators are looking for parallels with life cycle stages in other *Hepatozoon* species and their arthropod vectors. Large variations have been observed after the entry of gamonts in tick gut, consisting mainly in tissues where gametogony and sporogony occur and the presence or lack of intracellular development. In the first experimental research on the life cycle of *Hepatozoon spp.*, pairs of *Hepatozoon muris* gamonts grouped in the gut lumen of the tick *Laelaps echidninus* have been described (22). The mobile zygotes (ookinetes) penetrate via the gut wall in the tick haemocoel, where sporogony takes place. Cx 1981 Krampitz have studied the development of *Hepatozoon erhardovae*, parasitising bank voles (*Clethrionomys glareolus*), in the rat flea (*Xenopsylla cheopis*) (23). The gamonts released in flea gut, migrate through the gut wall into the cells of the abdominal fat body, where they differentiate into macro- and microgametes. Oocysts are also formed in fat body cells of fleas. Mathew et al. investigated the sporogony of *Hepatozoon americanum* in the tick *Amblyomma maculatum* and described the penetration of gametes through the gut wall, and the subsequently formed oocysts protruded to the tick haemocoel (24). Contrary to the described intracellular development of *Hepatozoon spp.*, in other mammals, gametogony and sporogony of the parasite occur extracellularly. An extracellular development of sexual and sporulating stages of *Hepatozoon balfouri*, parasitising rodents from the *Jaculus* genus, in two tick species (*Haemolaelaps sp.*) is described (25). Others (26) have neither found out intracellular stages throughout their studies on the sporogony of *Hepatozoon griseisciuri*, infecting the grey squirrel, in the tick *Haemogamasus reidi*.

Having studied the development of *Hepatozoon canis*, Wenyon supposed that its sexual cycle was similar to that of

*Hepatozoon muris* (14, 22). The parasitic stages passed through the gut epithelium and continued their development near the gut folds (14). In ticks, experimentally infected by subcutaneous injection of blood infected with *Hepatozoon canis*, Baneth et al established that gametogony outside the tick gut was also possible – in tick's haemocoel (21). The rapid development and enlargement of oocysts (quadrupled their average diameter) after nymph moulting was also confirmed by other researchers having observed a rapid growth of oocysts in many *Hepatozoon* species (24, 25, 26, 27).

The oocyst wall of *Hepatozoon spp.* appears thin and subtle in adult ticks and could be easily destroyed. In *Hepatozoon griseisciuri* and *Hepatozoon americanum*, the oocyst wall is disrupted in the presence of bile and permits the encystment of sporozoites (24, 26). Most possibly, this is the mechanism of encystment of other *Hepatozoon* species, infecting mammals, including *Hepatozoon canis*.

The dog becomes infected with *Hepatozoon canis* by eating ticks or parts of tick body containing oocysts (11, 12). The pathway of distribution of sporozoites released in the host gut is unknown. The spread of sporozoites in internal organs occurs probably via two routes: haematogenous, by passing through the gut wall and transportation by blood or by ingestion from phagocytising cells and transportation with the lymph or the blood. In the haematogenous route, the primary schizogony would appear in the liver, where the venous blood from the gut passes through prior to reach the bloodstream. Thus, the schizogony in the other organs would be secondary. In the intracellular dissemination, the primary schizogony would take place in the gut lymph tissue and the mesenterial lymph nodes (21). Numerous studies have demonstrated that the primary site for schizogony, merozoite penetration in leukocytes and their development to gamonts is the bone marrow (10, 15, 28). Schizonts are detected in the lungs, heart, skeletal muscles, liver, spleen, lymph nodes (11, 12). The sporozoites penetrated in endothelial cells and the cells of the phagocytic system of these organs become transformed into schizonts with an almost round shape and diameter of 20–30 µm. Two types of schizonts are formed: macroschizonts containing 2-4 macromerozoites and microschantons with 20-30 micromerozoites. The released micromerozoites penetrate in neutrophils and differentiate into gamonts

with oval shape, large granulated nucleus and a size of 8-12/3-6  $\mu\text{m}$  (11, 12, 21).

The investigations with other *Hepatozoon* species showed the simultaneous presence of two types of schizonts (27, 29, 30, 31, 32), and in some of them it was demonstrated that macroschizonts preceded the development of microschizonts (33, 34). The past studies in *Hepatozoon canis* evidence that both types of schizonts are simultaneously formed. If we could accept the hypothesis that primary schizogony occurs in the liver and gut lymphatic tissue and that schizogony in other tissues is secondary, the model with macromerozoites followed by micromerozoites could be true for *Hepatozoon canis* too (21).

Together with schizonts, monozytic cysts are detected in the tissues of infected dogs. After *Hepatozoon canis* infection, the cysts are detected in the spleen and their dimensions are 20-26/15-21  $\mu\text{m}$  (35). The role of these cysts is not known. Similar, but larger cysts are observed in the muscles of dogs infected with *Hepatozoon americanum* (36, 37). Monozytic and dizoic small cysts have been reported in other *Hepatozoon* species as well (32, 38, 39, 40, 41).

Gamontogony – the invasion of leukocytes by micromerozoites and their subsequent transformation into gamonts occurs within four weeks. By the 28<sup>th</sup> day of the infection, mature gamonts could be found in the blood of affected animals, and by the 26<sup>th</sup> day of the infection there were only free merozoites in the bone marrow and merozoite-like cells penetrating the leukocytes. The penetration of leukocytes probably takes place in tissues, because in naturally and experimentally infected dogs, neutrophils exhibited only mature gamonts. Another possible mechanism of gamontogony in the spleen, lymph nodes, liver and other tissues is the accumulation of inflammatory cells at the sites of occurring schizogony, exactly at the moment of schizonts' rupture and death of host cells. Merozoites released at that moment, could penetrate the neutrophils and monocytes and turn into gamonts (21). This is the presumed mechanism of development of *Hepatozoon americanum*, where schizogony occurs in the muscle tissue (42, 43).

## CLINICAL FINDINGS

The infections with *Hepatozoon canis* in dogs could occur in three forms: subclinical – probably the commonest one; acute –

developing about one week before the death and chronic – with phases of clinical expression and remission (44, 45, 46). The symptoms are very various, but non-specific.

In several studies in dogs, experimentally and spontaneously infected with *Hepatozoon canis*, the most frequently observed clinical signs were anaemia, emaciation and intermittent fever (11, 12, 13, 21, 28, 47, 48, 49, 50). On many occasions, cachexia, depression, muscle hyperaesthesia, purulent conjunctivitis and rhinitis were reported. Less frequently, diarrhoea (often bloody), anorexia, paraparesis and parapalysis were observed. The low parasitaemia with gamonts in less than 5% of neutrophils is the most commonly encountered extent of infection. It is generally related to asymptomatic or mild illness. The severe clinical signs are characteristic for high parasitaemia reaching 100% and often, is associated with marked leukocytosis (up to 150,000 / $\mu\text{l}$ ) (13).

The underdeveloped immune system in young animals, the immunosuppression caused by infectious and non-infectious agents or immunodeficiency states have an impact on the pathogenesis of a new or persisting *Hepatozoon canis* infection (5, 49, 51, 52). Treatments with immunosuppressive agents such as prednisolone are followed by parasitaemia in dogs, experimentally infected with *Hepatozoon canis*. In the literature, severe co-infections of *Hepatozoon canis* with other pathogens are reported: parvovirus (5), *Ehrlichia canis* (10), *Toxoplasma gondii* (53), *Leishmania infantum* (54).

## PATHOLOGICAL FINDINGS

The principal gross pathological finding in dogs, infected with *Hepatozoon canis* is cachexia. Muscle atrophy is most frequent and most visible in the temporal region. Also, anaemia, mildly icteric mucous coats and slightly enlarged spleen and liver are observed. Congestive changes in the lungs and the gastric mucous coat, lymphadenopathy and pale kidneys are also communicated (11, 12, 13, 19).

Histologically, schizonts are observed in the skeletal and cardiac muscles, lymph nodes, the spleen, liver, kidneys etc. (11, 12, 13, 19). Two types of schizonts are detected: microschizonts containing micromerozoites that are larger and macroschizonts filled with macromerozoites. Microschizonts in the various organs are observed more frequently and at a higher extent. At the time of schizont formation, there is usually no cellular

reaction. However, at the time of merozoites release, an intensive cell response is detected, consisting of equal amounts of macrophages and neutrophils and a varying number of eosinophils.

In the skeletal muscle, cysts with diameter of 250 µm are detected, determined by some authors as developing microsclerites and clusters of smaller structures with a diameter of about 10 µm, determined as microsclerites (12). In muscles, they form clusters of neutrophils that are probably the cause for the pain, fever and periosteal proliferations. These proliferations are localized at the sites of attachment of muscles to the vertebra, pelvis, radius, ulna, humerus, femur, fibula and tibia. Bone lesions are not present in all affected animals, but are more common in young dogs (less than 1 year of age) (12).

## EPIDEMIOLOGY

The *Hepatozoon canis* infection among dogs is widespread. Its distribution corresponds to that of the vector tick *Rhipicephalus sanguineus*: Africa, South Europe, South America and Asia, including the Middle East, the Pacific and Indian ocean islands (11, 12, 13, 19).

Hepatozoonosis is manifested as an enzootic disease with a variable prevalence.

The initial studies on the seroprevalence of *Hepatozoon canis* in dogs showed a variety of percentages: 36% in Portugal, 17.6% in Nigeria, 2.5% in India, 2.3% in Israel, 2.1% in Thailand (11). Because of its frequently asymptomatic course, the determination of the extensity and intensity of *Hepatozoon canis* infection require systematic, direct and indirect studies of this parasite in dogs.

The prevalence of *Hepatozoon canis* infection in the different regions is also considerably varying. Circulating *Hepatozoon canis* gamonts in blood have been detected in 39% of dogs in the rural areas of Rio de Janeiro state in Brazil (9), in 22% of dogs in Zaria, Nigeria (6) and in 1.2% of dogs in Malaysia (7). Similar to other tick-borne diseases as ehrlichiosis, babesiosis, Lyme disease etc., the contact with *Hepatozoon canis* in endemic regions is much more frequent than the clinical incidence of illness. Most dogs infected with *Hepatozoon canis* probably develop subclinical infections. Among the dogs surveyed for the presence of *Hepatozoon canis* antibodies in Israel, 33% were seropositive, only 3% had gamonts in the blood and 1% exhibited severe clinical

signs of infection (55).

The homogeneity of the different *Hepatozoon canis* isolates was not studied in detail by PCR. So far, a phylogenetic analysis of parts of 18S rRNA of canine *Hepatozoon* isolates from Japan and Israel has been performed, showing up to 99% identity with *Hepatozoon canis* and differing from *Hepatozoon americanum* (56).

The distribution of hepatozoonosis is closely related to that of the definitive tick host -*Rhipicephalus sanguineus* (2, 14, 20). It is a three-host tick and is considered to be one of the most prevalent species worldwide (16), adaptable to different environmental conditions and found in warm and temperate regions. *Hepatozoon canis* has a transphasic (trans-stage) transmission from nymph to imago. Transovarial transmission has not been observed. Experimentally, ticks could be infected by percutaneous injection of gamont-containing blood (20). In Japan, potential and additional tick vectors – *Haemaphysalis longicornum* and *Haemaphysalis flava* were found (57).

The principal route of infection in dogs is ingestion of a tick containing mature oocysts, but alternative routes are also reported. As with other coccidia, *Toxoplasma gondii* and *Neospora caninum*, a vertical transmission from the mother to the offspring was observed in *Hepatozoon canis* (58). Experimental infections were not successful with parenteral inoculation of tissues or blood from infected dogs, but could be achieved with inoculation of tick tissue emulsion (16). Some *Hepatozoon* species infecting snakes, lizards and frogs, could be transmitted by ingestion of the cysts in tissues of intermediate hosts (3).

No connection was established between the infection of dogs with *Hepatozoon canis* and their gender and age. Animals from neonatal to adult age are infected (3, 10, 55). Dogs from rural regions become more commonly infected compared to those living in cities, probably because of the closer contact with tick vectors (10).

Most cases of *Hepatozoon canis* infections are detected in hot seasons, when the activity of the vector is higher. However, illnesses were observed during the cold months too, most likely due to persisting infections (10).

The host range of *Hepatozoon canis* in carnivores is not yet elucidated, except for the domestic dog. Many morphologically similar *Hepatozoon* species have been established in other carnivores: fox – *Vulpes vulpes* (59, 60,

61), black-backed jackal – *Canis mesomelas* (62), golden jackal – *Canis aureus* (63), African wild dog – *Lycaon pictus* (64), hyena – *Crocuta crocuta*, cheetah – *Acinonyx jubatus*, leopard – *Panthera iridus*, lion – *Panthera leo* (17). The potential of *Hepatozoon canis* to infect also other species is considerable and these are possibly species genetically close to domestic dogs. This could be confirmed by experimental transmissions or genetic typization and comparison of obtained isolates.

## DIAGNOSIS

### Clinical

Anaemia is the commonest, primary haematological sign observed in most cases (13). Usually, it is normocytic, normochromic, regenerative in particular.

The leukocyte counts are often within the normal range, when the parasitaemia is low and increase in highly parasitaemic animals (up to 150,000/μl). One third of dogs, infected with *Hepatozoon canis* exhibited thrombocytopaenia, but in some instances, it is connected to co-infections such as ehrlichiosis. The changes in some serum biochemical parameters are clearly manifested and include hyperglobulinaemia and hypoalbuminaemia, increased creatine kinase and alkaline phosphatase activities (10, 50).

### Parasitological

The microscopic detection of *Hepatozoon canis* gamonts in blood smears stained according to Romanovski-Giemsa, Pappenheim or with Hemacolor is the commonest diagnostic approach to this infection. The protozoa concentration is directly related to the severity of the illness. Gamonts are with an oval shape, dimensions of 8-12/3-6 μm and are detected in the cytoplasm of neutrophils and rarely in that of monocytes (13).

Schizonts of *Hepatozoon canis* could be observed in histological or touch impression preparations from lymph nodes, spleen, and bone marrow. Schizonts are round or oval, with a diameter of about 30 μm and contain 2 or 4 macromerozoites or over 20 micromerozoites. Histologically, microschorizonts with the so-called “wheel-spoke” shape could be observed (13).

The *Hepatozoon canis* infection could be detected also in the tick vector. Large oocysts could be observed by microscopy in wet preparations or Romanovski-Giemsa

stained preparations of haemocoel, salivary glands or other internal organs. Within the oocysts, numerous oval sporocysts are observed (13).

### Serological

For serodiagnostic purposes, indirect immunofluorescent antibody test (IFAT) and ELISA are applied. Previously prepared antigen of *Hepatozoon canis* gamonts is used. These assays are performed mainly in epidemiological studies (65, 66).

## THERAPY AND PREVENTION

The primary drug used nowadays in the therapy of canine *Hepatozoon canis* infection is *Imidocarb dipropionate*. It is applied at 5-6 mg/kg, subcutaneously or intramuscularly at 14-day intervals until the disappearance of gamonts in blood. Usually, one or two injections are sufficient, but in severe infections, an 8-week or longer treatment could be necessitated (10).

In order to treat the possible co-infections transmitted by the tick vector, often *Imidocarb dipropionate* is combined with *Doxycycline* at daily oral doses of 10 mg/kg for 21 days. The percentage of healed dogs with low *Hepatozoon canis* parasitaemia is high and mostly depends on other accompanying diseases. The prognosis in animals with high parasitaemia rates is guarded. In experimental studies, 48% of dogs with high parasitaemia are reported to survive but after almost 2-month specific therapy (10).

The prevention of *Hepatozoon canis* infection is based upon the effective control of ticks on dogs and in the environment. This is done by application of various acaricides. They only kill the ticks, and the regular cleaning and combing of animals would prevent them from eating a tick. For prevention of congenital transmission, infected dogs should be treated prior to being mated. Because of the probability of transmission of *Hepatozoon canis* infection by infected tissues, dogs from endemic regions should not be fed raw meat (13).

## PUBLIC HEALTH

The range of natural *Hepatozoon canis* hosts is not completely studied. There is only one report for *Hepatozoon spp.* infection of a man in the Philippines (19). The patient was anaemic and icteric. Gamonts were found in his blood. Parasites were not present in the liver and the bone marrow.

All available data up to now suggest that *Hepatozoon canis* is a pathogen with no importance for the immunocompetent man, but it is essential to take precautions in working with infected dogs or when removing ticks from their bodies.

## STUDIES IN BULGARIA

In Bulgaria, there are only two reports on *Hepatozoon canis* infections in dogs. Ivanov and Kanakov reported for the first time in Bulgaria a clinical case of canine *Hepatozoon canis* infection (67). The physical examination showed a marked cachexia, weakness of the hindlimbs, winding gait, anorexia, pale icteric conjunctivas, strong infestation with ticks. Haematological changes consisted in erythropenia, oligocytaemia, oligochromaemia, hyperleukocytosis with neutrophilia and left shift to metamyelocytes and lymphopenia. The microscopic observation of blood smears showed gamonts in neutrophils (42%), in monocytes and in blood plasma.

In 2008, Tsachev et al. observed a co-infection with *Ehrlichia canis* and *Hepatozoon canis* in a dog (68). The animal was in a poor clinical state, could not stand on its feet, was anorectic, with pale mucous coats, myalgia and ticks over the body. The blood analysis revealed anaemia, thrombocytopenia, leukocytosis, hypoalbuminaemia, hyperglobulinaemia, uraemia, creatinaemia and bilirubinaemia. Serum activities of ASAT, ALAT, ALP and CK were elevated. The microscopy showed gamonts in 13,75% of neutrophils and cytoplasmic bodies in single monocytes, identified as *Ehrlichia canis*. The serological ELISA test confirmed the monocytic ehrlichiosis. The instituted two days therapy: Ringer, 5% glucose, Imizol, Injectavit, Ketozal, nandrolone, doxycycline and blood transfusion was made but no improvement was observed, on the contrary, a profuse vomiting with blood, melena and a lethal issue occurred.

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