Veterinary Parasitology: Regional Studies and Reports 8 (2017) 90–93

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr

Original Article

Hematological and epidemiological characterization of *Hepatozoon canis* infection in dogs from Buenos Aires, Argentina

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ARTICLE INFO

Article history:
Received 16 September 2016
Received in revised form 6 February 2017
Accepted 26 February 2017
Available online 04 March 2017

Keywords:
Canine hepatozoonosis
Seasonality
Hematological abnormalities
Epidemiology
Argentina

ABSTRACT

Canine hepatozoonosis caused by *Hepatozoon canis* is widespread in America. In Argentina, since the first finding of the disease in Buenos Aires in 1999, several isolated cases were reported in other six provinces. However, there is no information regarding hematological and epidemiological characterization of the disease in the country. A total of 100,123 canine blood samples obtained during the period 2002–2013 from Southern Greater Buenos Aires were examined by light microscopy. Overall prevalence was 2.3%, with high parasitemia levels (>800 gamonts/μl) in 680 samples, mild (100–800) in 1088, and low (<100) in 433 patients. Among parasitemic dogs, anemia (mostly non-regenerative) was present in 56.9%. Inflammatory leukogram, defined as neutrophilia with or without leukocytosis, with or without left shift and left shift with or without neutrophilia, was the main hematological abnormality and was present in 74.1% of positively tested dogs. A clear seasonal tendency was observed, with maximum values in summer and minimal in winter, and an increasing prevalence was recorded during the study decade. Young, male and mixed breed dogs showed higher prevalence values. Our findings strongly suggest that canine hepatozoonosis is endemic and expanding in the region.

1. Introduction

Canine hepatozoonosis is a tick-borne disease caused by the apicomplexan protozoa of the genus *Hepatozoon*. *Hepatozoon canis* is transmitted mostly by the brown dog tick *Rhipicephalus sanguineus* (Baneth et al., 2001, 2007) and has also been shown to be transmitted by *Amblyomma ovale* (Rubini et al., 2008). Transmission of the infection to canine hosts takes place by ingestion of ticks, or parts of ticks containing mature *H. canis* oocysts (Baneth et al., 2001, 2007). Merogonic stages are located in hematopoietic and lymphatic tissues in vertebrate host, where asexual generations take place with production of new meronts and tissue damage as a consequence. Merozoites are released from the meronts and can initiate a new merogonic generation or invade the cytoplasm of the white blood cells (neutrophils and monocytes) as new forms called gamonts. The number of gamont-infected neutrophils and monocytes per microlitre of blood determines the level of host parasitemia. Traditionally, it is proposed that these levels of parasitaemia relate to clinical manifestations of the infected dogs. Infection ranges from being asymptomatic in dogs with low parasitemia to a severe life-threatening illness with fever, lethargy, anemia and emaciation in dogs with high parasitemia (Baneth and Weigler, 1997; Gavazza et al., 2003).

Hepatozoonosis caused by *H. canis* is widespread and well-known in Southern Europe, Asia and Africa (Baneth et al., 2001). Additionally, *H. canis* has also been identified infecting dogs throughout America: Brazil (O'Dwyer et al., 2001; Ramos et al., 2015), USA (Allen et al., 2008), Colombia (Ardila et al., 2007), Venezuela (Criado-Fornelio et al., 2007) and Argentina (Eiras et al., 2007).

In Argentina, canine hepatozoonosis was first described in Buenos Aires in 1999 (Esarte et al., 1999; Silva et al., 1999) and in 2007 molecular characterization of *H. canis* was performed (Eiras et al., 2007). There are several recent reports of the disease in the other six provinces, namely Santa Fe, Salta, Chubut, San Luis, Mendoza y Entre Ríos (Linares, 2011; Ruiz et al., 2013; Varisco et al., 2013). To our knowledge, there is no information dealing with hematological or epidemiological characterization of the hepatozoonosis in the country. The aim of this study is to present these findings obtained during the past decade in the Southern Greater region of Buenos Aires.

2. Material and methods

A total of 100,123 canine blood samples were collected from owned dogs by veterinary practitioners from Southern Greater

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http://dx.doi.org/10.1016/j.vprsr.2017.02.008
2405-9390/

Buenos Aires between October 2002 and May 2013, and submitted to the DIAP laboratory (Diagnóstico en Animales Pequeños) in the locality of Banfield for routine testing. Blood was drawn from the cephalic vein, collected in EDTA tubes and stored at 4 °C until it was processed 12–24 h later.

Thin blood smears were stained by May Grünwald-Giemsa and examined by light microscopy. The microscopic detection was made by several veterinarians equally trained and qualified. The presence and level of *Hepatozoon* parasitemia were determined by observing 100 microscopic fields per smear at ×1000 magnification. Absolute parasitemia was calculated by multiplying the percentage of parasitized neutrophils and monocytes by the total number of neutrophils and monocytes per μl (Eiras et al., 2007). Parasitemia levels (expressed in gamonts per μl) are traditionally divided in patients into low or high parasitemia and was classified in low (<100), mild (100–800) and high (>800).

Hematological parameters were evaluated by combining manual and automatic techniques. A cell counter Abacus (Diatron, Austria) was used to calculate hematocrit and total leukocyte count. Relative leukocyte differentiation was performed by microscopic observation and white blood cell absolute counts calculated. The abnormalities in the leukogram were recorded as follow: leukocytosis (>18,000 leukocytes/μl), leukopenia (<6000 leukocytes/μl), eosinophilia (>1250 eosinophils/μl), and monocytosis (>1350 monocytes/μl). In addition, an inflammatory leukogram was defined as neutrophilia with or without leukocytosis, neutrophilia with or without a left shift (i.e. >300 immature and band neutrophils/μl) and a left shift with or without neutrophilia. Finally, the reticulocyte index was calculated in anemic dogs in order to discriminate between regenerative (1–2), non-regenerative (<1) and highly regenerative (>2) process. Normal reference values were established with data obtained from clinically healthy dogs at DIAP laboratory and by comparing classical veterinary laboratory literature (Meyer and Harvey, 2004).

Epidemiological findings were assessed according to the availability of appropriate data; data from June 2008 to May 2010 were not available to assess temporal patterns, and risk factors (breed, age and gender) and data on mixed infections with other vector borne pathogens (microfilariae and merozoites of piroplasms) were not available prior to June 2010. In the study area, *Dirofilaria immitis* (Vezzani et al., 2011) and *Babesia vogeli* (Eiras et al., 2008) are present at a low prevalence. In order to describe the temporal patterns of the infection, the annual and seasonal prevalences were calculated. Prevalence rates calculated for breed, age and gender categories were compared using the ×2 test for independent proportions (Fleiss et al., 2003). Statistical comparisons were performed using the WINPEPI software (Abramson, 2004).

3. Results

Overall prevalence of *H. canis* during the study period was 2.3% (2328/100123). Gamonts were observed in neutrophils and rarely in monocytes. Among positively tested dogs, the parasitemia was high in 680 samples (29.2%), mild in 1088 (46.6%), and low in 433 (18.6%). In addition, 127 positively tested dogs recorded panleukopenia (<1000–2000 leukocytes/μl) however in these cases, the level of parasitemia was not recorded.

Anemia was present in 1313 positively tested dogs (56.9%). Among them, it was regenerative, non-regenerative and high regenerative in 244 (18.6%), 915 (69.7%), and 154 cases (11.7%), respectively. Regarding the leukogram, 849 (36.3%) patients showed leukocytosis and 175 (7.5%) leukopenia. In those with leukocytosis, 810 (97.4%) recorded neutrophilia with left shift. Among 1304 patients with normal total leukocyte counts, 211 (16.2%) showed neutrophilia with left shift. According with the definition mentioned above, among the 2328 parasitemic dogs, an inflammatory leukogram was present in 1724 (74.1%). 1080 (46.4%) patients presented with eosinophilia and 414 (17.8%) monocytosis.

Regarding temporal variations of prevalence, a clear seasonal tendency was observed, with maximum values in summer and minimal in winter (Fig. 1). In addition to these marked annual peaks, an increasing prevalence was recorded over the study decade. Maximum values ranged 1.5–3.3% during the summers of 2003–2008 and between 3.4 and 5.9% in the last three summers. Accordingly, winter values increased from 0.1–0.5% to 0.7–0.9%.

Risk factors assessed in the period June 2010–May 2013 resulted in 2.6% positively tested dogs among 41,905 samples. The prevalence was significantly higher in male and mixed breed categories (Table 1). The age of the dog was also associated with *Hepatozoon* prevalence ($x^2_{(2)} = 68.29$, $P < 0.001$), with adult dogs less infected ($P < 0.001$). Co-infections with microfilariae of *D. immitis* and merozoites of *B. vogeli* were observed in 7 and 14 cases, respectively.

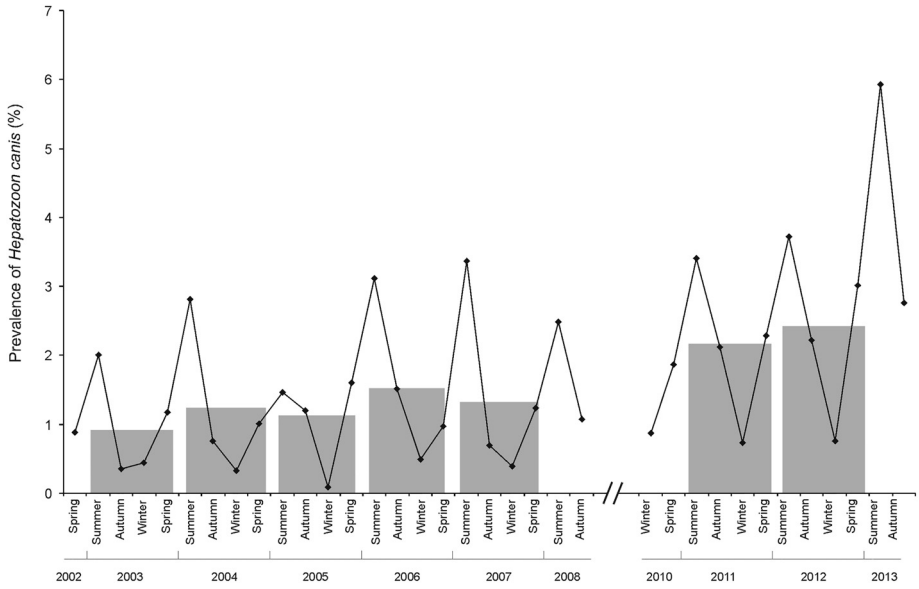

Fig. 1. Seasonal (lines) and annual (bars) prevalences of dogs with *Hepatozoon* parasitemia identified by blood smear microscopy during the study period in Buenos Aires (Argentina).

Table 1
Statistical comparisons of *Hepatozoon canis* prevalences between breed, age and gender categories.

	Categories		
Gender	Female	Male	
	2,09% (488/23369)	3,17% (588/18536)	$x^2 = 48.548$ $P < 0.001$
Breed	Pure	Mixed	
	1,28% (312/24367)	4,36% (764/17538)	$x^2 = 385.650$ $P < 0.001$
Age	<3 months	3–11 months	>11 months
	3,48% (31/891)	4,49% (176/3921)	2,34% (869/37093)

4. Discussion

This is the first epidemiological and hematological study of canine hepatozoonosis carried out in Argentina. Our findings demonstrate that canine hepatozoonosis is endemic in Buenos Aires, and strongly suggest that there is an increasing prevalence trend.

The most important hematological findings in parasitemic dogs were a non-regenerative anemia and an inflammatory leukogram. The inflammatory process was characterized mainly by the detection of left shift in >70% of the patients. This information is in agreement with previous studies globally and is associated with the level of parasitemia (Baneth and Weigler, 1997). In our study, most positively tested dogs had mild or high parasitemia levels. As *H. canis* could not be completely removed by available drugs, level of parasitemia can be used as a marker of merogonic activity. Declining parasitemia levels following treatment could indicate a good prognosis in a patient.

The seasonal distribution of infection from the time of the first diagnosis presumably paralleled the tick season and warmer months like in other parts of the world; e.g. in Italy (Gavazza et al., 2003). The infection was more prevalent in puppies, males and mixed-breed dogs. These findings are also in agreement with one report from Brazil on 115 infected dogs (Mundim et al., 2008). Puppies have been reported previously as more susceptible to infection than adults because the immature immune system in animals younger than 4–6 months (Baneth et al., 1997). The higher prevalence in male and mixed-breed dogs may be related with behavioral characteristics that increase the exposure to ticks (Mundim et al., 2008).

Although only owned dogs were surveyed, 2.3% of the 100,123 samples evaluated were positive for *Hepatozoon* by blood smear microscopy. The prevalence of infection in stray and rural dogs is likely to be higher to that observed in owned dogs, and it should be matter of future studies. In addition, the true prevalence of infection in the region is undoubtedly even higher considering that blood smear microscopy underestimates infection values. A study from Turkey demonstrated that detection of *H. canis* by PCR (25.8%) is considerably more sensitive than light microscopy (10.6%) (Karagenc et al., 2006). In another study from southern Brazil, 11.3% of 150 dogs were positive by blood smear evaluation and 53.3% by PCR (Rubini et al., 2008).

Regarding mixed infections, *Dirofilaria* and *Babesia* were observed with *Hepatozoon* but in a very few dogs, which preclude any analyses of the potential effect of the co-infection on hematological values or risk factors. There are other two vector borne pathogens of dogs in Argentina, *Ehrlichia canis* and *Anaplasma platys*, recently described for the first time (Eiras et al., 2013). In this paper, co-infection with *Hepatozoon* was recorded in some cases but during a short period of time. Unfortunately, in the current research these bacteria were not actively tested for. Co-infections of vector borne pathogens of dogs are likely to have compounding effects on hematological and clinical outcomes and must too be included as an aspect of future studies.

In conclusion, *H. canis* infection is endemic in the Southern Greater Buenos Aires. Most positively tested dogs appear with some hematological abnormalities with mostly mild or high parasitemia levels. Due the risk of infection, it would be advisable to implement tick control from the beginning of the spring to the end of the summer. Hepatozoonosis must be considered as a part of routine diagnosis especially in warmer months when ticks are more prevalent. Despite *R. sanguineus* ticks being recorded in the study area since the 1940s (González et al., 2004; Guglielmone and Nava, 2005), there is notably little information regarding ticks as vectors of this and other tick-borne diseases in the region. Further studies focused on vectors will contribute to reach a more comprehensive understanding of the epidemiology of canine hepatozoonosis in Argentina.

Acknowledgments

The authors thank veterinary practitioners from the southern part of Greater Buenos Aires for provide blood samples.

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