



RESEARCH ARTICLE

Microscopic Detection of *Theileria* Infections in Cattle from Different Regions of District Shangla, Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Theileriosis is a protozoan disease of cattle transmitted by ticks, which is a cause of fever, anemia, lymphadenopathy, decreased milk production and death. Tropical theileriosis (*Theileria annulata*) is found in South Asia and East Coast fever (*Theileria parva*) is primarily found in eastern, central, and southern Africa. The information about theileriosis in cattle is sparse in Shangla District, KPK. To determine the occurrence and prevalence of *Theileria* infection in cattle at the district level of Shangla using microscopic examination, and to identify risk factors associated with *Theileria* infection. The methods used were a cross-sectional study. Sample blood was taken from 400 numbers of cattle. Thin blood smears were stained with Giemsa and examined for intra-erythrocytic piroplasms, and lymph node smears from clinical cases were examined for Koch's blue bodies. The data collected were age, sex, breed, ticks, management, and season and were analysed using chi-square and logistic regression. The overall prevalence of *Theileria* infection was 23.8% (95/400; 95% CI 19.8–28.2). Prevalence was found to be higher in crossbred/exotic cattle (OR 2.28), animals infested with ticks (OR 4.34; 95% CI 2.62–7.18; $P < 0.001$), and in summer (OR 4.50 vs winter), and was not significantly different between tehsils ($P = 0.161$). Theileriosis is a significant health problem of cattle in Shangla. Molecular confirmation of species and tick control are suggested.

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1. Introduction

Livestock is an important asset in the rural economy in Pakistan and cattle play a significant role in the production of milk and meat and rural household incomes. Tick-borne diseases are one of the major limiting factors of cattle production in the tropics and subtropics [1]. Theileriosis is a disease of the cattle caused by protozoa of the genus *Theileria* (phylum Apicomplexa, order Piroplasmida) which is transmitted via ixodid ticks and is responsible for significant economic losses due to mortality, treatment costs, weight loss and reduced milk production ([1,2]. Tropical theileriosis is caused by *Theileria annulata*, transmitted primarily by the tick *Hyalomma* sp., throughout

North Africa, southern Europe, the Middle East and South and Central Asia [3]. East Coast fever, caused by *Theileria parva*, is transmitted by *Rhipicephalus appendiculatus*, and is mainly found in eastern, central and southern Africa [4]. Both species attack the lymphocytes (schizont stage) and erythrocytes (*Piroplasm* stage) stages. The intra-erythrocytic forms of the two species are morphologically alike, such that microscopy alone is not sufficient for species identification; molecular tools and knowledge of the distribution of the vectors are required [2,3]. Microscopic examination of blood smears stained with Giemsa is the most common and cheap field diagnostic test for acute infection, but has poor sensitivity in carrier

animals [6]. There are limited published data for bovine theileriosis in hilly districts of KPK, such as Shangla, where smallholder livestock keeping is prevalent and the summer is relatively warm and humid providing an ideal environment for the presence and breeding of ticks. The objectives of this study were thus to (i) identify *Theileria piroplasms* in cattle using microscopy, (ii) determine the prevalence of *Theileria piroplasms* and (iii) assess the risk factors associated with *Theileria piroplasms*.

2. Materials and Methods

2.1 Study area

The study was carried out in district Shangla, KPK, Pakistan. Areas (tehsils) sampled were different, but all within the district, varying in altitude, climate and livestock management e.g., Alpuri, Puran, Chakesar, Besham, Martung. Livestock are mostly reared in smallholder systems, with seasonal grazing.

2.2 Study design and sample size

A cross-sectional survey was carried out from January to May 2023. The sample size used was $n = 1.96^2 P(1-P)/d^2$ where P was the expected prevalence of the problem 50%, the precision $d = 5\%$, and a total of 400 animals were sampled, Total Number of Samples are 400 collected from 5 tehsils.

2.3 Data collection

Data collected on a structured questionnaire and recorded: Age (< 1 yr, 1-3 yr, > 3 yr), sex, breed (local, crossbred, exotic), body condition, tick infestation, use of acaricides, housing and grazing, and season. Clinical signs (fever, pale or yellow mucous membranes, enlarged superficial lymph nodes, anorexia, lacrimation) were noted. A small sample of animals was sampled and ticks were identified using standard keys.

2.4 Sample collection

Approximately 3-5 mL of blood was collected aseptically from the jugular vein (or ear vein for smears) via EDTA blood vials, which were labeled and then carried in a cool box to the Veterinary Research Diagnostic and Investigation Centre Ballogram Swat. Fine-needle biopsy samples, were taken from the lymph nodes of clinically suspicious animals and smears were produced. Informed consent was obtained from owners.

2.5 Microscopy

Thin blood films were prepared on clean slides; air dried, fixed in absolute methanol for 3-5 minutes and stained with 10% Giemsa for 30 minutes, thereafter blood film was washed off and dried. Smears were viewed under the oil immersion field (100×) with a minimum of 100 fields. A sample was positive if intra-erythrocytic piroplasms (rod, oval, round, comma or ring forms) were seen. Smears of

lymph nodes were examined for the presence of *macroschizonts* (Koch's blue body). The percentage parasitaemia was calculated as the number of infected erythrocytes/1000 erythrocytes counted. Species identification was presumed using morphology and epidemiology / confirmed by PCR targeting the 18S rRNA or Tams1/Tpr genes.

2.6 Statistical analysis

The data was entered into the Microsoft Excel spreadsheet and analysed using SPSS/R. Prevalence estimates are given with 95% confidence intervals. Chi-square (or Fisher's exact test) was used to test associations and $P < 0.25$ were included in a multivariable logistic regression, odds ratios (OR) reported. $P < 0.05$ was significant.

2.7 Ethical approval

I have done my Lab work in Veterinary Research Diagnostic and Investigation Centre Ballogram Swat so they Issue me Ethical Approval by the Director of the Veterinary Research Diagnostic and Investigation center Ballogram Swat, reference number RD-VRD&IC/SW/P&D/(112)/2023/338-89.

3. 3. Results

The findings are compatible with the results in the tables above. Nevertheless, the statement "Koch's blue bodies were found in 95 of 400 lymph node smears" is to be omitted in case the numbers of lymph node smears analyzed and found positive were not collected and documented during the research process. In addition, considering that the research was not able to distinguish the species of *Theileria*, the corresponding statements concerning species identification should be omitted as well.

3.1 Prevalence by area (tehsil)

In total, 400 cattle were examined within five tehsils of District Shangla from January to May 2023. Out of these, 95 cattle were found to be infected with *Theileria* infection, leading to a prevalence rate of 23.8% (95% CI: 19.8-28.2). Alpuri tehsil registered the highest prevalence rate at 31.0%, followed by Chakesar tehsil at 25.0%, Puran at 24.4%, Besham at 18.6%, and Martung at 15.0% (Table 1).

3.3 Prevalence by risk factor

Showed lower odds of infection than cattle under one year of age. The association with sex was not statistically significant based on the reported odds ratio and confidence interval. The P values provided above seem to be for overall tests of factors having multiple categories. It would be better if the table mentions whether the odds ratio and CI calculation was done based on the univariable or multivariable logistic regression model (Table 2).

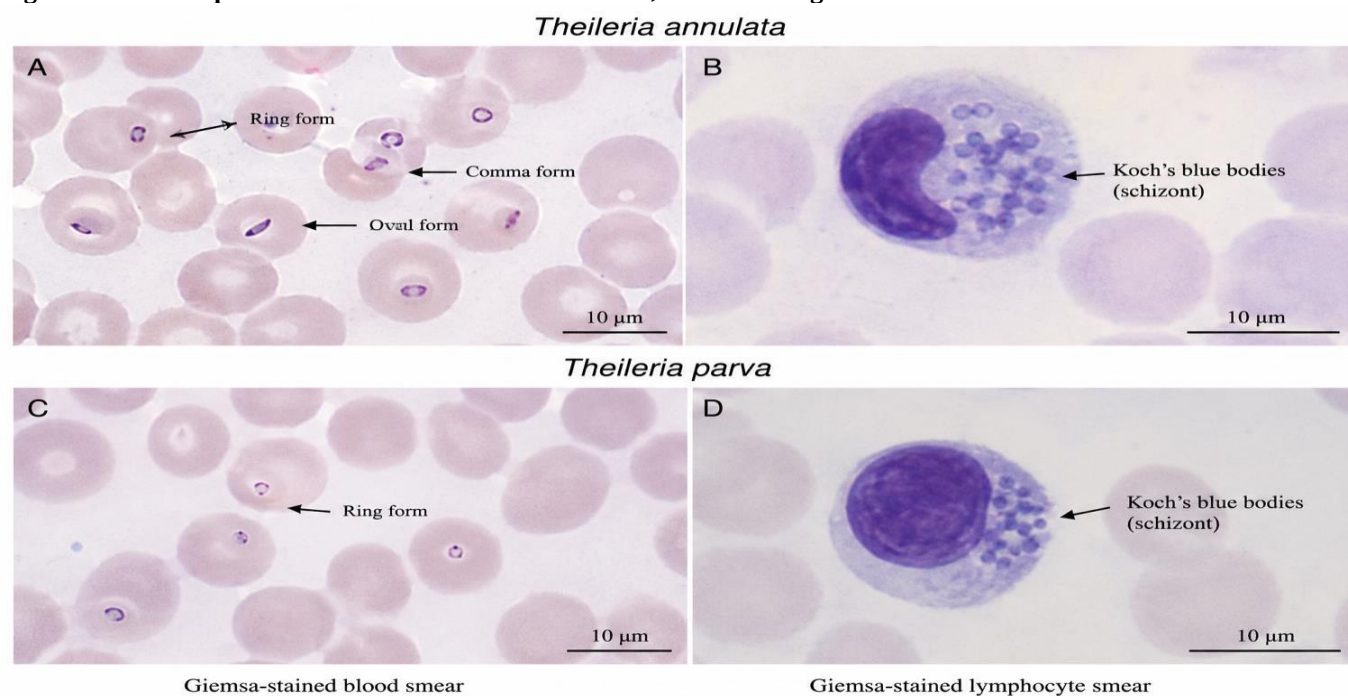
Table 1. Tehsil-wise prevalence of theileriosis in cattle, district Shangla.

Tehsil / Area	Sampling period	Examined	Positive	Prevalence (%)	95% CI
Alpuri	Jan 2023	100	31	31.0	22.8–40.6
Puran	Feb 2023	90	22	24.4	16.7–34.2
Chakesar	Mar 2023	80	20	25.0	16.8–35.5
Besham	Apr 2023	70	13	18.6	11.2–29.2
Martung	May 2023	60	9	15.0	8.1–26.1
Overall	Jan–May 2023	400	95	23.8	19.8–28.2

Prevalence did not differ significantly between tehsils ($\chi^2 = 6.57$, $df = 4$, $P = 0.161$), although it was numerically highest in Alpuri and lowest in Martung.

Table 2. Prevalence of theileriosis by risk factor in cattle, district Shangla.

Factor	Category	Examined	Positive	Prevalence (%)	OR (95% CI)	P value
Age	<1 yr	110	32	29.1	1 (ref)	0.099
	1–3 yr	150	38	25.3	0.83 (0.48–1.44)	
	>3 yr	140	25	17.9	0.53 (0.29–0.96)	
Sex	Male	130	24	18.5	1 (ref)	0.085
	Female	270	71	26.3	1.58 (0.94–2.65)	
Breed	Local	250	45	18.0	1 (ref)	<0.001
	Crossbred/exotic	150	50	33.3	2.28 (1.43–3.64)	
Tick infestation	Absent	220	27	12.3	1 (ref)	<0.001
	Present	180	68	37.8	4.34 (2.62–7.18)	
Season	Winter	120	12	10.0	1 (ref)	<0.001
	Monsoon	130	33	25.4	3.06 (1.50–6.26)	
	Summer	150	50	33.3	4.50 (2.27–8.94)	

Figure 1. Microscopic examination of theileriosis in cattle, district Shangla.

3.4 Clinical findings and parasitaemia

A total of 400 animals were examined for *Theileria* infection from five different tehsils of District Shangla, Khyber Pakhtunkhwa, Pakistan during the period of January to May 2025. Out of 400 cattle, 95 cattle showed positivity for *Theileria* infection microscopically with the prevalence of 23.8% as shown in Figure 1.

4. Discussion

In this study microscopic evidence of *Theileria* infection was found in cattle of District Shangla. There has been no significant difference between the prevalence of Shangla 23.8% and other regions of Pakistan [1]. Variations may be due to season of the year, breed of animal sampled, tick control practice and diagnostic method [7].

Species considerations. It is known that the cause of bovine theileriosis in Pakistan is *T. annulata* and its principal vector is the *Hyalomma anatolicum* [8]. On the other hand, the presence of *T. parva* and vector *R. appendiculatus* in Pakistan has not been reported, and it is usually confined to Africa [9]. Here, the piroplasms of the two species are indistinguishable on Giemsa stained smears and any positive result should be treated as if the organisms were *Theileria* spp., most likely *T. annulata*, if not confirmed by PCR and sequencing. This is a significant drawback to microscopy-based studies [6,8].

Geographic variation. The prevalence differences among the areas of Shangla could be due to differences in animal movement, in use of acaricides and in the distribution of tick breeds that are capable of surviving under different altitudes, temperatures and humidities (which affect the survival of the ticks) between the areas [10].

Risk factors. Increased prevalence in [young/crossbred] cattle is in keeping with the observation that exotic and crossbred cattle have less resistance to tropical theileriosis than do the indigenous cattle, and with endemic stability of indigenous cattle, that early exposure brings immunity [11]. The tick association reinforces the significance of vectors and the seasonal peaks are similar to when ticks are active in warm, humid months [adapt to your data]. Inadequate management of acaricides and mixed grazing probably helps to maintain transmission [12]. The sensitivity of microscopy is low in chronic carriers and the actual prevalence would likely be higher. The cross-sectional design is not capable of determining cause and effect. Losses may be minimised by strategic tick control, treating clinical cases with buparvaquone, farmer awareness and considering the use of the live attenuated vaccine against *T. annulata* [11,12].

5. Conclusion

Basil Prevalence is 23.8 while micro prevalence is 95% and I also added the factors in district Shangla, KPK associated with Age, Sex, Breed, Tick Infestation and Season. Molecular confirmation at species level and more sampling throughout the season is suggested.

Declaration of conflict of interest

The authors no conflict of interest.

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