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HEPATOZOON CANIS: THE PREVALENCE OF ANTIBODIES AND GAMETOCYTES IN DOGS IN ISRAEL

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ABSTRACT

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A survey for the prevalence of antibodies to *Hepatozoon canis* and for intraneutrophilic *H. canis* gametocytes in the peripheral blood neutrophils of dogs in Israel showed that 33.1% were seropositive, while only 1% of the dogs sampled had detectable parasites in their blood smears. Exposure to *H. canis* is widespread but it appears that most infected dogs undergo a subclinical infection and only a small proportion develop clinical disease.

Keywords: antibody, dog, gametocyte, *Hepatozoon canis*, immunofluorescence, Israel, prevalence

Abbreviations: IFAT, indirect fluorescent antibody test

INTRODUCTION

Hepatozoon canis is a tick-borne protozoan of the Phylum Apicomplexa which infects dogs. The infection is spread worldwide (Dissanaike, 1961; Klopfer *et al.*, 1973; McCully *et al.*, 1975; Craig *et al.*, 1978; Rajamanickam *et al.*, 1985; Kontos and Koutinas, 1990) by the tick vector *Rhipicephalus sanguineus*.

Natural infection with *H. canis* is acquired through ingestion of an infected tick containing mature oocysts (Craig, 1990). The oocysts rupture and release sporocysts, which then release sporozoites in the dog's intestinal lumen. The sporozoites penetrate the wall of the gut, invade phagocytic mononuclear cells, and are carried via lymph or blood to target organs, such as the spleen, bone marrow, skeletal muscle, liver, kidneys and lungs.

Schizonts develop in these organs and rupture to release macromerozoites and micromerozoites. Following several cycles of schizogony, the micromerozoites invade leukocytes and transform to gametocytes. To complete the life cycle, the tick has a blood meal, gametocytes are released from the leukocytes to undergo gametogony in the tick's body cavity, oocysts are formed, and mature sporozoites develop within them.

Infection with *H. canis* may range from subclinical to clinically severe and life-threatening (Barton *et al.*, 1985; Baneth *et al.*, 1995). Clinical disease is characterized by leukocytosis, anaemia, muscular hyperaesthesia, severe lethargy, anorexia and weight loss (Craig, 1990). Diagnosis of *H. canis* infections in dogs is made by detection

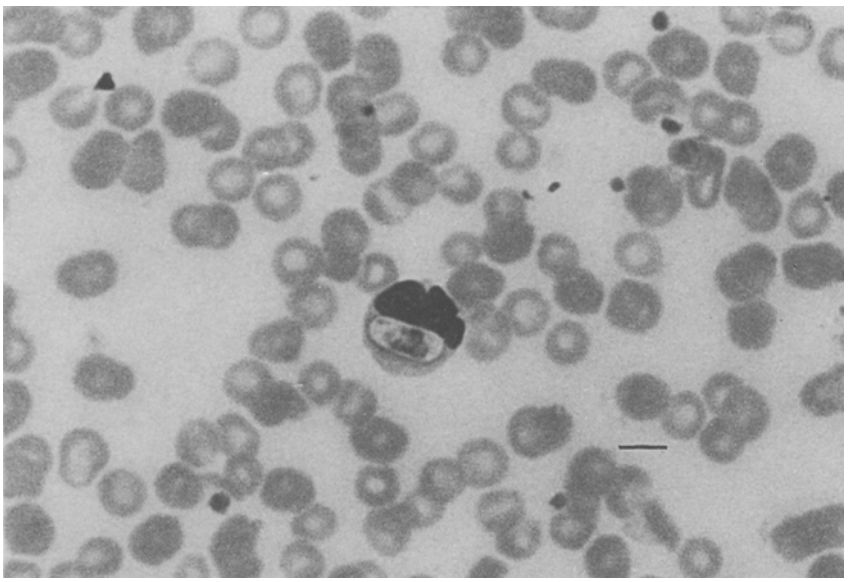

Figure 1. Photomicrograph of a *Hepatozoon canis* gametocyte in a leukocyte in a blood smear. Giemsa stain; bar=5 μm

of gametocytes within leukocytes in blood smears (Figure 1) and visualization of parasitic stages in tissue impression smears or histological samples taken from various organs. Recently, an indirect fluorescent antibody test (IFAT) has been described for the detection of specific antibodies against *H. canis* (Shkap *et al.*, 1994).

We present here a survey studying the exposure of dogs in Israel to *H. canis* based on demonstration of gametocytes in peripheral blood smears and on detection of serum antibodies to *H. canis*, as demonstrated by IFAT. This paper describes the first serological survey for detection of antibodies to *H. canis* in dogs.

MATERIALS AND METHODS

Dogs

Of the 286 dogs that were sampled, 161 were from municipal shelters or from shelters of humane societies for animals, and 125 from privately owned households. Samples were also taken from 14 beagles housed in a tick-free breeding colony, which served as a control group.

Blood and serum collection

Blood was obtained from all the dogs by cephalic venepuncture into tubes containing EDTA. Thin blood films were air dried, fixed in methanol and stained with Giemsa. Forty microscopic fields were examined under high dry magnification ($\times 400$) for *H. canis* gametocytes in the cytoplasm of neutrophils. Whole blood samples were collected into tubes, allowed to clot and centrifuged. The serum samples so obtained were frozen at –20°C until assayed.

Indirect fluorescent antibody test

In all, 174 serum samples were tested by an IFAT technique (Shkap *et al.*, 1994). Of these, 122 originated from shelter dogs, 38 were from privately owned dogs, and 14 were from the tick-free colony. Serum samples were tested in 2-fold dilutions from 1:16 on *H. canis* gametocyte antigen slides. A positive reference serum from a naturally infected dog with a demonstrable parasitaemia and a titre of 1:4096 to *H. canis* by IFAT was obtained in an earlier study (Shkap *et al.*, 1994). Both negative and positive control samples were included with each test series. Serum samples positive at a dilution of $\geqslant$1:32 were considered positive, in agreement with the findings from a previous study (Shkap *et al.*, 1994), where 1:32 was the lowest titre measured in dogs confirmed as having been infected with *H. canis*. The data were analysed using the chi-square test.

RESULTS

Prevalence of gametocytes

Intraneutrophilic gametocytes were detected in 3 out of 286 dogs (1%). Of these, 2 positive blood films were detected from dogs kept in shelters and 1 from a privately owned household dog (Table I). In the privately owned dog and one of the shelter-kept dogs, a parasitaemia of 1% of the neutrophils was found, while the other positive shelter-kept dog exhibited a 10% parasitaemia. When the latter dog was examined 3 months later, the parasitaemia had increased to 53% and was accompanied by severe clinical signs of lethargy, extreme weight loss and pale mucous membranes. The breeding colony of Beagles kept in tick-free conditions had no detectable intraneutrophilic gametocytes in their blood smears.

Prevalence of antibodies

Out of 160 shelter and private dogs (Table I), 53 (33.1%) had IFA titres $\geqslant$1:32. None of the 14 control Beagles was seropositive to *H. canis*. The prevalence of seropositivity did not differ statistically between dogs from animal shelters (32%) and private household

TABLE I.
The prevalence of seropositivity to *Hepatozoon canis* and the detection of the intraneutrophilic parasites in the peripheral blood of dogs from animal shelters and private households in Israel

Source of dogs	Gametocytes in blood		Seropositivity	
	n^a	INF (%)[b]	n^a	INF (%)[b]
Private households	125	1 (0.8)	38	14 (36.8)
Animal shelters	161	2 (1.2)	122	39 (32)

[a] *n*=the number of samples examined

[b] INF=the number and percentage of samples diagnosed positive

TABLE II.
The prevalence of seropositivity to *Hepatozoon canis* in dogs of different gender and breeds

Variable	Seropositivity	
	n^a	INF (%)[b]
Gender		
Male	91	27 (29.7)
Female	69	26 (37.7)
Breeds		
Mixed	111	37 (33.3)
German Shepherd	26	7 (26.9)
Other breeds	24	9 (37.5)

[a] *n*=the number of samples examined

[b] INF=the number and percentage of samples diagnosed seropositive

dogs (36.8%). The lowest titre (1:32) was found in 36 (68%) of the positive samples; a titre of 1:64 was found in 13 (24.5%); and the highest titre (1:256) was detected in 4 (7.5%) of these samples.

Breeds and gender

The dogs were categorized into three groups according to their breed: mixed breed, German Shepherd, and 'other breeds', which included breeds that were not represented by more than three individual dogs. As shown in Table II, no statistical difference was observed in the seroprevalence in these groups. The prevalence of seropositivity did not differ statistically between males and females (Table II).

DISCUSSION

The results of the survey showed that a significant proportion (33%) of the canine population of Israel has been exposed to *H. canis*. It is evident that antibodies to *H. canis* gametocytes are not necessarily associated with obvious clinical signs in dogs (Shkap *et al.*, 1994). The presence of antibody titres without microscopically detectable gametocytes in blood leukocytes may be the result of a previous exposure that has resolved, a persisting infection with an intermittent parasitaemia, or a very low number of circulating gametocytes which are not readily detectable. Cross-reactivity between *H. canis* and other organisms has not been reported.

Both the dogs in this study that had a parasitaemia of 1% had titres of 1:64; the dog with a 10% parasitaemia had a titre of 1:256. Shkap and colleagues (1994) reported that dogs with a patent demonstrable parasitaemia had higher mean titres than dogs that had had a parasitaemia 4 months earlier. This suggests that antibodies decrease with time when no gametocytes are seen microscopically. However, there is no specific information concerning the rate of decay of serum antibodies following an infection.

Infections with *H. canis* may be accompanied by severe clinical manifestations but the fact that a high proportion of the dogs are seropositive in the presence of a much lower number of evidently parasitaemic dogs suggests that a considerable number of the exposed dogs may undergo a subclinical infection. It appears that a small proportion of the exposed dogs go on to develop a widespread dispersion of tissue parasites, with severe systemic clinical disease. Infections may be persistently subclinical but, in the presence of an immune-suppressing state or another infectious agent, may re-erupt with enhanced schizogony and subsequently release of gametocytes into the blood.

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