Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2022.012

RESEARCH ARTICLE

First Molecular Evidence of *Ehrlichia* Infection: An Emerging Pathogen of Small Ruminants in Pakistan

Muhammad Abdul Basit[1], Muhammad Ijaz[1], Rao Zahid Abbas[2], Jawaria Ali Khan[1] and Kamran Ashraf[3]

[1]Department of Veterinary Medicine, University of Veterinary and Animal Sciences, Lahore-Pakistan
[2]Department of Parasitology, University of Agriculture, Faisalabad-Pakistan
[3]Department of Parasitology, University of Veterinary and Animal Sciences, Lahore-Pakistan
*Corresponding author: mijaz@uvas.edu.pk

ARTICLE HISTORY (21-452)

Received: November 12, 2021
Revised: January 28, 2022
Accepted: February 08, 2022
Published online: February 15, 2022

Keywords:
Ehrlichia
Hematology
Phylogenetic analysis
Prevalence
Risk factors

A B S T R A C T

The emergence and inter-species dissemination of new tick-borne pathogens have become a serious health hazard in domestic animals. Ehrlichiosis caused by *Ehrlichia* (*E.*) *ruminantium* in small ruminants is becoming an emerging and prevailing issue adversely affecting the health and production of sheep and goats. The current study was designed to investigate the *Ehrlichia* infection load in the sheep and goat population of South Punjab, Pakistan. The phylogenetic analysis of local isolates along with risk factor analysis and comparative hematological analysis was also conducted. A total of 192 blood samples consisting of sheep (n=96) and goat (n=96) were subjected to microscopy and PCR. The study results revealed an overall prevalence of 04.69 and 7.81% based on microscopy and PCR respectively. A higher molecular prevalence of *Ehrlichia* was observed in goats (9.38%) as compared to sheep (6.25%). The phylogenetic tree constructed by the maximum likelihood method exhibited the identity of study isolate with *E. ruminantium* isolated from various countries. The risk factor analysis showed a significant association of previous tick history, tick infestation, presence of shrubs, and tick control strategies adaptation with the disease occurrence. The hematological analysis observed a significant reduction in RBCs, hemoglobin, and hematocrit in infected animals compared to healthy animals. The study concludes that *Ehrlichia* infection is an emerging issue of small ruminants. The hematological findings can be helpful in early detection of disease in field conditions but molecular confirmation by PCR is necessary for accurate diagnosis of *Ehrlichia* infection while risk factor analysis will provide the relevant information to develop control measures for preventing the spread of disease.

To Cite This Article: Basit MA, Ijaz M, Abbas RZ, Khan JA and Ashraf K, 2022. First molecular evidence of *Ehrlichia* infection: An emerging pathogen of small ruminants in Pakistan. Pak Vet J, 42(2): 208-214. http://dx.doi.org/10.29261/pakvetj/2022.012

INTRODUCTION

Tick-borne hemoparasitic diseases (TBHDs) caused by protozoans (*Babesia, Theileria*) and bacteria (*Ehrlichia, Anaplasma*) are considered a serious constraint in the livestock health and productivity in tropical and sub-tropical ranges where the host, disease, and vector distributions join together (Jongejan *et al.*, 1991; Ali *et al.*, 2020). The pathogens that cause tick-borne diseases are of zoonotic impact as the introduction of new and the re-emergence of previously treated tick-borne pathogens has a significant influence on veterinary and human medicine (Ceylan *et al.*, 2021). However, due to a growing concern about the socio-economic importance of small ruminants (Yin *et al.*, 2007), more attention is now being paid to pathogens of sheep and goats because the losses associated with TBHDs include production losses, mortality, veterinary diagnosis/treatment costs, and tick control strategies (Ghaffar *et al.*, 2020).

Ehrlichiosis is a disease caused by the rickettsial organism *Ehrlichia* (*E.*) *ruminantium* and is mainly transmitted by ticks of the Genus Amblyomma. It affects a wide range of ruminants including cattle, sheep, goats, and certain wild animals (Allsopp, 2010). In small ruminants, TBHDs typically manifest as subclinical infections and a long-lasting carrier state also develop in animals that survive an acute stage of illness, and it is associated with significant productivity and economic

losses in the long run. *Ehrlichia* infected goats show a variety of clinical signs including serous nasal discharge, pyrexia, inappetence, lethargy, decreased alkaline phosphatase, and neutropenia (Loftis *et al.*, 2008). *Ehrlichia* multiplies in monocytes and macrophages, as well as peripheral blood neutrophils after infecting the host, and spreads to phagocytic cells of various organs such as the liver, spleen, lungs, and lymph nodes (Zhang *et al.*, 2017). The recovered animals remain chronically infected for several months after exposure to *Ehrlichia* infection. Clinical signs of *Ehrlichia* infection, tick history, lab tests including serological assays, and DNA detection by PCR are considered to be the most reliable techniques to diagnose Ehrlichiosis (Peter 2020; Selim *et al*., 2021). However, the diagnosis of Ehrlichiosis remains still unconfirmed due to varying clinical symptoms of the disease (Zhang *et al.*, 2017).

The epidemiology of Ehrlichiosis in small ruminants is not well understood. In Ehrlichiosis endemic areas, where extensive livestock systems exist and ticks are not controlled or restricted, ticks numbers are high and animals that are continuously exposed to ticks, are likely to be infected with *E. ruminantium* (O'Callaghan *et al.*, 1998). Some investigators say that endemic stability of ruminant and tick-borne infections, in general, may depend on the infection of very young hosts by tick infections during periods of reduced susceptibility to clinical disease (Perry and Young, 1995). To date, comprehensive data on disease transmission and control of ticks and tick-borne diseases is inadequate in Pakistan. Various serological techniques have been used in many countries of the world including China, Kenya, Gambia, and Zimbabwe for detecting Ehrlichiosis in sheep and goats. This is the first report on molecular evidence of *Ehrlichia* infection in small ruminants of Pakistan to highlight this neglected pathogen as well as to control this disease by adopting different control strategies.

MATERIALS AND METHODS

Sampling strategy: From August 2020 to July 2021, a total of 192 blood samples from small ruminants (n=96 goats, and n=96 sheep) were collected from various veterinary hospitals, farms, and diagnostic laboratories of South Punjab, Pakistan (Fig 1). The animals manifesting tick infestation or any clinical sign of pyrexia, nasal discharge, dyspnoea, anorexia, coughing, nervous signs or petechial hemorrhages were included in the study regardless of breed, age, and sex. A questionnaire was designed to analyze the association of numerous animal-based and farm-based assumed risk factors with the *Ehrlichia* infection in small ruminants. The risk factors considered in this study include age, sex, previous or recent tick infestation history, tick control strategies, farm hygiene, and housing type.

For screening of the animals, thin blood smears (in triplets) from all the suspected animals were prepared, air-dried, and later on processed for Giemsa staining and microscopy. For molecular confirmation of *Ehrlichia* and to check its effect on various hematological parameters of sheep and goats, 3ml of blood from each animal was collected aseptically by jugular venipuncture. The EDTA-coated vacutainers containing the blood samples were dispatched to the laboratory at 4ºC and processed for further analysis.

DNA extraction and PCR analysis: All collected blood samples were subjected to DNA extraction using the kit method (GeneAll® Exgene™ Blood SV mini 105-101). After the extraction of DNA following the manufacturer's guidelines, the concentration of DNA in samples was measured using Nano-Drop at 260/280nm. The extracted DNA samples were stored at -20ºC until further processing by PCR.

The molecular confirmation of *Ehrlichia* spp. was done by targeting 304bp DNA fragments of *sodb* gene. The forward and reverse primers (F=TTTAATAATGCTGGTCAAGTATGGAATCAT; R=AAGCGTGTTCCCATACATCCATAG) were used to amplify the *sodb* gene of *Ehrlichia* (Qurollo *et al.*, 2014). The steps of PCR reaction include 5 minutes of initial denaturation at 95ºC followed by 30 sec of denaturation at 95ºC, annealing at 57ºC for 30 sec, and extension at 72ºC for 1 minute. The final elongation was done for 10 minutes at 72ºC. The PCR products were run on gel electrophoresis and visualized under a UV illuminator (Fig. 2).

Sequencing and phylogenetic analysis: The purification of *Ehrlichia* positive PCR bands was done by using a gel extraction kit (GeneAll® Expin™ Gel SV 102-150) and purified samples were shipped for sequencing. The obtained sequences were further analyzed by various bioinformatics tools. The nucleotide sequences of the study isolate obtained after sequencing were evaluated using the Basic local alignment search tool (BLAST) of NCBI. All the sequences showed high similarity so a representative sequence from 15 positive samples was used to check the identity of local isolates with already reported *Ehrlichia* isolates. Mega X (Molecular evolutionary genetic analysis) software version 10.1.6 was used for constructing the phylogenetic tree using the Maximum Likelihood (ML) method.

Hematological analysis: The hematological parameters were conducted on a total of 10 small ruminants (n=5 goats; n=5 sheep) positive for *Ehrlichia* spp. The hematology of healthy animals (n=5 goats; n=5 sheep) was also performed for comparison. Various hematological parameters like total erythrocyte count (TEC), packed cell volume (PCV), hemoglobin (Hb) level, platelet count, and total leukocyte count (TLC) were evaluated for any type of variation associated with *Ehrlichia* infection.

Data analysis: The statistical analysis of study data was done by using statistical software SPSS version 20.00. To assess the association of various assumed risk factors with the *Ehrlichia* infection in small ruminants, a Chi-square test, and logistic regression were used. The data on hematological parameters were analyzed using an independent sample *t*-test with a 95% confidence interval. The variables having a *p*-value less than "0.05" and odds ratio more than "1.00" were considered significant and potential risk factors towards the disease occurrence.

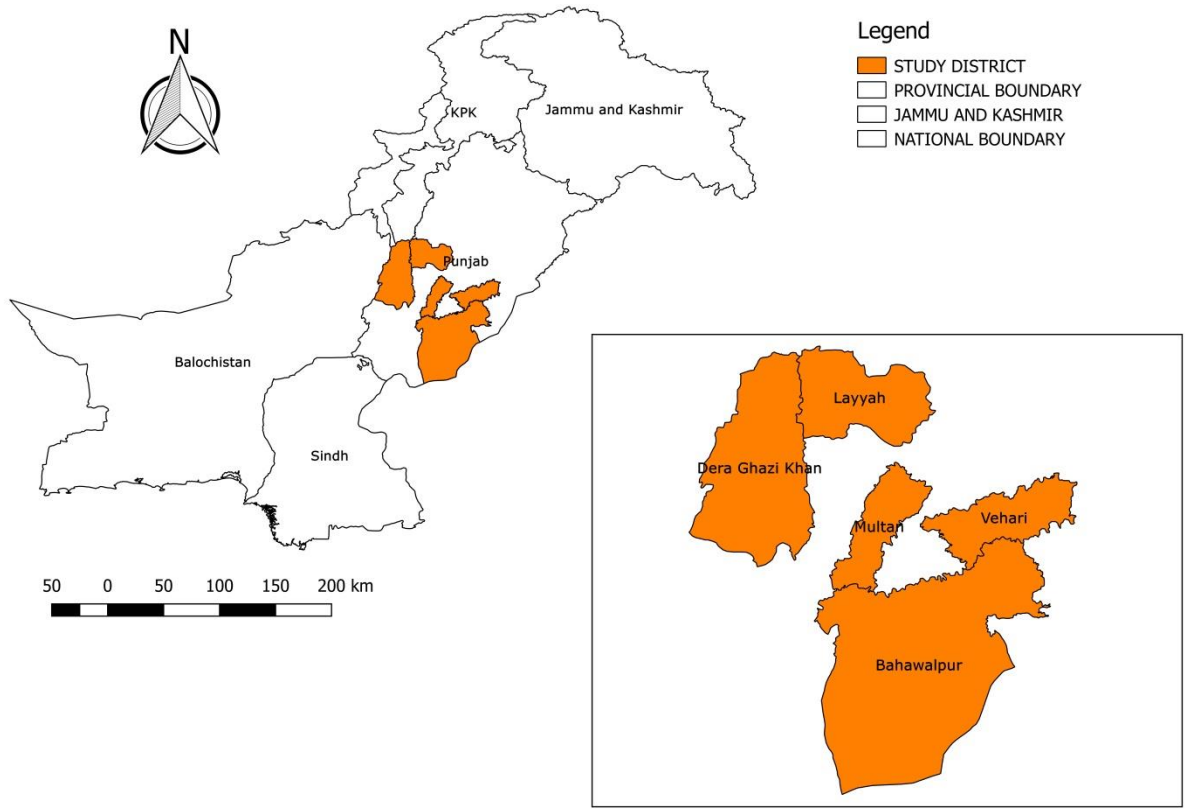

Fig. 1: QGIS map showing sampling area for the current study.

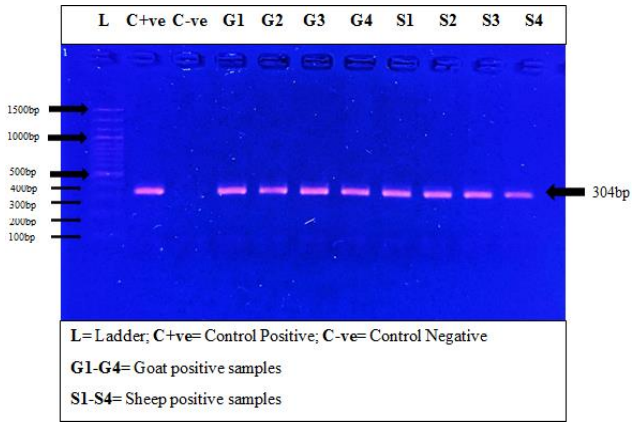

Fig. 2: PCR results of small ruminants samples positive for *sodb* gene of *Ehrlichia.*

RESULTS

Prevalence of *Ehrlichia* infection in small ruminants: The findings of the current study revealed an overall prevalence of 04.69% in small ruminants based on microscopy results in which goats exhibited a higher prevalence of 05.21% as compared to 04.17% in sheep. While the current study found an overall 7.81% (15/192) molecular prevalence of Ehrlichiosis in domestic small ruminants. Among the domestic small ruminants, goats were found more prone to disease showing a prevalence of (9.38%) compared to sheep (6.25%) as shown in Table 1.

The area-wise molecular prevalence showed a higher infection rate in district Bahawalpur (14.29 %) followed by Layyah (12.50%), Multan (10.26%), DG khan (2.56%), and Vehari (2.13%) (Table 2).

Risk factor analysis: The data regarding various animal-based and management-based factors was collected to evaluate the association of various assumed risk factors with Ehrlichiosis in domestic small ruminants. The risk factor analysis revealed that recent or previous tick infestation history, presence of shrubs in the housing of animals, and adoption of ticks control strategies showed significant ($P<0.05$) association with the disease based on chi-square analysis. Sex of the animal was proved a non-significant determinant with female animals having a greater prevalence (8.13%) than males (7.25%). The age of animals was non-significantly ($P>0.05$) associated with Ehrlichiosis showing more prevalence (9.41%) of disease in younger small ruminants (sheep and goat) compared to the older ones (6.54%). The housing type, farm hygiene, methods, and acaricide interval were found non-significant ($P>0.05$) determinants towards disease (Table 2).

Afterward, the variables having ($P<0.2$) were also analyzed by the logistic regression model. Tick infestation in the animals was a significant risk factor towards the occurrence of disease and the animals having tick infestation were 5.44 times more at risk of disease occurrence while the animals showing tick infestation in the past were 3.259 times more at risk of acquiring this disease. Similarly, the animals with poor hygienic conditions at the farm level were 3.33 times more affected as compared to animals with good hygienic measures at the farm level while the prevalence of Ehrlichiosis was 3.13 times more in the animals having shrubs around their housing. The findings of tick control status also showed an association with the occurrence of disease in such a way that the animals at which control measures are not adopted at farm level were 10.672 times more infected as compared to animals having proper tick control practices (Table 3).

Table 1: Overall prevalence of Ehrlichiosis in small ruminants of South Punjab

Ruminants *spp.*	No. of animals examined	Microscopy		PCR	
		No. positive	Prevalence (%)	No. positive	Prevalence (%)
Sheep	96	04	04.17	06	06.25
Goat	96	05	05.21	09	09.38
Total	192	09	04.69	15	07.81

Table 2: Risk factors associated with Ehrlichiosis in small ruminants

Study Variables	Category	Positive (%)	Negative	*p*-value
Species	Sheep	06 (06.25)	90	0.42
	Goat	09 (09.38)	87	
Area	DG Khan	01 (02.56)	38	0.13
	Bahawalpur	05 (14.29)	30	
	Layyah	04 (12.50)	28	
	Vehari	01 (02.13)	46	
	Multan	04 (10.26)	35	
Sex	Male	05 (07.25)	64	0.82
	Female	10 (08.13)	113	
Age	<2 Years	08 (09.41)	77	0.46
	>2 Years	07 (06.54)	100	
Housing type	Open	08 (10.67)	67	0.23
	Congested	07 (05.98)	110	
Tick infestation	Yes	12 (13.79)	75	0.05
	No	03 (02.86)	102	
Shrubs around the house	Present	10 (12.66)	69	0.036
	Absent	05 (04.42)	108	
Previous tick history	Yes	11 (11.96)	81	0.04
	No	04 (04.00)	96	
Tick control status	Yes	13(16.25)	67	<0.01
	No	02 (01.79)	110	
Method of acaricide application	Topical	02 (03.85)	50	0.22
	Parenteral	00 (00.00)	11	
	NA	13 (10.08)	116	
Interval of acaricide use (days)	Less than 30	01 (02.86)	34	0.104
	More than 30	01 (02.50)	39	
	NA	13 (11.11)	104	
Hygiene at farm	Good	03 (03.61)	80	0.056
	Poor	12 (11.11)	96	

P<0.05 shows a significant effect

Table 3: Risk factors included in a logistic regression model

Variables	Variable levels	Odd ratio	95% C.I.		S.E	*p*-value
			Lower	Upper		
Tick control status	No	10.672	2.335 – 48.762		0.775	0.002
	Yes	1				
Hygiene at farm	Poor	3.33	0.909 – 12.225		0.663	0.069
	Good	1				
Previous tick history	Yes	3.259	1.000 – 10.628		0.603	0.05
	No	1				
Shrubs around house	Yes	3.13	1.026 – 9.548		0.569	0.04
	No	1				
Tick infestation	Yes	5.44	1.483 – 19.957		0.663	0.01
	No	1				

p<0.05 and OR>1 show a significant effect.

Phylogenetic analysis: The current study isolates showed resemblance with *sodB* gene of *E. ruminantium* present in the Genbank database by Basic Local Alignment Search Tool (BLAST). The alignment of sequences by Clustal W multiple alignment tool by bio-edit software revealed that the current study isolate (Ehrlichia SR12) showed significant substitutions at almost 50 different base pair positions in comparison with reported sequences (Fig. 3). The current study sequence showed significant variation with reported sequences as compared to dissimilarity among themselves.

A representative sequence was selected from the PCR-positive study isolates and a phylogenetic tree was constructed using the Maximum Likelihood method (Fig. 4). The results revealed that the study isolate showed more resemblance with the *E. ruminantium* isolates of the USA and Nicaragua. However, the isolate made a separate clade by showing less similarity with *E. ruminantium* of South Africa, Comoros, Mozambique, and Uganda.

Hematological analysis: The hematological analysis was performed on infected and non-infected sheep and goats to access the alterations in hematological parameters associated with the *Ehrlichia* infection. The comparative hematological analysis revealed a non-significant increase in white blood cells and lymphocytes in both sheep and goats infected with *Ehrlichia* infection. Furthermore, infected sheep and goats had significantly lower red blood cells, hemoglobin, and hematocrit compared to the healthy animals (Table 4 and 5). The platelet count was found lower in both animal species.

DISCUSSION

In human and veterinary medicine, the recently emerged as well as the previously controlled but re-emerging tick-associated pathogens are of higher significance (Dantas-Torres *et al*., 2012).

Fig. 3: Blast Alignment of Nucleotide sequences positive for *sodb* gene of *Ehrlichia.*

Table 4: Comparative hematological analysis in healthy and *Ehrlichia*-infected sheep

CBC Parameter	Healthy (Mean±SD)	Diseased (Mean±SD)	F-value	Confidence Interval	*p*-value
WBC (× 10^3/µL)	8.100±1.298	10.28±1.915	0.210	- 0.2018 to 4.5698	0.068
RBC (× 10^6/µL)	8.100±1.365	5.620±1.851	0.147	-4.8525 to -0.1075	0.042
Hb conc. (g/dl)	12.540±1.7210	8.180±0.389	6.267	-6.180 to -2.539	0.001
HCT (%)	30.600±3.305	12.220±3.961	1.058	-23.701 to -13.059	0.000
Platelets (x 10^3/µL)	675.20±175.22	226.60±30.435	2.328	-632.01 to -265.18	0.000
Lymphocytes (x 10^3/µL)	4.620±1.654	6.276±1.314	0.238	-0.523 to 3.835	0.118

P<0.05 shows a significant effect.

Table 5: Comparative hematological analysis in healthy and *Ehrlichia*-infected goats

CBC Parameter	Healthy Mean±SD	Diseased Mean±SD	F-value	Confidence Interval	*p*-value
WBC (× 10^3/µL)	8.480±1.478	9.994±0.938	1.106	-0.2921 to 3.3201	0.324
RBC (× 10^6/µL)	10.780±1.064	4.600±2.283	5.185	-8.777 to -3.583	0.001
Hb conc. (g/dl)	10.540±1.128	7.040±1.244	0.020	-5.232 to -1.768	0.002
HCT (%)	25.860±3.462	13.680±7.018	4.735	-20.250 to -4.109	0.008
Platelets (x 10^3/µL)	592.80±169.67	373.18±283.54	0.779	-560.387 to 121.147	0.176
Lymphocytes (x10 ^{3}/µL)	4.740±0.702	5.820±0.835	0.003	-0.045 to 2.205	0.058

p<0.05 shows a significant effect.

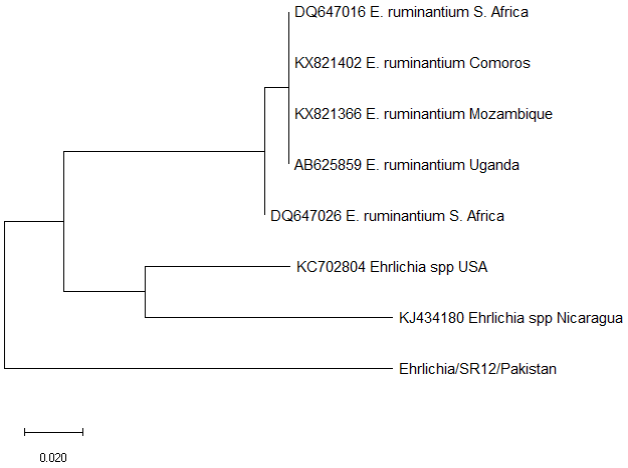

Fig. 4: Phylogenetic analysis representing the *Sodb* gene diversity in *Ehrlichia* spp. isolated from domestic small ruminants in comparison to already reported sequences from Gene bank.

Ehrlichiosis, caused by *E. ruminantium*, with threatened clinical outcomes is considered the most economically devastating rickettsial disease in cattle, goats, sheep, and wild ruminants (Allsopp, 2010).

The present study found an overall prevalence of 04.69% and 7.81% based on microscopy and PCR respectively. A higher molecular prevalence was observed in goats (9.38%) as compared to sheep (6.25%). The current findings were in agreement with the study reported by (Ringo *et al.*, 2018) who revealed an overall infection rate of *E. ruminantium* to be 14.3% with a higher prevalence of 19.7% in goats and lesser in sheep (3.3%) based on PCR results. Various serological studies have also reported the occurrence of Ehrlichiosis in sheep and goats. A study in Tanzania indicates 64.7% and 68.6% seroprevalence of mean antibody titer for *E. ruminantium* in sheep and goats respectively (Swai *et al.*, 2009). Similarly, the antibody seroprevalence of *E. ruminantium* in sheep was found to be 62% in the pastoral community area in Kenya (Wesonga *et al,* 2006). A study conducted in Gambia, West Africa, reported 51.6% seroprevalence of *E. ruminantium antibodies* in sheep (Faburay *et al.*, 2007). Similarly, other studies like in Zambia found 40% serological prevalence in goats (Ahmadu *et al.*, 2004), as well as serological evaluation in free-range goat flocks of Zimbabwe, revealed 67 to 100% prevalence in range as described previously (Peter *et al.*,

2001; Kakono *et al.*, 2003). Moreover, another study reported 51 and 28% seroprevalence in sheep and goats respectively with the help of polyclonal competitive ELISA (PC-ELISA) as narrated by (Sumption *et al*., 2003). The variation in seropositivity of *E. ruminantium* infection between studies could be due to variation in small ruminant management systems, diagnostic tests, or the investigator's technical skills. However, in *Ehrlichia* infection, the goats have been known to be more affected by the peracute form of the disease, whereas sheep are more likely to be affected by an acute or chronic type. The discrepancy in infection among goats and sheep could be linked to the feeding behavior of both species, with goats being mostly browsers and sheep being grazers and wool of sheep which is helpful in easy adherence of ticks as compared to goats hair (Wesonga *et al*., 2006). In serological evaluation, a critical examination of the ELISA technique is required. Moreover, in small ruminants, the PCR technology with better specificities has proved a more reliable and specific technique in the confirmatory diagnosis of *Ehrlichia* infection (Faburay *et al.*, 2007).

The results of the phylogenetic analysis revealed more similarity of the current study isolate with the *E. ruminantium* isolates reported from different countries. The movement of animals from one grazing area to another may increase their exposure to pathogenic diseases (Macpherson, 1995). Moreover, the type of management system may also be an important variable that increases the risk of cattle and sheep infection with tick-borne pathogens like *E. ruminantium*, which has been reported to identify the host-vector contact time and transmission dynamics (Zaid *et al.*, 2019). *Ehrlichia* spp. isolated from Pakistan may show variability from the reported strains of other countries due to biological variability and the occurrence of genetic recombination among different *Ehrlichia* species (Allsopp, 2010). So, new strains are continuously arising in the field. The presence of *E. ruminantium* in small ruminants might be due to improper farming systems in Pakistan which can be a significant risk factor for tick-borne diseases in animals.

The present study has shown that animals having a recent or previous history of tick infestation and no tick control practices were more prone to disease in comparison with the animals having no tick history. This might be because Ehrlichiosis is a tick-borne malady. The higher the number of ticks, the higher will be the chances of developing Ehrlichiosis (Randolph, 2004; Lorsirigool and Pumipuntu, 2020). The hematological analysis revealed an increase in WBC count and a significant decrease in erythrocytes, hemoglobin, and hematocrit. The increased leukocyte count revealed in the current findings was in line with the conclusions of a study conducted in China that reported a significant increase in WBCs count in goats infected with *Ehrlichia* (Zhang *et al.*, 2017). Similar hematological variations have also been reported in studies conducted in various other *Ehrlichia* infected domestic animal species (Oliveira *et al.*, 2000; De Castro *et al*., 2004; Dixit *et al*., 2012). The current findings might be associated with the reduced erythropoiesis or increased erythrocytes destruction due to *Ehrlichia* infection or due to epistaxis, severe anemia, and petechial hemorrhages (Bharadwaj, 2013).

Conclusions: The current study is the first molecular investigation of *Ehrlichia* infection, an emerging issue of small ruminants of Pakistan. The phylogenetic analysis revealed similarities of study isolate more towards the *E. ruminantium* which might be due to possible transmission of the pathogen from cattle to small ruminants especially in mixed farming system. The risk factors analysis revealed that *Ehrlichia* infection is associated with previous or present tick infestation history and hence demands strict tick-control strategies to be adopted. The study concluded that risk factor analysis, hematological evaluation, and molecular detection tools can aid in the early diagnosis and treatment of this notorious infection. Moreover, this emerging issue must be addressed properly by further molecular studies to design effective control strategies.

Acknowledgments: The authors are thankful to the Medicine Research laboratory, University of Veterinary and Animal Sciences Lahore for the provision of the laboratory during the study.

REFERENCES

Ahmadu B, Lovelace CEA, Samui KL, *et al*., 2004. Some observations on the sero-prevalence of heartwater and tick infestation in Zambian goats. Onderstepoort J Vet Res 71:161–4.

Ali S, Ijaz M, Ahmed A, *et al*., 2020. Prevalence and associated risk factors of bovine babesiosis in Lahore, Pakistan. Agrobiol Rec 2:17-23.

Allsopp BA, 2010. Natural history of *Ehrlichia ruminantium*. Vet Parasitol 167:123–35.

Bharadwaj R, 2013. Therapeutic management of acute canine monocytic ehrlichiosis. Indian Vet J 90:138–9.

De Castro MB, Machado RZ, De Aquino LPCT, *et al.*, 2004. Experimental acute canine monocytic ehrlichiosis: Clinicopathological and immunopathological findings. Vet Parasitol 119:73–86.

Ceylan O, Uslu A, Ceylan C, *et al*., 2021. Predominancy of rhipicephalus turanicus in tick-infested sheep from Turkey: A large-scale survey. Pak Vet J 41:429–33.

Dantas-Torres F, Chomel BB and Otranto D, 2012. Ticks and tick-borne diseases: A One Health perspective. Trends Parasitol 28:437–46.

Dixit AK, Dixit P and Shukla PC, 2012. Canine monocytic ehrlichiosis and its therapeutic management in a dog. Intas Polivet 13:140–1.

Faburay B, Geysen D, Munstermann S, *et al.*, 2007. Longitudinal monitoring of *Ehrlichia ruminantium* infection in Gambian lambs and kids by pCS20 PCR and MAP1-B ELISA. BMC Infect Dis 7:1–11.

Ghaffar A, Ijaz M, Ali A, *et al*., 2020. First Report on Molecular Characterization of Anaplasmosis in Small Ruminants in Pakistan. J Parasitol 106:360–8.

Jongejan F, Thielemans MJC, De Groot M, *et al.*, 1991. Competitive enzyme-linked immunosorbent assay for heartwater using monoclonal antibodies to a *Cowdria ruminantium*-specific 32-kilodalton protein. Vet Microbiol 28:199–211.

Kakono O, Hove T, Geysen D, *et al*., 2003. Detection of antibodies to the *Ehrlichia ruminantium* MAP1-B antigen in goat sera from three communal land areas of Zimbabwe by an indirect enzyme-linked immunosorbent assay. Onderstepoort J Vet Res 70:243–9.

Loftis AD, Mixson TR, Stromdahl EY, *et al.*, 2008. Geographic distribution and genetic diversity of the Ehrlichia sp. from Panola Mountain in Amblyomma americanum. BMC Infect Dis 8:1–7.

Lorsirigool A and Pumipuntu N, 2020. A retrospective study of dogs infected with *Ehrlichia canis* from 2017-2019 in the Thonburi area of Bangkok province, Thailand. Int J Vet Sci 9:578-80.

Macpherson CNL, 1995. The effect of transhumance on the epidemiology of animal diseases. Prev Vet Med 25:213–24.

O'Callaghan CJ, Medley GF, Peter TF, *et al*., 1998. Investigating the epidemiology of heartwater (Cowdria ruminantium infection) by means of a transmission dynamics model. Parasitology 117 Pt 1:49–61.

Oliveira D, Nishimori CT, Costa MT, *et al*., 2000. Anti-Ehrlichia canis

antibodies detection by dot-ELISA in naturally infected dogs. Rev Bras Parasitol Vet 9:1–5.

Perry BD and Young AS, 1995. The past and future roles of epidemiology and economics in the control of tick-borne diseases of livestock in Africa: the case of theileriosis. Prev Vet Med 25:107–20.

Peter TF, O'callaghan CJ, Medley GF, *et al.*, 2001. Population-based evaluation of the *Ehrlichia ruminantium* MAP 1B indirect ELISA. Exp Appl Acarol 25:881–97.

Peter SG, 2020. Zoonotic *Anaplasma* and *Ehrlichia* infections and their potential reservoirs: a review. Int J Vet Sci 9:1-9.

Qurollo BA, Riggins D, Comyn A, *et al.*, 2014. Development and validation of a sensitive and specific sodB-based quantitative PCR assay for molecular detection of Ehrlichia species. J Clin Microbiol 52:4030–2.

Randolph SE, 2004. Tick ecology: Processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. Parasitology pp:129.

Ringo AE, Adjou Moumouni PF, Taioe M, *et al.*, 2018. Molecular analysis of tick-borne protozoan and rickettsial pathogens in small ruminants from two South African provinces. Parasitol Int 67:144–9.

Selim A, Abdelhady A and Alahadeb J, 2021. Prevalence and first molecular characterization of Ehrlichia canis in egyptian dogs. Pak Vet J 41:117–21.

Sumption KJ, Paxton EA and Bell-Sakyi L, 2003. Development of a polyclonal competitive enzyme-linked immunosorbent assay for detection of antibodies to *Ehrlichia ruminantium*. Clin Diagn Lab Immunol 10:910–6.

Swai ES, Moshy W, Mtui PF, *et al.*, 2009. Serological survey of antibodies to *Ehrlichia ruminantium* in small ruminants in Tanzania. Trop Anim Health Prod 41:959–67.

Wesonga FD, Wesongah JO, Chemuliti J, Wanjala K, Munga L GP, 2006. Seroprevalence of *Ehrlichia ruminantium* (heartwater) in small ruminants in a pastoral production system in Narok district, Kenya. 54:23–33.

Yin H, Schnittger L, Luo J, *et al.*, 2007. Ovine theileriosis in China: a new look at an old story. Parasitol Res 101:191–5.

Zaid T, Ereqat S, Nasereddin A, *et al.*, 2019. Molecular characterization of Anaplasma and Ehrlichia in ixodid ticks and reservoir hosts from Palestine: a pilot survey. Vet Med Sci 5:230–42.

Zhang H, Chang Z, Mehmood K, *et al.*, 2017. First report of Ehrlichia infection in goats, China. Microb Pathog 110:275–8.