

RISK FACTORS ASSESSMENT AND MOLECULAR CHARACTERIZATION OF *THEILERIA* IN SMALL RUMINANTS OF BALOCHISTAN

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ABSTRACT

In Pakistan, theileriosis is an important disease of small ruminants due to favourable climatic conditions for ticks. The northern highlands of Balochistan were never been previously explored for theileriosis, so the present one year study (June 2012-May 2013) was designed to determine epidemiology and molecular characterization of *Theileria* in domestic small ruminants in two regions (Northern Highlands and Suleiman Mountain) of Balochistan, Pakistan. The domestic small ruminant flocks (n= 2200 sheep; n=670 goats) were visited for data collection and blood sampling. The samples were examined microscopically for the presence of *Theileria* in RBCs. The association of various risk factors with the disease were tested through Chi square test. The Prevalence of Theileriosis was higher in sheep than goats (20.81% vs 9.70%; p<0.05). We did not find any significant difference in the prevalence of disease in Northern Highlands and Suleiman Mountain Region of Balochistan (p>0.05). A clear trend of seasonal pattern (p<0.05) came to record with highest in summer season (30.30%) followed by autumn (19.07%), spring (14.52%) and winter (7.61%). The disease was significantly higher (p<0.05) in above two years age group (22.17%) followed by between 1-2years (15.85%) and lowest in less than one year (7.99%). Gender was not a significant risk factors for theileriosis (18.92% male vs 17.92% female; p>0.05). The molecular characterization of positive samples through 18S ribosomal RNA revealed the presence of *Theileria lestoquardi* and *Theileria ovis* in the infected animals. *T. lestoquardi* was dominated in both sheep (73.80%) and goats (69.23%) while *T. Ovis* accounted for only 26.19% of sheep infection and 30.76% in goats. The observed prevalence can be explained by the farming system of the rural livestock of Balochistan, lack of awareness of community, absence of diagnostic facilities, expensive acaricidal drugs and poor economic condition of farmers.

Key words: Theileriosis, *T. lestoquardi*, *T. ovis*, Balochistan, Small ruminants

INTRODUCTION

Theileriosis is a tick borne parasitic infection responsible for heavy economic losses to livestock industry due to high morbidity and mortality associated with it (Schnittger *et al.* 2000; Naz *et al.* 2012). There are several species of genus *Theileria* (*T. lestoquardi*, *T. ovis*, *T. separate* and newly identified *T. china1*) which vary in their virulence reported to cause theileriosis in small ruminants (Chaet *et al.* 1999; Li *et al.* 2014). The disease is characterized with fever, weakness, anorexia, conjunctival petechiae, swollen lymph nodes, anaemia and cough. The animals are also seen with nasal and ocular discharges, pyrexia, leucopenia, paleness of mucus membrane (Naz *et al.* 2012). The diseased animals may die within 3-4 weeks after getting lymphocytolysis if left untreated (Ahmed *et al.* 2015). The later stages of the disease are associated with diarrhea, dysentery, recumbency and increase risk for viral, bacterial and fungal infections. (Schnittger *et al.* 2000; Ahmed *et al.*

2015). Malignant Ovine theileriosis caused mostly by *T. lestoquardi* is considered one of the major constraints to domestic small ruminant industry of the tropical and subtropical countries where environmental factors are suitable for ticks (El-Azazy *et al.* 2001; Saeed *et al.* 2015; Razmi *et al.* 2006; Sayin *et al.* 2009; Durrani *et al.* 2011; Jabbar *et al.* 2015).

Theileriosis is diagnosed primarily on the basis of history of signs and symptoms followed by microscopic examinations of blood smears for the detection of parasites (Kirvar *et al.* 1998; Durrani *et al.* 2011). However, microscopy is not reliable in detection of carrier animals and piroplasm differentiation of *Theileria* species due to morphological similarities (Aktas *et al.* 2006). The development of advanced PCR based assay is considered as highly sensitive and specific for the detection of species of *Theileria* (Aktas *et al.* 2006; Durrani *et al.* 2011).

In Pakistan, theileriosis has been reported from small ruminants but never been extensively explored on large scale for prominent risk factors for the disease

(Durrani *et al.* 2011; Naz *et al.* 2012). The reported prevalence and impact of risk factors mentioned in previous studies is a subject of debate as they were conducted on small scale with small sample sizes (Durrani *et al.* 2011; Naz *et al.*, 2012) while on contrary to this the same disease is more extensively studied in large ruminants (Jabbar *et al.* 2015). To the best of our knowledge the northern highlands of Balochistan were never being investigated for this disease. So the present study was designed for comprehensive investigation of epidemiology and molecular characterization of theileriosis in small ruminants of northern highlands of Balochistan.

MATERIALS AND METHODS

Study Areas: Balochistan province is located in the north west of Pakistan, shares borders with Punjab and the Federally Administered Tribal Areas (FATA) to the northeast, the Arabian Sea to the south, Iran to the west, Sindh to the southeast, and Afghanistan to the north. The province has unique climate with very cold winters in Northern highlands and hot summer in Suleiman Mountains. The source of revenue for the poor rural communities of these regions is substantially from livestock farming (Weiss *et al.* 2012). The sheep and goats are reared under range management system with their abundance in Northern highland pastures (38% of total area of province) which are rich in their natural resource for grazing (Kakar, 2009). Three union councils of Northern Highlands (Kuchlak, Hanna Urak and Aghberg) and Soleiman Mountains (Zingiwai Jogazai, Kach Amagzai, Sinjavi) were randomly selected to screened domestic small ruminants for theileriosis (Figure 1).

Data Collection and Sampling: The small ruminant flocks in villages and pastures were visited during March 2012 to February 2013 for data collection and sampling. The data collection was carried out through self developed questionnaire which includes information *viz* species, gender, age, season, flock size, flock composition. Blood samples were taken from animals symptomatic for theileriosis in EDTA vacutainer tubes through ear tip puncture and stored in ice box before processing in laboratory (Benjamin, 1986).

Diagnosis of theileriosis: In the laboratory thin blood smear was prepared and processed on clean glass slides according to previously described procedure by Benjamin (1986). The presence of *Theileria* in the RBCs confirmed the diagnosis of theileriosis (Urquhart *et al.* 2002).

Molecular Characterization of *Theileria*: In order to characterize *Theileriaspp.* from positive samples, genomic DNA was extracted by GeneAll DNA extraction kit (GeneAll Biotechnology Co. Ltd, Banseok Bldg., 128

Ogum-dong, Songpa-gu, Seoul, Korea 138-859). The extracted DNA was quantified through spectrometer, Nanodrop 2000 (Thermo Scientific, USA). For the specific detection and identification of *Theileria* species i.e. *T. lestoquardi* and *T. ovis*, two primer sets were designed to amplify 340 bp and 300 bp fragments respectively of their 18S ribosomal RNA gene.

For identification of *T. lestoquardi*,

Forward [T.Les_F] 5 - CGGAGTTTCTTTGTCTGA-3

Reverse [T.Les_R] 5 -GAAGGAGTCGTA AAAAC

TGA-3 For identification of *T. ovis*, Forward [T.Les_F]

5 - GCTTTTGCTCCTTTACGA -3 Reverse [T.Les_R]

5 - ACGACCTCCAATCTCTA GTC-3 were used.

PCR reactions was performed in BioRad thermocycler in a total reaction volume of 25 μ l containing 5 μ l of 100ng/ μ l genomic DNA, 3 μ l of 1.5 mM MgCl₂, 2 μ l of 10 X PCR buffer [100mM Tris-HCl (pH = 9), 500 mM KCl, 1% Triton X-100], 2.5 μ l of 200 μ M of each dNTPs, 1 μ l of 10pM each forward and reverse primer, 0.5 μ l of 1.50 U Taq DNA polymerase (Fermentas) and 10 μ l water. The PCR condition was optimized under following program: for *T. lestoquardi* specific primer was an initial denaturation at 95°C for 5 minutes followed by 35 cycles, 45 s for denaturation at 95°C, 45 s for annealing at 52°C and 45 s for extension at 72°C and with a final extension step at 72°C for 10 minutes. The PCR conditions for the *T. ovis* specific primer were similar except annealing step which was performed at 54°C for 45 s. The PCR products were run on 1.5% agarose gel, 10 μ L of PCR product was mixed with 5 μ L 3 X bromophenol blue dyes and the electrophoresis was done at constant voltage 100V for 40-50 minutes in 1X TAE buffer.

Statistical analysis: The sample size for survey was calculated using EpiInfo™ 7 (Centers for Disease Control, Atlanta, GA, USA). Microsoft Excel (2007) was used to calculate the prevalence and corresponding 95% Confidence Interval. To test the association of risk factors (host species, region, breed, gender, age and season) with theileriosis Chi square test was performed on EpiInfo™ 7 (Centers for Disease Control, Atlanta, GA, USA). With reference to the age, the animals were classified into three age groups (<1 year, 1-2 years and >2 years). The strength of association of all risk factors with theileriosis was estimated through odds ratio (OR) and the corresponding 95% confidence intervals calculated on EpiInfo™ 7. p value less than 0.05 was considered as significant.

RESULTS

A total of 2870 small ruminants were screened during entire study among them 523 were diagnosed positive for theileriosis giving an overall prevalence of 18.22% (95% CI: 16.81%-19.64%) in small ruminants. The prevalence of theileriosis was higher in sheep

(N=458/2200; 20.82%; 95% CI: 19.12%-22.51%) as compared to goats (N=65/670; 9.70%; 95% CI: 7.46%-11.94%) with a significant difference between them ($\chi^2=42.59$, $p<0.05$) (Table 1). The prevalence of disease was 19.19% (95% CI: 17.00%-21.39%) in Northern Highlands and 17.48% (95% CI: 15.64%-19.33%) in Sulaiman Mountain Range without any statistical association of disease with regions ($\chi^2=1.38$, $p>0.05$). The distribution of disease in different union councils and breeds of host species are illustrated in Table 2 and 3. The prevalence of theileriosis was highest in summer (30.30%; 95% CI: 27.39%-33.21%), followed by autumn (19.07%; 95% CI: 15.52%-22.61%), spring (14.52%; 95% CI: 11.38%-17.67%) and lowest in winter (7.61%; 95% CI: 5.93%-9.29%). As regards age, above two years age group (22.17%; 95% CI: 20.09%-24.25%) was at highest risk, than between one to two years age group (15.85%; 95% CI: 13.56%-18.14%) whereas less than one year age group (7.99%; 95% CI: 5.20%-10.78%) was relatively less exposed ($\chi^2=13.81$ & 37.66 , $p<0.05$). There was no difference in the prevalence of theileriosis in male

(18.92%; 95% CI: 16.31%-21.52%) and female (17.92; 95% CI: 16.24%-19.60%) animals ($\chi^2=0.40$, $p>0.05$).

The DNA of all positive samples was successfully amplified during molecular characterization of *Theileria spp* (Figure 2). In 523 positive cases, *T. lestoquardi* (N=383/523; 73.23; 95% CI: 69.44%-77.03%) was found significantly higher than *T. ovis* (N=140/523; 26.77%; 95% CI: 22.97%-30.56%) in small ruminants of Northern Highlands and Suleiman Mountain Region of Balochistan ($\chi^2=225.81$, $p<0.05$). Among positive cases of sheep, *T. lestoquardi* accounted for 73.80% (N=338/458; 95% CI: 69.77%-77.83%) and remaining 26.20% (N=120/458; 95% CI: 22.17%-30.23%) were caused by *T. ovis* with a significant difference between them ($\chi^2=207.53$, $p<0.05$). *T. lestoquardi* (N=45/65; 69.23%; 95% CI: 58.01%-80.45%) was also dominating causative species of theileriosis in goats than *T. ovis* (N=20/65; 30.77%; 95% CI: 19.55%-41.99%) ($\chi^2=6.52$, $p<0.05$). As regards the studied regions both species were reported from Northern highlands and Suleiman Mountain region.

Table 1. Prevalence and associated risk factors of Theileriosis in Small Ruminants in Northern Balochistan.

Variable	Number of Samples examined	Number of Positive	Prevalence (%)	OR value	χ^2 value	P-Value
Species						
Sheep	2200	458	20.82	2.45	42.59	0.00000
Goats	670	65	9.70			
Sex						
Male	867	164	18.92	0.94	0.400	0.5270
Female	2003	359	17.92			
Age						
<1year	363	29	7.99		Reference category	
1-2year	978	155	15.85	2.17	13.81	0.000201
>2year	1529	339	22.17	3.28	37.66	0.000000
Season						
Spring	482	70	14.52		Reference category	
Summer	957	290	30.30	2.55	42.55	0.00000001
Autumn	472	90	19.07	1.38	3.529	0.0603
Winter	959	73	7.61	0.48	17.14	0.000034

Table 2. Regional Prevalence of Theileriosis in Small Ruminants in Northern Balochistan.

Region	District	Union Council	Number of Samples examined	Number of Samples Positive	Prevalence (%)	OR value	χ^2 value	P-Value
NH	Quetta		1240	238	19.19	1.12	1.38	0.24
SMR	Loralai		1630	285	17.48			
NH	Quetta	Kuchlak	480	109	22.71	Reference category		
		Hanna Urak	380	59	15.53	0.62	6.96	0.0083
		Aghberg	380	70	18.42	0.77	2.36	0.12
SMR	Loralai	ZangiwalJogezai	600	119	19.83	0.84	1.32	0.251
		KachAmaqzai	540	88	16.30	0.66	6.70	0.0096
		Sinjavi	490	78	15.92	0.64	7.18	0.007

Table 3. Prevalence of Theileriosis in four breeds of Sheep and Khurasani breed of goat in Northern Balochistan.

Breeds of Sheep	Total Samples	No. of Positive	Prevalence (%)	Odds Ratio	95% CI	t ²	P-value
Shinwari	709	174	24.54		Reference category		
Karakul	104	36	34.61	1.63	1.05-2.53	04.84	0.028
Bibrik	692	134	19.36	0.74	0.57-0.95	05.41	0.020
Harnai	695	114	16.40	0.60	0.46-0.79	14.15	0.002
Khurasani	670	65	09.70	0.33	0.24-0.45	52.59	0.00000001

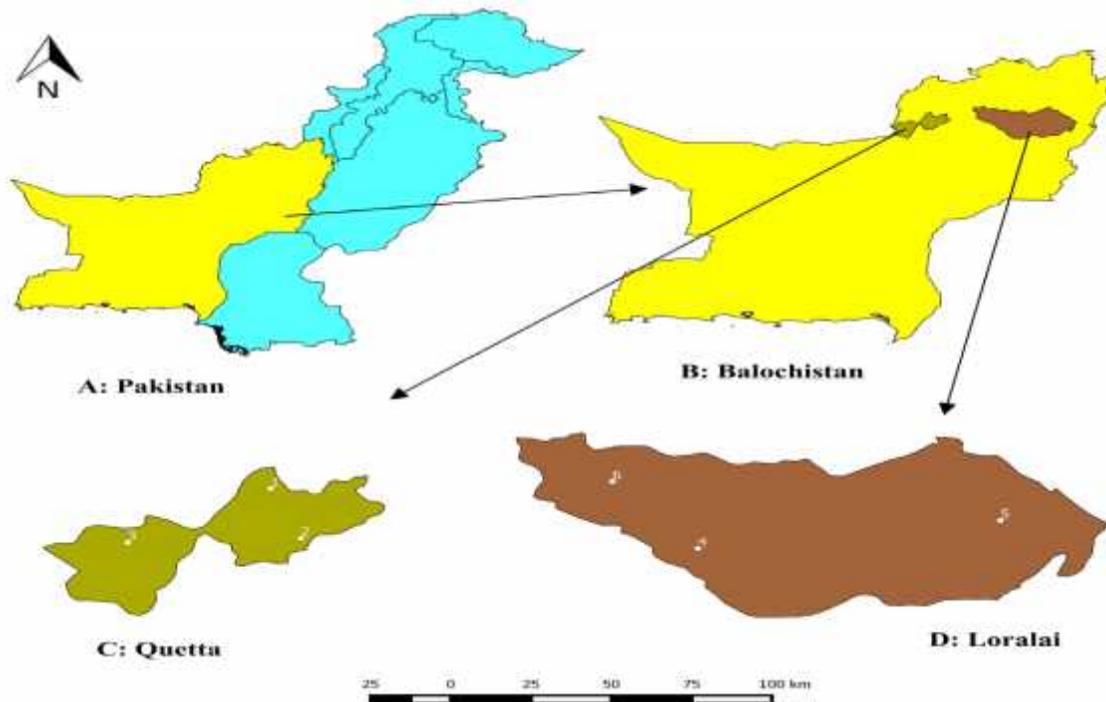


Figure 1. Two targeted areas showed in the map are Quetta (C) and Loralai (D) of Balochistan, where the samples were collected. The white circle showing the target regions Kuchlak (1), Unna Urak (2), Aghberg (3) in district Quetta, while Sinjavi (4), Kach Amakzae (5), Zingiwal Jogazae (6) in district Loralai.

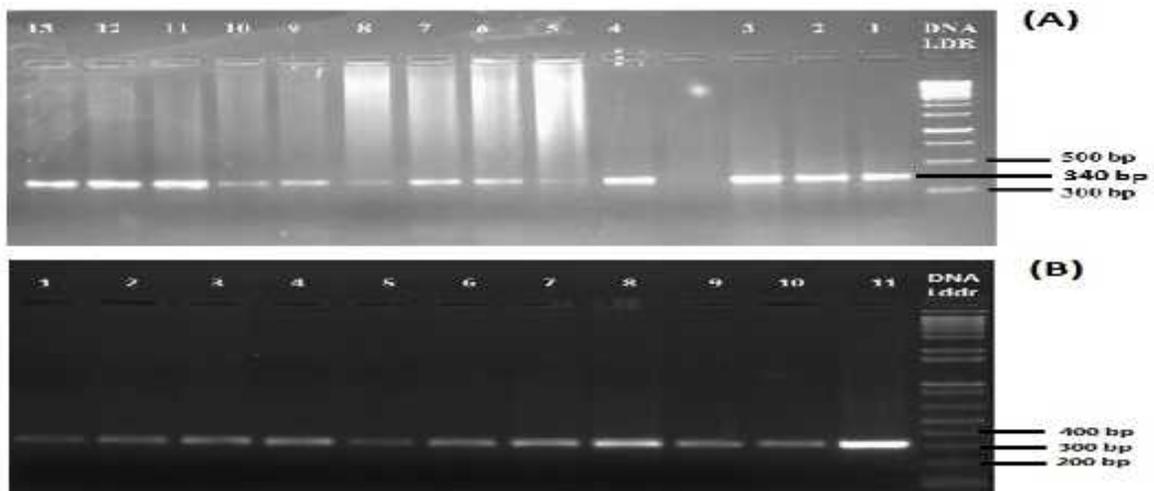


Figure 2. Agarose gel electrophoresis of amplified products achieved from *T. lestoquardi* (A), *T. ovis*(B) using species specific primers.

DISCUSSION

In Pakistan, several studies have been revealed the *Theileria* species are endemic in small ruminants in various part of the country (Durrani *et al.* 2011; Nazet *et al.* 2012; Durrani *et al.* 2012). The current study is pioneer observation in the most important livestock production regions, where this sector is central to the livelihoods of local people. The results on prevalence in present study in sheep (20.82%) and goats (9.70%) are in line with previous reports conducted in other regions of Pakistan. The results of Razmi and Yaghfoori (2013) and Ali *et al.* (2013) were also found in agreement with the present investigations. The results of Yas *et al.* (2014) showed 20.8% prevalence of *Theileria* in sheep and 4% in goats. Altay *et al.* (2007) reported 18.29% infection rate in sheep and 2.88% in goats. The results in contrast with present investigations were also reported by Naz *et al.* (2012) where they found 11.2% disease prevalence in small ruminants. A study from Turkey by Sayin *et al.* (2009) reported 64.19 % prevalence of infection in sheep and 12.36 % in goats much higher than present investigations. Nagore *et al.* (2004) reported 64.7% prevalence in sheep; these differences in prevalence were due to management and environmental conditions of the area. Data on regional prevalence revealed non-significant difference ($p>0.05$) in the disease in two regions of Balochistan. Both sheep and goats were found equally affected with Theileriosis however; in Northern highland region slightly higher rate of infection was found. This difference was due to the introduction of different species and breeds of the animal in the area as Quetta district is the major market of the province. The comparison of the prevalence of the disease at union council level showed significant difference ($p<0.05$). Data showed highest prevalence (22.71%) in Union Council Kuchlak district Quetta and Union Council Zangiwal Jogezi (19.83%) in District Loralai. This difference in prevalence was due to the reason that these Union Councils are the main markets for animal trade. Animals are routinely brought here from different parts of the country and Afghanistan for trade. This causes introduction of the infection in susceptible animals. In the present study Theileriosis was found a disease seriously affecting sheep in two regions of Balochistan. Many researchers reported the results in line with the present investigations from different parts of the world. In India, Velusamy *et al.* (2015) studied the prevalence in sheep and goats in North Western part of Tamil Nadu. The prevalence was found significantly higher in sheep as compared to goats. Although different rates of infection were reported but the researchers were convinced with the fact that sheep was the primary specie of small ruminants affected. The wool of the sheep and grazing behaviour favours vector of *Theileria* to propagate and transmission of infection. The difference in prevalence

rate at different geographic locations indicates the difference in management and environment. The present study showed highest prevalence of theileriosis in Karakul breed of sheep followed by Shinwari, Bibrik and Harnai. The results indicated the difference in susceptibility of breeds of sheep for Theileriosis. This difference of prevalence was reported first time in these breeds of sheep from Balochistan, Pakistan. The Harnai and Bibrik are the native breeds of sheep comparatively resistant to such infection. In the present study the infection rate was significantly lower ($p<0.05$) than exotic breed like Karakul. Chaisi *et al.* (2013) reported that the variation in infection rates of ruminants with tick borne pathogens is related to several factors out of which breed of the animal was found important because some breeds of animals were found very resistant to tick transmitted diseases. Karakul breed of sheep is introduced in Balochistan from Central Asia hence not resistant to infections like Theileriosis. The seasonal prevalence in sheep and goats showed significantly higher ($p<0.05$) rates of infection in summer (June-August). This difference was due to the maximum exposure of the animals to tick vectors. Moreover, during these months animals travel long distances for grazing and mixed with other flocks in grazing fields hence, share transmission of infections. The lower rates during winter months were due to the reason that animals were kept in captivity and there were less population of ticks. These results were in line with Irshad *et al.* (2010) and Sayin *et al.* (2009) who reported the highest prevalence of ticks during the summer season (May-July). The high prevalence during the summer months is attributed to hot and humid climate during summer season as ticks infestation was reported influenced by rainfall, temperature and humidity (Gosh *et al.* 2007). Data revealed significant difference ($p<0.05$) in rate of infection of three age groups. It was noted that infection increases with age of the animal. Adult animals above 2 year of age showed highest prevalence of disease compared with animals 1-2 year and below 1 year of age. RATHERA and Taka (2015) reported higher infection rate with *Theileria*, *Babesia* and *Anaplasma* of Cattle, sheep and goats in old ages as compared to age below 1 year. The results in line were also reported by Saleem and colleagues (2014) in cattle from Faisalabad, Pakistan. The less prevalence of the disease in young animals in Balochistan attributes to the fact that young ones are kept indoor during grazing hence less exposed to tick infestation. Adult animals more frequently exposed to hot humid conditions in rangelands during grazing season. Sex related prevalence showed non-significant difference ($p>0.05$) in both sheep and goats. These results were in agreement with Naz *et al.* (2012), which reported that there was no sex related effect on rate of infection with Theileriosis in sheep and goats. They also emphasized the view that both male and female animals have equal

chance of infection due to the same environment and management.

In sheep, the infection rate of two infectious agents *T. lestoquardi* was 73.80% and *T. ovis* was 26.19%. Heiderpour-Bamiet *et al.* (2010) also found the highest infection rate of *T. lestoquardi* 55.3% as compare to *T. ovis* 44.7% in sheep in Iran. The positive samples revealed high prevalence of infectious agents was recorded 65.96% in domestic sheep as compared to goat 34.04% in the study. It is much higher than the previous studies in the country, where Saeed *et al.* (2015) was reported 4.5% in sheep, 32% in Southern Punjab and Khyber Pakhtoon Khwa by Iqbal *et al.* (2013), 8.16% in Multan, Bahawalnagar, DG Khan, Layyah and Muzaffargarh by Fatima *et al.* (2015), whereas Durrani *et al.*, (2011) reported 35% in Lahore district of Punjab. Hence, the molecular prevalence of *T. lestoquardi* (73.83%) was much higher than *T. Ovis* (26.19%) in sheep in this study. However, our results conflicting with previous report in Lahore, where Durrani *et al.* (2011) was obtained 79% of *T. ovis* and 21% *T. lestoquardi* in sheep. The molecular prevalence obtained in goat was 69.23% of *T. lestoquardi* and 30.76% of *T. ovis* with overall prevalence of 9.7%. It was higher than 5% previously obtained by Iqbal *et al.* (2013) in Southern Punjab and Khyber Pakhtoon Khwa. In other studies, Saeed *et al.* (2015) and Fatima *et al.* (2015) obtained 2.5% and 0% in goat infected by *T. lestoquardi*. Similarly in sheep, the *T. lestoquardi* (69.23%) was more prevalent than *T. ovis* (30.76%) in goats.

Conclusions: The present study outcomes provide important information that should increase understanding of the epidemiology of *Theileria* species and assist the implementation of control measure of theileriosis transmission in small ruminants in the region. We observed that the molecular prevalence of *Theileria* species was significantly different in the domestic ruminants. The *T. lestoquardi* was highly prevalent in both sheep and goats than *T. ovis* in the region. The ticks were observed more attributed to sheep than goats due to unknown nature. However, it is assumed that the sheep's wool might be playing an important role in carrying the infectious agent.

REFERENCES

- Ahmed, H., El Imam, M. Khalid and M. Taha (2015). Malignant Ovine Theileriosis (*Theileria lestoquardi*) A Review. *Jordan. J. Biol. Sci.* 8(3): 165-174
- Aktas, M., K. Altay and N. Dumanli (2006). PCR-based detection of *Theileria ovis* in *Rhipicephalus bursa* adult ticks. *Vet. Parasitol.* 140(3-4):259-63.
- Ali, M. B., H. Ehsan, and R. Mostafa (2013). Theileriosis in Grazing Sheep and its Interrelation with the Reptiles Ticks. *Global Veterinaria* 10 (5): 599-606.
- Altay, K., M. Akta , and N. Dumanli (2007). *Theileria* Infections in Small Ruminants in the East and Southeast Anatolia. *Türkiye Parazitolo Dergisi*, 31 (4): 268-271.
- Benjamin, M. M. (1986). *Outline of Veterinary Clinical Pathology*, 3rd Ed. The Iowa State Uni. Press, Ames, Iowa, USA. Pp: 7-8, 29-30.
- Chae, J., B. A. Allsopp, S. D. Waghela, J. Park, T. Kakuda, C. Sugimoto, M. P. Allsopp G. G. Wagner and P. J. Holman. (1999). A study of the systematics of *Theileria* spp. based upon small-subunit ribosomal RNA gene sequences. *Parasitol. Res.* 85: 877-883.
- Chaisi, M. E., N. E. Collins, F. T. Potgieter, and M. C. Oosthuizen (2013). Sequence variation identified in the 18S rRNA gene of *Theileria mutans* and *Theileria velifefera* from the African buffalo (*Syncerus caffer*). *Vet. Parasitol.* 191: 132-137.
- Durrani, A. Z., M. Younus, N. Kamal, N. Mehmood, A. R. Shakoori (2011). Prevalence of ovine *Theileria* species in district Lahore Pakistan. *Pakistan J. Zool.* 43:57-60.
- Durrani, S., Z. Khan, R. M. Khattak, M. Ali, H. Hameed, A. Taqddas, M. Faryal, S. Kiran, M. Riaz, S. Hussain, R. S. Shiek, M. Ali and F. Iqbal (2012). A comparison of the presence of *Theileria ovis* by PCR amplification of their ssurRNA gene in small ruminants from two provinces of Pakistan. *Asian Pacific J. Trop. Dise.* 2:43-47.
- El-Azazy, O. M. E., T. M. El-Metenawy and H. Y. Wassef (2001). *Hyalomma impeltatum* (Acari: Ixodidae) as a potential vector of malignant theileriosis in sheep in Saudi Arabia. *Vet. Parasitol.* 99: 305-309
- Fatima, M., S. Saeed, R. S. Shaikh, M. Ali, and F. Iqbal (2015). A Study on Molecular Detection of *Theileria lestoquardi* by PCR Amplification in Apparently Healthy Small Ruminants from Five Districts of Southern Punjab. *Pakistan J. Zool.* 47(2), 441-446.
- Gebrekidana, H., A. Hailu, A. Kassahun, I. Rohousová, C. Maiac, D. Talmi-Franka, A. Warburge and G. Baneth (2010). *Theileria* infection in domestic ruminants in northern Ethiopia. *Vet. Parasitol.* 200, 31-38
- Gosh, S., G. C. Bansal, S. C. Gupta, D. Ray, M. Q. Khan, H. Irshad, M. Shahiduzzaman, U. Seitzer, and J. S. Ahmed (2007). Status of tick distribution in Bangladesh, India and Pakistan. *Parasitol. Res.* 101: 207-216.

- Heidarpour-Bami, M., P. Khazrainia, H. R. Haddadzadeh and B. Kazemi (2010). Identification of *Theileria* species in sheep in the eastern half of Iran using nested PCR-RFLP and microscopic techniques. *Iranian J. Vet. Res.* 11:262–266.
- Iqbal, F., R. M. Khattak, S. Ozubek, M. N. K. Khattak, A. Rasul, and M. Aktas (2013). Application of the reverse line blot assay for the molecular detection of *Theileria* and *Babesia* sp. in sheep and goat blood samples from Pakistan. *Iranian J. Parasit.* 8: 289-295.
- Irshad, N., M. Qayyum, M. M. Hussain and M. Q. K. Khan (2010). Prevalence of Tick Infestation and Theileriosis in Sheep and Goats. *Pakistan Vet. J.* 30 (3): 178-180.
- Jabbar, A., T. Abbas, Z. D. Sandhu, H. A. Saddiqi, M. F. Qamar and R. B. Gasser. 2015. Tick-borne diseases of bovines in Pakistan: major scope for future research and improved control. *Parasites and Vectors* 8:283.
- Kakar, A. R. (2009). Assessing the potential of the indigenous livestock breeds of Balochistan. A Dry net Sci Tech Expertise Technical report.
- Kirvar, E., T. Ilhan, F. Katzer, G. Wilkie, P. Hooshmand-Rad, and C. G. D. Brown (1998). Detection of *Theileria*lestoquardi (hirci) in ticks, sheep and goats using the polymerase chain reaction. *Ann. New York Acad. Sci.* 29, 52–62.
- Li, Y., Z. C. Z. Liu, J. Liu, J. Yang, Q. Li, Y. Li, S. Cen, G. Guan, Q. Ren, J. Luo and H. Yin. (2015). Molecular identification of *Theileria* parasites of northwestern Chinese Cervidae. *Parasites and Vectors* 7: 225.
- Nagore, D., J. Garcia, Sanmartin, P. Al Garcia, R. A. Juste, and A. Hurtado (2004). Identification, genetic diversity and prevalence of *Theileria* and *Babesia* species in a sheep population from northern Spain. *Int. J. Parasitol.* 9:1059-1067.
- Naz, S., A. Maqbool, S. Ahmed, K. Ashraf, N. Ahmed, K. Saeed, M. Latif, J. Iqbal, Z. Ali, K. Shafi, and A. Nagra (2012). Prevalence of Theileriosis in Small Ruminants in Lahore. *J. Vet. Anim. Sci.* 2: 16-20.
- Rathera, S. A. H., and D. K. K. Taka (2015). Seroprevalence of Haemoprotozoan diseases in ruminants of Kashmir valley. *Agricultural Advances* 4(11): 122-128.
- Razmi, G., and S. Yaghfoori (2013). Molecular surveillance of *Theileria* ovis, *Theileria* lestoquardi and *Theileria* annulata infection in sheep and ixodid ticks in Iran. *Onderstepoort J. Vet. Res.* 80: 76-83.
- Razmi, G. R., H. Eshartic, and M. Rashtibaf (2006). Prevalence of *Theileria* spp. Infection in sheep in South Khorasan Province, Iran. *Vet. Parasitol.* 140: 3-4.
- Rehman, Z. U., Khan, M. S., Avais, M., Aleem, M., Shabbir, M. Z. & Khan, J. A. (2010). Prevalence of theileriosis in sheep in Okara District, Pakistan. *Pakistan J. Zool.* 42: 639-643.
- Saeed S, M. Jahangir, M. Fatima, R. S. Shaikh, R. M. Khattak, M. Ali and F. Iqbal (2015). PCR based detection of *Theileria*lestoquardi in apparently healthy sheep and goats from two districts in Khyber Pukhtoon Khwa (Pakistan). *Trop. Biomed.* 32(2): 225–232.
- Saleem, M. I., A. Tariq, A. Shazad, and S. A. Mahfooz (2014). Clinical, epidemiological and therapeutic studies on bovine tropical theileriosis in Faisalabad, Pakistan. *Iraqi J. Vet. Sci.* 28(2): 87-93.
- Sayin, F., S. Dincer, Z. Karaer, A. Cakmak, A. Inci, B. A. Yukari, H. Eren, Z. Vatansever and S. Nalbantoglu (2009). Studies on the epidemiology of tropical theileriosis in cattle in central Anatolia, Turkey. *Trop. Anim. Health Prod.* 35: 521-539.
- Schnittger, L., H. Yin, L. Jianxun, W. Ludwig, P. Shayan, S. Rahbari, A. Voss-Holtmann and J. S. Ahmed (2000). Ribosomal small-subunit RNA gene-sequence analysis of *Theileria* lestoquardi and a *Theileria* species highly pathogenic for small ruminants in China. *Parasitol. Res.* 86: 352–358.
- Urquhart, G. M., J. Armour, J. L. Duncan, A. M. Dun, and F. W. Jennings (2002). *Veterinary Parasitology*, 2nd Ed. Blackwell Science Co, London. 254-255.
- Velusamy, R., N. Rani, G. Ponnudurai, and P. Anbarasi (2015). Prevalence of intestinal and haemoprotozoan parasites of small ruminants in Tamil Nadu, India. *Vet. World* 6: 1205-1209.
- Weiss, A. M., and M. M. Aurangzeb (2012). Pakistan, Louis Kotzé and Stephen Morse (eds), *Berkshire Encyclopedia of Sustainability*, Vol. 9. Great Barrington, MA: Berkshire, 236-240.
- Yas, A., D. Noaman, and N. Aaiz (2014). Prevalence of Theileriosis in sheep in Wasit province. *AL-Qadisiya J. Vet. Med. Sci.* 13 (2): 47-55.