Bulgarian Journal of Veterinary Medicine (2003), 6, No 1, 43 – 46

CASE REPORT

FIRST CASE OF CANINE HEPATOZOONOSIS IN BULGARIA

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Hepatozoon canis James, 1905 (*Apicomplexa* phylum, *Sporozoa* class, *Haemogregarinidae* family) is a parasite on dogs, wild canids and rarely on felids.

Hepatozoonosis is encountered in South Europe, Africa, Asia, including the Middle East, the Pacific and Indian ocean islands, inhabited by the carrier tick *Rhipicephalus sanguineus* (Beaufils and Martin-Granel, 1988 a, b). Cases of canine hepatozoonosis in the USA are encountered only on Texas coast (Mercer *et al.*, 1988) that also corresponds with the prevalence of the vector (ticks).

The infection of dog is realized via ingestion of ticks carrying *Hepatozoon canis* oocysts in their body. Oocysts are about 100 μm in diameter and contain 30–50 sporocysts each containing about 12–24 spoorozoites. Sporozoites released in the gastrointestinal tract of the dog penetrate in blood and lymph and enter the mononuclear phagocytes and the endothelial cells of the spleen, lungs, liver and muscles, where the schizogony takes place. Macro- and microschizonts are formed, containing macro- and micromerozoites. Freed macromerozoites become schizonts again, while micromerozoites enter the leukocytes and differentiate to form gametes. They are encountered primarily in neutrophils and monocytes. Their shape is oval, their dimensions are 8–12/3–6 μm and the nucleus is well visible. Some of schizonts formed in muscles, spleen, lungs and liver remain and are called endogenic cysts similarly to those seen in toxoplasmosis.

The ticks become infected (in the larval or nymph stage) when sucking blood from infected canids. They ingest gametes with host's blood. The gametogony occurs in gastrointestinal tract of the tick where the sporogony takes place. Oocysts with sporocysts and sporozoites are formed.

According to Barton *et al.* (1985) and Beaufils and Martin-Granel (1988 a, b), the infection of the dog with *Hepatozoon* could be manifested in three forms: subclinical, acute – that develops a week prior to death and chronic with stages of clinical expression and stages of remission. The symptoms are various but nonspecific.

Most commonly, during the clinical investigation of the different forms of the disease, Graig *et al.* (1978) and Elias and Homans (1988) reported mainly anaemia, exhaustion and intermittent fever. In many cases, cachexia, depression, muscular hyperaesthesia, purulent conjunctivitis and rhinitis are also observed. Less frequently, the authors observed diarrhea (often bloody), anorexia, paraparesis and paraparalysis. The pain in the lumbar region is similar to that resulting from traumatic or degenerative vertebral changes. The development of the disease is often accom-

panied with periods of remission followed by new episodes of fever and pain .

Leukocytosis (from 20 to 200 G/L) with neutrophilia and presence of gametes and regenerative anaemia are typical for the infection (Gaunt *et al.*, 1983). The percentage of invaded cells is from 0.1% to 80% (Gervey, 1993).

The diagnosis of hepatozoonosis is based on several findings: leukocytosis (from 20 to 200 G/L), regenerative anaemia, presence of gametes in neutrophils, monocytes and blood plasma, detection of macro- and microschizonts (endogenic cysts) in muscles, spleen and liver (Craig, 1990, Gervey, 1993).

On August 18, 2001, a 7-year-old male Collie was referred to the Clinics of the Faculty of Veterinary Medicine, Trakia University – Stara Zagora. The history showed that the vaccinations were regularly scheduled. The dog was bred outdoor in a rural region. The onset of the disease was three months ago. Initially the dog refused to eat, was febrile and depressed. Afterwards, its condition improved but later the forementioned signs reccurred. The animal was treated in a private clinic for a week with antibiotics and tonic drugs, but despite the treatment its status did not improve.

The clinical examination of the dog revealed a marked cachexia, impaired locomotion, weakness of the hindlegs, winding gait, arexia with limited water consumption. The conjunctives were pale, with an icteric tint and distinct enophtalm. The examination revealed subnormal body temperature (36.5 ^{o}C), weak, soft and rhythmic pulse (88 min^{-1}). The peripheric blood vessels were collapsed. A weak and imperceptible heartbeat was present. The respiration was slow and superficial (10 min^{-1}). The faeces were hypercholic. The animal was strongly infested with lice and ticks.

The haematological analysis (automated haemoanalyzer Serono-System 150+, USA) and the differential white blood cell counts (on blood smears stained according to Romanovski-Giemsa) are presented in Table 1.

Table 1. Haematological data in a dog, referred to the clinics of the Faculty of Veterinary Medicine – Stara Zagora on August 18, 2001

Determination	Units	Values
Erythrocytes	T/L	2.60
Leukocytes	G/L	38.5
Haematocrit	%	19.5
Haemoglobin	g/L	69
Mean corpuscular volume		74.9
Differential white blood cell counts:		
Eosinophils	%	2
Metamyelocytes	%	5
Band neutrophils	%	20
Segmented neutrophils	%	59
Lymphocytes	%	11
Monocytes	%	3

The results of the haematological analysis could be interpreted as eryhtropenia, oligocitaemia, oligochromemia, hyperleukocytosis with neutrophilia, a regenerative left shift to metamyelocytes and lymphopenia.

The microscopic examination of blood smears revealed multiple oval cells inside the neutrophils, monocytes and in the blood plasma (Fig. 1). Their dimensions were 9–11/4–6 μm and had a with a well visible nucleus. These features including the dimensions and morphological structure, matched *Hepatozoon canis* gametes, described in the literature (Craig, 1990; Gervey, 1993). The percentage of infected leukocytes was 42%.

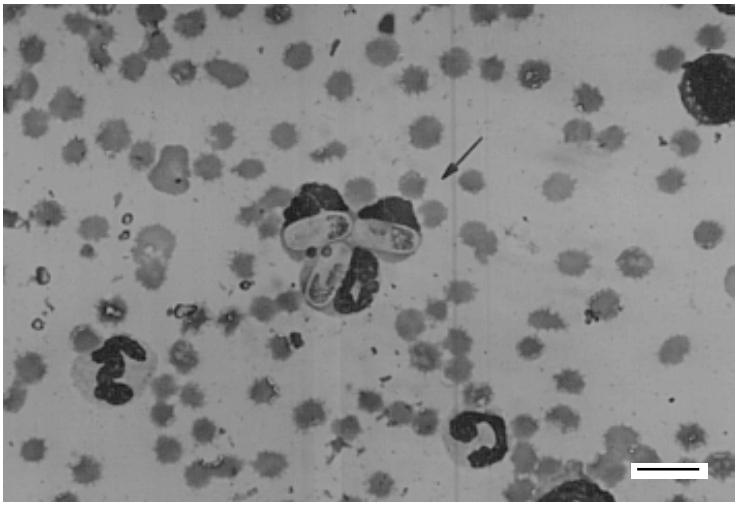

Fig. 1. Three leukocytes containing *Hepatozoon canis* gametes. Romanovski-Giemsa staining. Bar = 10 μm.

The clinical signs (arexia, cachexia, icterus, anaemia, massive infection with ticks and alternating episodes of expression and remission) and the detection of gametes in blood smears were indicative for diagnostizing hepatozoonosis.

Because of the severe anaemia, a blood transfusion was performed and hypertonic glucose, vitamins and hepatoprotective drugs were intravenously infused.

The data from the clinical examination of the dog: cachexia, weak and soft pulse, superficial respiration and subnormal body temperature suggested that the animal was in the last stage of the disease, when the prognosis was poor.

As a result of our studies, we conclude that hepatozoonosis on canids occurs in Bulgaria. The presence of ticks (*Rhipicephalus sangineus*) and susceptible to the *Hepatozoon* hosts are factors determining the existence of this infection in Bulgaria, although it was not diagnozed until now.

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Paper received 19.12.2001; accepted for publication 14.11.2002

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