

## Epidemiological Survey of Bovine Babesia and Theileria Parasites in Buffaloes in District Dera Ismail, Khyber Pakhtunkhwa

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### Recommended Citation

Ullah, W., Khan, A., Ali, A., Shah, I. A., Aimen, U. e., Khan, I., Jan, A., & Rustam, S. (2023). Epidemiological Survey of Bovine Babesia and Theileria Parasites in Buffaloes in District Dera Ismail, Khyber Pakhtunkhwa, *Journal of Bioresource Management*, 10 (1).

ISSN: 2309-3854 online

(Received: Dec 24, 2022; Accepted: ; Published: Mar 30, 2023)

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### Cover Page Footnote

The authors are thankful to the Government of Khyber Pakhtunkhwa, for providing the grant and human resource to execute this research project.

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## EPIDEMIOLOGICAL SURVEY OF BOVINE BABESIA AND THEILERIA PARASITES IN BUFFALOES IN DISTRICT DERA ISMAIL, KHYBER PAKHTUNKHWA

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### ABSTRACT

Piroplasmosis (babesiosis and theileriosis) are the leading blood parasites responsible for significant economic losses to the dairy enterprises. These parasites are transmitted by Ixodid ticks from a diseased animal to a healthy animal. This study was executed to determine the prevalence of Theileria and Babesia in water buffaloes in District Dera Ismail Khan for their risk assessment. During this trial, 350 random blood samples were obtained from buffaloes within the region of District Dera Ismail Khan and examined for Babesia and Theileria parasites using Giemsa staining technique under an oil immersion lens. Forty-eight blood samples were found positive and the prevalence of Babesiosis was 7.43 %, followed by the prevalence of Theileriosis (6.28 %). Babesiosis had a significantly higher ( $p < 0.05$ ) prevalence among both males and females (6.9 % and 7.7 %, respectively) followed by Theileria (5.38 % and 6.8 %, respectively). The significantly highest ( $p < 0.05$ ) prevalence of Theileria was seen in calves younger than 2 years (16.7 %). Babesiosis was most prevalent ( $p < 0.05$ ) in calves aged 2 to 5 (11.7 %). The Kundhi buffalo breed had the highest ( $p < 0.05$ ) prevalence of babesiosis, followed by the Nili Ravi breed, while, the Nili Ravi breed had the highest frequency ( $p < 0.05$ ) of Theileria. Piroplasmosis prevalence significantly ( $p < 0.05$ ) peaked in the summer season. Piroplasmosis the major risk factor in endangering the general health of the buffalo's population, therefore, stringent control measures are recommended for the management of the tick population.

**KEYWORDS:** Babesia, hemonchus, parasitemia, Ruminants infections, theileria.

### INTRODUCTION

Hemoparasites are intracellular pathogens that infect vertebrate hosts' erythrocytes (Suarez and Noh, 2011). Water buffaloes can be infected with tick-borne hemoparasites and continue to spread the infections, posing an infectious danger to the more susceptible animals (Obregon et al., 2019).

Babesiosis is caused by the Babesia, which resembles the malaria parasite, belongs to the class protozoan and is the second most prevalent after

Trypanosoma in livestock. In regions with a mild winter, they pose a significant threat to the health of domesticated animals. Human babesiosis is uncommon, although the number of cases has increased in recent years due to increased medical awareness (Herwaldt et al., 2010). Theileriosis is caused by Theileria, a parasitic protozoan of the Apicomplexa. Two species of Theileria, namely *T. annulata* and *T. parva*, are responsible for causing theileriosis. Tropical theileriosis is caused by *T. annulata*, whereas East Coast fever is caused by *T. parva* (Brown, 2008).

Even though buffaloes are generally more resistant to infectious and parasitic diseases than cattle, these animals are more often infested with ticks (Abbasi et al, 2017; Rehman et al., 2017). The incidence of these diseases renders cause huge economic losses to the farmers and the national economy in the form of treatment costs and case fatality rates (Ibrahim et al., 2013). In Pakistan, piroplasmosis caused by *Theileria annulata*, *Babesia bovis*, *B. bigemina*, *B. divergens*, *Anaplasma marginale* and *A. centrale* is the common tick-borne infections of both water buffaloes (*Bubalus bubalis*) and cattle (*Bos indicus* and *Bos taurus*) (Durrani et al., 2008, Khan et al., 2022). Cattle are the natural reservoir host for *B. bovis*, *B. bigemina* and *A. marginale*, water buffaloes and other ruminants are also susceptible to infections from these hemoparasites (Chauvin et al., 2009; Kocan et al., 2010). *Theileria annulata* and *Theileria parva* cause severe clinical manifestations of theileriosis in cattle (Bishop et al., 2004). Theileriosis is endemic in Pakistan's tropical regions. The predisposing environmental circumstances of tropical and subtropical climate enhance the prevalence, growth, and dissemination of ticks, which result in animal infections (Sajid, 2007).

The tick fauna consists of around 30 species of ixodid ticks, which are

known to carry a number of deadly infectious diseases including Theileria and Babesia parasites. The *Dermacentor marginatus*, *Hyalomma marginatum*, *Hyalomma anatolicum*, *Hyalomma asiaticum*, and *Hyalomma scupense* have epidemiological relevance. Ticks carry Crimean-Congo hemorrhagic fever, tick-borne encephalitis, babesiosis and theileriosis, Q fever and Lyme borreliosis (Sultankulova et al., 2022).

Therefore, this study was executed to determine the prevalence of Theileria and Babesia in District Dera Ismail Khan to assess the risk of piroplasmosis in water buffaloes and to further design their management strategies to curtail the economic and health losses in livestock and dairy enterprise.

## MATERIALS AND METHODS

### Study Design

The research was conducted in ten regions (Table 1) of District Dera Ismail Khan and 35 blood samples were collected from the buffaloes of each region at random. A total of 350 buffalos were selected in this study (Khan et al., 2017), and the data was recorded on the approved questionnaire.

**Table 1: The sample collection locale and species**

S. No	Area	Specie, sample type	No. of samples collected
1	BastiMaheer	Buffalo, blood	35
2	Indus society	Buffalo, blood	35
3	Imamia colony	Buffalo, blood	35
4	Diwala	Buffalo, blood	35
5	Nawab Ada	Buffalo, blood	35
6	BastiUstrana	Buffalo, blood	35
7	Mallana	Buffalo, blood	35
8	Muryali	Buffalo, blood	35
9	Madina colony	Buffalo, blood	35
10	Kech	Buffalo, blood	35
<b>Total</b>			<b>350</b>

### Analysis of Samples

Blood samples were collected from the jugular vein of buffaloes and transferred to the labeled EDTA-coated vacutainers. The vacutainers were stored in the ice box. The sampled animals ranged in age from 1 to 5 years. Then these samples were transported to the laboratory facility at Veterinary Research and Disease Investigation Centre, Dera Ismail Khan, for further analysis.

### Giemsa Staining

Thin blood smears were prepared, and after air drying, the smears were fixed in methanol for 2 minutes, then stained with Giemsa stain for 20 minutes and examined microscopically under an oil immersion lens to diagnose Babesia and Theileria parasites based on parasite morphology (Khan et al., 2022).

### Data Analysis

The positive samples were recorded on the computed record sheets, and the data between the treatment groups were analyzed using one-way ANOVA including Tukey's test and Chi-square tests.

## RESULTS

Our findings revealed that forty-eight (48/350) blood samples of buffaloes were found positive for haemoparasites. The prevalence of Babesia was 7.43 %, followed by Theileria with a frequency of 6.28 % (Table 2). The results indicated

that there was a non-significant difference ( $p > 0.05$ ) between Babesia and Theileria in the total samples collected. The prevalence of Babesia was found to be 6.9 % in 130 male buffaloes, while the prevalence of Theileria was found 5.38 % in buffaloes and in 220 females, the prevalence of babesiosis was 7.7 % and the prevalence of Theileria was 6.8 %. There was a statistically significant ( $p < 0.05$ ) variation in prevalence by age group (Table 3). The prevalence of Theileria was 16.7 % in young buffaloes of age one to two years, whereas Babesia prevalence was found 2.22 % (Table 4). The prevalence of Babesia in calves aged 2 to 5 years was 11.7 %, while the prevalence of Theileria was 3.33 %. Buffaloes of age 5 years and older had a prevalence of Babesia of 7.14 % and Theileria was 2.14 %. There was a highly significant ( $p < 0.05$ ) difference between the prevalence in each age group. The incidence of Babesia in the Nili Ravi breed was 8.48 %, and the prevalence of Theileria was 6.6 %. (Table 5). The Kundhi breed had a prevalence of Babesia of 6.56 and Theileria was 7.29 %, while the non-descriptive breed had a prevalence of Babesia of 6.25 and Theileria was 2.08 %. There was a statistically significant ( $p < 0.05$ ) difference between the two groups. Season-wise prevalence was also recorded: March (6 %), April (8 %), May (8 %), June (10%), July (8 %), and August (12 %) had the highest incidence of Babesia, whereas March (4 %), April (6 %), May (6 %), June (8 %), July (10 %), and August (10 %) had the highest incidence of Theileria (Table 6).

**Table 2: Overall prevalence of Babesia & Theileria in Buffalos sampled from district Dera Ismail Khan**

Incidence	No. of samples collected	No. of Positive samples	Prevalence	Mean	SD	SE	p-value
Babesia	350	26	7.43%	5.2	2.167	0.9695	0.596
Theileria		22	6.28%	4.4	2.408	1.077	

**Table 3: Sex-wise comparison prevalence of Babesiosis & Theileriosis in Buffalos sampled from district Dera Ismail Khan.**

Sex	No.of samples collected	Positive Babesia	samples for	Positive Theileria	samples for	Mean	SD	p-value
	350	Positive cases	Prevalence %	Positive cases	Prevalence %			
Male	130	9	6.9	7	5.38	1.4	0.894	0.0138*
Female	220	17	7.7	15	6.8	1.3	0.707	

**Table 4: Prevalence of Babesiosis in different age groups & Theileriosis in Buffalos sampled from district Dera Ismail Khan.**

Age	No.of samples collected	Positive Babesia	samples for	Positive samples for Theileria	samples for	Mean	SD	SE	p-value
	350	Positive cases	Prevalence %	Positive cases	Prevalence %				
Less Than 2 years	90	2	2.22	15	16.7	3	0	0	0.0000*
2-5 years	120	14	11.7	4	3.33	0.8	0.447	0.2	
Above 5 years	140	10	7.14	3	2.14	0.6	0.547	0.244	

**Table 5: Breed-wise prevalence of Babesiosis & Theileriosis in Buffalos sampled from district Dera Ismail Khan**

Breed	No.of samples collected	Positive Babesia	samples for	Positive samples for Theileria	samples for	Mean	SD	SE	p-value
	350	Positive cases	Prevalence %	Positive cases	Prevalence %				
Nilli Ravi	165	14	8.48	11	6.66	2.2	0.836	0.374	0.001*
Kundi	137	9	6.56	10	7.29	2	0.707	0.316	
Non-Descriptive	48	3	6.25	1	2.08	0.2	0.447	0.2	

**Table 6: Month-wise prevalence of Babesiosis & Theileriosis in Buffalos sampled from district Dera Ismail Khan.**

Month	No. of samples collected	Positive samples for Babesia	Positive samples for Theileria
	350	Positive cases	Prevalence %
March	50	3	6
April	50	4	8
May	50	4	8
June	50	5	10
July	50	4	8
August	50	6	12

## DISCUSSION

A similar methodology for the diagnosis of hemeprotzoans including Theileria and Babesia under the light microscope was followed in a similar

nature study conducted by Khan et al. (2022); Farooqiet al., (2017) and Niaziet al. (2008). Farooqiet al. (2017) reported aprevalence of 10% in three climatic zones of Khyber Pakhtunkhwa province, while Zulfiqaret al. (2012) also reported

comparable findings. Theileriosis had a prevalence of 6.28 percent among buffaloes, but Kala et al. (2018) estimated the incidence of Theileriosis reached 9.33 percent. Females (6.9 %) had a higher Babesiosis infection rate than males (7.9 %). Similarly, the prevalence of Theileriosis was found to be higher in girls (6.8 %) than in males (5.38 %). The results were compared with the research conducted by Khan et al. (2021). Babesiosis was more prevalent in age groups older than 2 to 5 years. The incidence of Theileriosis was higher in calves younger than 2 years of age. Maharana et al. (2016) reported the susceptibility of adult buffaloes to babesiosis and theileriosis. While Mohsin et al. (2022) evaluated the susceptibility of young animals (below 1 year) to theileriosis based on their age, this study analyzed the susceptibility of older animals. The Kundhi breed was found to be at high risk for babesiosis, followed by the Nili Ravi breed, these results were also supported by Siddique et al. (2020). While Theileriosis was more prevalent in the Nili Ravi breed, the findings were correlated with Farooqi et al. (2017). The incidence of piroplasmosis increased as the summer season progressed from March onwards. Our findings were also supported by Khan et al. (2022), Mohsin et al. (2022), Siddique et al. (2020), and Khan et al. (2017).

Giemsa staining, revealed 19.35 %, 23.08 %, and 21.93 % for babesiosis and 15.89 %, 15.25 %, and 15.16 % for theileriosis, whereas, PCR-based surveillance revealed 35.43 %, 36.40 %, and 36.95 % for babesiosis and 42.21 %, 41.01 %, and 41.52% for theileriosis in SSZ, CPZ, and NAZ. For *B. bigemina* and *T. annulata*, the length of the PCR product was observed to be 278 bp and 393 bp, respectively. Overall, PCR-based detection yielded a substantially greater prevalence than Giemsa staining. This tendency has been found in numerous earlier studies, demonstrating that PCR is a more sensitive

diagnostic method than Giemsa staining (Zaman et al., 2022). Our findings were correlated with the studies conducted in different regions of Pakistan, reporting that prevalence estimates for TBP in Pakistan were 18.42 % in Lahore, 29 % in Okara, 6.57 % in Rawalpindi, and 25.26 % in Chakwal (Nasir et al., 2000; Chaudhry et al., 2010; Hassan et al., 2018).

## CONCLUSION

This study concluded that District Dera Ismail Khan is endemic for Piroplasmosis (babesiosis and theileriosis) and a major risk factor in endangering the general health of the buffaloes population and that ticks are the main source of the transmission of this pathogenic agent, so stringent control measures are recommended for the management of the tick population. Plans and their implementation by the Livestock Department of Khyber Pakhtunkhwa, for effective measures and proper control of these lethal infections are the dire need of the day.

## AUTHORS' CONTRIBUTION

All the authors contributed equally to this research trial.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ACKNOWLEDGMENT

The authors are thankful to the Government of Khyber Pakhtunkhwa, for providing the grant and human resources to execute this research project.

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