

Full Article

***Babesia* infection in urban and rural dogs in Ahvaz district, Southwest of Iran**

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Abstract

Canine Babesiosis is an important worldwide, tick-borne disease caused by apicomplexan hemoparasitic from genus *Babesia*. The aim of the present survey was to identify the current state of *Babesia* infection in urban and rural dogs in Ahvaz district, southwest of Iran. For this reason, 200 rural dogs from 5 village around Ahvaz and 200 urban dogs (stratified random sampling) referred to the veterinary hospital of Shahid Chamran University were examined for the presence of *Babesia* species within 2 years. The studied dogs were classified based on age, sex, breed and season. Blood samples were taken from cephalic or saphenous vein and then peripheral thin blood smears were prepared and stained with Giemsa for parasitological examination. Among 400 dogs, 15 samples (3.75%) were infected with *Babesia canis*. The present study showed that from 200 rural dogs, 11 samples (5.5%) and from 200 urban dogs, 4 samples (2%) were positive for *B. canis*. Infection rate was higher in adult dogs 3-6 years-old (4.46; 5 out of 112) compared with young's less than 3 years (3.59; 7 out of 195) and above 6 years (3.85; 3 out of 78). The infection was higher in female dogs (4.29%; 6 out of 140) than males (3.46%; 9 out of 260) and in warm season (5.15%; 12 out of 233) compared with cold season (1.8%; 3 out of 167), nevertheless, there was not significant relationship between sex, age and season in urban dogs ($P>0.05$), but significant difference was revealed between season and infection in rural dogs population ($P<0.05$). Although the infection rate of this parasite was low, but transmission of the protozoan to dogs should be intentioned. This is the first report indicating the presence of *B. canis* in dogs of Ahvaz district; however, the sources of infection in these dogs are not clear. The role of dogs in the epizootiology of *B. canis* infection needs to be further explored.

Keywords: Infection rate, *Babesia canis*, Dog, Ahvaz

INTRODUCTION

Canine Babesiosis is a vector-borne disease caused by intra-erythrocytic protozoa that induces anaemia,

fever, jaundice, splenomegaly, thrombocytopenia and occasionally haemoglobinuria. *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

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species, the large *Babesia canis* (4–5 μm) and the small *Babesia gibsoni* (1–2.5 μm). 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 rossii* 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). Recently, it has gained increasing attention as an emerging zoonosis in humans. People who have had a splenectomy or who are older (more than 55 years) are especially at risk (Neer & Harrus 2006). Diagnosis of canine *Babesiosis* is made by the microscopic detection of parasites in peripheral blood smears or by serological tests, flow cytometry and polymerase chain reaction (PCR). There are many strains of canine *Babesia species* (Kjemtrup et al 2000), with *Babesia canis* and *B. gibsoni* being two organisms that be caused clinically indifferent disease manifestations but can be differentiated by microscopy (Ayoob et al 2010, Garc 2006), serology and PCR. Packed cell volumes (PCVs) in *Babesia* infections are reported to be between 8 and 28% in hyperacute cases and between 35 and 41% in chronic cases (Abdullahi et al 1990), with other haematological changes such as thrombocytopenia, anisocytosis and neutropaenia (Ayoob et al 2010, Mathe et al 2006, Zygner et al 2007). Infections may be asymptomatic but the disease sometimes presents or degenerates into hyper-acute septic shock-like syndromes, which are highly fatal in dogs (Matijatko et al 2009) with mortality rates of 10–15% compared with rates of less than 2% in uncomplicated infections. Various authors describe seasonal peaks in *Babesia* prevalence related to vector and other transmission dynamics (Leschnik et al 2008, Maia et al 2007) in many countries. The seasonal trends of *Babesia* infection in dogs in Ahvaz district has not been previously studied or reported,

though it is commonly thought to increase during the rainy season when the vector burdens also increase. There is no published literature on the prevalence of the parasite in this area, despite there being numerous cases of the infection that are diagnosed microscopically. This study is thus designed to show information on the infection rate and epidemiology of canine *Babesiosis* in the blood samples of urban and rural dogs in Ahvaz district, Southwest of Iran.

MATERIALS AND METHODS

Study area and sample size. The present study was performed in Ahvaz district that is located at an elevation of 12 meters above sea level and with warm-humid climate. In this study, a total number of 400 dogs of different age groups and by stratified random sampling were examined for the presence of *B. canis* in blood smears. Urban dogs were referred cases to Veterinary Teaching Hospital of Shahid Chamran University of Ahvaz from November 2008 to March 2010, but rural dogs were from 5 villages around Ahvaz. Most of the dogs were apparently healthy. Information about dogs were taken from their owners and recorded. Samples were conveniently obtained from pets whose owners agreed to the sampling and data captured on a form. Informed consent was sought from owners to participate in the survey and collect blood from their dogs. Patient data recorded at the time of blood collection included age, sex, breed, season, color of mucous membranes, PCV, vital signs, body condition score and presence or absence of lymphadenopathy. The examined dogs were grouped in four age groups (<1 year, 1- 3 years, 3-6 years and > 6 years). Blood samples were collected from cephalic or saphenous vein of 400 dogs (260= male and 140= female). Age was estimated by dental formula and owner information's. Breeds of the studied dogs were mostly German Shepherds, Doberman Pinschers and Mixed.

Parasitological procedures: The samples were prepared and allowed air drying. Ethylene Di amine tetra acetic acid (EDTA)- anticoagulated blood was

collected from the cephalic or saphenous vein of the dogs for Giemsa-stained thin blood smear examination to detect *Babesia* species in RBC. The smears were fixed in methanol for 5 min and stained by 10% Giemsa. Two blood smears were made from each sample and a minimum of 100 fields of each stained blood smear were examined under oil immersion to determine parasitaemia (Figures 1 and 2).

Statistical analysis: Dogs were grouped based on age, sex, breed and season to determine whether these factors were associated with the prevalence of *B. canis*. Chi-square test and fisher's exact test were under taken. Statistical evaluations were carried out using SPSS 16.0. Differences were considered significant when $P < 0.05$.

Table 1. *Babesia canis* in urban and rural dogs of different age groups and sexes, in Ahvaz district, Southwest of Iran, 2008-2010

Age Sex	< 1 year		1- 3 years			> 6 years		
	Neg. Pos.		Neg. Pos.	Neg. Pos.	Neg. Pos.			
Male*	49	0	45	0	26	0	4	0
Female*	17	1	37	0	13	0	5	1
Male**	7	0	28	4	48	5	44	0
Female**	3	2	9	0	25	0	25	2
Total	76	3	119	4	112	5	78	3

*urban **rural

Table 2. *Babesia canis* in urban and rural dogs of different age groups and season, in Ahvaz district, Southwest of Iran, 2008-2010

Age Season	< 1 year		1- 3 years		3-6 years		> 6 years	
	Neg. Pos.		Neg. Pos.	Neg. Pos.	Neg. Pos.	Neg. Pos.		
Cold*	34	2	25	0	31	0	3	0
Warm*	32	1	59	0	6	0	6	1
Cold**	6	0	21	0	25	0	19	1
Warm**	4	0	14	4	50	5	50	1
Total	76	3	119	4	112	5	78	3

*urban **rural

RESULTS

Fifteen out of 400 blood samples (3.75%) were infected with *B. canis*. The results revealed that from 200 rural dogs, 11 samples (5.5%) and from 200 urban dogs, 4 samples (2%) were infected with *B. canis*.

Morphological characteristics of the *Babesia* in positive samples indicated that all were of the large-sized *B. canis* infection (Figures 1 and 2).

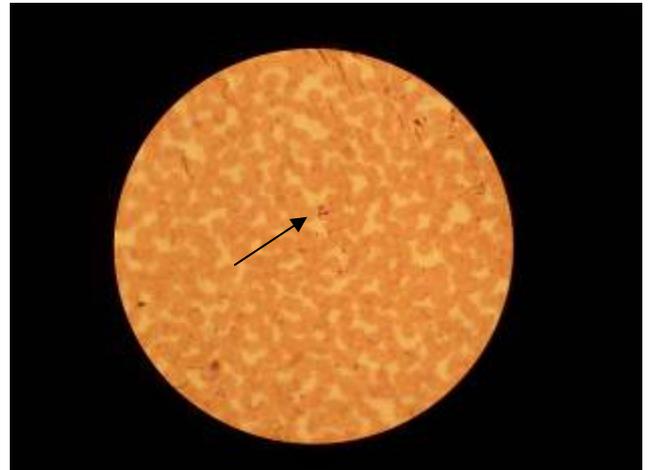


Figure 1. The presence of *B. canis* (Binary form) in thin blood smears stained with Giemsa ($\times 1000$).

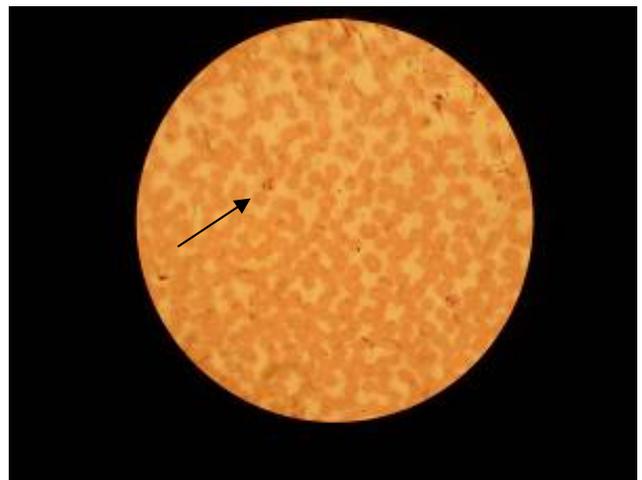


Figure 2. The presence of *B. canis* (Quaternary form= Crosslike form) in thin blood smears stained with Giemsa ($\times 1000$)

PCVs in *Babesia* positive dogs ranged from 17% to 58% and were non-specific in these *Babesia*-positive dogs. Infection rate was higher in adult dogs 3-6 years-old (4.46; 5 out of 112) compared with young's less than 3 years (3.59; 7 out of 195) and above 6 years (3.85; 3 out of 78). The infection was higher in female dogs (4.29%; 6 out of 140) than male dogs (3.46%; 9 out of 260) and in the warm season (5.15%; 12 out of 233) compared with cold season (1.80%; 3 out of 167) also, nevertheless there was not significant relationship

between sex, age and season in urban dogs regard to infection with *Babesia canis* ($P>0.05$), but significant difference was revealed between season and infection with *B. canis* in rural dogs population ($P<0.05$). The most susceptible breeds were mixed dogs, followed by German shepherd.

DISCUSSION

This is the first report in urban and rural dogs in Ahvaz district, Southwest of Iran, in which the infection rate of canine Babesiosis was evaluated and confirmed. The data collected showed that 3.75% of dogs were infected with *B. canis*. The low relatively infection of *B. canis* is in agreement with the scarcity of reports of these parasites in other regions of Iran based on morphological observations (Ashrafi *et al* 2001). There has been very limited research and few publications on Babesiosis in dogs from Iran. In a survey by Niak *et al* (1973) on blood parasites of 155 dogs and one fox (*Vulpes vulpes*) undertaken in the north of Iran, *Babesia canis* was found in only one splenectomized dog. *B. gibsoni* was found only in the single fox examined. Bigdeli *et al* (2012) noted that the seroprevalence of canine Babesiosis was 0.36% (only 1 out of 280) using PCR. Large variations in seroprevalence of infection have been found due to certain epidemiological factors, especially geographical distribution of biological vectors, the average age, lifestyle and clinical status of the examined population (Neer & Harrus 2006). Studies indicate that the incidence of Babesiosis can vary greatly between different countries and regions. The results of our study are similar to findings by Dantas-Torres and Figueredo (2006) in Brazil and different to finding by Oduye and Dipeolu (1976) in Nigeria, who found a very high prevalence of 41–53%. The differences with the findings in Iran may be due to climatic differences. Ahvaz district has a longer warm season and the unfavorable climatic conditions that result, such as high ambient temperatures, may induce ticks to undergo diapause and inhibit reproduction and questing behavior and, thus, consequently reduce chances of

infecting hosts (Maia *et al* 2007) and thus lower canine *Babesia* prevalence. Findings of annual average *Babesia* prevalence of 3.75% by microscopic examination in laboratory blood smears in this study are lower than findings by other researchers in Australia, who found *Babesia* seroprevalence of 35.7% in hospital populations (Trapp *et al* 2006). This can be attributed to the fact that serology is a more sensitive method of detection of previous, current and sub-clinical infection than microscopy and is thus likely to detect more animals as being positive, so more designed studies are needed to be done.

Babesiosis has been occurred with the highest incidence in summer in canines. Most cases were diagnosed during the spring periods. Infected dogs were found from February until the beginning of December; peak numbers occurred from September to November (Jacobson 2006). Mathe *et al* (2006) observed more cases of Babesiosis in the spring and autumn. In our survey, the prevalence of *Babesia* in clinical blood samples had not difference with that found in healthy dog survey samples. Peaks in the proportion of *Babesia*-positive dogs are observed in warm season (5.15%) compared with cold season (1.80%). This can be probably attributed to the dog breeding season, which occurs during these months; dogs are more likely to roam in search of mates and this can increase their likelihood of contact with tick vectors as well as being involved in dog fights and contacting the infection (Bashir *et al* 2009). Our study showed that the prevalence of *Babesia* in female dogs in Ahvaz was higher than in males, nevertheless the difference was not significant. This lack of sex bias concurs with the findings by other researchers (Martinod *et al* 1986) but differs from findings by others (Bashir *et al* 2009), who found that male dogs have a significantly higher prevalence than female dogs. The age distribution of dogs that were positive for *Babesia* in this study was similar to the findings of Oduye and Dipeolu (1976) in Nigeria and Bashir *et al* (2009) in Pakistan. *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 (Sandor *et al* 2006). It has also been reported that age do not have any influence on the animals' susceptibility to the disease. Non-specific or innate factors (genetic or age) possessed by the hosts can act as natural protective elements (Martinod *et al* 1986). In the present study, it was found that mid-age dogs (3-6 years) were more likely to have the infection (4.46%) than young's less than 3 years (3.59%) and above 6 years (3.85%), of-course without significant difference. It is interesting to note that certain breeds have opposite degrees of susceptibility (for instance, Spaniel vs. Beagle or Teckel vs. Pekingese). 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 breeds in Ahvaz district was mixed dogs, followed by German shepherd. The unremarkable differential PCV found in *Babesia*-positive dogs was similar to findings by other researchers (Niwetpathomwat *et al* 2006). The mean vital signs were in normal range in *Babesia*-positive dogs also. Our study only demonstrated the large-sized *Babesia*, which is similar to findings by other researchers in other areas (Abdullahi *et al* 1990; Jacobson 2006). This large-sized form, when diagnosed microscopically, is assumed to be *B. canis*, although recent evidence suggests that, other than the three known subspecies of *B. canis*, namely *B. canis canis*, *B. canis vogeli* and *B. canis rossi*, there is another distinct large form but yet unnamed (Ayoob *et al* 2010). A *Babesia* epidemiological survey will be a useful follow-up study to give an indication of the seroprevalence of *Babesia* in Iran dogs. Blood smears will have the advantage of indicating current exposure (Inokuma *et al* 2004). It will thus be desirable to do

molecular studies on the archived collected samples to also determine molecular prevalence and the exact subspecies of *Babesia* present in Iran. A combination of a serosurvey and molecular study will overcome shortcomings that arise from serosurveys or blood smear examinations alone. In conclusion, this study shows that the infection rate of *Babesia* in dogs in Ahvaz district, as determined by blood smear examination, is relatively low and that it seems to increase during the warm season particular in rural dogs population. More clinical samples and data will need to be collected and analyzed to understand the importance of Babesiosis.

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