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ORIGINAL RESEARCH ARTICLE

PREVALENCE OF TICK BORNE BABESIOSIS IN ANIMALS OF DISTRICT SIALKOT, PAKISTAN

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ABSTRACT

Keeping in view the relevance of tick borne diseases to the economic losses to livestock sector, the present study was conducted to evaluate the prevalence of infection caused by *Babesia* parasite in District Sialkot. Total 150 blood samples were collected from suspected host animals. Blood samples were analyzed by light microscopy after staining of blood smears. Total 50 animals (33%) were found to be infected with three species of genus *Babesia* including *Babesia bigemina*, *Babesia bovis* and *Babesia divergens*. Among all three detected species *B. bigemina* showed highest prevalence (54%) followed by *B. bovis* (36%) and *B. divergens* (10%) respectively. Prevalence of infection was highest in cows (37.5%) as compared to Buffaloes (15%) and Sheep (20%). The highest number of positive samples was collected during summer season followed by spring, autumn and winter seasons respectively.

INTRODUCTION

Babesiosis is one of the most prevalent disease of animals which is transmitted through ticks (Jabbar *et al*., 2019). This disease is fatal for the output of livestock sector in Asian countries including Pakistan (Abbas *et al*., 2021). A huge loss of crossbred cow takes place due to tick borne infections including babesiosis (Baqir *et al*., 2021; Yousaf *et al*., 2017). Domestic animals including goats, horses, dogs, cats and sheep are also affected by babesiosis (Ahmad *et al*., 2014). Babesiosis is caused by a pathogenic parasite from genus *Babesia* which causes both chronic and acute infection in livestock animals (Babar *et al*., 2021). There are about 100 species in genus *Babesia* but mortality and morbidity in livestock sector is significantly caused by *Babesia bovis* and *Babesia bigemina* (Babar *et al*., 2021; Baloch *et al*., 2021). Signs and symptoms of babesiosis include fever, anemia, hemolysis, jaundice and hemoglobinuria (Baqir *et al*., 2021). Babesia parasite divides rapidly in erythrocytes leading to their massive destruction and it can cause death in case of advanced disease in animals due to below than 20% packed cell volume (Hussein *et al*., 1991). This may cause the death of the animal within a few days during which the packed red cell volume falls below 20%. Babesiosis also has the potential to become zoonotic (Naazir *et al*., 2021). Human beings are infected by *Babesia divergens* and *Babesi microti* species of genus *Babesia* (Baqir *et al*., 2021). Main vectors for transmission of babesiosis belongs to genus *Rhipicephalus* (Panhwar *et al*., 2021). Ticks transmit both *B. bovis* and *B. bigemina* from diseased animals to healthy animals (Jamali *et al*., 2021). Tick vectors are significantly distributed in countries present in tropical and subtropical regions particularly Pakistan, Bangladesh and India because this region provides favorable temperature and climate for development and growth of ticks (Bilawal *et al*., 22021; Yousaf *et al*., 2021). It is necessary to diagnose and treat babesiosis to reduce the risk of economic and medical losses to livestock sector (Herrera *et al*., 2017; Soomro *et al*., 2021). Several reports on prevalence of babesiosis have been published from different parts of Pakistan but the prevalence from district Sialkot has not been published so far. Therefore, this study was conducted to examine the prevalence of babesiosis in different host animals including cows, buffaloes and sheep from district Sialkot, Pakistan.

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METHODOLOGY

A total of 150 blood samples were collected in EDTA coated tubes, from different suspected animals exhibiting symptoms of babesiosis. Out of these 150 blood samples, 120 samples were from Cows, 20 from buffalos and 10 were from sheep. After collection blood samples were stored in ultralow temperature freezer at -80°C.

Microscopic examination

Microscopic examination of collected blood samples was performed for the identification of infected samples and to find out the morphological differences among

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different species of genus *Babesia*. Field stain was used for the staining of thin blood smears. Thin blood smear was prepared by placing a drop of blood on a clean slide and by placing the edge of another clean slide on drop at an angle of 45º to spread the drop on slide. It was allowed to air dry for 15 minutes. For fixation, smear was treated with methanol and stained by field staining. Finally, the stained slides were observed under oil immersion lens (100X objective) of light microscope.

Statistical analysis

For statistical analysis IBM SPSS statistics version 21 software was used. One sample T-test was used to perform statistical analysis for all the parameter included in this study.

RESULTS

Overall Prevalence

The present study was conducted from January 2018-June 2019 in different areas of District Sialkot. A total 150 blood samples were examined in District disease diagnostic laboratory, Sialkot. Out of 150 samples, 50 samples were detected as positive which depicts an overall rate of 33% (Table 1).

Table 1: Prevalence of babesiosis in different host animals.

Host Animal	Total blood samples	Positive blood samples	Prevalence (%)	P-value
Cow	120	45	37.5%	0.071
Buffalo	20	3	15%	
Sheep	10	2	20%	
Total	**150**	**50**	**33%**	

Prevalence of Babesiosis in different Host Animals

Out of 50 positive samples 45 positive samples were from Cows, 3 samples were from Buffalo and 2 samples were from Sheep which indicated the highest prevalence in Cows. Overall there was a non-significant difference in prevalence of babesiosis in different host animals (P>0.05).

Prevalence of Different species of *Babesia*

In the present study *B. bigemina* (Figure 1), *B. bovis* (Figure 2) and *B. divergens* (Figure 3) species of genus *Babesia* were identified by microscopic examination. Trophozoites of different *Babesia* species were identified by morphological keys provide by (Levine, 1961). Species wise rate of prevalence is given in (Table 2) according to which *B. bigemina* was the prevalent specie of genus *Babesia* with a prevalence of 54% followed by 36% of *B.bovis* and 10% for *B. divergens*. Overall there was a non-significant difference in prevalence of different *Babesia* species (P>0.05) (Table 2).

Table 2: Species wise prevalence of *Babesia* in collected samples.

Babesia species	Total no. of positive samples	Positive samples	Rate (%)	P-value
B.bigemina	50	27	54%	0.121
B. Bovis	50	18	36%	
B.divergens	50	5	10%	

Seasonal Prevalence of *Babesia*

Different number of samples was collected in four different seasons which shows the different rate of prevalence in all seasons as shown in (Table 3). During sample collection it was also identified that more cases of babesiosis were seen in summer season followed by spring season, but the number of cases was gradually decreased with decreasing temperature as in autumn and winter season (Fig 4). Overall, there was a non-significant difference in prevalence in different seasons (P>0.05) (Table 3).

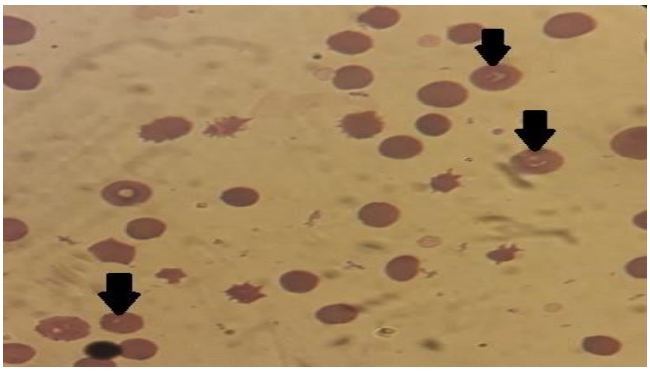

Figure 1: Trophozoites of *B. bigemina* inside RBCs (Black arrows)

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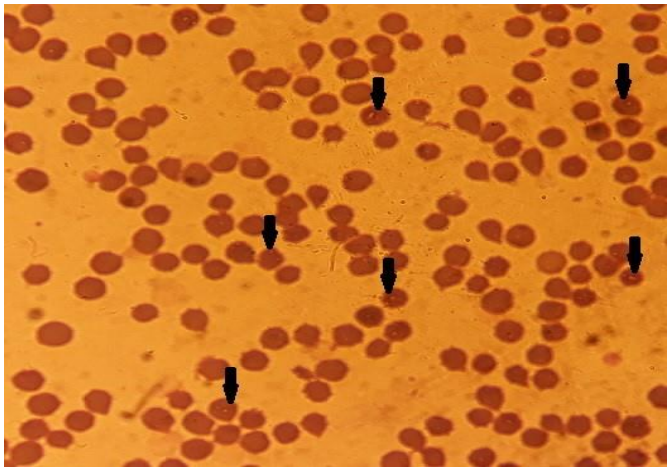

Figure 2: Trophozoites of *B. bovis* inside RBCs

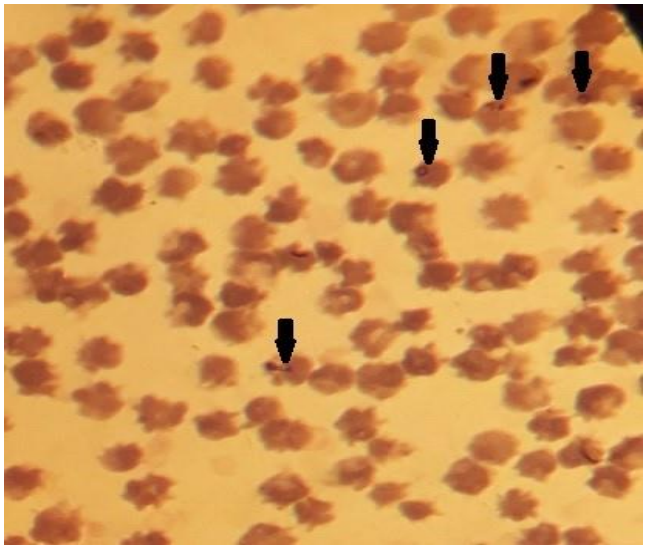

Figure 3: Trophozoites of *B. divergens* inside RBCs

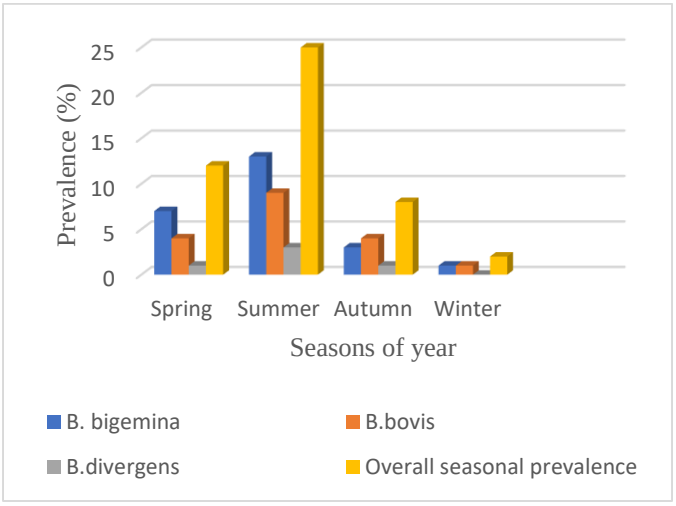

Figure 4: Variation in number of infected samples in different seasons

Table 3: Overall prevalence of *Babesia* species in different seasons of year.

***Babesia* species**	**Spring**	**Summer**	**Autumn**	**Winter**	**P-value**
B.bigemina	7	13	4	2	0.072
B.Bovis	4	9	5	1	
B.divergens	1	3	1	0	
Total	12	25	10	3	
	24%	50%	20%	6%	

DISCUSSION

In present study out of 150 animals, 45 positive samples were identified from cow with 37.5% infection, while the prevalence (%) in buffalo and sheep was 15% and 20%, respectively. Almost similar results were reported by (Mahaveer *et al*., 2022) in different areas of Sindh and by (Irshad *et al*., 2010) in Southern Punjab, who reported the 35.46% and 35% rate of prevalence respectively. Another study conducted by (Hussain *et al*., 2019; Yousaf *et al*., 2017) in Khyber Pakhtunkhwa showed 61% prevalence. A study carried out by (Khattak *et al*., 2017) in cattle of Afghan refugees in Mohmand Agency revealed an overall prevalence of 10%. This greater frequency in District Sialkot can be attributed to the warm and humid climatic conditions which favors the development and growth of tick vectors (Khan *et al*., 2021; Yousaf *et al*., 2021).

The rate of prevalence was also determined for different host species. It showed that the prevalence of babesiosis was higher in cows (37.5%) as compared to buffaloes (15%) and sheep (20%). Similar results of incidence of babesiosis in cattle were reported in Pakistan by (Afzal *et al*., 1991; Afzal *et al*., 1999 and Khan *et al*., 2005). Comparable results have also reported from other countries of world by (Gueye *et al*., 1994; Lima *et al*., 1999; Sharma and Bansal, 1984 and Metenawy. The results revealed that there was a high incidence of disease in cows which are more susceptible to disease than sheep and buffaloes in Pakistan and other countries (Fereig *et al*., 2017; Shams *et al*., 2013 and Jabbar *et al*., 2015).

In this study species wise prevalence was also studied which revealed the rate prevalence of *B.bigemina* was 54% and that of *B. bovis* and *B.divergens* was 36% and 10% respectively. A study conducted by (Khattak *et al*., 2017) has also reported the species wise prevalence as 6.05% for *B. bovis* and 3.94% for *B. bigemina.* Similarly, many other studies from Pakistan by (Khan *et al*., 2005; Afzal *et al*., 1991) have also reported the occurrence of B. bovis and B. bigemina in cattle of Pakistan

Rate of infection was also studied in different seasons (Khan *et al*., 2021; Yousaf *et al*., 2021). Prevalence of infection was highest during summer which was followed by the rate of infection in spring season, autumn season and winter season where the prevalence was lowest. This is because there is a linear relationship between increased temperature and increased activity of ticks which may results in the increased rate of infection during summer season and increased humidity is also associated with the increased rate of infection (Mathan *et al*., 2020; Zhou *et al*., 2019).

CONCLUSION

Babesiosis is a prevalent tick borne disease in district Sialkot. Cows are at high risk of becoming infected with babesiosis. This infection is most prevalent in summer season with highest number of infections leading to the great losses to economy due to high mortality rate of infected animals.

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