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HEPATOZOONOSIS IN FOXES FROM PORTUGAL

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ABSTRACT: *Hepatozoon* sp. is described for the first time in foxes (*Vulpes vulpes silacea*) in Portugal. Of 301 foxes examined, 143 (48%) were infected. The gametocyte was the predominant stage of the life cycle and was found in every organ except the bone marrow, where schizonts were the most abundant stage. The morphological similarity of this parasite's gametocytes to *Hepatozoon canis* is emphasized.

Key words: *Hepatozoon* sp., European fox, *Vulpes vulpes silacea*, *Hepatozoon canis*, survey, life history stages.

INTRODUCTION

Several species of the genus *Hepatozoon* are reported from mammals, reptiles and birds (Levine, 1973). *Hepatozoon canis*, a parasite of the domestic dog, was the first described species. The dog tick, *Rhipicephalus sanguineus*, is the most probable vector. Dogs acquire the infection through ingestion of ticks harboring sporozoites (Christophers, 1907; Levine, 1973).

During a study on the wild reservoirs of leishmaniasis in Portugal (Abranches et al., 1982, 1983a, b, 1984), we found a species of *Hepatozoon* in foxes (*Vulpes vulpes silacea*). *Hepatozoon* sp. has been reported previously in foxes (Rau, 1926; Rioux et al., 1968; Maede et al., 1982) but the high prevalence of infection in our study resulted in a better understanding of the biology of this parasite in the vertebrate host and in the vector. Whether or not the species of *Hepatozoon* we recovered from foxes is *H. canis* remains to be resolved.

MATERIALS AND METHODS

In January and February (season for hunting foxes) from 1979 to 1985 biological specimens were collected from 301 animals in the Lisbon and Alcácer do Sal regions of Portugal (Abranches et al., 1982, 1983a, 1984). Samples of blood, spleen, liver, tibial bone marrow and sometimes popliteal lymph node were collected. These were processed as described by Abranches et al. (1982, 1983b).

Parasites were identified by direct microscopic examination of blood, bone marrow and tissue impression smears. Some pieces of liver and spleen were fixed in 10% formol–saline and sent to England for histological examination (P. C. C. Garnham, Imperial College, Silwood Park, Ascot, Berkshire SL5 7PY, England). For vector studies, 51 ticks were collected and smears were prepared from their hemolymph.

Representative specimens of *Hepatozoon* sp. are deposited in the collection of the British Museum of Natural History (London SW7 5BD, England; accession numbers 1987:7:27:1–4).

RESULTS

Of the 301 foxes examined, 143 (48%) were infected with *Hepatozoon* sp. Of the organs examined the number of foxes infected/the number examined for spleen, bone marrow, lymph node and blood were 131/138 (95%), 33/56 (59%), 7/17 (41%) and 44/125 (35%), respectively. Thus the spleen and the bone marrow were the most heavily parasitized organs. The parasites were mostly intracellular, but some occurred extracellularly in the spleen, liver and bone marrow.

In the blood, gametocytes were observed mostly inside neutrophils (Fig. 1). Occasionally, gametocytes were seen in other blood cells, but the large size of the parasite (8–12 × 4–6 μm) which often obscured the cell nucleus made correct identification of the host cell difficult. The gametocytes were the most frequently observed stage in all organs except for the bone marrow. They were morphologically identical to those of *H. canis* (Wenyon,

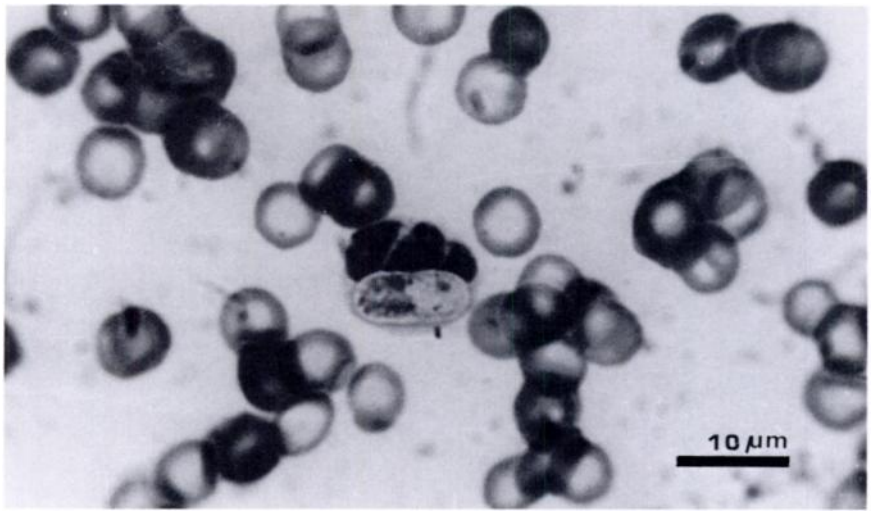

FIGURE 1. Gametocyte of *Hepatozoon* sp. in a neutrophil from a blood smear of the European fox.

1926; Soulsby, 1968; Levine, 1973). The schizonts were numerous in the bone marrow, but were scanty in the spleen and liver and were never observed in the blood. The schizonts in the bone marrow were predominantly oval and measured from 15.8 × 7.1 µm to 10.3 × 4.4 µm. The number of nuclei was not established (Fig. 2). On one liver impression smear, several extracellular curved merozoites were seen.

Histologically, two different kinds of schizonts were seen in the liver of the same fox. There were immature schizonts (12 µm) with four nuclei, as well as mature schizonts (16 µm) with about 25 merozoites arranged peripherally and many others at different planes of the section resulting in a total number of ≥100. Additionally, a ruptured schizont with about 12 merozoites (variable length but averaging 5.8 µm) with pointed ends and a sometimes eccentric nucleus was seen. The host cell was probably one of the reticuloendothelial series.

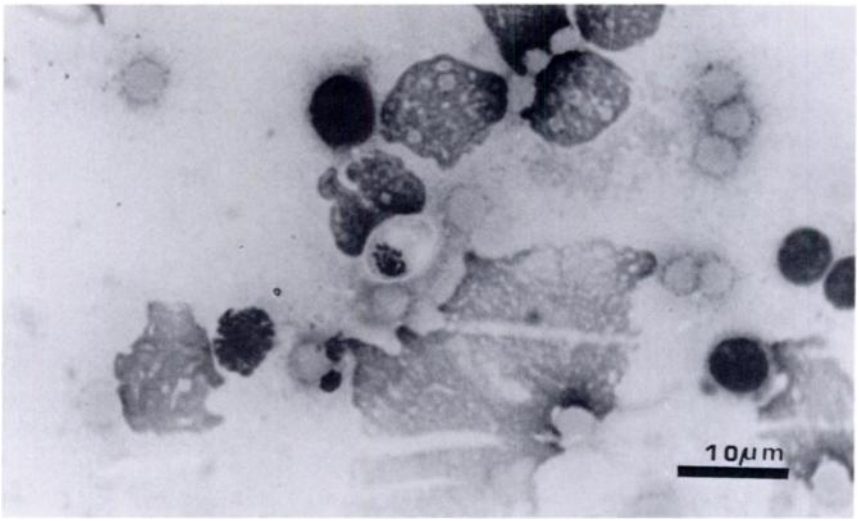

FIGURE 2. Schizont of *Hepatozoon* sp. in a bone marrow impression smear from the European fox.

Ticks collected in this study were identified as *Rhipicephalus sanguineus* (11 specimens), *R. pusillus* (26), *Ixodes festai* (8), *I. hexaporus* (4) and *I. ricinus* (2). In hemolymph smears from one tick, two gametocytes similar to those in the fox tissue were seen, but sporogonic forms of the parasite were not observed.

DISCUSSION

The parasite described in this study is similar to that described by Wenyon (1926) as *Hepatozoon canis*. Prior to this study, *Hepatozoon* sp. in foxes from Europe has been reported only in France (Rioux et al., 1968). *Hepatozoon* sp. was found also in *Vulpes bengalensis* from India (Rau, 1926), and more recently in *Vulpes vulpes schrenki* from Japan (Maede et al., 1982). The latter two authors suggested the parasite was *H. canis*.

The high prevalence of *Hepatozoon* sp. that we found in foxes (48%, 143 of 301) contrasts with the much lower prevalence in dogs (3%, 50 of 1,752) from the same area. The prevalence found in dogs was from examination of blood smears (for comparison, the prevalence in fox blood smears was 22% or 44 of 197). The difference was statistically significant ($P < 0.001$) with chi-square analysis on prevalence data. This indicates that wild Canidae may be an important reservoir for this parasite as suggested by Davies et al. (1978). As a consequence of its peridomestic habits, the fox could represent a possible health hazard to domestic dog populations.

The pathogenicity of the *Hepatozoon* sp. remains controversial. Some authors suggest that this parasite is not very pathogenic (Rioux et al., 1964; McCully et al., 1975), while others report some cases of splenomegaly, progressive emaciation and in one case, lumbar paralysis (Levine, 1973). The prevalence of vulpine hepatozoonosis without clinical signs, even in the cases with concomitant infections (three with *Leishmania infantum* and one with microfilaria), suggests that this parasite is harmless to foxes.

The gametocyte in this parasite is the predominant form, observed in all organs with the least number seen in the bone marrow. Immature schizonts were numerous in the bone marrow, but were seen rarely in the spleen and liver. This infers that schizogony in fox infections occurs mainly in the bone marrow. In our experience all the schizonts observed in the bone marrow seemed to be at a similar developmental stage.

The search for *Hepatozoon* sp. in the ticks was unsuccessful. Examination of oocysts and sporocysts which is essential to determine the role of ticks as vectors of *Hepatozoon* sp. was not possible.

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