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EPIDEMIOLOGICAL INVESTIGATION OF THEILERIOSIS IN GOATS AND ITS EFFECT ON HEMATOLOGICAL PARAMETERS IN DISTRICT KARAK

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ABSTRACT

Theileriosis is an emerging issue of wild ungulates, especially in tropical and subtropical areas globally. The current study was designed to investigate Thelerial infection in goats using microscopic identification and its effect on haematological parameters. A total of 300 blood samples were randomly collected from asymptomatic goats using a convenient sampling technique. All the blood samples were examined in the laboratory of Clinical Medicine and Parasitology laboratory using simple microscopy. The result showed that in total three hundred samples 34 % were positive. The highest prevalence was recorded in female goats (55.88%) as compared to males (44.1%) and the result was statistically significant (P<.04). The highest prevalence was recorded in the region of Lawaghar Chani Khel (25.49%), followed by Kamran Chowk (20.58%), Mitha Khel (16.66%), Karak city (13.72%), while the lowest prevalence was recorded in the region of Shaheedan Banda (11.76%). The result was statistically significant (P<.04). The non-significant difference was observed where the p-value was (P<.0000). Regarding the alterations in haematological parameters the highest increase was observed in the region of Kamran Chowk (KC) 22/102×100. while the number of Mitha Khel (MK) 11/102×100 was seriously decreased.

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1. Introduction

Pakistani natives have incorporated meat as a significant component of their dietary habits to meet supplemental nutritional needs in this developing nation. About 64% of the meat consumed in Pakistan comes from buffaloes, with goat meat coming in second at 27%, chicken at 6%, and sheep at 3% (Joshi, Singh *et al*. 2001). Goat skin can be utilized to make a variety of goods, including boots, gloves, and other items that need a soft hide. The finest cashmere wool in the world is made by cashmere goats, whose hair grows below the guard tufts of hair and is incredibly soft and fine. Mohair is used to make dolls' hair and sweaters, among other products. The Persian word pashm, which means soft, is where the word pashmina originates. These goats are known as pashmina goats in South Asia, where cashmere is referred to as pashmina (Rizal 2010).

The most crucial sector for Pakistan's economy is the livestock industry (Qudus, Ahmad *et al*., 2013). Livestock has historically been the primary source of food, protein, nutrition, and financial income for small holders. One of the main way that dairy farming lowers the unemployment rate in by placing educated and uneducated workers in appropriate both indoor and exterior field jobs (Hagmann 2012). The primary instrument for reducing poverty and raising the socioeconomic standing of rural residents is livestock. The approximate net revenue from livestock exports is Rs. 53 billion (Khan and Ashfaq 2010; Qudus, Ahmad *et al.* 2013) and livestock's value-added contribution to agriculture was 58.55 percent, while in 2015–16, it contributed 11.8 percent to Pakistan's GDP (Aslam, Zaheer *et al.* 2016). In 2015, livestock contributed 11.8 percent to Pakistan's GDP, with net export earnings of approximately Rs 53 billion. Livestock also contributed 58.55 percent to the value addition of agriculture. Their gross milk production is 16 20143 million tons and 34122 million tons annually, respectively, among which 27298 million tons and 16115 million tons are utilized by humans. Considering that 2085 million tons of beef are produced annually (Joshi, Singh *et al*. 2001; Khan and Ashfaq 2010).

The most significant and prevalent blood protozoan disease affecting goats is theileriosis. *Theileria ovis*, *T. recondita*, and *T. separata* are associated with subclinical illness in goats, whereas Theileria lestoquardi, T. luwenshuni, and *T. uilenbergi* represent the transmissible causes of

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malignant illness (Schnittger, Yin et al. 2004; Guo, Mu et al. 2007). There are just two species of theileria known to cause caprine theileriosis in Pakistan: *T. lestoquardi* as well as *T. ovis* (Durrani, Younus *et al.* 2011; Riaz and Tasawar 2017).

Ticks have been recognized as ectoparasites of animals since 400 B.C., according to historical knowledge. One of Aristotle's most well-known writings, Historia Animalium contains his discussion of ticks as repulsive ectoparasites that are produced by grass (Hussain and Durrani 2009). Since ticks feed on blood, cattle that are heavily infested with ticks become malnourished and weak. Ticks cause stress, immunodepression, weight loss, skin value degradation, hypersensitivity, and toxicosis in cattle (Lorusso, Picozzi *et al*. 2013). A major concern on a global scale, tick infestation impacts approximately 80% of the livestock population (Kemal, Muktar *et al.* 2016). Tick infestation is estimated to cause economic losses of $14,000–18,000 million annually worldwide, while tick-borne diseases (TBDs) are responsible for 498.7 million in annual costs in India (Minjauw and McLeod 2003). Worldwide, losses from tick-borne illnesses range from 13.9 to 18.7 billion US dollars annually. Worldwide, losses from tick-borne illnesses range from 13.9 to 18.7 billion US dollars annually (Leger, Quiedeville *et al*. 2013).

The predominant clinical symptoms include petechial hemorrhages on the conjunctival mucosa, enlarged lymph nodes, anemia, because of intra-erythrocytic parasitism by piroplasms, coughing, and fever, general debility, and weight loss (Horbar, Rogowski *et al.* 2001; Van Rooij, Sutherland *et al.* 2007). Animals with theileriosis may die from the disease, but with proper care, a speedy recovery is also feasible. This depends on the animal's immunity and tolerance as well as the parasite strains. Changes in hematological and clinical parameters can be used to gauge the severity of the illness, the diagnosis, and the prognosis (Col and Uslu 2007; Saeb, Baghshani *et al.* 2010).

As a result, the current study was designed to meet the following goals: to determine the effects of theileriosis on goats in Karak, Pakistan's urban and peri urban areas.

2. Materials and Methodology

Study Area

The Pakistani district of Karak is part of Khyber Pakhtunkhwa and is situated between latitudes 70.40° and 70.30°N and longitudes 32.48° and 33.23°E. At 340 meters above sea level, it is

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situated. The 2017 census report (GOP 2007) stated that 7,06,299 people called the district home. 150 miles from Peshawar, on the Indus Highway N-55 (which connects Peshawar and Karachi), is District Karak in Khyber Pakhtunkhwa, Pakistan. Karak district receives 330 millimeters of rainfall annually despite its geographical aridity. The absence of water and the arid climate in some areas limit commercial activity. Though, if the necessary facilities are available, some big and small animals may be obtained in the towns. The arid and semi-arid climate of the district Karak prevents a large and varied green flora, resulting in only 2.1% of the district's land area being covered by xerophytic vegetation.

Selection of Animals

Variety of caprine breeds were selected for the microscopy and hematological examination for the prevalence of Theileriosis in District Karak. A total of 300 (n=300) samples were taken from 8 various localities goats were checked randomly for caprine theileriosis. 50 goats of both gender were checked per region. All the regions are located in Tehsil Karak. During collection of samples special care was made considering equal ratio of number and gender.

Sample Collection and Preservation

Blood samples were taken with a 10 mL BD Luer-LokTM 302995|BD syringe from the jugular vein, which is situated on both sides of the neck. The syringe was filled with 3 mL of blood. Not long after the extraction, the blood was moved into an EDTA containing tube. In the laboratory of Medicine and Parasitology at the College of Animal Husbandry and Veterinary Sciences, Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa, labeled EDTA tubes were refrigerated until further use.

Blood samples

The blood samples were collected from 300 asymptomatic goats. Each blood sample is sent to the lab in an ice box after being stored in EDTA tubes. Information about various animal characteristics taken into consideration during sample collection, such as age, feeding habits, breed, tick infestation, sex, season, and herd size.

Processing of samples

Using 20 ml syringes, blood samples were taken and then added to EDTA solution. The blood samples underwent examination and analysis at the CVS & AH AWKUM

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Microscopy of Blood Samples

All the blood samples were examined under microscope in the College of Animal parasitology and Veterinary Sciences, AWKUM, Laboratory of Medicine and Parasitology for identification.

Blood sample microscopy was carried out using a traditional technique, as stated by Iqbal et al (2006). The process that followed was applied.

Preparation of Geimsa stain

Blood smears were stained using the Giemsa stain, one of the Romanowisky series of stains. Examining stained slides and identifying parasites: Using keys, as explained by Soulsby (1982), the identification of parasites was accomplished.

Step 1	Microscopic glass slides were cleaned properly to avoid any contamination or debris.
Step 2	Drop of blood samples was placed on the slide held in one hand and then spread over slide with edge of other slide hold at 45 degree with other hand.
Step 3	Smears were then air dried and fixed in absolute methyl alcohol for 1-2 minutes
Step 4	Fixed smears were then stained with 10% solution of Giemsa stain for 5 minutes.
Step 5	Slides were rinsed in running tap water to remove excessive stain and air dried.
Step 6	Smears were mounted with cover slips and examined under oil immersion (100X) lens.

Table I Preparation and staining of blood smear

Hematological examination

A hematological analyzer was used to examine all of the blood samples, both positive and negative, in order to determine how the disease affected the goat's various physiological biomarkers, such as the number of red blood cells (RBCs), hemoglobin (Hb) levels, packed cell volume (PCV), and lymphocytes.

Data analysis

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Microsoft Excel is used to store data, which can also be used to analyze basic descriptive statistics. Computation of descriptive statistics was done using IBM-SPSS version 24.0. In the statistical analysis, a P-value of less than 0.05 was deemed significant for the variation in the prevalence of theleria among risk variables, with a 95% confidence level maintained.

3. Results

The present study was conducted to investigate the prevalence of *Caprine* Theileriosis at eight different regions of southern area of Karak. Blood samples were collected from 300 animals Goats (n= 300). Among these (50%) were male while (50%) were female goats for detection of the disease. The samples were collected and processed in the laboratory of Medicine and Public Health, College of Animals Husbandry and Veterinary Sciences (CAH and VH), Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa.

Prevalence:

A hematological analyzer was used to examine all of the blood samples, both positive and negative, in order to determine how the disease affected the goat's physiological biomarkers (Red Blood Cells (RBC), Hemoglobin (Hb) levels, Packed Cell Volume (PCV), and Lymphocytes).

Overall Prevalence in Goats

Data analysis from various sites showed that 102 samples were positive out of 300 (34%). Among these 34%, 44.12% (45/102) were male positive cases while 26% (55.88/102) were female cases.

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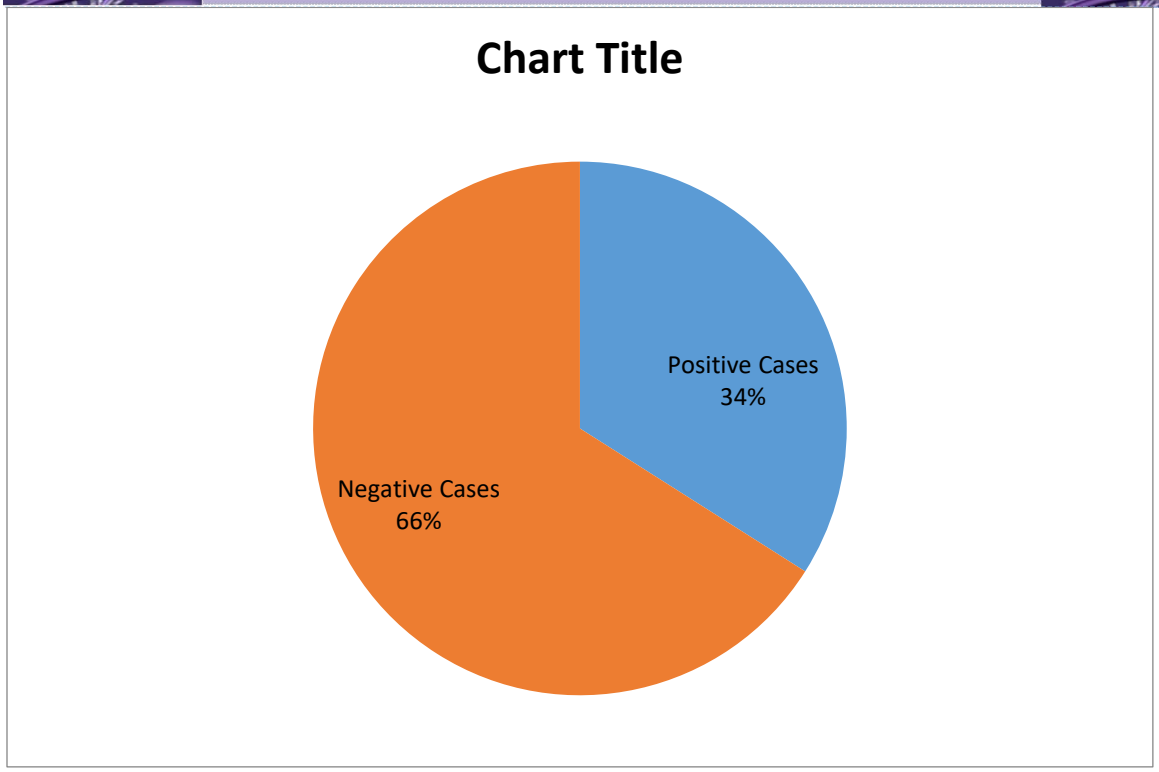

Figure 1: Overall Prevalence.

Area Wise Prevalence:

The highest prevalence was observed in the region of Kamran Chowk (KC) 22/102×100, while the lowest prevalence is screened in the region of Mitha Khel (MK) 11/102×100. Other prevalence was observed in Karak City (KKC) 21/102×100, Mitha Khel (MK)11/92×100, Kamran Chowk (KC) 22/102×100, Lawaghar Chani Khel (LCK) 21/102×100, Ghundi Mirkhan Khel (GMK) 15/102×100 and Shahidan Banda (SB) 11/102×100. T-test Analysis for means showed non-significant (P>0.05) difference in the prevalence of the disease in two regions.

S. No	Area	Sample Tag	Prevalence	%age	P-value
1	Karak City	KKC-001-50	14/102 ×100	13.72%	0.04

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2	Mitha Khel	MK-001-50	17/102×100	16.66%	0.04
3	Kamran Chowk	KC-001-50	21/102×100	20.58%	0.04
4	Lawaghar Chani Khel	LCK-001-50	26/102×100	25.48%	0.04
5	Ghundi Mir Khan Khel	GMK-001-50	12/102×100	11.76%	0.04
6	Shaheedan Banda	SB-001-50	12/102×100	11.76%	0.04
	G. Total	300	102	34%	

Table 2.: Area wise prevalence

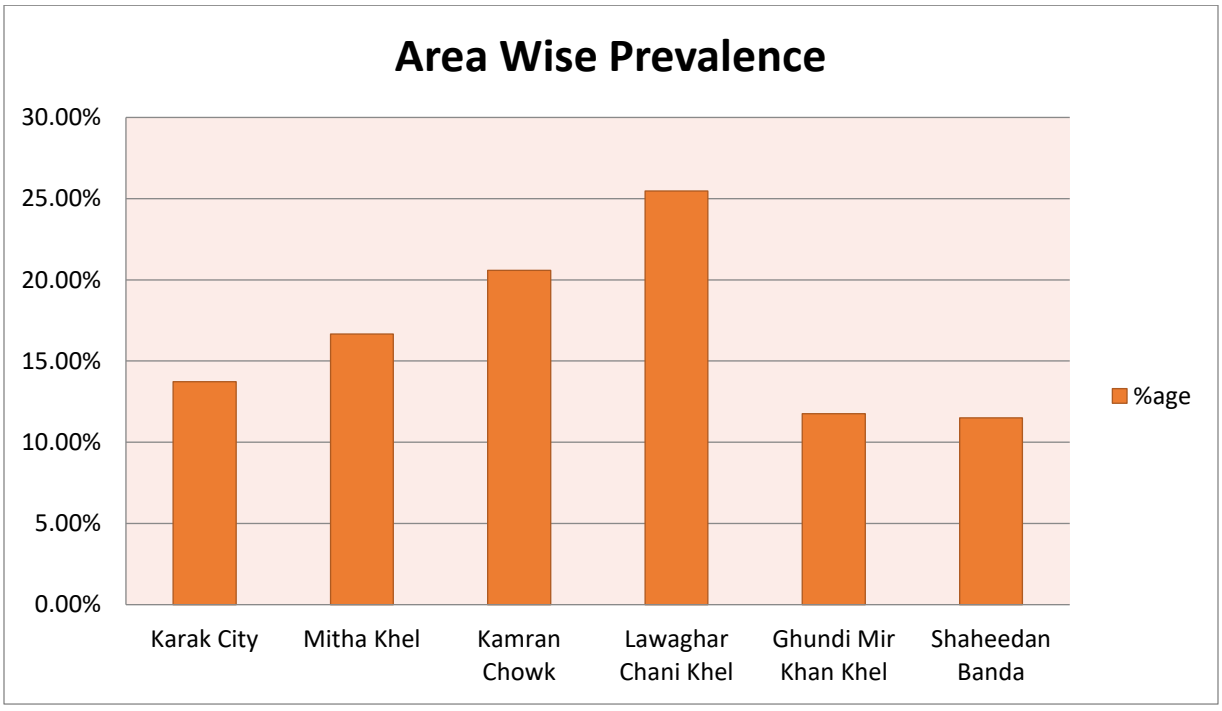

Figure I 4.2: Overall Prevalence.

Sex Wise Prevalence

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By microscopic examination of blood and Hematocrit blood count of infected was different to that of normal one. It was confirmed that female goat were more infected as compared to male. A total of 57/ 102×100 female goat were infected than male 45/102. Results showed significant decrease (P<0.01) in RBC, Hg and Hct. There was significant increase (P<0.01) in WBC in Theleria Annulata infected than non-infected young.

S.No,	Region	Male	%age	Female	%age	P-Value
1	Karak City	05/102	4.9%	09/102×100	8.82%	0.0000
2	Mitha Khel	09/102	8.82%	08/102×100	7.84%	0.0002
3	Kamran Chowk	08/102	7.84%	13/102×100	12.74%	0.004
4	Lawaghar Chani Khel	12/102	11.76%	14/102×100	13.72%	0.315
5	Ghundi Mir Khan Khel	06/102	5.88%	06/102×100	5.88%	0.012
6	Shaheedan Banda	05/102	4.9%	07/102×100	6.86 %	0.219
	G. Total	45/102	44.18%	57/102×100	55.82%	

Table 3. Area wise Prevalence:

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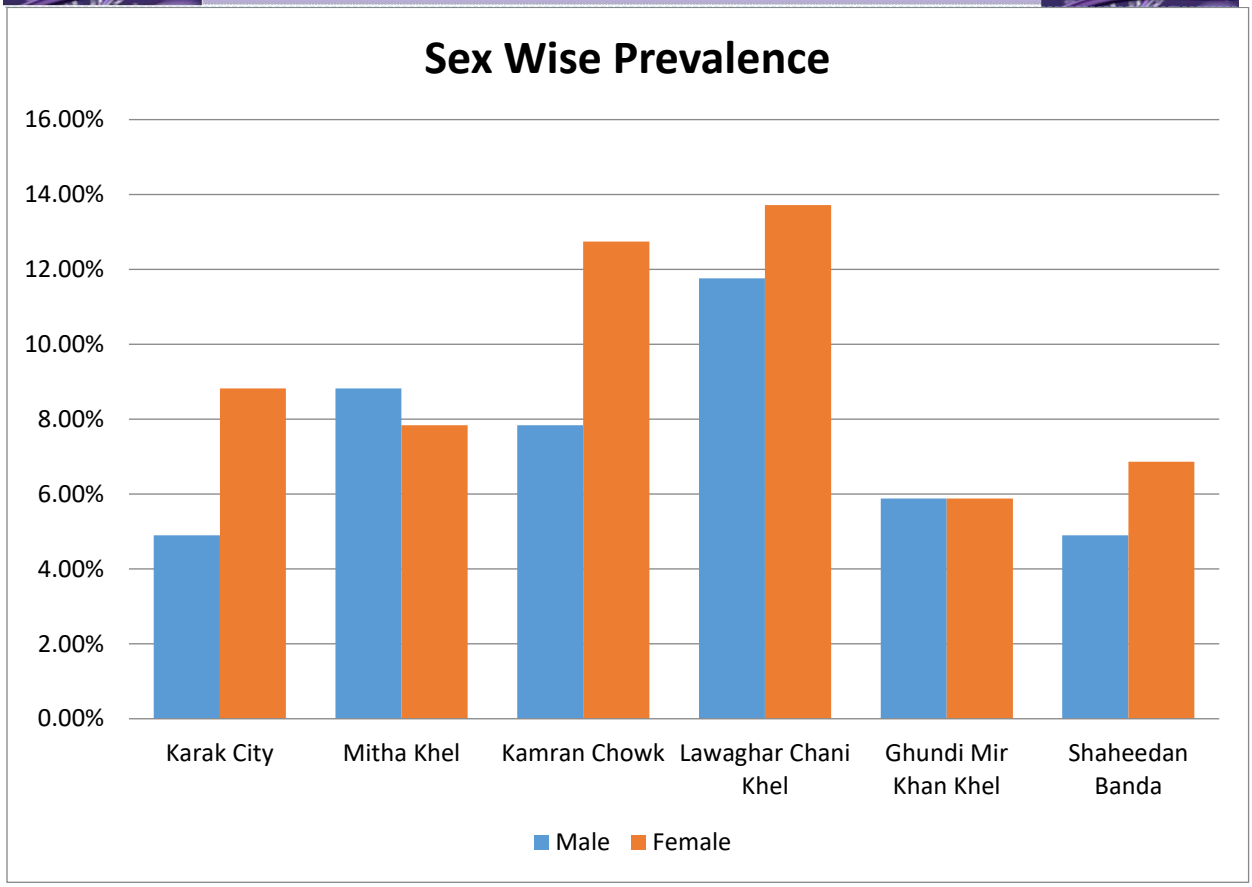

Figure 3 :Sex wise Prevalence

S.No	Parameter	Healthy Goat	Infected Goats	P-Value
1	Total WBC ($x10^3$/µl)	4-14	18.2	0.0000
2	Total RBC ($x10^6$/ µl)	8.02 -18.02	6.44	0.0002
3	Hgb (g/dL)	9-13	7.1	0.004
4	Lymphocyte (%)	22,00-10,000	12,500	0.315
5	Monocyte (%)	0-660	930	0.012
7	Granulocyte (%)	1300-7300	36,50	0.219

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8	Platelets (x10^3/ µl)	399.48	580.5	0.00001

Table II: Effect on Hematological Parameters

4. Discussion

The current study was conducted to ascertain the prevalence of theileriosis associated risks and effect on blood parameter in different locations of Karak. The effects of various housing systems, seasonal variation, age, and gender on the health of animals was also recorded. Theileriosis is vector-borne disease caused by Theileria spp. Once susceptible animals remain carriers of infection, caused a continuous threat to animal populations due to its zoonotic potential. After the completion of laboratory diagnosis, it was found that 102/300 (34%) goats were positive. The suitable ecological condition favors the transmission of Theileria species due to its eggs development and also through the large population of its vectors.

The lack of proper health status checks up and treatment leads to the increase in prevalence rate. In severe case it lead to the economic loss of the goats. Based on clinical results such as weakness, blood deficiency, abnormal gait, stiff neck muscles, abnormal posture, droopy eyes, infertility, dermatitis, and tick manifestation throughout the body. Durrani *et al.,* (2006) conducted a case study involving 600 animal blood samples, resulting in the appearance of 17.8% of the disease. Furthermore, it was noted that the disease rate in July 2003 was 15.6%, and at the end of the monsoon season in August and September 2003 it was 20.6% and 17.4%, respectively. He discovered an 18% incidence. Because of the hygienic state of that area, the ratio is lower than it is in current research work of district Karak area.

Based on an epidemiological study, Gachohi *et al.,* (2012) reported that a number of factors, including the host's immune status, the appearance of the vector population, the fatality rate, and the animals affected, can influence the occurrence of theileriosis. Other risk factors include living situations, housing conditions, environmental variation, immune status of the host, and sensitivity to the diseases. Treating different genotypes as separate units (species) further complicates an investigation of parasite epidemiological studies because it can obscure additive or competitive consequences between genotypic populations. But when it comes to spreading the infection to ticks as a species, they are crucial (Chaisi *et al.,* 2011 and Mans *et al.,* 2011b). Karak

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agro-agricultural regions offer the ideal climate for the spread of *Haylomma* ticks, which are the primary vector of tropical theileriosis throughout most of the district. This is the main reason for the higher prevalence ratio.

During the cross sectional study of caprine population in district karak various geographical region it was found that theileriosis cause heath abnormalities. Due tom unhygienic ecological factors goats get infected due to *theileria* species. *Theileria annulata* not only cause blood cells breakdown but also lead to serious economic loss if remain un treated. In lactating goats is cause reduction in the production of milk. Theileria infection can also cause reproductive issues, abortion and reduced fertility due to effect on endocrinology of the body. During the study it was noted that female were more exposed to infection of theileriosis compared to male due to their weak immune system and exposure to heterogeneous environment.

5. Conclusion

Theileriosis is an emerging issue of wild ungulates, especially in the tropical and subtropical areas of the globe. The current study had been designed to investigate the goats for *Theleria* infection using microscopic identification and hematological analysis. During this study a total of 300 blood samples collected randomly from asymptomatic goats using convenient sampling technique. The blood samples collected with the help of disposable syringesand preserved in EDTA tubes till analysis. All the blood samples examined in the laboratory of clinical medicine and Parasitology laboratory, college of veterinary sciences, Abdul Wali Khan University Mardan. The blood samples were examined under microscope after proper staining. After microscopic analysis it was found that 102 (34%) out 300 were positive. Out of 34% 44.1% (45/102) were male positive cases while 55.88% (57/102) were female cases. During examination regional prevalence showed highest prevalence in the region Lawaghar Chani Khel (LCK) 26/102, while the lowest prevalence is screened in the region Shaheedan Banda (SB) 12/102. Other prevalence is Karak City (KKC) 14/102, Mitha Khel (MK) 17/92, Kamran Chowk (KC) 21/102, Lawaghar Chani Khel (LCK) 26/102, Ghundi Mirkhan Khel (GMK) 12/102 and Shaheedan Banda (SB) 12/102. Chi-square analysis showed non-significant ($p< 0.05$) difference in the prevalence of the disease in two regions. A well-designed questionnaire designed showing various risk factors such as age, sex, breed, presence of ticks or not, season, month of collection of sample, name and address of the

owner, management, stall feeding or grazing. After microscopic examination the same blood samples analyzed auto hematological analyzer for identification of blood parameters. At the end of study prevalence, risk factors and hematological parameters determined using t-test and P value remain lower than 0.05.

6. Conflict of Interest

The authors do not have any conflict of interest to declare

7. Consent for Publication:

Written informed consent was taken from the patient for case report and publication. None of personal information will be disclosed in the final publication.

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