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 hepatozoonosis, 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 (Ezeokoli 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 leukocytosis, 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).

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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

Table 1 Haematological findings

Hematologic findings	Patient	Reference values ^a
Hematocrit (%)	22.6	43.3–59.3
Hemoglobin (g/dl)	6.2	14.1–20.0
Red blood cell ($\times 10^6$ 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 ($\times 10^3$ µl)	151	164–510
White blood cells ($\times 10^3$ µl)	6.75	6.02–16.02
Mature neutrophils ($\times 10^3$ µl)	4.99	3.23–10.85
Lymphocytes ($\times 10^3$ µl)	0.54	0.53–3.44
Monocytes ($\times 10^3$ µl)	0.81	0.0–0.43
Eosinophils ($\times 10^3$ µl)	0.405	0.0–1.82
Parasitaemia of neutrophils(%)	2	–
Parasitised neutrophils (µl)	135	–

^a From Willard and Tvedten 2004

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

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.

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

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

Table 2 Serum biochemistry results

Biochemistry findings	patient	Reference values ^a
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

^a From (Willard and Tvedten, 2004)

al. 1991; Panciera et al. 1997; Baneth 2001; Shaw et al. 2001; Voyvoda et al. 2004).

According to Gondim et al. (1998), canine hepatozoonosis 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

Fig. 3 Merogonous stage of *H. canis* (arrowhead) in the spleen (haematoxylin and eosin $\times 640$, bar=25 μm)

Fig. 4 Encysted form of *H. canis* (arrowhead) in the skeletal muscle (haematoxylin and eosin $\times 320$, bar=25 μm)

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 hepatozoonosis 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 hepatozoonosis 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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References

- Baneth G (2001) Canine hepatozoonosis. In: Service MW (ed) Encyclopedia of Arthropod-transmitted infections of man and domesticated animals. CABI, Wallingford, pp 215–220
- Baneth G, Weigler B (1997) Retrospective case-control study of hepatozoonosis in dogs in Israel. J Vet Intern Med 11:365–370
- Baneth G, Harmelin A, Presentez BZ (1995) *Hepatozoon canis* in two dogs. J Am Vet Med Assoc 206:1891–1894
- Baneth G, Shkap V, Presentey BZ, Pipano E (1996) *Hepatozoon canis*: the prevalence of antibodies and gametocytes in dogs in Israel. Vet Res Commun 20(1):41–46
- Baneth G, Breitschwerdt EB, Hegarty BC, Pappalardo B, Ryan JA (1998) A survey of tick-borne bacteria and protozoa in naturally exposed dogs from Israel. Vet Parasitol. 74(2–4):133–142
- Baneth G, Mathew JS, Shkap V, Macintire DK, Barta JR, Ewing SA (2003) Canine hepatozoonosis: two disease syndromes caused by separate *Hepatozoon* species. Trends Parasitol 19(1):27–31
- Barton CL, Russo EA, Craig TM, Green RW (1985) Canine hepatozoonosis: a retrospective study of 15 naturally occurring cases. J Am Anim Hosp Assoc 21:125–134
- Craig TM (1990) Hepatozoonosis. In: Greene C (ed) Infectious diseases of the dog and cat. 2nd edn. W. B. Saunders, Philadelphia, pp 778–785
- Ezeokoli CD, Ogunkoya AB, Abdullahi R, Tekdek LB, Sannusi A, Ilemobade AA (1983) Clinical and epidemiological studies on canine hepatozoonosis in Zaire, Nigeria. J Small Anim Pract. 24:445–460
- Gondim LF, Kohayagawa A, Alencar NX, Biondo AW, Takahira RK, Franco SR (1998) Canine hepatozoonosis in Brazil: description of eight naturally occurring cases. Vet Parasitol. 74(2–4):319–323
- Macintire DK, Vincent-Johnson N, Dillon AR, Blagburn B, Lindsay D, Whitley EM, Banfield C (1997) Hepatozoonosis in dogs: 22 cases (1989–1994). J Am Vet Med Assoc. 210(7):916–922
- Murata T, Shiramizu K, Hara Y, Inoue M, Shimoda K, Nakama S (1991) First case of *Hepatozoon canis* infection of a dog in Japan. J Vet Med Sci 53(6):1097–1099
- Murata T, Shimoda K, Inoue M, Shiramizu K, Kanoe M, Taura Y, Nakama S (1993) Seasonal periodical appearance of *Hepatozoon canis* gamont in the peripheral blood. J Vet Med Sci 55: 877–879
- O'Dwyer LH, Massard CL, Pereira de Souza JC (2001) *Hepatozoon canis* infection associated with dog ticks of rural areas of Rio de Janeiro State, Brazil. Vet Parasitol 94(3):143–150
- Paludo GR, Dell'Porto A, de Castro e Trindade AR, McManus C, Friedman H (2003) *Hepatozoon* spp.: report of some cases in dogs in Brasília, Brazil. Vet Parasitol 18(3–4):243–248
- Panciera RJ, Gatto NT, Crystal MA, Helman RG, Ely RW (1997) Canine hepatozoonosis in Oklahoma. J Am Anim Hosp Assoc 33:221–225
- Shaw SE, Day MJ, Birtles RJ, Breitschwerdt EB (2001) Tick-borne infectious diseases of dogs. Trends Parasitol. 17:74–80
- Smith TG (1996) The genus *Hepatozoon* (Apicomplexa: Adeleina). J Parasitol 82(4):565–585
- Vincent-Johnson NA, Macintire DK, Lindsay DS, Lenz SD, Baneth G, Shkap V, Blagburn BL (1997) A new *Hepatozoon* species from dogs: description of the causative agent of canine hepatozoonosis in North America. J Parasitol 83(6):1165–1172
- Voyvoda H, Pasa S, Uner A (2004) Clinical *Hepatozoon canis* infection in a dog in Turkey. J Small Anim Pract. 45(12):613–617
- Willard MD, Tvedten H (2004) Small animal clinical diagnosis by laboratory methods. Saunders, Philadelphia, pp 417–418