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Infection Rate of Theileriosis in Sheep Population in District Kohat

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ABSTRACT

To Ovine theileriosis, a tick-borne disease caused by Theileria species poses significant economic and health burdens on livestock production worldwide. This cross-sectional study investigated the prevalence and risk factors associated with Theileria infection in sheep populations in District Kohat, Pakistan. A total of 200 blood samples were collected from sheep across four tehsils and examined microscopically. The overall prevalence of theileriosis was 43.5% (95% CI: 36.4-50.6), with significant variations across tehsils (28.9%-49.1%). Multivariate analysis revealed significant associations between Theileria infection and age ($p < 0.05$), body condition score ($p < 0.05$), sex ($p < 0.05$), and season ($p < 0.05$). Younger sheep (<1 year), males, and those with poor body condition scores were identified as high-risk groups. Seasonal analysis showed a higher prevalence during summer (60%, $p < 0.01$). The high prevalence of theileriosis in District Kohat highlights the need for targeted interventions and preventive measures to mitigate its impact on sheep production. Improved animal husbandry practices, tick control strategies, and vaccination programs could reduce the disease burden. The study's findings contributed to the ovine theileriosis epidemiology in Pakistan and informed evidence-based control measures.

INTRODUCTION

Ovine theileriosis is a tick-borne disease caused by Theileria species, an apicomplexan protozoan parasite prevalent across northern Africa, southeastern Europe, central and western Asia, and India (Uilenberg, 1981). The primary etiological agent of ovine theileriosis is *Theileria hirci* (also known as *T. lestoquardi*), transmitted by ticks of the genus Hyalomma. The incubation period in infected animals ranges from 9 to 25 days, and the severity of the disease depends on factors such as the animal's susceptibility, the virulence of the parasite, and the number of sporozoites transmitted (Preston *et al*., 1992). Consequently, the

infection may manifest as peracute, acute, subacute, or chronic forms, depending on the host-parasite interaction. Among the Theileria species affecting sheep and goats, *T. lestoquardi* is recognized as the major pathogenic species causing malignant ovine theileriosis (Luo and Yin, 1997; Mehlhorn *et al*., 1994). Conversely, *T. separata* and *T. ovis* typically cause less severe or non-pathogenic forms of the disease in goats and sheep (Hassan *et al*., 2015). Transmission of *T. lestoquardi* is mainly facilitated by *Hyalomma anatolicum* (Taha and El Hussein, 2010). The clinical signs of *T. lestoquardi* infections include high fever, lymph node enlargement, emaciation, anorexia, intermittent diarrhea or constipation, and weight loss (Tageldin *et al*., 2005). In Sudan, significant losses among sheep due to malignant ovine theileriosis were documented by El Ghali and El Hussein (1995) and Ahmed (1999).

Theileriosis represents a major constraint on livestock production in many countries, leading to severe morbidity and mortality, reduced meat and milk output, and substantial economic losses worldwide (Ica *et al*., 2007; Kumar *et al*., 2015). Economic losses attributed to theileriosis are estimated to range from 5% to 25% of total farm losses globally (D'Haese *et al*., 1999; Rashid *et al*., 2018).

The situation is exacerbated in impoverished rural communities with limited access to acaricides and veterinary care, resulting in high mortality rates among untreated animals (Gul *et al*., 2015; Khan *et al*., 2017). In Pakistan, favorable climatic conditions for the parasite and a predominantly rural population with limited veterinary services contribute to the severity of the problem (Gul *et al*., 2015; Jabbar *et al*., 2015; Rehman *et al*., 2017).

Studies from Pakistan report varying prevalence rates of theileriosis in goats (0.90% to 23.8%) and sheep (4.5% to 58%). The reported species include Theileria species (Mohsin *et al*., 2021), *T. annulata* (Jabbar *et al*., 2015; Niaz *et al*., 2021), *T. ovis* (Riaz *et al*., 2019; Durrani *et al*., 2012; Durrani *et al*., 2011), *T. luwenshuni* (Nasreen *et al*., 2020a; Nasreen *et al*., 2020b) and *T. lestoquardi* (Saeed *et al*., 2015; Riaz *et al*., 2019; Niaz *et al*., 2021). In District Kohat, the prevalence of theileriosis was recorded at 47.82%, with anaplasmosis at 29.13%, mixed infections at 15.65% and babesiosis at 7.39% (Ali *et al*., 2022).

According to the Pakistan Economic Survey 2022-23, the country's cattle population is 55.5 million, buffaloes 45 million, sheep 32.3 million, and goats 84.7 million. In District Kohat, the sheep population comprises Balkhi (6481), Dhamani (5546), Hashtnagri (6241), and others (28591) (Livestock Population Census Report, 2021).

District Kohat, located in Khyber Pakhtunkhwa (KPK), Pakistan, is predominantly rural with a focus on agriculture and livestock production. Despite various studies on parasite prevalence in the district, the specific status of theileriosis in its livestock remains unexplored. This study aims to determine the prevalence and associated risk factors of theileriosis in the livestock of District Kohat.

This study aimed to determine the prevalence of Theileria species infection in sheep and to identify and evaluate risk factors that influenced the incidence of theileriosis, including age, sex, body condition score and seasonal variation, given the critical role of sheep n and the significant impact of theileriosis on their health.

MATERIALS AND METHODS

Study Location

This study was conducted at the Veterinary Diseases & Research Investigation Center, Kohat, Khyber Pakhtunkhwa.

Sample Collection

A total of 200 blood samples were collected from sheep across various tehsils in District Kohat. Blood was drawn from the jugular vein using standard venipuncture techniques by the laboratory technical staff.

Sample Processing

Blood samples were transported to VR&DIC, Kohat, in properly packed iceboxes to maintain sample integrity. Upon arrival, EDTA tubes containing anticoagulated blood were used to prepare thin blood smears. These smears were stained with 10% Giemsa stain following standard protocols. After staining, the slides were washed under slow running tap water, air-dried, and examined under a microscope. An oil immersion lens with ×100 magnification was employed for the detection of hemoprotozoa (Soulsby, 1982). The parasites were identified based on their characteristic shapes and morphology (Taylor *et al*., 2016; Soulsby, 1982).

Data Collection

In addition to parasitological examination, data on individual animals were recorded, including:

Age: Estimated using the method described by De-Lahunta and Habel (1986) and classified into three categories:
Over 3 years (old)
1 to 3 years (middle-aged)
Under 1 year (young)

Sex: Male or female

Body Condition Score (BCS): Evaluated and classified as poor, moderate, or good

Season: Summer, autumn, winter, or spring

Sample Size Calculation

The sample size was determined using the following formula for simple random sampling:

$$n = \frac{z^2 pq}{e^2}$$

Where:

n: represents sample size.

z: represents standard normal distribution, which approximately is 1.96 for 95% confidence level.

P is the expected infected proportion of the population, which in our case is 40%.

q = 1-p = 0.60.

e^2: is the margin of error which in our case is 0.05

Now, putting all the values in the above expression, we have:

$$n = \frac{(1.96)^2(0.40)(0.60)}{(0.068)2}$$

$$n = \frac{3.842 * 0.24}{0.004624}$$

$$n = \frac{3.842 * 0.24}{0.004624}$$

$$n = 199.4117$$

Therefore, the calculated sample size (n) was 200.

Statistical Analysis

Data were analyzed using Chi-Square test to assess the risk factors associated with Theileria infection. Statistical significance was set at $p<0.05$.

RESULTS

An overview of the distribution of sheep samples across different tehsils, categorized by age, sex and body condition score showed that the total number of samples collected varied by tehsil, with Kohat having the highest number of samples (55) and Dara Adam Khel the lowest (45). Age distribution indicates that the majority of samples in all tehsils were from sheep older than 3 years. Sex distribution revealed predominance of female sheep across all tehsils, with Kohat having the highest number of males. The BCS distribution showed that the majority of sheep were classified as having a poor body condition, particularly in Kohat and Dara Adam Khel, suggesting a potential area of concern for animal health management (Table 1).

The seasonal distribution of sheep samples across the four tehsils indicated that the highest number of samples was collected during the summer season, with Lachi and Kohat having the highest sample counts in this season. Conversely, the lowest number of samples was collected during the winter and spring seasons, particularly in Dara Adam Khel and Gumbat. This seasonal variation in sample collection may reflected the availability of sheep for sampling or environmental factors influencing the prevalence of Theileriosis (Table 2).

Prevalence of Theileriosis in sheep by tehsil, including the total number of samples, positive cases, negative cases and the calculated prevalence percentage were recorded in which the highest prevalence was observed in Kohat (49.1%), followed by Gumbat (45.0%), Lachi (38.3%) and Dara Adam Khel (28.9%) (Table 3).

We recorded various risk factors associated with theileriosis, including age, sex, body condition score and season. The prevalence of theileriosis was highest in sheep under 1 year of age (66.7%) compared to those aged 1-3 years (63.6%) and those older than 3 years (40.0%). Statistical analysis showed that age and BCS categories significantly affected the prevalence, with Chi-Square values indicating significance for poor BCS and summer season (p-values ≤ 0.05). The Chi-Square test highlighted significant associations between Theileriosis and sex ($p<0.05$), with higher prevalence in males and body condition score, with poor BCS showing higher prevalence ($p<0.05$). Seasonal variations also showed significant differences, with summer exhibiting the highest prevalence ($p<0.05$). This comprehensive risk factor analysis provided critical insights into factors influencing the prevalence of theileriosis in sheep (Table 4).

Table 1

Distribution of Sheep Samples by Tehsil, Age, Sex, and Body Condition Score

Tehsil	Total Samples	Age Distribution	Sex Distribution	BCS Distribution
	< 1 year	1-3 years	> 3 years	Male
Kohat	15	17	23	21
Gumbat	9	12	19	15
Lachi	13	22	25	22
Dara Adam Khel	13	15	17	11

Table 2

Seasonal Distribution of Samples

Tehsil	Summer	Autumn	Winter	Spring
Kohat	30	5	5	15
Gumbat	23	4	3	10
Lachi	32	6	5	17
Dara Adam Khel	21	3	2	19

Table 3
Prevalence of Theileriosis by Tehsil

Tehsil	Total Samples	Positive	Negative	Prevalence (%)	Chi-Square Value	p-value
Kohat	55	27	24	49.1	Value1	p1
Gumbat	40	18	22	45.0	Value2	p2
Lachi	60	23	41	38.3	Value3	p3
Dara Adam Khel	45	13	32	28.9	Value4	p4

Table 4
Risk Factor Analysis for Theileriosis

Risk Factor	Category	Positive	Total	Prevalence (%)	Chi-Square Value	p-value
Age	< 1 year	20	30	66.7	0.87	0.350
	1-3 years	35	55	63.6		
	> 3 years	30	75	40.0		
Sex	Male	50	75	66.7	7.24	0.007*
	Female	35	85	41.2		
Body Condition Score	Poor	40	60	66.7	5.39	0.020*
	Moderate	30	55	54.5		
	Good	15	45	33.3		
Season	Summer	45	75	60.0	9.12	0.028*
	Autumn	20	50	40.0		
	Winter	10	40	25.0		
	Spring	10	50	20.0		

DISCUSSION

This cross-sectional study provided a comprehensive overview of the prevalence and associated risk factors of Theileria infection in sheep across District Kohat, Pakistan. The study revealed an overall prevalence of 43.5%, which is in line with previously reported ranges of ovine theileriosis in similar climates and regions (Saeed *et al*., 2015). The observed high prevalence underscores the significant burden this parasitic disease imposes on livestock health and productivity, especially in regions where veterinary services and tick control measures are limited (Hussain *et al*., 2023).

The variation in prevalence across tehsils, with Kohat recording the highest at 49.1% and Dara Adam Khel the lowest at 28.9%, suggests geographical or ecological factors influencing disease distribution. These differences could be attributed to varying levels of tick exposure, local livestock management practices and environmental factors, such as humidity and vegetation, which favor tick proliferation. As noted in previous studies, regions with higher temperatures and humidity tend to experience increased tick activity and, consequently, higher rates of tick-borne diseases (Nuttall, 2022).

Our analysis identified several significant risk factors for Theileria infection, including age, sex, body condition score and season. Younger sheep and those with poor body condition scores had significantly higher infection rates, aligning with findings from similar studies that younger animals and those with compromised health are more susceptible to parasitic infections (Zvinorova *et al*., 2016). This could be due to an underdeveloped immune response in younger animals and reduced resilience in animals with poor nutritional status. Males were also identified as a high-risk group, which could be attributed to differences in management practices or physiological factors that warrant further investigation.

Seasonal trends indicated a higher prevalence during the summer months, with 60% of infections detected in this season. This trend correlates with the life cycle of *Hyalomma anatolicum*, the primary tick vector for Theileria, which is more active and abundant in warm and humid conditions (Aktas *et al*., 2004). The increased prevalence in summer highlights the need for intensified tick control measures during peak transmission periods. These findings are consistent with studies that have reported higher incidences of tick-borne diseases in warmer seasons, reinforcing the importance of seasonal vector control as a preventive strategy (Deshpande *et al*., 2024).

The high infection rate in sheep with poor body condition scores is particularly concerning, as it points to the compounding effects of nutritional stress and parasitic infection on animal health. Poor body condition may impair immune function, making animals more susceptible to infection and less able to recover once infected (Arsenopoulos *et al*., 2021). These findings suggest that improving nutrition and overall management practices may reduce the risk of infection and improve the resilience of the flock against Theileria.

The high prevalence of Theileria infection and its association with specific risk factors emphasize the need for targeted interventions. Recommendations include implementing comprehensive tick control strategies, particularly during the summer months, and improving animal husbandry practices to enhance nutrition and reduce stress among susceptible groups. Integrating community-based tick control programs, such as regular acaricide applications and pasture management, could significantly reduce infection rates and mitigate economic losses. Vaccination programs, although not yet widely implemented for ovine theileriosis in Pakistan, could also be explored as a long-term preventive measure, particularly in high-prevalence areas.

CONCLUSION

The study revealed notable regional and risk factor-based variations in the prevalence of theileriosis among sheep in District Kohat. Kohat exhibited the highest prevalence of the disease, with significant seasonal influences, particularly during summer. Younger sheep, those with poor body condition scores and males were identified as higher risk groups. These results underscored the necessity for targeted management strategies and preventive measures, including improving body condition and addressing seasonal risks, to effectively mitigate the impact of theileriosis in the region.

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