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Burden of babesiosis among domestic cattle of southern Khyber Pakhtunkhwa, Pakistan

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Abstract
A study was carried out to find out the prevalence of *B. bigemina* and *B. boves* in house hold dairies of district Bannu and Lakki Marwat, southern part of Khyber Pakhtunkhwa, Pakistan. Blood samples were collected in a span of one year from January 2012 to December 2012. Thick and thin blood smear was examined under light microscope. The overall prevalence of babesiosis showed that 20.66% were positive for *B. bigemina* and 34% *B. boves*. Along the age high prevalence was found in the lower age group animals. Also female cattle showed high prevalence for both *B. bigemina* 23.85% and *B. boves* 38.07% than male cattle. Summer season was more prone to this disease than other season of the year.

Keywords: Bannu, *B. bigemina*, *B. boves*, KPK

1. Introduction
Babesiosis is economically the most important tick-borne disease of cattle [1], which causes extensive monetary loss to the cattle industry in the developing countries [2]. The economic impact of babesiosis also occurs in other domestic animals, including horse, sheep, goats, pigs and dogs [3]. Clinical signs of this disease are characterized by fever, anemia and hemoglobinuria in the infected cattle [4], which may causes abortion in pregnant ones and reduced fertility in males especially bulls [5].
Babesiosis is caused by two intra-erythrocytic protozoan parasite of the genus *Babesia* (Family: Babesiidae) namely *Babesia bovis* and *Babesia bigemina* [4]. Generally *B. bovis* is more pathogenic than *B. bigemina* [3], however both can cause clinical disease and carrier infections [6]. Ticks (*Boophilus* species) are the major vector for the transmission of both these spp. of *Babesia* [3], which are widely spread in tropical and subtropical countries particularly in Pakistan, Bangladesh and India as the environmental conditions favor the growth and development of many tick species [7].
Babesiosis has a great monetary impact due to mortality, loss of meat, beef and milk products of infected animals as well as this disease also have great influence on international dairy trade [1, 5]. It is essential to detect the disease for the control and prevention of the spreading of Babesiosis. *Babesia* spps can be detected easily through microscopic technique in the red blood cells of the host at the acute stage of Babesiosis. Polymerase chain reaction (PCR), which is more sensitive and specific technique, offers an alternative approach for the diagnosis of Babesiosis [8] in every age group of animals. Keeping in view, the importance of the disease due to economic losses to the dairy industries the research work was designed to study the epidemiology of Babesiosis in house hold dairies in district Karak and district Chat of Khyber Pakhtunkhwa.

2. Materials and methods
2.1 Blood samples collection
This study was carried out in two southern districts of Khyber Pakhtunkhwa (Bannu, Lakki Marwat). The study was carried out from March, 2012 to September, 2012. Questionnaire was also designed to obtain the desired information in the study area particularly about the disease awareness, age, sex, diagnosis, medication, control measures etc. 300 blood sample were collected from clinically suspected domestic cattle, 150 from each district. About 5ml blood samples was collected from jugular vein in sterilized vacutainer tubes and placed in ice jar and transported to the molecular parasitology and virology laboratory, department of Zoology KUST for further process.

2.2 Microscopic examination

Fixed thin and thick blood smears with absolute methanol for one minute and was stained by Giemsa stain. Observed the stained slides under microscope (Olympus Japan) having 100x magnification to detect *B. bovis* and *B. bigemina*. Pictures were taken from positive slides. *B. bovis* is smaller in size and having paired form at an obtuse angel to each other. While *B. bigemina* is larger in size and having paired structure at an acute angel to each other. However it is difficult to distinguish the two species of *Babesia* by microscopic examination.

2.3 Ethical approval

Ethical and moral clearance was obtained from the institutional review board of the Kohat University of Science and Technology Kohat.

2.4 Data analysis

SPSS statistical software version 18 was utilized for data entry and analysis. P value was calculated, where 0.05 was considered significant at the 95% CI (Confidence Interval) level.

3. Results

This study was carried out to find out the prevalence of babesiosis diseases among the southern district of KPK, Pakistan. The present study indicated that at overall 164(54.66%) were positive for babeioses, out of which 62(20.66%) were positive for *B. bigemina* and 102(34%) for *B. boves*. District Bannu showed high rate of infection 61.33% (22% for *B. bigemina* and 39.33% for *B. boves*) than Lakki Marwat 48% (19.33% for *B. bigemina* and 28.66% for *B. boves*). Chi-square was used for data analysis (P=0.005).

Table1: Overall prevalence of *B. bigemina* and *B. boves* among domestic cattle's

Localities	No. of sample	B. bigemina%	B. boves%	total/%	pvalue
Bannu	150	33(22)	59(39.33)	92(61.33)	0.0056
Lakki marwat	150	29(19.33)	43(28.66)	72(48)	
Subtotal	300	62(20.66)	102(34)	164(54.66)	

To know the relationship between prevalence of babesiosis with age, cattle were divided into four age group. Cattle having age >1 years were 53 in number, out of which 35(66.03%) were positive for babesiosis in which 15(28.30%) and 20(37.73%) positive for *B. bigemina* and *B. boves* respectively. Cattle in age group 1>3 years were 67 in number out of which 37(55.22%) were positive in which 14(20.89%) and 23(34.32%) positive for *B. bigemina* and *B. boves* respectively. Cattle in age group 3>6 years were 79 in number out of which 41(51.89%) were positive for babesiosis in which 15(18.98%) positive for *B. bigemina* and 26(32.91%) for *B. boves*. Age group 6>9 years comprised of 101 cattle out of which 51(50.49%) were positive for babesiosis in which 18(17.82%) and 33(32.67%) were positive for *B. bigemina* and *B. boves* respectively.

Table 2: Age wise Prevalence of babesiosis among cattle

Age group (Years)	No. of sample	B. bigemina%	B. boves%	total/%	p value
>1	53	15(28.30)	20(37.73)	35(66.03)	
1>3	67	14(20.89)	23(34.32)	37(55.22)	0.0002**
3>6	79	15(18.98)	26(32.91)	41(51.89)	
6>9	101	18(17.82)	33(32.67)	51(50.49)	

**One way ANOVA

Table 3 showed the sex wise prevalence of babesiosis among domestic cattle. And the present study indicated that out of 113 male 42(37.16%) were positive in which 15(13.27%) and 27(23.89%) positive for *B. bigemina* and *B. boves* respectively. Out of 197 female 122(61.92%) were positive for babesiosis in which 47(23.85%) were positive for *B. bigemina* and 75(38.07%) were positive for *B. boves*.

Table 3: Gender wise prevalence of babesiosis

Gender	No. of sample	B. bigemina%	B. boves%	total/%	p value
Male	113	15(13.27)	27(23.89)	42(37.16)	0.0545*
Female	197	47(23.85)	75(38.07)	122(61.92)	

*Chi-square test

The entire year consisting of four basic seasons, such as winter, spring, summer and autumn. The babesiosis infection occurred throughout the four seasons. The present study recorded that low rate of babesiosis infection was in winter, in spring from low to medium, in summer at peak and in autumn the rate of infection decreases to low.

Table 4: Season wise prevalence of babesiosis

Season	No. of sample	B. bigemina%	B. boves%	total%	p value
Winter	50	5(10)	15(30)	20(40)	
Spring	100	14(14)	21(21)	35(35)	0.0068**
Summer	100	33(33)	53(53)	86(86)	
Autumn	50	10(20)	13(26)	23(46)	

**One way ANOVA

4. Discussion

One of the most widespread diseases affecting and causing massive losses to cattle diligence is the babesiosis [9]. About 1.2 billion cattle are affected by babesiosis in many countries including Australia, South and Central America, Asia and United States [5]. While in Pakistan babesiosis infects cattle and buffaloes about 5.5-42.8% [10]. So it is necessary to diagnose and treat this disease well in time in order to minimize the monetary losses of the cattle industry.

The present study indicated that at overall babesiosis infection in two districts Bannu and Lakki Marwat was 54.64% (164/300). Among these, district Bannu 61.33% (92/150) showed high prevalence than Lakki Marwat 48% (72/150). *B. boves* 34% (102/300) infection was high than *B. bigemina* 20.66% (62/300) were found by microscopy during the present study.

Similar results were found in Punjab where its prevalence rate was 66.66% for *B. bigemina* and 33.33% for *B. boves* [11], in Qadirabad 18% for *B. bigemina* and 11% for *B. boves* [3]. Our result is also in line with other researchers like Oliveira-Sequeira *et al.,* 2005 in Brazil [12], Adham *et al.,* 2009 in Egypt [13], Ghirbi *et al.,* 2008 in Tunisia [14], and Zulfiqar *et al.,* 2012 in Southern Punjab [5].

Small differences in the prevalence rate might be due to difference in environmental conditions of the areas, samples size, and different bread under study or may be due to difference in protocols used.

To know the relation of babesiosis with the age of the cattle, the present study showed high prevalence rate of both *B. bigemina* and *B. boves* in the cattle of lower age group. However this rate decreases along the age. This high incidence in the lower age group of cattle may be due to low immunity level, their soft and thinner skin which helps the ticks (vector) to transmit the infection easily. Similar result was also observed by Ahmad *et al.,* 2014 [15].

The highest prevalence of both *B. bigemina* 23.85% and *B. boves* 38.07% was found in female cattle, while male cattle were less prone this disease (13.27% for *B. bigemina* and 23.89% *B. boves*). The high prevalence of babesiosis in female is may be due to the fact that contaminated needles are commonly used for injecting drugs for milk let down, or for treatment of a disease. The results of the present study were not in agreement with Ahmad *et al.,* 2014 [15]. However in line with Ayaz *et al.,* 2013 [16].

Season wise prevalence of babesiosis indicated that high prevalence of babesiosis was found in summer season 86% followed by spring 35%, autumn 23% and winter 20%. More infestation in these season/months is due to the association of an enhanced tick's activity with increased hotness and dampness of the environment. Same finding was also observed by AZ, Durrani *et al.,* 2008 [11].

5. Acknowledgement

6. Reference

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