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A study on prevalence of canine babesiosis in and around Jimma Town, Western Ethiopia

Kebede Shanko Hordofa* and Dereje Adugna

College of Veterinary Medicine, Haramaya University, Ethiopia.

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Cross sectional study was carried out from November, 2014 to March, 2015 to investigate the prevalence of canine babesiosis, associated risk factors and the species of *Babesia* affecting dogs in eight peasant associations found in and around Jimma town Western Ethiopia. Blood examination conducted on 384 randomly selected dogs showed an overall prevalence of 15.9% (61/384). The prevalence of canine babesiosis in and around Jimma town was 10.8 and 20.6%, respectively. The species of *Babesia* encountered in the current study were *Babesia canis and Babesia gibsoni which* accounted for 9.9% and 6% of the overall infection, respectively. The prevalence of canine babesia infection did not show any significant difference between dogs of different ages, origin and sex groups (*P* > 0.05 in each case). There was a significant (*P*< 0.05) association between canine *Babesia* infection to body condition score (BCS). The significant impacts of *Babesia* infection on dogs should not be neglected. Therefore, a large survey and other highly sensitive and specific molecular diagnostic tools are recommended in Ethiopia to investigate the prevalence of *Babesia* infection, and its associated risk factors in different areas of the country.

Key words: Babesiosis, canine, Ethiopia, Jimma, prevalence.

INTRODUCTION

Babesiosis is a tick borne blood protozoan disease of domestic and wild animals, which occurs in the southern USA, central and South America, Africa, Asia and Europe. *Babesia* species are tick-transmitted apicomplexan parasites that infect a wide range of vertebrate hosts. The identification of individual species has traditionally been based on the host specificity and on the morphology of the intra-erythrocytic forms (piroplasms) (Taboda, 1998; Uilenberg, 2006).

Members of the genus *Babesia* readily parasitize the red blood cells of dogs. Canine *Babesia* are morphologically classified into large (3.0 to 5.0 µm) and small (1.5 to 2.5 µm) forms, both exhibiting a worldwide distribution. *Babesia canis* , *Babesia vogeli* and *Babesia rossi* are large *Babesia* spp. detected in the USA while *Babesia gibsoni* and *Babesia annae* are small *Babesia* spp. that have been documented to infect dogs (Birkenheuer et al., 2004**).** The three main species of large *Babesia* are antigenically distinct, transmitted by different vectors and differ widely in pathogenicity and geographic distribution (Uilenberg et al., 1989).

B. vogeli is the least pathogenic. It occurs in France,

*Corresponding author. E-mail: sh.kebeko@yahoo.com.

Australia, Japan, Brazil, South Africa and the USA, and usually causes mild disease in adult dogs, but severe disease in some puppies (Matjila et al., 2004). *B. rossi* occurs predominantly in southern Africa, and is ostensibly the most virulent of the subspecies. Improved polymerase chain reaction (PCR) techniques have lately allowed for better definition of these parasites (Matjila et al., 2008). The smaller parasite, *B. gibsoni* occurs principally in the Middle East, southern Asia, Japan, Africa, and South America and is an emerging infectious disease in the USA, as well as having been detected lately in Italy, Hungary and Australia (Muhlnickel et al., 2002). A more virulent subspecies of *B. gibsoni* has recently been identified in California. A *Babesia microti* like piroplasma, *B. annae* (also known as *Theileria annae*) has been found to be endemic in dogs in northwest Spain (Camacho et al., 2003).

Various species of ticks such as *Rhipicephalus sanguineus*, Dermacentor *spps*, *Haemaphysalis ellipticum* can transmit the large *Babesia* of dogs, whereas *B. gibsoni* is transmitted by *Haemaphysalis bispinosa* and *Haemaphysalis longicornis*. *Babesia annae* is thought to be transmitted by ixodes hexagonus (Lobetti, 2006). Both transtadial and transovarial transmission can occur, and ticks are believed to remain infective for several generations. Babesia spp. can also be transmitted by blood transfusion. Strong circumstantial evidence exist that *B. gibsoni* is transmitted by dog bites (Birkenheuer et al., 2005), whilst transplacental transmission from dam to offspring has recently been proven as an additional mode of transmission (Fukumoto et al., 2005).

A small subset of dogs presents with high haematocrits (relative haemo concentration), despite vigorous haemolysis, due to presumed shifting of fluid from the intravascular to the extra vascular component. These dogs are at increased risk of developing ARF or cerebral complications, as well as other organ failures (Welzl et al., 2001).

Large surveys on canine babesiosis are scarce, but numbers of reports suggest that the parasite infects dogs worldwide. In India, a variable prevalence of canine babesiosis has been reported viz. 0.66 to 8.9% in referral clinics canines in Uttar Pradesh (Chaudhuri, 2006); 21.7% in Assam (Chandhuri and Varshney, 2007), 5.4% in Hissar, Haryana (Bansal et al., 1985), and 3.17% of *B. gibsoni* and 1.26% *B. canis* in Punjab (Eljadar, 2010). However, the prevalence of canine babesiosis in the study area is not yet known. Therefore, this study is crucial to know the status of the disease and its associated risk factor, and even tremendously very important to take measures to control the disease in the study area. The objectives of the study were:

1. To investigate the prevalence of canine babesiosis in and around Jimma town.

2. To assess major associated risk factors of canine babesiosis.

MATERIALS AND METHODS

Study area

The study was conducted in Jimma zone, southwestern part of Ethiopia at Jimma town. Jimma town is the capital city of Jimma zone located in Oromia region. It is located 352 km south west of Addis Ababa at latitude of about 7°13' to 8°56'N, and longitude of about 35°52' to 37°37'E and at elevation ranging from 880 to 3360 m above the sea level. The study area receives a mean annual rain fall of about 1530 mm, which comes from the long and short rainy seasons. The annual mean minimum and maximum temperature during the study period were 12.7 and 26.7°C, respectively. The study was conducted in eight kebeles/Peasant associations such as Furstale, Bosa kitto, Sato samora, Ifa bula, Dedo dima, Bebella kera, Gudeta bula and Doyo bikila.

Study animals

The study was conducted on dogs randomly selected from four kebeles in Jimma town: Furstale, Bosa kitto, Sato samora and Ifa bula and other four PAs around Jimma town such as: Dedo dima, Bebella kera, Gudeta bula and Doyo bikila from November, 2014 to March, 2015.

Study design

A cross-sectional (observational) study was conducted from November, 2014 to March, 2015 to determine the prevalence of babesiosis in the study animals. The animals were selected randomly and restrained by owners for sampling. Blood sample was collected from cephalic vein, collected into heparinized tubes and examined under with light microscope.

Sampling method

Sample size determination

384 samples were collected from the study area by using simple random sampling methods and 95% confidence interval with required 5% precision, the sample size was determined by the formula of Thrusfield (1995).

$$n = \frac{1.96^2 P_{exp}(1-P_{exp})}{d^2}$$

Where; n = required sample size, Pexp = expected prevalence, d = required precision

The expected prevalence of canine babesiosis was 50% to get the maximum number because there was no previous work of canine babesiosis in the study area. The precision was decided to 5 (0.05) to 95% confidence level. By substituting the value in the above formula, the study got the sample size:

$$n = \frac{1.96^2 \times 0.5(1-0.5)}{(0.05)^2}$$

= 384 dogs

Sampling strategy

A total of 384 samples were collected during the study period from animals in and around Jimma town by using simple random sampling. The species of animals sampled were only dogs of any age. Blood samples were collected randomly from dogs, thin blood smear was made on clean slide and PCV determination was performed. The collected blood samples were put in iceboxes box and were transported to Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) veterinary parasitology for immediate laboratory examinations.

Study methodology

Blood examination

Blood samples were collected aseptically from cephalic vein in vials containing anticoagulant (EDTA and study design above). A thin blood smear was prepared for each sample, a drop of blood was placed on a clean glass slide, air dried, fixed in methanol, stained with Giemsa (Coles, 1986) and examined under light microscope by using the oil immersion objective to identify and to examine the morphology. *Babesia* species were identified by morphological characteristics using thin blood smear.

Statistical analysis

The data were collected from the study area, result obtained from blood examination was recorded in the format developed for this purpose and later on entered into Microsoft Excel 2007. Dogs were grouped based on age, sex, origin and body condition to determine whether these factors were associated with the prevalence of canine babesiosis. Statistical evaluations were carried out using statistical package for the social sciences (SPSS) 20.0 and the mean PCV of infected and non-infected dog was compared using independent T-test at 95% confidence level ($p<0.05$). Differences were considered significant when $p < 0.05$.

RESULT

Blood examination conducted on 384 randomly selected dogs showed prevalence of babesiosis of 15.9% (61/384).The prevalence of canine babesiosis was not statistically significant association with sex and age ($p>0.05$) but a statistically significant association was seen ($p<0.05$) with body condition score (BCS) of dogs (Table 1). The distribution of *B. canis* and *B. gibsoni* were identified in each kebeles (Table 2). The prevalence of canine babesiosis in eight PAs is shown in Table 3. This difference in prevalence was statically not significant ($p>0.05$) (Table 3). To assess the relationship between *babesia* infection and packed cell volume (PVC) determination using hematocrit and also the mean PCV of infected and non-infected dogs were calculated. The association of infection with anemia was found to be statistically significantly associated ($p<0.05$) (Table 4).

DISCUSSION

This is the first report in dog in and around Jimma town, in which infection rate of canine babesiosis was evaluated and confirmed. From a total of 384 blood samples collected from dogs, 61 samples were positive for canine babesiosis with light microscopy. The data collected show that the overall prevalence of canine babesiosis based on blood smear evaluation was 15.9 % in and around Jimma town. The result of this study is different from findings by Oduye and Dipeolu (1976) in Nigeria, who found a very high prevalence of Canine babesiosis (47%).

In this study the prevalence of babesia in female dogs (18.12%) in and around Jimma town was not significantly higher than in male dogs (14.47%) (Table 1). This means both male and female are equally susceptible and are equally exposed to the disease. This finding differs from finding by others (Bashir et al., 2009), who found that male dogs have significantly higher prevalence than female dogs.

Babesia can infect dogs of all ages thus in the present study, it was found that young dogs ($\leq$ 1 year) have prevalence rate of 12.37%, while the infection rate of adult (>1 and $\leq$3 years) and old (>3 years) dogs were 19.04 and 20.93% respectively (Table 1). In this study dogs that were $\leq$ 1 year were affected by *Babesia canis* (11.22 %) while *Babesia gibsoni* affects adult (6.8 %) and old dogs (11.63 %). The age distribution of dogs that were positive for babesia in this study was different from the finding of Oduye and Dipeolu (1976) in Nigeria and Bashir et al. (2009) in Pakistan (which the prevalence was 3.59, 4.46 and 3.85% in young, adult and old dogs, respectively). This might be due to the habit formed by the young animals of playing on the grasses around where they pick up a waiting tick that was ready to attach itself to scavenging host as observed during the study.

There was a significant difference in prevalence rate among poor and good body condition that is the result observed revealed the marked effect of babesiosis on body condition of dogs (Table 1). The prevalence of canine babesiosis in Dedo dima, Bebella kera, Gudeta bula, Doyo bikila, Furstale, Sato samora, Bosa kitto and Ifa bula were 20.45, 23.64, 20.83, 17.31, 14.04, 2.94, 2.56 and 18.18%, respectively (Table 3). Based upon this, the prevalence of canine babesiosis was higher in Bebella kera and lower in Bosa kitto kebele. Peaks in the proportion of babesia positive dogs were observed in around Jimma town (20.6 %) compared with in Jimma town (10.81 %). This can be due to different reasons. In Jimma town most of the dogs rather than stray dogs were kept in door, while the dogs found around Jimma town 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 contracting the infections (Bashir et al., 2009).

During the study period, the mean PCV of infected and non-infected dog was compared using student t-test at 95% confidence level ($p<0.05$). Dogs with PCV value <37 % were considered as anemic and those with PCV value

Table 1. Prevalence of canine babesiosis with sex, age and body condition score.

Risk factor		No. examined	No. of positive	Prevalence (%)	X^2	P-value
Sex	Male	235	34	14.47	0.911	0.340
	Female	149	27	18.12	-	-
	Total	384	61	15.9	-	-
Age	Young	194	24	12.37	-	-
	Adult	174	28	19.04	3.712	0.156
	Old	43	9	20.93	-	-
	Total	384	61	15.9	-	-
BCS	Poor	180	53	29.44	46.619	0.000
	Good	204	8	3.92	-	-
	Total	384	61	15.9	-	-

Table 2. Prevalence of *B. canis* and *B. gibsoni* in eight peasant associations.

Origin/Kebele	Species of *Babesia* (n= 61)		Total
	B. canis	*B. gibsoni*	
Dedo dima	3	6	9
Furstale	4	4	8
Sato samora	1	0	1
Bosa kitto	1	0	1
Ifa bula	7	3	10
Bebella kera	9	4	13
Gudeta bula	6	4	10
Doyo bikila	7	2	9
Total	38 (62.3%)	23 (37.7 %)	61 (100%)

Table 3. Prevalence of canine babesiosis in different origins.

Origin/Pas	No. examined	No. positive	Prevalence (%)	X^2	P-value
Dedo dima	44	9	20.45	-	-
Furstale	57	8	14.04	-	-
Sato samora	34	1	2.94	-	-
Bosa kitto	39	1	2.56	13.925	0.053
Ifa bula	55	10	18.18	-	-
Bebella kera	55	13	23.64	-	-
Gudeta bula	48	10	20.83	-	-
Doyo bikila	53	9	17.31	-	-
Total	384	61	15.9	-	-

≥37% were taken as normal (Kamani et al., 2011). The result of the present study showed that a mean PCV of 30.56 and 42.45% for infected and noninfected dogs respectively. It was generally accepted that PCV value is affected by many factors other than babesiosis. However, these factors likely affect both infected and non-infected dogs. The PCV values for positive and negative samples were compared and the result was found statistically strongly associated. The mean PCV value of studied animals was statistically significantly different ($p<0.05$)

Table 4. Association of the mean PCV of dogs with Babesia infection.

Condition	PCV range (%)	Mean PCV (%)	SD	CI	T-test	P-value
Infected	<37	30.56	4.786	10.55-13.23	17.18	0.000
Non-infected	37-55	42.45	4.988	10.53-13.25	-	-

between positive and negative dogs (Table 4). The prevalence of infected dog with PCV value <37% is 15.9%, while the prevalence of non-infected dog with PCV value <37% is 2.08%. The mean PCV value of the infected dogs was significantly lower than those of non-infected dogs. This finding agrees with the works of Kamani et al. (2008) and Shitta (2009) who observed a lower mean PCV value in infected dogs than non-infected dogs. The appearance of babesia negative dogs with PCV value <37% may due to inadequate detection method, other hemoparasite disease, helminthes parasite and delayed recovery of anemia after treatment.

There has been very limited research and no publications on babesiosis from Ethiopia. Finding of annual average babesia prevalence of 15.9% by microscopic examination in laboratory blood smear in this study is lower than findings by other researchers in Australia, who found babesia seroprevalence of 35.7% (Trapp et al., 2006). This can attributes to the fact that serology is more sensitive method of detection of previously, current and sub clinical infection than microscopic examination.

CONCLUSION AND RECOMMENDATIONS

The present study revealed that canine babesiosis is a major problem in the study area, and the result confirms the disease was more common around Jimma town rather than in Jimma town. The prevalence of Canine babesiosis was associated with several risk factors. Canine babesia infection was more likely associated with body condition of dogs. Those dogs affected by babesia parasite become poor in body condition, and also they become prone to anemia. The study also concludes that *B. canis* and *B. gibsoni* were the two species of babesia identified in the study area, strategic tick control should be practiced at the study area. Also the following recommendations are forwarded:

1. Since there was no publication on canine babesiosis in Ethiopia, a large survey is necessary in Ethiopia to investigate the rate of *babesia* infection and its associated risk factors in different areas of the country.
2. Detection of *babesia* parasite by serology and molecular examination should be more sensitive to find the parasite in blood.
3. Strategic tick control should be done to prevent canine babesiosis.
4. The stray dogs should be controlled so as to know the sample frame of the study animals in the study area.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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