Short Communication

A Preliminary Parasitological Survey of *Hepatozoon* Spp. Infection in Dogs in Mashhad, Iran

**AA Rahmani Amoli¹, *J Khoshnegah¹, GhR Razmi²**

¹. Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
². Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

*Corresponding author: Tel.: 0098 511 8803762, Email: jkhoshnegah@gmail.com

(Received 27 Mar 2012; accepted 15 Oct 2012)

ABSTRACT

**Background:** We attempted to determine the prevalence of *Hepatozoon* spp. infection in Mashhad, northeast of Iran, via blood smear parasitology.

**Methods:** The prevalence was investigated by examination of blood smear parasitology, using blood samples collected from 254 dogs (51 strays and 203 privately owned-dogs).

**Results:** Two stray dogs (2/51; 3.92%) and two privately-owned dogs (2/203; 0.98%) were infected with *Hepatozoon* spp. Therefore, as per blood smear parasitology, the prevalence of *Hepatozoon* spp. infection was 1.57% (4/254). Sixteen out of 254 dogs (6.29%) were infested with ticks; all of which were *Rhipicephalus sanguineus*. One of the dogs infected with *Hepatozoon* spp. exhibited ticks at the time of examination. Concurrent infection with *Ehrlichia canis* and *Leishmania infantum* was not detected in the four *Hepatozoon* spp. infected dogs.

**Conclusion:** This is the first epidemiological study on the prevalence of *Hepatozoon* spp. infection in dogs in Iran.

**Keywords:** *Hepatozoon*, Prevalence, Blood, Parasitology, Dog, Iran

Introduction

*Hepatozoon* is a tick borne protozoan parasite, classified in the Phylum Apicomplexa and is closely related to *Plasmodium* spp. and *Piroplasms* (1). The parasite is primarily transmitted by the brown dog tick, *Rhipicephalus sanguineus* via the ingestion of ticks, or parts of ticks containing mature *Hepatozoon* spp. oocysts (2). In dogs, the infection results in the development of schizonts...
within various tissues and gamonts in the peripheral blood (3-5). *Hepatozoon* spp. infection produces variable manifestations. The infection can result in an asymptomatic state, with low parasitemia, or can culminate into a severe, life-threatening illness, resulting in fever, lethargy, anemia, and emaciation, with high levels of parasitemia (6). Hepatozoonosis is mainly diagnosed by the observation of intracellular *Hepatozoon* gamonts within neutrophils in Giemsa-stained peripheral blood smears (7). Although canine hepatozoonosis was first reported in India in 1905 (8), it has only recently been noted in Iran (9).

The epidemiology of *Hepatozoon* spp. infection remains to be established and, thus, this preliminary epidemiological study was attempted to determine the prevalence of *Hepatozoon* spp. infection in the Mashhad, northeast of Iran, via blood smear parasitology.

**Materials and Methods**

**Dogs and blood smear parasitology**

The sampling was performed from June 2010 to July 2011. Blood samples were collected from 51 stray dogs, captured and kept in the municipality shelter located in Mashhad, Iran. In addition, 203 privately owned dogs were admitted to the Ferdowsi University of Mashhad Veterinary Teaching Hospital and were screened for the presence of *Hepatozoon* spp. All the dogs were examined for the presence of fever, depression, anorexia, weight loss, oculonasal discharge, lymphadenopathy, skin and hair coat changes and membranous pallor. Blood samples (5 ml) were drawn from the cephalic vein of each dog and were placed into plain and Ethylenediaminetetraacetic acid (EDTA) tubes. A thin blood smear was prepared for each sample, fixed with methanol and stained with Giemsa. Complete blood counts were performed manually for all dogs, and the presence of hematological disorders was recorded for each animal. The parasitemia was calculated manually by counting 500 neutrophils under oil immersion field (14).

This research proposal has received ethical approval by Ferdowsi University of Mashhad Research Office.

**Diagnosis of concurrent infections**

The sera were separated off and frozen at 20 °C for serological assays. All sera were examined for the presence of the antibodies against *Ehrlichia canis* and *Leishmania infantum* by indirect immunofluorescence antibody (IFA) test kit (Mega Screen® FLUO; MegaCor, Horbranz, Austria) in accordance with the manufacture’s recommendations. The stained slides were read under 400 magnification fluorescence microscope (Olympus, BH-2). Evaluation was carried out with green and red filter.

**Results**

**Blood smear parasitology**

Blood smear parasitology, revealed 1.57% (4/254) of the subjects were infected by *Hepatozoon* spp. (Fig. 1). These included two stray (2/51; 3.92%) and two privately-owned dogs (2/203; 0.98%). The parasitemia ranged from 1 to 3% with the highest parasitemia (3%) found in a 15-year-old male privately-owned dog.

![Gametocyte of Hepatozoon spp. in a neutrophil from peripheral blood smear (Giemsa’s stain ×1,000)](image-url)
Tick infestation
Out of 254 dogs, 16 (6.29%) were infested with ticks; all of which were *R. sanguineus*. The ticks were found at the surface of the skin on one of the four infected dogs.

Clinical and hematological findings
Anorexia was observed in two infected dogs. Anemia was detected in 67 of the 254 dogs (26.37%), two of which were *Hepatozoon* spp. infected. Table 1 summarizes the clinical and hematological results from the four *Hepatozoon* spp. infected dogs.

Concurrent infections
Twenty-two of 254 (8.67%) serum samples had antibodies to *L. infantum*. Also, a very low prevalence of anti-*E. canis* antibodies (0.8%; 2/254), was detected among studied dogs (unpublished data). In the four *Hepatozoon* spp. infected dogs, concurrent infection with these pathogens were not detected.

Table 1: Clinical and hematological findings of the four dogs infected with *Hepatozoon* sp.

| Dog No. | Breed         | Age (yr) | Sex | Clinical sign                                | Tick infestation | Parasitemia(%) | PCV% | Total WBC (/μL) | Neutrophils (/μL) | TP (g/dL) |
|---------|---------------|----------|-----|---------------------------------------------|------------------|----------------|------|----------------|------------------|-----------|
| 1       | Undetermined  | 15       | M   | Anorexia, superficial pyoderma              | +                | 3              | 52   | 7500           | 4950             | 7         |
| 2       | Terrier       | 1.5      | F   | -                                           | -                | 1              | 34   | 7000           | 4060             | 6.8       |
| 3       | Mixed breed   | 3        | M   | Broken hind limb Anorexia, pale mucous mem-  | -                | 1              | 41   | 10150          | 7105             | 6.1       |
| 4       | Mixed breed   | 3        | M   | -                                           | -                | 1              | 37   | 11000          | 9020             | 7.9       |

Reference values (24) (Willard and Tvedten 2004); PCV: 37-55, Total WBC (/μL):6000-17000, Neutrophils (/μL): 3000-11500

Discussion
In this preliminary epidemiological survey, we attempted to reveal the prevalence of *Hepatozoon* spp. infection among dogs living in Mashhad, northeast of Iran. As per this study, the prevalence of *Hepatozoon* spp. infection was 1.57% by examination of blood smear parasitology. In contrast to the present study, others have reported a rather high prevalence of *Hepatozoon* spp. infection. Garcia de Sá et al. examined 31 dogs from rural areas and identified 7 dogs (22.6%) positive by blood smear examination (10). Rubini et al. reported a prevalence of 11.3% (17/150) using Giemsa-stained blood smears (11).

In the present study, we used peripheral blood smears to detect *Hepatozoon* spp. gamonts. Buffy coat smears, however, may have significantly increased the sensitivity of the detection of *Hepatozoon* spp. gamonts compared to peripheral blood smears as shown previously for the detection *E. canis* morulae by microscopy (12). There is also evidence that direct observation is less sensitive than IFAT since the prevalence of *H. canis* infection investigated by blood smear parasitology was only 1%, as compared to IFAT yielding a 33.1% finding (13). Karagenc et al. showed that the number of *Hepatozoon* positive animals detected by IFAT were significantly higher than that determined by PCR and microscopy (14). The absence of gamonts in IFAT positive animals may be due to low or intermittent parasitemia or arrest of parasite development at the meront stage in visceral organs (6,13,15). Alternatively, anti-*Hepatozoon* spp. antibodies may have persisted for months after parasitemia can no longer be detected (16, 17).
In the present study, one of the Hepatozoon spp. infected dogs was old (15 yr) and the other three were young (1.5 to 3 yr). Hepatozoon spp. infection was found in dogs from 9 months to 7 years old (18). Hepatozoon spp. infection was the most prevalent in dogs less than 6 months and in dogs 5 to 10 years old (6). Similar to the present study, it has also been reported that Hepatozoon spp. infection can be seen in both sexes (18). The distribution of Hepatozoon is tied closely to its primary vector, R. sanguineus (2). This tick is considered as an abundant tick species in northeast of Iran (19).

In the present study, the infected dogs were asymptomatic, with low parasitemia, ranging from 1% to 3%. A low level of Hepatozoon spp. parasitemia with gamonts found in less than 5% of neutrophils is the most common form of infection and is usually associated with an asymptomatic or mild disease. A high level of parasitemia, however, would be associated with significant clinical signs (3,6). The present study indicates that the exposure to Hepatozoon spp. was very low amongst the dogs, in our area. In addition, it appears that most of the infected dogs were asymptomatic and only a relatively small number of animals (12.2%), developed the severe form of the disease. Previously, Khoshnegah et al. described a severe form of hepatozoonosis occurred in an 11-year-old male dog. Clinical signs included anorexia, weight loss, depression, nasal and ocular discharge, in coordination of the posterior limbs, a mildly painful hind limb, peripheral lymphadenopathy, pale mucous membranes but afebrile (9). In cases where gamonts are detected upon blood smear parasitology, the various clinical signs exhibited by the infected dogs are attributed to concurrent infections due to other, more potent pathogens such as Babesia canis, Ehrlichia spp., Leishmania, etc. (20-23). In the present study, concurrent infection with other pathogens (including E. canis and L. infantum) were not detected in the four Hepatozoon spp. infected dogs. There is also the possibility that an interaction between the different pathogens might exist, which in turn might lead to further deterioration in the infected dogs’ clinical condition (6).

Conclusion

The present study demonstrates a low prevalence of Hepatozoon spp. infection among dogs residing in the northeast of Iran. One limitation of the direct observation is that gametocytes are not always detectable since parasitemia may be intermittent or the number of circulating gametocytes very low. From a clinical perspective, practitioners should be aware of Hepatozoon spp. and should include its screening in their routine diagnostic panel. Misdiagnosis may lead to inappropriate treatment and relapses. As soon as the disease is diagnosed, practitioners should know that since elimination of gametocytes from the peripheral blood is slow, an 8-week treatment is always required (1). Canine hepatozoonosis in Iran may be caused by H. canis or by a new species of Hepatozoon and could be considered endemic. Further work based on PCR and serological tests is necessary to confirm the species causing this disease in Iran. Most importantly, strict control measures against ticks applied in dog kennels and shelters may prevent the spread of tick-borne diseases.

Acknowledgment

This study was supported by the research fund of Ferdowsi University of Mashhad (No. 3/14948 - 1389/05/10). The authors wish to thank Mr. H. Eshrati for excellent technical assistance. The authors declare that there is no conflict of interest.

References

1. Baneth G, Mathew JS, Shkap V, Macintire DK, Barta JR, Ewing SA. Canine hepatozoonosis: two disease syndromes caused by separate Hepatozoon spp. Trends Parasitol. 2003; 19(1):27–31.
2. Baneth G, Samish M, Alekseev E, Aroch I, Shkap V. Transmission of Hepatozoon canis to dogs by naturally-fed or percutaneously-injected Rhipicephalus sanguineus ticks. J Parasitol. 2001; 87(3):606–611.

Available at: http://ijpa.tums.ac.ir
3. Barton CL, Russo EA, Craig T.M, Green RW. Canine hepatopiroplasmosis: a retrospective study of 15 naturally occurring cases. J Am Anim Hosp Assoc. 1985; 21: 125–134.

4. Baneth G, Harmelin A, Presentey BZ, Hepatozoon canis infection in two dogs. J Am Vet Med Assoc. 1995; 206(12):1891–1894.

5. O’Dwyer LH, Massard CL, Pereira de Souza JC. Hepatozoon canis infection associated with dog ticks of rural areas of Rio de Janeiro State, Brazil. Vet Parasitol. 2001; 94(3):143-50.

6. Baneth G, Weigler B. Retrospective case-control study of hepatopiroplasmosis in dogs in Israel. J Vet Intern Med. 1997; 11(6):365–370.

7. Elias E, Homans PA. Hepatozoon canis infection in dogs: clinical and haematological findings treatment. J Small Anim Pract. 1988; 29: 55–62.

8. James SP. On a parasite found in the white corpuscles of the bloods of dogs. Sci Mem Offrs Med Sanit Deps India. 1905;14: 1-12.

9. Khoshnegah J, Mohri M, Movassaghi AR, Kazemi Mehrjerdi H. The first report of Hepatozoon canis infection of a dog in Iran. Comparative Clinical Pathology. 2009; 18: 455–458.

10. Garcia de Sá A, Figueiredo Cerqueira AM, O’Dwyer LH, Abreu FS, Ferreira RF, Muller Pereira A, Velho PB, Rubini AS, Pereira Almosny NR. Detection of Hepatozoon spp. in Naturally Infected Brazilian Dogs by Polymerase Chain Reaction. Int J Appl Res Vet Med. 2007; 5(2):49-51.

11. Rubini AS, dos Santos Paduan K, Von Ah Lopes V, O’Dwyer I H. Molecular and parasitological survey of Hepatozoon canis (Apicomplexa: Hepatozoidae) in dogs from rural area of Sao Paulo state, Brazil. J Parasitol Res. 2008; 102(5):895-9.

12. Mylonakisa ME, Koutinasa AF, Billinisb C, Leontides LS, Kontosd V, Papadopoulosb O, Ralliab T, Fytiano A. Evaluation of cytology in the diagnosis of acute canine monocytic ehrlichiosis (Ehrlichia canis): a comparison between five methods a comparison between five methods. Vet Microbiol. 2003; 91: 197–204.

13. Baneth G, Shkap V, Presentey BZ, Pipano E. Hepatozoon canis: the prevalence of antibodies and gametocytes in dogs in Israel. Vet Res Commun. 1996; 20(1):41–46.

14. Karagenc TI, Pasa S, Kirli G, Hosgor M, Bilgic HB, Oz YH. A parasitological, molecular and serological survey of Hepatozoon canis infection in dogs around the Aegean coast of Turkey. Vet Parasitol. 2006; 135: 113–119.

15. Murata T, Shimoda K, Inoue M, Shiramizu K, Kanoe M, Taura Y, Nakama S. Seasonal periodic appearance of Hepatozoon canis gamont in the peripheral blood. J Vet Med Sci. 1993; 55: 877–879.

16. Baneth G, Shkap V, Samish M, Pipano E, Savidtsky I. Antibody response to Hepatozoon canis in experimentally infected dogs. Vet Parasitol. 1998; 74(2-4):299–305.

17. Gonen L, Strauss-Ayali D, Shkap V, Vincent-Johnson N, Macintire DK, Baneth G. An enzyme-linked immunosorbent assay for antibodies to Hepatozoon canis. Vet Parasitol. 2004; 122(2):131–139.

18. Gavazza A, Bizzeti M, Papini R. Observations on dogs found naturally infected with Hepatozoon canis in Italy. Revue Méd Vét. 2003; 154: 565-571.

19. Razmi GR, Naghibi A, Aslani MR, Dastjerdi K, Hossieni H. An epidemiological study on Babesia infection in small ruminants in Mashhad suburb, Khorasan province, Iran. Small Rumin Res. 2003; 50(1):39-44.

20. Rioux JA, Golvan YJ, Honin R. Mixed Hepatozoon canis and Leishmania canis infection in a dog in the Sets area, France. Ann Parasitol Hum Comp. 1964; 39:131-135.

21. Ogunkoya AB, Adeyansu JB, Aliu YO. Experiences with the use of Imizol in treating canine blood parasites in Nigeria. J Small Anim Pract. 1981; 22: 775–777.

22. Gossett KA, Gaunt SD, Aja DS. Hepatozoonosis and ehrlichiosis in a dog. J Am Anim Hosp Assoc. 1985; 21: 265–267.

23. Kontos VJ, Koutinasa AF. Clinical observations in 15 spontaneous cases of canine babesiosis. Canine Pract. 1997; 22: 30–34.

24. Willard MD, Tvedten H (2004). Small animal clinical diagnosis by laboratory methods. Saunders, Philadelphia, pp 417–418.