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EPIDEMIOLOGICAL AND VECTOR IDENTIFICATION STUDIES ON CANINE BABESIOSIS

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ABSTRACT

Canine babesiosis is increasing in incidence and prevalence and is now a major problem in dogs. During this study, a total of 6204 dogs were examined for babesiosis over a 12 month period from January to December, 2006 in Lahore and 2.62% were found positive. The dogs were grouped on the basis of their age, sex and breed and season of the year. The male dogs were more prone to disease than female dogs (3.39 vs. 1.32%), whereas the incidence of disease was higher in younger dogs (6.9%) than older age groups. Crossbreds were more prone to the infection (10.9%) than purebreds. However, none of them were completely resistant. Warm and humid season played a key role in the spread of disease. Predominant vector of the disease was found to be Rhipicephalus species.

Key words: Babesia, epidemiology, vector, dog.

INTRODUCTION

Babesia species are tick-transmitted apicomplexan parasites infesting a wide range of wild and domestic animal hosts (Kuttler, 1988). Canine piroplasms belong to two distinct species, the large (4–5 µm) *Babesia canis* and the small (1–2.5 µm) *Babesia gibsoni*. Differences in geographical distribution, vector specificity and antigenic properties subdivided the former species into three subspecies, namely *Babesia canis canis* transmitted by *Dermacentor reticulatus* in Europe, *B. canis vogeli* transmitted by *Rhipicephalus sanguineus* in tropical and subtropical regions and *B. canis rossi* transmitted by *Haemaphysalis leachi* in South Africa (Uilenberg *et al*., 1989). *B. gibsoni* occurs in Asia, North America, Northern and Eastern Africa, Australia and Europe (Birkenheuer *et al*., 1999; Muhlnickel *et al*., 2002; Criado-Fornelio *et al*., 2003). The purpose of the present study was to find out the disease pattern of canine babesiosis in relation to various parameters (age, sex, and breed of the dogs and season of the year) for future prophylaxis and to identify responsible vector species.

MATERIALS AND METHODS

Experimental animals

Epidemiological and vector identification studies were undertaken through January 2006 to December 2006 at the Pet center, University of Veterinary and Animal Sciences, Lahore, Pakistan. A total of 6204 dogs showing signs of babesiosis (i.e. pale mucus membrane, depression, pyrexia, anorexia, weight loss etc.) were included in the study. Pertinent signalment (breed, age, sex of the patient) and history from each individual dog was collected. During clinical examination, the presence of ticks on the coat of the dog was determined. Metrological data for the study period was also obtained. Epidemiology of the disease was studied on the bases of the age {Group1 (<2 years), Group 2 (2 to 4 years old), Group 3 (4 to 6 years old) and Group 4 (>6 years of age), sex (male and female), breed (Bull Terrier, Russian, Labrador, Alsatian, Doberman, German Shepherd, Pointer, and crossbred) and season.

Collection of samples

Blood sample were collected from the jugular vein of all experimental dogs. Two thin blood smears of each blood sample were prepared. The blood smears were stained by the standard Giemsa's staining method and were cover-slipped using a permanent mounting solution (e.g. DPX) for later examination.

Ticks were collected from 100 dogs and fixed in 70% ethanol solution. Ticks from the same animal were stored in the same bottle. These bottles were transported to Australia for further studies. Ticks were identified in the laboratory following the method described by Soulsby (1982).

Statistical analysis

The data pertaining to the epidemiological study (age, sex, breed and season) was compared on the basis of odds ratio (Steel and Torrie, 1982).

RESULTS

Epidemiological study

Statistical analysis of the data (Table 1) revealed that dogs up to two years of age (group 1) were 10.6 times more prone to the disease, followed by group 4 (above six years of age; 3.63 times) and group 2 dogs (2-4 years old; 1.92 times) than the referent group i.e., group 3 (4-6 years old). Male dogs were 2.63 times more prone to the disease than female dogs (Table 2). The highest percentage of the disease positive cases was found in Crossbreds, whereas lowest percentage was found in Doberman (Table 3). The disease was

more prevalent during the months of January, July, August, September and November compared to February, March, April, May, October and December (Fig. 1).

Vector identification

From a total of 507 ticks, 491(96.84%) ticks were identified as Rhipicephalus, 14(2.76%) as Dermacentor. Only 2(0.40%) ticks were belonging to Haemaphysalis.

DISCUSSION

The results of the present study indicate that dogs <2 years of age were more likely infected with Babesia species than the dogs of other ages. *B. gibsoni* was transmitted transplacentally and not by the trans-mammary route (Fukumoto *et al*., 2005). Brikenheuer *et al*. (1999) also diagnosed *B. gibsoni* in puppies as young as 10 days of age, a time interval that is shorter than the prepatent period following tick transmission (Birkenheuer, 1999). This over-representation may be due to an increased susceptibility to infection or less immunity. In some protozoan infections, most of the neonates of chronically infected mothers show a high degree of immunity to the homologous parasites compared to those born of normal mothers (Palmer, 1978).

Babesiosis can infect dogs of all ages, although most infected dogs are less than three years old. On the other hand, the older dogs were also prone to babesia infection. Older animals are predisposed for babesial complications. Seropositivity for babesia infection first increased and then declined with age, reaching a maximum in case of 3.1-to 5-year-old dogs (Sándor *et al*., 2006). It has also been reported that age do not have any influence on the animals' susceptibility to the disease (Martinod *et al*., 1986). Non-specific or innate factors (genetic or age) possessed by the hosts can act as natural protective elements (Johnston *et al*., 1978; Levy *et al*., 1982).

The present study indicated that male dogs were more prone to disease than the females, which suggested bite wounds or blood transmission during fighting contact in male dogs as possible routes of transmission for *B. gibsoni*. Male dogs are most commonly associated with organized dog fighting in Pakistan. Similar observation was reported by Vanzyl (1995), where out of 904 cases, 57% were males and 43% were females. However, other studies showed that the sex ratio was the same in the sick and in the total population of dogs, there was no difference in susceptibility to *B. canis* between males and females (Martinod *et al*., 1986).

Table 1: Occurrence of canine babesiosis in relation to various age groups

Groups	Age (Yrs.)	Total No. of dogs	Positive No.	Positive %	OR[1]	Lower 95% CI[2]	Upper 95% CI[2]
1	<2	1966	119	6.1	10.60	5.54	20.28
2	2<-4	2166	25	1.2	1.92	0.92	4.01
3	4<-6	1655	10	0.6	1.00	-	-
4	>6	417	9	2.2	3.63	1.46	8.99

[1]OR: Odds Ratio, [2]CI: Confidence interval

Table 2: Occurrence of canine babesiosis in relation to sex

Sex	Total No. of dogs	Positive No.	Positive %	OR[1]	Lower 95% CI[2]	Upper 95% CI[2]
Male	3923	133	3.39	2.63	1.77	1.77
Female	2281	30	1.32	1.00	-	-

[1]OR: Odds Ratio, [2]CI: Confidence interval

Table 3: Occurrence of canine babesiosis in relation to breeds

Breeds	Total No. of dogs	Positive No.	Positive %	OR*	Lower 95% CI*	Upper 95% CI*
Bull Terrier	1756	27	1.5	2.24	1.05	4.78
Russian	1299	9	0.7	1.00	-	-
Labrador	908	14	1.5	2.24	0.97	5.21
Alsatian	633	36	5.7	8.64	4.14	18.06
Doberman	539	0	0	-	-	-
German Shepherd	512	24	4.7	7.05	3.25	15.27
Pointer	327	28	8.6	13.42	6.27	28.74
Cross bred	230	25	10.9	17.48	8.04	37.98

*OR: Odds Ratio, *CI: Confidence interval

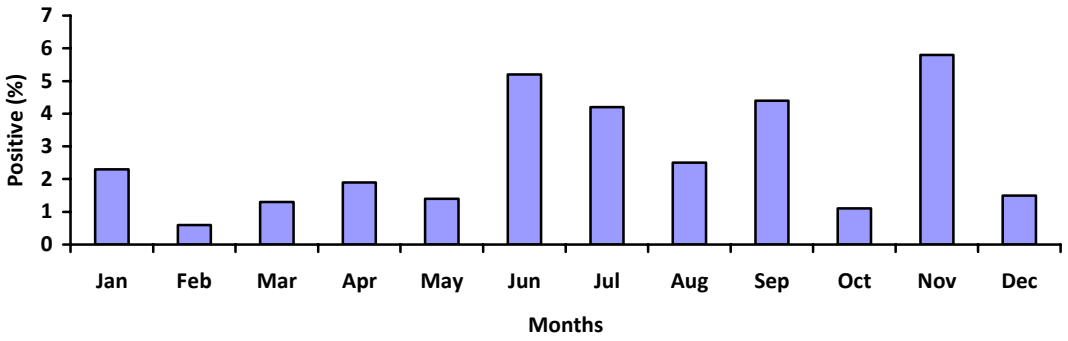

Fig. 1: Seasonal occurrence of canine babesiosis

It is interesting to note that certain breeds with the same ethology have opposite degrees of susceptibility (for instance, Spaniel vs. Beagle or Teckel vs. Pekinese). Likewise, size does not appear to be a decisive factor: animals of the same size, (i.e. Teckel and Pekinese) have different levels of susceptibility and animals of different sizes (Porcelain and Teckel) have the same susceptibility (Martinod *et al*., 1986). The results of the present study showed that the most susceptible breed in Pakistan was crossbred dogs, followed by the Pointer. The most common breed was mixed breed (Jacobson, 2006). Prevalence of antibodies to *B. canis* was significantly higher among German Shepherds and Komondors, suggesting a genetic predisposition of German Shepherd dogs to chronic babesiosis (carrier status) with long-term maintenance of their seropositivity (Sándor *et al*., 2006).

Higher incidence of babesiosis in the present study was observed during the months of January, July, August, September and November. The winter is usually at its peak in January in Pakistan, and people use heaters to keep the premises of their animals warm, which might influence the activity of the ticks, and thus may result in high incidence of the disease. Whereas, the remaining months may be considered as the tick active months (Afzal *et al*., 1991). Babesiosis occurred with the highest incidence in summer in canines (Jacobson, 2006). Most cases were diagnosed during the spring periods (Porchet *et al*., 2007). Infected dogs were found from February until the beginning of December; peak numbers occurred from September to November (Shen *et al*., 1997).

Ticks have been considered second only to mosquitoes in ability of transmission of disease agents such as blood protozoans (Hillyard, 1996). Ticks attach securely to their hosts, facilitating not only effective transmission of infectious agents, but also the spread of both ticks and microorganisms to different geographical habitats via traveling pets. Pathogens ingested by ticks can be spread transtadially and/or trans-ovarially (Susan *et al*., 2001). Arthropodes in general and ticks in particular, have evolved as ectoparasites of wild animals (Hillyard, 1996). Only a minority of tick species, generally those with wide host range, transmit diseases to domestic animals and humans. The increasing prevalence of tick-transmitted diseases of pets and their owners has been associated with increased accessibility of traditional wilderness environments and an increase in the reservoir of wild host species that now have a closer association with human activity. *Rhipicephalus sanguineus, Boophilus microplus, B. annulatus, Hyalomma anatolicum anatolicum, H. isaacii, H. aegyptium* and *Dermacentor marginatus* were identified from cattle, buffaloes, sheep, and goats (Kaiser and Hoogstraal, 1964). Although infestations with a single species of tick were recorded, most of the animals examined during the study had mixed infestation with different tick species. A similar trend of tick infestation has been observed by different workers (Drummond, 1967; Iqbal, 1971). However, variation in the rates of prevalence between single and mixed infestation exists, which can be attributed to the exposure of the animals and/ or innate resistance to some species of ticks (Daynes and Gutierrez, 1980).

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