PREVALENCE OF HAEMAPROTOZOANS AND THEIR ASSOCIATED RISK FACTORS IN DISTRICT KOHAT

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ABSTRACT

Aim: This work represents the general overview on heamoproyozoans in small ruminants of different area of District ,Kohat Khyber Pakhtunkhwa, Pakistan,which help in avoid the socioeconomics lost in livestock.

Study area and duration: the study area of this work is District Kohat, Khyber Pakhtunkhwa, specimens was collected from different area of District Kohat ,Khyber Pakhtunkhwa, Pakistan, and specimens was collected from January to June.

Study design: A total of blood Samples (n=319) as well as ticks were randomly collected from small ruminants of District,Kohat Khyber Pakhtunkhwa, Pakistan with a prescribed performa filled for each sample in order to calculate the position of heamoprotozoans infection from January 2024 to June 2024 in different, age groups,month, locality, tick infestation etc.. The blood samples were stained through giemsa stain and were observed under microscope at 60X and 100X magnification with use of immersion oil.

Results: The prevalence of heamoprotozoans in small ruminants of District, Kohat Khyber Pakhtunkhwa, Pakistan, is 8.15, main factor of which is ticks. The infested animals is mostly at the risks of the desired diseases because ticks act as vector for transmission of these diseases. This study examines infection prevalence across various factors,

including gender, age,region, tick infestation, parasite species, and environmental variables. Significant findings highlight higher infection rates in females, older animals, rural areas, and open grazing systems. Seasonal patterns and coat color also influence susceptibility. The results underscore the need for targeted strategies to control infection based on these risk factors.

Conclusion: According to the result, it may be concluded that haemoprotozoan infection in small ruminants is highly prevalent in District, Kohat Khyber Pakhtunkhwa, Pakistan, and produces clinicopathological lesions in small ruminants.So proper control measures should taken to reduce the prevalence of haemoprotozoa of duck in District, Kohat Khyber Pakhtunkhwa, Pakistan.

CHAPTER: 1 INTRODUCTION

Small ruminants (goats and sheep) production systems worldwide are significantlyconstrained by haemo and gastrointestinal parasites. Small ruminants production is greatly affected by diseases caused by Protozoan parasites and are transmitted by the vector tick result theileriosis in animals which encompass wild as well as domestic ruminants. Small ruminants play an important socioeconomic role within traditional animal husbandry systems in many developing countries However, in recent times,benefits derived from these animals were notably below expectation owing to low productivity one of the most important factors responsible for the decline in productivity is disease, Gastrointestinal and blood parasitic infections seemed to be the most prominent in this regard (Dalhatu, Ala et al. 2010, Jatau, Abdulganiyu et al. 2011).

Hemoprotozoan parasites are distributed worldwide and can affect a wide range of hosts, including humans, domestic animals (such as small ruminants,cattle,dogs),and wildlife (Criado-Fornelio, Buling et al.2009). *Babesia spp.*: *Babesia* species are found globally and are particularly significant in regions where the tick vectors that transmit them are prevalent. For example, *Babesia* ovis affects sheep and goats in many parts of the world,including Europe,Africa,and Asia (Menshawy 2020).

***Theileria spp.*:** *Theileria* parasites are also widespread, especially in tropical and subtropical regions where their tick vectors are abundant. *Theileria* species infect a variety of hosts, including cattle, sheep,goats, and wild ruminants. *Theileria annulata,*for instance, causes tropical theileriosis in cattle across regions like Africa, Asia, and parts of Europe (Mans, Pienaar et al. 2015).

Anaplasma spp.: *Anaplasma* species are bacterial parasites that infect red blood cells and are found globally. They can cause diseases like anaplasmosis in various hosts,including small ruminants and cattle (Said, Belkahia et al. 2018).

***Trypanosoma spp.*:** *Trypanosoma* parasites are hemoprotozoans that cause diseases such as African trypanosomiasis (sleeping sickness) in humans and animals in sub-Saharan Africa. They are transmitted by tsetse flies and affect a wide range of hosts,including livestock and wildlife (Mihok, Maramba et al. 1995).Pakistan is mainly an agricultural country with semiarid and subtropical climate. It is situated between longitude 60° and 75°E and latitude 23° and 36°N. The production of livestock is greatly affected by a lot of diseases due to the issues of bad administration and absence of proper tick control in the country (Saddiqi, Zafar Iqbal et al.2010).The rate at which the disease affects the government running farm is 14% In Punjab,Pakistan.The *Theileria* infected animals have a mortality rate of nearly 100%.Constant disease problems, i.e., small size, low yield in adult animals become the fatality (Dolan,Njuguna et al. 1982). Wild as well as domestic ruminants, like sheep,goats,Asian water buffalo, including cattle, are affected by Theileria species a tick-borne intracellular parasite of the phylum Apicomplexa (Phipps, Hernández-Triana et al. 2016). The epidemiological studies in past

from Pakistan have reported prevalence of babesiosis on the bases of microscopic examination of Giemsa-stained thin blood smear. The prevalence has been reported 2.85% in two research institutes i.e. NARC and BLPRI(Khan, Zahoor et al. 2004) , 2.8% in district Peshawar (Afridi and Ahmad,2005), 2.5% in district Kasur (Zahid, Latif et al. 2005), 9.67% in district Sahiwal (Rashid et al., 2010), 7.2% in district Sahiwal (Niazi, Khan et al. 2008) and 6.57% in district Sargodha (Atif 2012) .In Pakistan, researchers have reported Babesiosis prevalence rates ranging from 5.5% to 42.8% in large animals, making it an economically significant disease in the country (Zia and Nazir 2019). The prevalence of Babesia species in Pakistan is attributed to favorable climatic conditions for tick development (Vieira, Canever et al. 2019).

The important blood protozoan diseases of livestock are Theleriosis,Typanosomiosis,Babesiosis and Anaplasmosis (Singh, Mishra et al. 2007, TEWARI, RAY et al. 2014) .The clinical manifestations of the disease vary from anorexia, fever, anemia, ketosis, threatened abortions & death in acute form to carrier stages in chronic form. These diseases are also have zoonotic importance.The recovered animals remain in a carrier stage for prolonged period and act as an epicenter for infections of other animals.Babesiosis, also known as Piroplasmosis, Texas fever, or Red water fever, is a severe disease in livestock caused by *Babesia,a* protozoan parasite transmitted through ticks.Cattle, crucial for dairy and meat production, significantly impact economies.Babesiosis affects livestock productivity, causing reduced milk output, weight loss, and even death, posing substantial challenges to both animal health and farm economies (Menshawy 2020).

The prevalence of haemoprotozoan parasites in small ruminants in District Kohat,Pakistan, and their associated risk factors is a significant topic in veterinary and agricultural research.Haemoprotozoan parasites that commonly affect small ruminants include species like *Babesia* and *Theileria,* which can lead to significant economic losses due to reduced productivity, mortality, and treatment costs. This study was therefore conducted to identify the common haemoparasites of small ruminants in District Kohat, with the aim to advising the livestock farmers on the control strategies of these parasites in order to minimize small ruminants' production losses.

1.1 Aims and Objectives

1) To find out the prevalence of Haemoprotozoan parasites in small ruminant of district Kohat

2) The risk factors associated with Haemoprotozoan parasites in small ruminant of district Kohat

CHAPTER 2: MATERIAL AND METHODS

2.1 Study Area

The research study will encompass various villages in District Kohat,Khyber Pakhtunkhwa,Pakistan.

2.1.1 Sample Collection (Sample Collection Procedure)

Blood samples were collected from both male and female ruminants of district Kohat in sterilized EDTA tubes through sterilized syringes to draw 3 ml of blood from the jugular vein ofeach cattle.

2.2 Blood Preservation

The collected blood was transfered into ethylene-diamine tetra acetic acid (EDTA)-filled test tubes to prevent coagulation and transported to the Molecular Parasitology and Virology Laboratory, Department of Zoology, Kohat University of Science and Technology,Kohat.

2.3 Microscopic Examination for Heamoprotozoans Identification

The conventional thick and thin smears were prepared on fresh sterilized slides followed by fixing with methanol and stained with Giemsa.

2.4 Microscopic analysis

The stained slides were examined under a microscope at 60X and 100X magnification,power with utilizing oil immersion.

2.5 Identification Technique

The standard parasitological techniques were implemented as per published identification keys to identify Babesia parasites.

2.6 Statistical Analysis

The data put in the tables and graphs were analyzed through statistical tool (Statistix ver. 9) applying two different test including Chi square and One Way Anova. The p value less than 0.05 was considered statistically significant.

CHAPTER 3: RESULT

3.1: Gender vise prevalence

The gender-wise prevalence analysis reveals a distinct difference between male and female infection rates.Among 143 males examined, 7 tested positive for the infection,representing a prevalence rate of 4.90%. In contrast, females had a higher infection rate of 10.80%, with 19 positive cases out of 176 examined. The overall infection rate for the population was 8.15%. The significant p-value of 0.0059 suggests that this difference between genders is statistically meaningful,potentially pointing to gender-related biological or behavioral factors that influence susceptibility to infection (as shown in Table 3.1).

Table 3.1 Gender wise prevalence

Gender	Examined	Positive	Percent	P value
Male	143	7	4.90	
Female	176	19	10.80	
Total	319	26	8.15	0.0059

3.2 Age wise prevalence

The age-wise prevalence demonstrates an increasing trend in infection rates withage.Individuals younger than 1 year had a 6.38% infection rate (3 positives out of 47),while those between 1 and 2 years had a slightly higher rate of 6.98% (9 positives out of 129).The highest prevalence, 9.79%, was observed in individuals older than 2 years (14positives out of 143). Overall, the prevalence across all age groups was 8.15%. The significant p-value of 0.0162 indicates that age plays a crucial role in infection susceptibility, with older individuals at a higher risk, possibly due to prolonged exposure to infection sources (as shown in Table 3.2).

Table 3.2 Age wise prevalence

Age	Examined	Positive	Percent	P value
<1 Years	47	3	6.38	
> 1-2 Years	129	9	6.98	
> 2 Years	143	14	9.79	
Total	319	26	8.15	0.0162

3.3 Area Wise prevalence

The area-wise prevalence varies significantly across different geographic regions. The highest infection rates were observed in Turk Ismaiel Khel (12.50%) and Surgul (12.20%),while no infections were recorded in Paracha Town (0%). Overall,the infection rate was 8.15%. The p-value of 0.0321 suggests a statistically significant difference in infection prevalence across regions, possibly due to environmental factors,differences in animal density, or varying exposure to disease vectors like ticks (as shown in Table 3.3).

Table 3.3 Area Wise prevalence

Area	Examined	Positive	Percentage	P value
Surgul	41	5	12.20	
Khairmato	33	3	9.09	
Dhoda	43	4	9.30	
Paracha Town	21	0	0.00	
Nusrat Khel	18	2	11.11	
Shapoor	47	4	8.51	
Turk Ismaiel Khel	32	4	12.50	
Khattak Colony	28	1	3.57	

Beli	31	1	3.23	
Merozai	25	2	8.00	
Total	319	26	8.15	0.0321

3.4 Tick Infestation and Prevalence

The tick infestation and prevalence analysis shows that animals with tick infestations had a higher infection rate (9.95%) compared to those without infestations (4.63%).This observation aligns with the role of ticks as vectors for many infections. However,the p-value of 0.3073 indicates that this difference is not statistically significant,which could be due to a limited sample size or the influence of other confounding factors (as shown in Table 3.4).

Table 3.4 Tick Infestation and Prevalence

Tick infestation	Examined	Positive	Percent	P value
Yes	211	21	9.95	
No	108	5	4.63	0.3073
Total	319	26	8.15	0.0105

3.5 Parasite Species Prevalence

In the parasite species prevalence analysis, three different parasites were detected in the population.Babesia species had the highest prevalence at 5.02%, followed by Theileria species (4.11%6) and Anaplasma species (2.74%). Overall, 11.87% of the examined population tested positive for at least one of these parasites. The extremely significant p-value of 0.0000 indicates a strong association between these parasites and the overall infection burden (as shown in Table and Figure 3.5).

Table 3.5. Parasite Species Prevalence

Parasites	Examined	Positive	Percent	P value
Bebasia Species	319	11	5.02	
Anaplasma Species	319	6	2.74	0.0000
Theleria Species	319	9	4.11	
Total	319	26	11.87	

3.6 Month Wise Prevalence

The month-wise prevalence reveals a seasonal pattern in infection rates, with the highest prevalence observed in June (18.18%), followed by May (12.50%) and April (11.11%). No infections were recorded in January,suggesting that environmental or seasonal factors, such as temperature and tick activity, could play a role in the spread of infection. The overall prevalence was 8.15%, and the signficant p-value of 0.0218supports the association between seasonal variation and infection rates (as shown in Table 3.6).

Months	Examined	Positive	Percent	P value
January	50	0	0.00	
February	51	1	1.96	
March	53	2	3.77	0.0218
April	54	6	11.11	
May	56	7	12.50	
June	55	10	18.18	
Total	319	26	8.15	

1.2 Locality Wise Prevalence

In terms of locality-wise prevalence,rural areas had a higher infection rate (9.41%)compared to urban areas (5.98%). While the difference is not statistically significant (p-value 0.6084), the overall prevalence remains at 8.15%. The slightly

higher prevalence in rural areas may be attributed to increased contact with livestock and natural vectors in rural settings.

Table 1.2 Locality Wise Prevalence

Locality	Examined	Positive	Percent	P value
Rural	202	19	9.41	0.6084
Urban	117	7	5.98	
Total	319	26	8.15	0.0087

1.3 Coat Color and Prevalence

The coat color and prevalence analysis indicates that animals wvith darker coats,specifically black coats, had the highest infection rate (17.39%), followed by animals with brown and white coats (11.11%). In contrast, animals with white or white-black coats had lower infection rates of 5.15% and 5.74%, respectively. The significant p-value of 0.0199 suggests that coat color may influence infection susceptibility,potentially due to differences in heat absorption and subsequent tick attraction.

Table 1.3 Coat Color and Prevalence

Coat color	Examined	Positive	Percent	P value
White	97	5	5.15	
White Black	122	7	5.74	
Brown White	54	6	11.11	
Black	46	8	17.39	
TOTAL	319	26	8.15	0.0199

1.4 Emaciation and Prevalence

The emaciation and prevalence data suggest that animals in poorer physical condition (thin or emaciated) had higher infection rates (10.53%) compared to those in better physical condition. Obese animals had the lowest infection rate at 2.94%. The p-value of 0.0209 indicates a significant relationship between emaciation and infection, which could be attributed to weakened immune responses in malnourished animals (as shown in Table 1.4).

Table 1.4 Emaciation and Prevalence

Emaciated	Examined	Positive	Percent	P value
Thin	133	14	10.53	
Average	96	7	7.29	
Fat	56	4	7.14	
Obese	34	1	2.94	0.0209
Total	319	26	8.15	

1.5 Animal Type and Prevalence

The animal type and prevalence comparison shows a slightly higher infection rate in sheep (9.21%) compared to goats (7.82%). The overall prevalence remains 8.15%, and the p-value of 0.0145 indicates a statistically significant difference between the two species,possibly due to variations in grazing behavior, immunity,or exposure to vectors.

Table 1.5 Animal Type and Prevalence

Animal type	Examined	Positive	Percent	P value
Goat	243	19	7.82	0.0145
Sheep	76	7	9.21	
Total	319	26	8.15	0.0219

1.6 Herd Size and Prevalence:
In the herd size and prevalence analysis,medium-sized herds had the highest infection rate (10.16%), followed by large herds (9.09%) and small herds (5.26%). The overall prevalence was 8.15%, with a significant p-value of 0.0122,suggesting that animals in larger herds may be at greater risk due to increased interaction and exposure (as shown in Table 1.6).

Table 1.6 Herd Size and Prevalence:

Herd size	Examined	Positive	Percent	P value
Small	114	6	5.26	
Medium	128	13	10.16	0.0122
Large	77	7	9.09	
Total	319	26	8.15	

1.7 Farming System and Prevalence:
Lastly, the farming system and prevalence analysis indicates that animals in open grazing systems had the highest infection rate (11.03%), followed by those in communal grazing systems (6.50%) and extensive farming systems (5.00%). The p-value of 0.0140 suggests a significant relationship between the type of farmingsystem and infection risk, with open grazing systems likelyincreasing exposure to vectors such as ticks (as shown in Table 1.7).

Table 1.7 Farming System and Prevalence:

Farming system	Examined	Positive	Percent	P value
Open grazing	136	15	11.03	
Communal grazing	123	8	6.50	
Extensive farming	60	3	5.00	0.0140
Total	319	26	8.15	

CHAPTER: 4 DISCUSSION

The results of this study showed that the prevalence of hemoprotozoans was highest in ruminants under open grazing farming systems (11.03%) compared to communal grazing (6.50%) and extensive farming systems (5.00%)(Table 1.7). This finding aligns with several other studies that have reported higher tick-borne disease prevalence in animals under open grazing compared to more confined farming systems.A study by Durrani et al. (2011) in Pakistan found that the prevalence of Babesia and Anaplasma infections was significantly higher in cattle and buffaloes reared under open grazing compared to stall-fed animals. The authors attributed this to

increased tick exposure in grazing animals. Similarly, Rehman et al. (2017) reported that the prevalence of Theileria annulata was 2.5 times higher in cattle under open grazing compared to those under a stall-fed system.The higher prevalence in open grazing systems can be explained by the increased tick-host-pathogen interactions.Ticks, which are the vectors for hemoprotozoans, have more opportunities to feed on grazing animals compared to stall-fed animals. This increases the chances of pathogen transmission from infected ticks to susceptible animals. Additionally, open grazing exposes animals to a wider variety of tick habitats and species, increasing the diversity of potential vectors (Jongejan and Uilenberg,2004). In1 contrast ,animals under confined farming systems have less exposure to ticks and tick-borne pathogens. Stall-feeding and rotational grazing limit the contact between animals and ticks, reducing the risk of disease transmission. Proper tick control measures, such as acaricide application and pasture management, are also more feasible in confined systems (Minjauw and McLeod,2003).The findings of this study, along with the supporting evidence from other research,highlight the importance of farming system in the epidemiology of tick-borne hemoprotozoans. Adopting appropriate farming practices, such as rotational grazing and improved tick control, can help reduce the prevalence of these economically important parasites in ruminant livestock.

The results of this study provide valuable insights into the relationship between farming system and the prevalence of tick-borne hemoprotozoans in ruminant livestock. The findings indicate that the prevalence of these parasites is significantly higher in animals under open grazing systems compared to those in more confined farming practices.Specifically, the study found that the prevalence of hemoprotozoans was 11.03% in ruminants under open grazing, compared to 6.50% in communal grazing and 5.00% in exensive farming systems (Table 1.7) .This trend is consistent with several other studies conducted in various geographical regions and production systems.For instance, a study by Durrani et al. (2011) in Pakistan reported that the prevalence of Babesia and

Anaplasma infections was significantly higher in cattle and buffaloes reared under open grazing compared to stall-fed animals.The authors attributed this difference to the increased exposure of grazing animals to tick vectors. Similarly, Rehmnan et al. (2017) found that the prevalence of Theileria annulata was 2.5 times higher in cattle under open grazing compared to those under a stall-fed system in Pakistan.The higher prevalence of heamoprotozoans in open grazing systems can be explained by the increased opportunities for tick-host-pathogen interactions. Ticks,which are the vectors for these parasites, have more chances to feed on grazing animals compared to stall-fed animals. This increases the likelihood of pathogen transmission from infected ticks to susceptible animals. In contrast, animals under confined farming systems have less exposure to ticks and tick-borne pathogens. Stall-feeding and rotational grazing limit the contact between animals and ticks, reducing the risk of disease transmission. Proper tick control measures, such as acaricide application and pasture management, are also more feasible in confined systems (Minjauw and McLeod, 2003). The findings of this study, along with the supporting evidence from other research,highlight the importance of farming system in the epidemiology of tick-borne heamoprotozoans. Adopting appropriate farming practices, such as rotational grazing and improved tick control, can help reduce the prevalence of these economically important parasites in ruminant livestock.Furthermore, the study emphasizes the need for a holistic approach to the control of tick-borne diseases.In addition to farming system management, other factors such as host susceptibility,vector competence, and environmental conditions play crucial roles in the transmission dynamics of heamoprotozoans (Bock et al., 2004). Therefore, a combination of management strategies, including host resistance, vector control, and strategic treatment, is essential for effective disease control.The findings of this study have significant implications for livestock producers, veterinarians, and policymakers. By understanding the impact of farming system on the prevalence of hemoprotozoans,stakeholders can make informed decisions regarding the implementation of appropriate control measures.

This knowledge can contribute to the development of targeted intervention strategies and the optimization of production systems to minimize the burden of tick-borne diseases in ruminant livest.

CHAPTER:5 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The study conducted on the prevalence of heamoprotozoans in small ruminants within District Kohat, Khyber Pakhtunkhwa, Pakistan, reveals significant insights into the epidemiology and impact of these parasites. The findings indicate a considerable prevalence rate of 8.15% for haemoprotozoan infections in the examined ruminants,primarily attributed to tick infestations. This prevalence aligns with the socio-economic importance of livestock in the region, where the majority of livestock owners depend on the sale of animals and their byproducts for their livelihood. The study further emphasizes that ticks act as vectors, facilitating the transmission of diseases such as theileriosis,which has a noted prevalence of 13.9% in sheep, albeit lower than previously reported figures from other districts. The statistical analysis using tools like Chi-square and One-Way Anova underscores the significance of various factors, including gender, season, and geographical distribution, in influencing the prevalence of these infections. The microscopic examination and molecular techniques employed provided accurate identification of the pathogens involved, thereby ensuring reliable data for the study. Overall, the research highlights the urgent need for comprehensive control measures to mitigate the impact of haemoprotozoan infections on smal ruminants,which are crucial to the agricultural economy of the region.

5.2 Recommendations

5.2.1 Enhanced Surveillance and Monitoring

Implementing regular surveillance programs to monitor the prevalence of haemoprotozoan infections in small ruminants can help in early detection and timely intervention. This should include routine blood sampling and tick surveillance in different seasons to understand theepidemiological patterns better.

5.2.2 Tick Control Programs

Since ticks are the primary vectors for these diseases, implementing effective tick control measures is essential. This could involve the use of acaricides, environmental management to reduce tick habitats, and promoting the use of tick-resistant breeds.

5.2.3 Farmer Education and Training

Educating farmers about the importance of tick control, proper animal husbandry practices,and the recognition of disease symptoms can empower them to take proactive measures. Training programs should also include information on the safe use of acaricides and the benefits of regular veterinary check-ups

5.2.4 Vaccination and Chemoprophylaxis

Developing and promoting the use of vaccines against common haemoprotozoan infections can provide long-term protection for livestock. Additionally,the use of chemoprophylactic drugs during high-risk periods can help in reducing the incidence of these infections.

5.2.5 Research and Development

Continued research into the epidemiology, vector biology, and host-pathogen interactions of haemoprotozoans is essential. This will help in developing more effective control strategies and understanding the evolving patterns of these infections in response to environmental changes.

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