Clinical *Ehrlichia canis* and *Hepatozoon canis* co-infection in a dog in Bulgaria

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**SUMMARY**

A clinical case of *Ehrlichia canis* and *Hepatozoon canis* co-infection in 7-year-old male Alaskan malamute is described. Clinical, haematological, blood biochemical, serological, parasitological, gross and histopathological examinations supporting the diagnosis have been performed.

**Keywords:** *Ehrlichia canis*, *Hepatozoon canis*, clinical sign, haematological and biochemical findings, gross and microscopic lesions.

**Case history**

*Ehrlichia canis* and *Hepatozoon canis* are the etiological agents of monocytic ehrlichiosis and hepatozoonosis in dogs. *E. canis* (a rickettsia) and *H. canis* (a protozoan) are vector borne diseases transmitted by the brown dog tick *Rhipicephalus sanguineus*. *E. canis* infection is widely prevalent at a worldwide scale and particularly in the Mediterranean basin. In Greece, it is the commonest tick-borne disease [32], whereas in Turkey, BATMAZ et al. [9] established 65% seropositive dogs. In Bulgaria, *E. canis* was clinically and serologically detected for the first time by TSACHEV [42] in a kennel nearby Plovdiv and later, retrospectively in many regions in South (30%) and North (37.5%) Bulgaria – Stara Zagora, Yambol, Plovdiv, Burgas, Blagoevgrad, Siliстра, Varna, Veliko Tarnovo, Pleven, Montana and Rousse [43, 44]. Hepatozoonosis is also prevalent at a global scale [7, 31, 35]. The seroprevalence of this infection in Turkey is 36.8% [30], in Israel - 33.1% [2], in Brazil - 5.9% [15], and in Japan –4.2% [28]. The *H. canis* infection is encountered in two forms: latent [5, 35] or with severe clinical symptoms [4, 36, 41]. In Bulgaria, *H. canis* has been detected for the first time in a patient by IVANOV and KANAKOV [29].

The *E. canis* and *H. canis* co-infection is frequently seen in the veterinary practice, but was poorly investigated [4, 22, 38]. In his studies, MYLONAKIS et al. [38] reported that in 65.2% of the cases of monocytic ehrlichiosis, *H. canis* antibodies were also present. BANET and al. [3] have observed 3 dogs infected with *E. canis* out of 7 dogs with hepatozoonosis.

In June 2007, a male 7-years old Alaskan malamute weighing 35 kg was referred to the University Hospital of the Faculty of Veterinary Medicine at the Trakia University – Stara Zagora. It was found and adopted by its present owners 10 days ago. By this time, the dog was in a good general condition with the exception of the extensive tick infestation. The owners had treated the dog externally with diazinon (Neocidol ®) and after that, the general condition of the animal rapidly worsened - it lied down, lost its appetite, experienced difficulties at standing up and exhibited an unsteady wobbling gait. The physical examination revealed: fever 39.5°C, heart rate of 140 beats/min, rhythmic and full, respiratory rate 20 beats/min, deep and rhythmic, bilateral amaurosis, a severe emaciation (Fig. 1), the superficial lymph nodes and blood vessels were normal, pale mucous coats. The animal was reluctant to stand up, exhibited muscle pain and difficult walking with ataxia and forelimb crossing steps and ataxia. Single ticks were observed on the skin. Blood samples were obtained from v. cephalica antebrachii for haematological and biochemical analysis on the first and second days. The haematological parameters - white blood cells (WBC), red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet counts (PLT) were determined on an automated blood analyzer (BC–2800 Vet Auto Hematology Analyzer, Mindray, China). The differential blood cell counts were determined on Giemsa-stained blood smears and...
the absolute counts of neutrophils, lymphocytes, eosinophils and monocytes were calculated. The haematological analysis showed a moderate anemia, aggravating on the second day (Table 1): RBC (x 10^{12}/l) - 3.49 ➞ 3.07; HGB- 75 ➞ 66 g/l; HCT- 19 ➞ 17 % respectively; a marked thrombocytopenia throughout both blood analyses 58 and 46 (x 10^9/l); the leukocytosis was on the account of lymphocytes on the first day 8.75x10^9/l, whereas on the second day – on the account of neutrophils 13.17x10^9/l). Blood biochemical analyses were performed with diagnostical tests (Roche Diagnostics GmbH, Germany) on a biochemistry analyzer (BA–88 Biochemistry Analyzer, Mindrey, China) and included determinations of the total protein, albumin, albumin/globulin (A/G) ratio, urea, creatinine, bilirubin, alkaline phosphatase...

TSACHEV (I.) AND COLLABORATORS

70

(AP), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), creatine kinase (CK) (Table 1). The data evidenced a normoproteinaemia 54 g/l with hypoalbuminaemia 17 g/l and decreased A/G ratio (0.32). Uraemia, creatinaemia and bilirubinaemia were also present. ASAT and ALAT activities were elevated, but AP and CK values were particularly high - AP = 1187 U/l and CK = 470 U/l. Blood smears for parasitological examination were made, stained with Haemacolor (Merck) and observed on a light microscope at 1000x. The microscopy clearly revealed oval cytoplasmic bodies in neutrophils. They were identified as gametocytes of *Hepatozoon canis* (mean length 11.1 µm, mean width 5.36 µm). (Fig. 2). The parasitaemia rate was determined to be 13.75 % (1000 neutrophils). In single monocytes, cytoplasmic inclusion bodies, identified as *Ehrlichia canis*, were observed. A serological study for vector-borne infections - *Ehrlichia canis*, *Borrelia burgdorferi*, *Dirofilaria immitis*, was done by means of ELISA test (IDEXX Snap® 3DXTM, Maine, USA). The result was positive only for monocytic ehrlichiosis.

On the basis of clinical signs and laboratory analyses, a diagnosis of monocytic ehrlichiosis and hepatozoonosis co-infection was put.

The instituted therapy was with intravenous infusions with Ringer solution 40 ml/kg, 5% glucose at 20ml/kg, s.c. Imizol 1.5 ml, Injectavit 4 ml, Ketozal 5 ml, nandrolone (Deca-

Durabolin 0.5-1.0 mg/kg); and orally doxycycline at 10 mg/kg once daily. On the next day, blood transfusion was made but no improvement was observed, on the contrary, a profuse vomiting with blood, melena and a lethal issue occurred.

A gross pathological examination of the body was performed and specimens from the liver, spleen, heart, lungs, kidneys, pancreas, thigh muscle and mesenterial lymph nodes were collected for histological study. The materials were fixed in 10% neutral formalin and embedded in paraffin. Cross sections of 5 µm were stained with haematoxylin and eosin (H/E). Gross lesions. The body condition was good. The visible mucous coats (conjunctival and oral) were icterically tainted. After opening of the abdominal cavity, multiple ecchymoses or striated omental haemorrhages were seen (Fig. 3). Such haemorrhages were also observed on serous coats of the pectoral and abdominal cavities and the organs within. An especially marked haemorrhagic diathesis was observed in mesenterial lymph nodes. Their cut surface had a marble-like appearance due to alternation of intact and blood-impregnated parenchymatous areas. Ecchymoses were also seen in the pancreas (Fig. 4). The spleen was moderately enlarged and hyperaemic. The liver was enlarged, hyperaemic, with rounded margins, stretched capsule and icteric (Fig. 5). In the gastrointestinal mucosa, multiple

FIGURE 7: Sinusoidal hyperaemia, activation of Kupffer cells, intercellular oedema and atrophied tubercles, deposition of bile pigments and haemosiderin the liver, H/E, bar = 4 µm.

FIGURE 8: A necrotic focus (N), haemorrhages and appearance of inflammation cell elements associated with *Hepatozoon canis* schizonts (arrows) in the liver, H/E, bar = 4 µm.

FIGURE 9: Haemorrhages, severe degenerative lesions in the epithelium of renal tubules and round cell infiltration (arrow) in the interstitium of the kidney, H/E, bar = 4 µm.

FIGURE 10: Hyperaemia and massive haemorrhages in the myocardium - a cross section, H/E, bar = 4 µm.
petechial bleedings or ecchymoses were observed and in some areas, they delivered a brownish colour of the content. The lungs were edematous, mottled with ecchymoses. The tricuspidal valves of the right cardiac half were embraced by ulcerative lesions. The kidneys were icteric and with a dense consistency. On the mucous coat of the urinary bladder, single petechiae were observed.

Microscopic lesions. In the lymph node parenchyma, there were haemorrhages, more apparent in the cortical zone, and atrophy of the white pulp manifested by reduced periarteriolar amounts of lymphoblasts and lymphocytes. In the periphery of lymph node follicle, a marked histiocytosis was present (Fig. 6). In the liver, sinusoidal hyperaemia, activated Kupffer cells, intercellular oedema and atrophy of liver tubercles, as well as deposits of bile pigments and haemosiderin were noticed (Fig. 7). Liver epithelial cells were embraced by a moderate fatty and parenchymatous dystrophy. The parenchyma of the organ was mottled by multifocal necrotic foci and amongst, haemorrhages and inflammation cell elements associated with *Hepatozoon canis* schizonts were shown (Fig. 8). The lungs and spleen revealed haemorrhages with various intensity, erythrophagocytosis, haemosiderosis and inflammation cell elements, primarily plasmocytes, and in adjacency, *Hepatozoon canis* schizonts. In kidneys, haemorrhages, severe degenerative lesions in the epithelium of renal tubules and round cell infiltration in the interstitium were observed (Fig. 9). Hyperaemia and massive bleedings were also present in thigh muscles and the myocardium (Fig. 10). The walls of the small arterial vessels in the parenchymal organs were also affected by inflammation (endarteritis).

**Discussion**

Tick-borne infections in dogs are incessantly becoming a global problem [19], necessitating a creative approach in prevention, diagnostics and control. These infections persist permanently in traditional endemic regions and frequently,
are transmitted as exotic infections in infection-free regions. Thus for instance, monocyctic ehrlichiosis was detected in England in dogs that have been abroad [37], and hepatozoonosis was established in Germany [18,23].

Tick-borne infections: ehrlichiosis, hepatozoonosis, anaplasmosis, rickettsiosis, Lyme disease, babesiasis etc., are frequently seen not as independent noxes, but as co-infections, because their epidemiology is characterized with the same vectors [10,12]. The fact, that wild carnivores are involved in the epidemiology of these infections, is little known. Thus, in a study in Israel on 84 foxes, 36% of them were positive for *E. canis* antibodies and 24% - for *H. canis* antibodies [19]. Another feature of these diseases is related to immunosuppression, provoked by aforementioned agents; in dogs, hepatozoonosis could even be provoked as opportunistic infection [22]. All these features, as highlighted by BREITSCHWERDT [11], are a serious diagnostical challenge for both practicing veterinarians and researchers.

The clinical manifestations observed by us as well as the alteration sin haematological and blood biochemical parameters could be encountered solely in either monocyctic ehrlichiosis or hepatozoonosis, as reported by BANETH and colleagues [22]. We assume, on the basis of the severe clinical course, the aggravating laboratory alterations and the lethal issue, that there was a synergism of co-existing agents, as hypothesized by other authors too [38]. *E. canis* and *H. canis* are pathogens of the haemolymphatic system. *E. canis* is thought to provoke a more serious clinical pathology and the disease could be hardly differentiated provided that another pathogen is also present [26].

Most probably, monocyctic ehrlichiosis was leading in our patient, because it is characterized by blindness and more commonly, by lethal issue, as present in this case. The observed bloody diarrhoea is also a characteristic clinical sign for ehrlichiosis and is less frequently seen with *H. canis* infections [14]. The detection not only of *E. canis* antibodies but also of morules evidenced that this was a case of acute monocyctic ehrlichiosis [13] as in chronic forms, morules were generally absent. The fever and the difficult breathing could be probably related to muscle inflammation, occurring throughout the rupture of schizonts and the release of merozoites [27]. Muscle pain is typical for hepatozoonosis.

On its own, hepatozoonosis with low-degree parasitic load as observed in our case includes a slight anaemia whereas the high-degree parasitaemia is accompanied by a severe course and continuous therapy [5,6]. In their studies in 16 dogs with hepatozoonosis, GAVAZZA et al. [22] observed leukocytosis only in 43.5% and reduced HCT in 31.25%, HGB in 16.25%, MCV in 16.25%, A/G ration in 37.5%. The anaemia, leukocytosis, thrombocytopenia, hypoalbuminaemia, increased alkaline phosphatase activity, observed by us, were reported by a number of authors [4,8,14,16,24,36,39]. The increased mean corpuscular hemoglobin concentration (394.7 → 388.2 g/l) is a marked compensatory mechanism of progressing anaemia. The anaemia and neutrophilia is attributed by many authors [21] to secondary necrosis and inflammation of the spleen, lymph nodes, lungs and liver, established throughout the histopathological study. In both diseases, thrombocytopenia is observed. The mechanism of thrombocytopenia is questionable. The observed hypoalbuminaemia with normal total protein concentrations is attributed to increased level of gamma globulins, observed by GAVAZZA et al. [22] that was on the account of β2- (100 %) and β1-hyperglobulinaemia (68.75 %), and γ-hyperglobulinaemia (37.5 %). The elevated alkaline phosphatase activity could result from osteoelastic activity or liver necroses [33,40]. The higher CK activity could originate from affected musculature, pointed out also by others [1,20].

The observed gross and histopathological changes in this case are usual and correspond to those, reported by numerous investigators [1,34].

**Conclusions**

The proved *E. canis* and *H. canis* co-infection is confirming its occurrence in Bulgaria already. The combination of these two infections could be lethal.

The differential diagnosis, especially in cases with tick infestation, should necessarily include monocyctic ehrlichiosis and hepatozoonosis. Further studies are need upon the combined *E. canis* and *H. canis* infection.

**References**

EHRLICHIA CANIS AND HEPAETOZOOON CANIS CO-INFECTION IN A DOG