The first report of *Hepatozoon canis* infection of a dog in Iran

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**Abstract** An 11-year-old male dog was presented with a 1-week history of inappetence, weight loss and hind limb paralysis. Physical examination revealed weakness, depression, incoordination of the posterior limbs, peripheral lymphadenopathy and pale mucous membranes. Laboratory analysis of blood samples revealed anaemia, thrombocytopenia and low serum albumin concentration. The diagnosis was confirmed microscopically, by demonstrating the presence of *Hepatozoon canis* gametocytes within neutrophils in Giemsa-stained peripheral blood smears and bone marrow smear. Also, schizonts of *H. canis* were seen in tissue sections of muscles, lymph nodes, spleen and liver. To the best of authors’ knowledge, this is the first description of *H. canis* infection in a dog in Iran.

**Keywords** *Hepatozoon canis*, Gametocyte, Schizont · Dog, Iran

**Introduction**

*Hepatozoon canis* is a tick-borne protozoan parasite of leukocytes and parenchymal tissues, which has been reported in dogs and other carnivores from many regions in the world (Craig 1990; Baneth 2001). The transmission of *H. canis* in dogs and other carnivores occurs by ingestion of a hematophagous arthropod, such as a tick, which is the definitive host and contains sporulated oocysts (Baneth et al. 1998). Schizogony occurs in various organs of intermediate vertebrate hosts, and merozoites invade leukocytes (in the case of *Hepatozoon* species that infect mammals or birds) and become gametocytes (Smith 1996). There is also a possibility that infection may occur by predation (Smith 1996).

*H. canis* infection, often referred to as Old World canine heptatozoonosis, varies from being subclinical and identified incidentally in apparently healthy dogs (Murata et al. 1993; Baneth 2001) to being a severe and life-threatening clinical disease (Baneth and Weigler 1997; Macintire et al. 1997; Shaw et al. 2001). The most common presentation of the infection in dogs is asymptomatic to mild disease, and it is usually associated with a low level of *H. canis* parasitaemia in which 1% to 5% of the animal’s neutrophils are infected (Baneth and Weigler 1997; Baneth et al. 2003). Some dogs exhibit high parasitaemia, often approaching 100% of the peripheral blood neutrophils, and severe illness characterised by fever, anorexia, weight loss, anaemia, ocular discharge, weakness of the hind limbs and signs of chronic debilitating disease (Ezekoki et al. 1983; Barton et al. 1985; Craig 1990; Baneth et al. 1995, Baneth and Weigler 1997; Macintire et al. 1997; Baneth 2001). Common abnormalities found through laboratory analysis include anaemia, neutrophilic leucocytosis, hypoglycaemia, hypoalbuminaemia and increased serum alkaline phosphatase activity (Craig 1990; Baneth et al. 1995; Macintire et al. 1997; Baneth 2001). *H. canis* infection is diagnosed mainly by finding *H. canis* gametocytes within neutrophils and monocytes in stained blood smears and/or by identifying a cyst-like structure containing *H. canis* organisms in biopsy specimens (Craig 1990; Macintire et al. 1997; Baneth 2001; Baneth et al. 2003).
Case history

In May 2008, an 11-year-old, male undetermined large breed dog weighing 51 kg was presented to Ferdowsi University of Mashhad Veterinary Teaching Hospital. The dog, from a rural area around Mashhad in northeastern Iran, had a 1-week history of anorexia, weight loss (not emaciated), depression and paralysis in hind quarters. No vaccinations had been performed. According to the owner’s information, the dog had been infested with ticks during the previous months, and this was evident at the present patient on the body. Physical examination revealed weakness, nasal and ocular discharge, respiratory alteration, incoordination of the posterior limbs, a mildly painful hindlimb, peripheral lymphadenopathy, pale mucous membranes with no fever (38.9°C). A *Rhipicephalus sanguineus* nymph and a larval tick were found on the dog. Radiograph of the limbs was normal. Haemogram revealed moderate normocytic hypochromic anemia with normal leukocyte count (Table 1). Giemsa-stained peripheral blood smears revealed ellipsoid, elongated, pale-staining cytoplasmic bodies (mean length 10.9 μm, mean width 4.2 μm) inside neutrophils (Figs. 1 and 2). These bodies were identified as *H. canis* gametocytes based on their morphological characteristics. The dog had a parasitaemia of 2% of the neutrophils with 135 parasitised neutrophils per microliter blood (Table 1). On the peripheral blood smears, there were no cells infected with other agents. Results of serum biochemical analyses indicated low albumin concentration (Table 2). Skin scrapings for scabies mites and a fecal sample for parasite ova were negative. Based on the clinical findings and laboratory results, *H. canis* was diagnosed. The dog was hospitalized, and treatment was initiated, but the owners elected euthanasia due to his failing health and deteriorating condition and allowed necropsy. At postmortem examination, there was mild enlargement of liver and spleen. Tissue samples from lymph nodes, skeletal muscles, spleen and liver were fixed in 10% buffered formalin, and sections were prepared for histological examination and stained with haematoxylin and eosin. Histologically, merogonous stages and encysted forms of the parasite were found in the spleen (Fig. 3) and the skeletal muscle (Fig. 4), respectively. There was also multifocal granulomatous hepatitis.

**Table 1** Haematological findings

| Hematologic findings                  | Patient | Reference valuesa |
|---------------------------------------|---------|-------------------|
| Hematocrit (%)                        | 22.6    | 43.3–59.3         |
| Hemoglobin (g/dl)                     | 6.2     | 14.1–20.0         |
| Red blood cell (×10⁶ μl)              | 3.57    | 6.15–8.70         |
| Mean corpuscular volume (fl)          | 63      | 63.0–77.1         |
| Mean corpuscular hemoglobin (pg)      | 17.4    | 21.1–24.8         |
| Mean corpuscular hemoglobin concentration (g/dl) | 27.4 | 29.9–35.6 |
| Platelets (×10⁵ μl)                   | 151     | 164–510           |
| White blood cells (×10³ μl)           | 6.75    | 6.02–16.02        |
| Mature neutrophils (×10⁵ μl)          | 4.99    | 3.23–10.85        |
| Lymphocytes (×10⁵ μl)                 | 0.54    | 0.53–3.44         |
| Monocytes (×10³ μl)                   | 0.81    | 0.0–0.43          |
| Eosinophils (×10³ μl)                 | 0.405   | 0.0–1.82          |
| Parasitaemia of neutrophils (%)       | 2       | –                 |
| Parasitised neutrophils (μl)          | 135     | –                 |

Fig. 1 Gametocyte of *H. canis* (arrowhead) in a neutrophil from peripheral blood smear (Giemsa’s stain ×1,000, bar=10 μm)

Fig. 2 Schizont of *H. canis* (arrowhead) in a bone marrow impression smear (Giemsa’s stain ×1,000, bar=10 μm)

Discussion

Canine hepatozoonosis caused by *H. canis* has been reported from many geographic areas, including Africa, southern Europe, Asia and the USA (Craig 1990; Murata et

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\[ ^{a} \text{From Willard and Tvedten 2004} \]
Table 2  Serum biochemistry results

| Biochemistry findings | patient    | Reference valuesa |
|-----------------------|------------|------------------|
| Total protein (g/dl)  | 5.22       | 5.3–7.6          |
| Albumin (g/dl)        | 1.9        | 3.2–4.7          |
| BUN (mg/dl)           | 8.6        | 7–32             |
| Creatinine (mg/dl)    | 1.12       | 0.5–1.4          |
| Glucose (mg/dl)       | 119        | 53–117           |
| Cholesterol (mg/dl)   | 187        | 116–317          |
| Bilirubin Total (mg/dl)| 0.67       | 0.1–0.6          |
| Alkaline phosphatase (IU/L) | 52    | 0–90             |
| Alanine aminotransferase (IU/L) | 16  | 10–94            |
| Aspartate aminotransferase (IU/L) | 22  | 10–62            |
| Creatinine kinase (IU/L) | 165       | 51–526           |
| Calcium (mg/dl)       | 7.31       | 9.0–11.9         |
| Magnesium (mg/dl)     | 1.3        | 1.36–2.9         |
| Phosphorus (mg/dl)    | 5.5        | 1.9–7.9          |

aFrom (Willard and Tvedten, 2004)

According to Gondim et al. (1998), canine hepatozoosmosis is characterised by clinical signs such as anorexia, pale mucous membranes, weight loss, pain, diarrhea, vomiting, gait abnormalities, fever, polyuria and polydipsia. These were accompanied by hematological findings including anemia, leukocytosis with neutrophilia and monocytosis. Hepatozoonosis observed in dogs from North America is characterised by gait abnormalities that range from stiffness to complete recumbence, generalised pain, deterioration of body condition and high white blood cell counts (Vincent-Johnson et al. 1997). In Israel and Brazil, it is considered an opportunistic infection, and white blood cell counts are within normal ranges (Baneth and Weigler 1997; Paludo et al. 2003). In Israel and Brazil, the prevalence of this agent, diagnosed by investigating the presence of gametocytes in blood smears, ranged from 1% to 22% (Baneth et al. 1996; Ezeokoli et al. 1983) and 0.5% to 3.0% (Gondim et al. 1998; O’Dwyer et al. 2001), respectively.

The dog described in this report had a parasitaemia of 2% of the neutrophils, clinical signs such as anemia and normal white blood cell counts, resembling the related infection in dogs from Israel and Brazil. It is possible that the reason for not finding the gametocytes was because at the moment of blood collection they were encysted. Another possibility could be that the parasitemia was so low that the gametocytes were missed during the laboratory proceedings (Vincent-Johnson et al. 1997).

Dogs with naturally occurring hepatozoosmosis infection often have concomitant infections of bacterial or viral origin that potentially weaken their immune defenses, including canine monocytic ehrlichiosis, canine distemper and canine parvovirus infection (Baneth et al. 1995; Baneth and Weigler 1997; Baneth 2001). Co-infection with Ehrlichia canis can be ruled out since no E. canis morulae were found on examination of blood smears. It is possible that canine distemper, canine parvovirus, leptospirosis or canine coronavirus infection may have played a role in the initial condition of the dog because of its lack of vaccination status. The hepatozoosmosis in Iran may be caused by H. canis or by a new species of Hepatozoon and could be considered endemic. Further work on muscle biopsies is necessary to confirm the species causing this disease in Iran.

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