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DETECTION OF BABESIOSIS AND IDENTIFICATION OF ASSOCIATED TICKS IN CATTLE

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Abstract: Babesiosis is a worldwide disease of live stock and different animal like sheep, pigs, horses and dogs. It is the cause of zoonotic disease in humans and in cattle transmitted by ticks worldwide and having different pathogenecities and clinical features. Keeping in views the importance of the disease, the present study was designed to determine the prevalence of babesiosis in cattles of Charsadda and Sawabi districts and to identify the associated ticks in Cows and Calves. For this study 100 ticks and 100 blood samples were collected from different Cows and Calves randomly.

Key Words: Babesiosis, *Babesia bigemina, Babesia bovis*, Giemsa stain and vector.

INTRODUCTION

Babesiosis is a worldwide disease of live stock and has economic importance due to heavy mortality and morbidity. *Bebasia spp.* is a protozoan intra erythrocytic parasites belonging to the class Apicomplexa. More than 100 species have been described so far, primarily based on morphological characteristics and the *Babesia spp* are strictly host specific (Palmer 2002). Babesiosis is transmitted by ticks in cattle worldwide having different pathogenecities and clinical features (Vial & Wright 1998). *B. bigemina* is more common than *B. bovis* but generally *B. bigemina* is less virulent than *B. bovis* and *B. divergens.* The percentage of erythrocytes (parasitaemia) in circulatory blood is less than 1% in acute cases. By comparing *B. bigeminia* to *B. bovis*, the parasitaemia exceed than 10% and may be sometime high than 30%.The symptoms of *B. bigeminia* mainly include fever, anemia, haemoglobinuria (Zintl *et al.,* 2003). But after infected with *Babesia* species some claves show uncommon medical symptoms of the disease (Bock *et al.,* 1998).

B. bovis and *B. bigemina* cause financially loss in the livestock industry in Africa, South America and Asia (Brockelmn 1989). Babesiosis caused by *B. bovis* is less sensitive to some *Babesia*cidal compounds making it hard to treat the infected animals (Tan & Sarathaphan 1991). In tropics and sub tropics regions both *B. bovis* and *B. bigeminia* are found usually transmitted by Boophilus ticks, while in Europe and Mediterranean, it is primarily transmitted by Ixodes ricinus ticks (Goff *et al.,* 2003). Keeping in views the importance of the disease the present study was designed to determine the prevalence of babesiosis in cattle of Charsadda and Swabi districts and to identify the associated ticks in Cows and Calves.

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MATERIALS AND METHODS

Blood and ticks samples were collected randomly from cows/calves in district Charsadda and district Swabi, Khyber Pakhtunkhwa, Pakistan. The study was carried out from January, 2010 to December, 2011 after obtaining ethical and moral approval from the Research and publication committee KUST, Kohat. Questionnaire was also designed to obtain the desired information in the studying area particularly about the ticks species, sex, age, localities and date.

Ticks collection

A total of 100 Ticks were randomly collected from cows and calves from the study area in small sterilized bottles for identification and brought to the Molecular Parasitology and Virology Laboratory, Department of Zoology, KUST, Kohat and were kept at 4°C. Then they were identified on the basis of external morphology described by Walker *et al.,* (2000) (Walker *et al.,* 2000), observing under microscope at 4X and 10X.

Blood sample collection

A total of one hundred blood samples were collected randomly from cows and calves from their jugular vein in sterilized vacutainer containing 0.25ml to 1ml and placed in ice jar and transported to the laboratory and were kept at 4°C until further process.

Microscopic blood smears examination

The thick and thin smears of the blood samples were made on the new particularly labeled glass slides. The dried blood smears were fixed in absolute methyl alcohol for one minute. Staining was performed using Giemsa Stain as described by

Benjamin (1986), (Benjamin 1086) i.e. the glass slides bearing thick and thin blood smears were stained with one fourth of dilution of commercially available Giemsa stain for four minutes and observed under oil immersion at 100X objective to detect the presence of *B. bigemina* and *B. bovis*. The measurements of the parasites were performed by micrometry using stage and ocular micrometers at 100X having a conversion factor of 1.0µm per unit space scale as described by Foreyt (2001) (Forety, 2001).

Prevalence rate

Prevalence rate was determined with the help of the following formula

P. Rate = No. of positive samples / No of total samples x 100

Table 1: Prevalence of different ticks in district Charsadda and Swabi

Ticks	No of Ticks in Charsadda n=50 (%)	No of Ticks in Swabi n=50 (%)	Total n=100 Over all percentage (%)
Hyalomma	19(38)	21(42)	40 (40)
Boophilus	15(30)	19(38)	34(34)
Heamphylas	13(26)	7(14)	20(20)
Rhipicephalus	3(6)	3(6)	6(6)

(%) showing percentage, n showed total number P<0.05 significant

RESULTS

During the study the blood smear examination showed overall 30% samples infected with babesiosis out of which 19% samples were positive for *B. bigemina* and 11% were positive for *B. bovis*. In district Charsadda the prevalence rate of *B. bigemina* was 18% while in Swabi district, it was 20%. Similarly the prevalence rate of *B. bovis* in district Charsadda was 14% and in district Swabi it was 8%. In Harichand the highest rate was recorded as 20% for *B. bovis* and in Umarzai 25% for *B. bigemina*, district Charsadda. Similarly in Turlandi, the locality of district Swabi the highest prevalence rate for *B. bigemina* was 27.27% and for *B, bovis* was 9.09%. The ticks i.e vectors of babesiosis showed that the prevalence of Hyalomma tick was highest (40%) followed by Boophilus (34%), Haemaphysalis (20%) and Rhipicephalus (6%) and for all of the results, the p<0.05 was considered significant during statistical analysis.

Identification and prevalence of ticks in district Charsadda and district Swabi

Ticks were identified on the basis of morphology as by Walker *et al.*, (2000) [8]. During the study n=100 specimens of ticks were identified. *Hyalomma* sp. was found 40%, *Boophilus* sp. 34%, *Haemaphysalis* sp. 20% and *Rhipicephalus* sp. was found 6% in district Charsadda and Swabi as shown in Table.1. In district Charsadda *Hyalomma* sp. were found 19/50 (38%), *Boophilus* sp. 15/50 (30%), *Heamphysalis* sp. 13/50 and Rhipicephalus 3/50 in Charsadda, While in Swabi *Hyalomma* sp. were 21/50 (42%), *Boophilus* sp. 19/50 (38%), *Heamphysalis* sp. 7/50 (14%) and *Rhipicephalus* sp. were 3/50 (6%). The ticks were found especially on the ventral side, head and upper parts of the legs, which were collected with the help of forceps for identification.

Area wise prevalence of nymphs (ticks) and adults ticks infected with Babesia sp. in district Charsadda and district Swabi

A total of 25 nymphs and 25 adult ticks were collected from different areas of district Charsadda, and Swabi in which 36% and 60% of nymphs and adult ticks were found positive for *Babesia spp.* respectively as cleared from Table 2.28% of nymphs and 48% of adult ticks were found infected with *Babesia* spp. collected from different localities of district Swabi. In the different localities of district Charsadda the prevalence rate of infected nymphs and infected adults were different. The prevalence rate of the infected nymphs in umarzai, Dakhi, Hrichand were 62.5%, 33.33% and 18.18% respectively while the prevalence of infected adults were 50, 66% and 20% respectively in the mentioned areas.

Table 2: Infected Nymphs and Adult (ticks) %age in districts Charsadda and Swabi

Districts Charsadda			Districts Swabi		
Locality	Infected Nymphs/n (%)	Infected Adults/n (%)	Locality	Infected Nymphs/n (%)	Infected Adults/n (%)
Umarzai	5/8 (62.5)	6/12 (50)	Dagai	5/11 (45.45)	4/12 (30.76)
Dakhi	2/6 (33.33)	4/6 (66)	Shaigh jana	2/5 (40)	02/5 (40)
Dairy farm Jamal Abad	0	0	Turlandi	0/4 (0)	03/4 (75)
Harichand	2/11 (18.18)	5/7 (20)	Taraki	0/5 (0)	3/3 (100)
Total	9/25 (36)	15/25 (60)	Total	7/25 (28)	12/25 (48)

n=total number, (%) percentage, *p*<0.05 significant

The prevalence rate of the infected nymphs in different localities of district Swabi i.e Dagai, contain 45.45% Shaigh Jana 40% while Turlandi and Taraki have no infected nymphs. Similarly the infected adult ticks were found 30.76% in Dagai, 40% in Shigh Jana, 75% in Turlandi and the Taraki has 100% which was the highest rate of prevalence of the infected adult ticks.

Area wise prevalence of Babesiosis in cattle

A total of 100 blood samples from cows and calves were collected from district Charsadda and Swabi. Through microscopic study the blood smear slides were examined. Out of 100 blood samples 30% were found positive for *Babesia spp*. A total of 11% samples were positive for *B. bovis* (Fig. A) and 19% were positive for *B. bigemina* (Fig B).

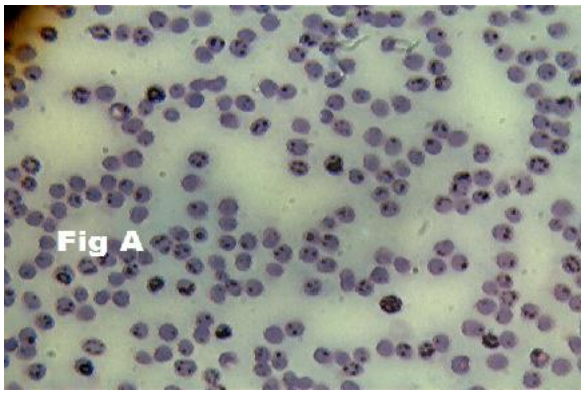

Figure A: Cattle's (cows and calves) blood infected with *B. bovis*.

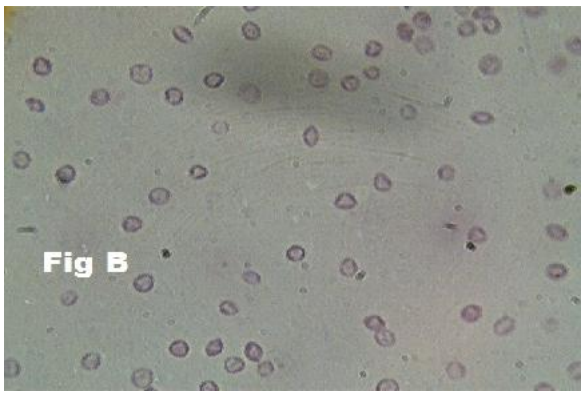

Figure B: Cattle's (cows and calves) blood infected with *B. bigemina*.

In different localities of district Charsadda, the prevalence rate of *B. bigemina* and *B. bovis* was 20% and 10% respectively in Umarzai 25% and 8.33% in Dakhi, 13.33% and 20% in Harichand. In Dairy farm Jamal Abad, the both species contained 15.38%.

Similarly in different areas of district Swabi the prevalence rate of *B. bigemina* and *B. bovis* were 21.23% and 7.14 % respectively in Dagai, 16.67% and 8.33% in Shaigh Jana, 27.27% and 9.09% in Turlandi and 15.38% and 7.69% in Taraki as shown in Table. 3.

Table 3: Area wise prevalence of babesiosis in districts Charsadda and Swabi

Districts Charsadda			Districts Swabi		
Locality	***B.bigemina* /n (%)**	***B. bovis*/n (%)**	**Locality**	***B.bigemina* /n (%)**	***B. bovis*/n (%)**
Umarzai	2/10 (20)	1/10 (10)	Dagai	3/14(21.23)	1/14 (7.14)
Dakhi	3/12 (25)	1/12(8.33)	Shaigh jana	2/12 (16.67)	1/12 (8.33)
Dairy farm Jamal Abad	2/13 (15.38)	2/13 (15.38)	Turlandi	3/11 (27.27)	1/11 (9.09)
Harichand	2/15 (13.33)	3/15 (20)	Taraki	2/13 (15.38)	1/13 (7.69)
Total	9/50 (18)	7/50 (14)	Total	10/50 (20)	4/50 (8)

n=total number, (%) percentage, $p<0.05$ significant

Prevalence of B. bovis and B. bigemina in different age groups of cattle

To know the relation of babesiosis with age, cows were divided into four age groups (I-IV). Over all prevalence of babesiosis in out of 35 cows (calves) in age group I, was (42.8%) and out of the 29 cows of age group II, 9 (31.04%) were detected positive for babesiosis. In age group III, out of 21 samples, 4 (19%) were positive, while in age group IV (>6 years), 2 (13.33%) cows were positive for babesiosis.

Out of 35 cows (calves) in age group (I), 6 (17.14%) were positive for *B. bovis* and 9 (25.71%) were positive for *B. bigemina*. Of the 29 cows of age group (II), 4 (13.79%) were detected positive for *B. bovis* while 5 (17.24%) were found positive for *B. bigemina*. In age group (III), out of 21 samples, 1 was positive for *B. bovis* and 3 were positive for *B. bigemina*, while in age group (IV), no sample was found positive for *B. bovis* while 2 were positive for *B. bigemina* which are shown in Table 4.

Table 4: Prevalence of *B. bovis* and *B. bigemina* in different age groups of the cattles (Cows and calves).

Groups	Age (Years)	Positive/Total No. (%)	Total B.bovis Positive (%)	Total B.bigemina Positive (%)
I	<1	15/35 (42.8)	06/35 (17.14)	09/35 (25.71)
II	1>3	09/29 (31.04)	04/29 (13.79)	05/29 (17.24)
III	3>6	04/21 (19)	01/21 (4.76)	03/21 (14.28)
IV	>6	02/15 (13.33)	0/15 (0)	02/15 (13.33)

(%) used for percentage, $p<0.05$, significant

Sex wise prevalence of babesiosis

Out of 60 female cows and calves, 28% were detected positive while out of 40 male cows 32% were found positive for babesiosis. A total no of 40 blood samples of male cattle were studied, out of which 5 were found infected with *B. bovis* and 8 were infected with *B. bigemina* while out of 60 blood samples of female cattle 6 were found infected with *B. bovis* and 11 were positive for *B. bigemina* which were mentioned in Table.5.

Table 5: Sex wise prevalence

Gender	Prevalence of Babesiosis/n (%)	B.bovis Positive/n (%)	*B. bigemina* +ive / n (%)
Male	13/40 (32)	05/40 (12.5)	08/30(26.66)
Female	17/60 (28)	06/60 (10)	11/60 (18.33)

n= total number, (%) used for percentage, p<0.05, significant

Months wise prevalence of babesiosis

Babesiosis occurs in cows/calves (cattle) throughout the year. In January and February, babesiosis infection in cattle recorded at low rate, in March and April from low to medium, in May, it was medium, while in June its rate of infection was high. In July, the infection rate of babesiosis reached to peak level. In August, the rate of infection of babesiosis was also high, while in September, it was from high to medium, and then from October to December, the rate of infection decreased gradually.

DISCUSSION

Babesiosis is a vector (tick) borne disease found throughout the world. *Babesia* species are intra erythrocytic protozoan. The present study was compared with the results of other researchers who worked on this disease. There are more than one hundred species which have different morphological characteristic, because these parasites are similar in shape and structure but the most important and usually occurred are *B. bovis* and *B. bigemina* (Palmer 2002). The microscopic result was different from the results of other researchers as described by Durrani and Kamal (2008) (Durrani & Kamal 2008) whose results were 66.66% for *B. bigemina* and 33.33% for *B. bovis*. The findings of present study also correlated with the findings of Guglielmone *et al.,* (1997) who reported the prevalence of *B. bovis* ranging from 7.6% to 18.2% in Argentina (Guglielmone 1997). Aulakh, (2003) reported the prevalence of babesiosis as 5.94% in India (Aulakh 2003). The minute difference may be due to different climatic conditions. The results of the present study were not in agreement with Chandrawathani *et al.,* (1994), Bell *et al.,* (2004) and Oliveira *et al.,* (2005) who reported that the prevalence range was 70 to 100%. This difference may be attributed to the application of highly sensitive tests like PCR, ELISA, and CFT for diagnostic purposes. According to Chaudry *et al.,* (2010), out of 100, 11% were positive for *B. bovis* and 18% for *B. bigemina* (Chaudhry *et al.,*2010) and Niazi *et al.,* (2008) result showed 7.2% prevalence among 415 crossbred cow calves (Niazi *et al.,*2008). Nazir (2002) reported 7% prevalence of babesiosis in cattle at Malakand Agency KPK, Pakistan (Nazir 2002) which was less than our 30% result.

Ticks act as a vector in the transmission of *babesiosis* from infected cattle to healthy animals. In the present study it was found that Hyalomma was 40%, Boophilus was 34% Haemaphysalis 20% and Rhipicephalus were 6%. Similar study was conducted by Durrani and Kamal, (2008) where the percentage of Hyalomma was 15%, Boophilus 12%, Haemaphysalis 5% and Rhipicephalus was 3%. (Durrani 2008).

CONCLUSION

It was concluded from the present study that *Babesia* was mainly caused by Hyalomma in district Charsadda and Swabi. The prevalence rate of babesiosis was very high in Umarzai and Harichand in district Charsadda while in distric Swabi, Dagai had greater prevalence rate of *Babesia* in cattle's (Cows and calves). The prevalence rate of *Babesia* in calves less than one year was very high 42.8% than other aged cows more than 1 year. It was suggested that some new techniques may be adopted for the detection of *Babesia spp*. like PCR and other sensitive tests. .

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