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Original article

Molecular surveillance of small ruminant anaplasmosis and phylogenetic investigations on zoonotic *Anaplasma capra* from distinct agro-ecological regions of Pakistan

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Abstract

Anaplasma infects diverse cell types in animals and humans, worldwide. The current study was aimed at evaluating the occurrence, risk parameters and phylogeny of *Anaplasma capra* in small ruminants from three agro-climatically distinct regions of Pakistan. A total of 600 blood samples were randomly gathered from asymptomatic sheep and goats from Toba Tek Singh, Khushab, and Dera Ismail Khan districts of Pakistan using a multistage cluster sampling technique from January 2023 to May 2024. The blood samples were analyzed for *Anaplasma* infection targeting the *16S rRNA* gene using the PCR followed by sequencing. A pre-tested questionnaire was used utilized to gather information regarding risk factors. The prevalence of anaplasmosis was revealed as 27.83% (167/600). The data suggested a higher frequency of disease in goats (30.37%) compared to sheep (24.82%). Analysis of multivariate logistic regression determined that cracks in walls ($p<0.0001$, OR=2.240, CI=1.439-3.487) and grooming practice ($p<0.01$, OR=1.793, CI=1.235-2.604) were the significant risk factors. The phylogeny of the *16S rRNA* gene identified two separate clusters of *A. capra* exhibiting 99-100% similarity with various geographic isolates. The isolates of the current study exhibited higher homology with isolates from France, Ghana, South Korea, Turkey, China, and Pakistan traced from ticks, sheep, water buffalo, cattle, and water deer, respectively. In conclusion, anaplasmosis is widespread in the study regions with indication of genetic diversity. Additional research is required on the clinicopathological and potential vectors of zoonotic *A. capra* for devising better treatment and control measures.

Keywords: Surveillance, small ruminants, *Anaplasma capra*, phylogenetic analysis, Pakistan

Introduction

Tick-borne diseases (TBDs) represent a significant economic danger to cattle productivity in arid and semi-arid regions globally. Various blood pathogens, including the louping ill, *Theileria*, *Borrelia*, *Anaplasma*, *Ehrlichia* and *Babesia*, are the predominant TBDs in small ruminants, resulting in significant financial losses (Berggoetz et al. 2014, Khan et al. 2019, Lihou et al. 2020, Khan et al. 2022, Basit et al. 2022). These ticks are blood-sucking parasites with the potential to transmit various viral, protozoal, and bacterial infections affecting livestock (goats, cattle, sheep and buffalo) and humans, worldwide. *Anaplasma*, *Babesia* and *Theileria* are the principal tick-borne diseases affecting small ruminants (Berggoetz et al. 2014).

Anaplasmosis is widely recognized as a significant vector-borne disease that affects humans, domestic animals and wild animals (Ceylan et al. 2021, Atif et al. 2023, Atif 2016). Based on sequencing of *16S rRNA* and *groEL* genes, six species have been grouped in the genus *Anaplasma* (A). The *A. capra*, *A. phagocytophilum*, *A. centrale*, *A. marginale*, *A. bovis* and *A. platys* are the major species of the genus *Anaplasma* (Dumler et al. 2001). These are tick-transmitted bacteria that grow inside blood cells causing disease in humans and animals (Guo et al. 2018, Kundave et al. 2018, Guimarães et al. 2019). The *A. capra*, *A. ovis*, and *A. phagocytophilum* are the major zoonotic pathogens with the potential to infect humans, small and large ruminants and wild mammals (Peng et al. 2018).

A novel zoonotic tick-borne bacterium, provisionally named as *A. capra*, has attracted the interest of public health researchers and veterinarians (Peng et al. 2021). This parasite was originally discovered in goats in northern and central China (Peng et al. 2021). Later on, the disease was observed among individuals at the Mudanjiang Forestry Central Hospital in Heilongjiang Province, China, and 28 cases were reported in northern China's Heilongjiang Province. This disease has not yet been classified as an exotic species. Unlike other *Anaplasma* species, *A. capra* represents an emerging zoonotic pathogen with distinct epidemiological characteristics (Li et al. 2015). The disease is characterized in humans by nonspecific symptoms such as fever, dyspnea, headache, rash, eschar, and dizziness (Li et al. 2015, Dahlgren et al. 2016). Infections attributed to *Anaplasma* species exhibit a certain degree of host specificity. Nevertheless, this characteristic is influenced by the identification of *Anaplasma* in other hosts, leading to further complications in the epidemiology of the disease. *A. capra* was primarily found in goats in southern and central China using *16S rRNA* and *msp4* genes (Peng et al. 2018). *A. capra* can infect domestic small ruminants (sheep and goats), dogs (Ceylan et al. 2021), wild animals (deer, Korean sambar, *Hydropotes inermis argyropus*; muntjac, *Muntiacus muntjak*; roe deer, *Capreolus capreolus*; Japanese antelope, *Capricornis crispus*; Persian, *Equus hemionus onager* (Sato et al. 2009, Jouglin et al. 2019, Remesar et al. 2022) and humans (Li et al. 2015). Further investigations have reported occurrence in a number of countries, such as Japan (Sato et al. 2009), Pakistan (Ishaq et al. 2022), France (Jouglin et al. 2019), Turkey (Altay et al. 2022), Spain (Remesar et al. 2022) and South Korea (Seo et al. 2019). In addition to reports from China and Europe, *Anaplasma capra* has also been molecularly detected in small ruminants in Eastern Turkey, highlighting its wider geographic distribution and potential epidemiological importance in the region (Oguz et al. 2024).

A. capra has been demonstrated in ticks *Dermacentor (D.) nuttalli*, *D. abaensis*, *Rhipicephalus microplus*, *H. qinghaiensis, Haemaphysalis longicornis*, and *Ixodes persulcatus*. Nonetheless, the potential vectors of *A. capra* are still unclear (Liu et al. 2012). Various host, environment and management factors contribute to the occurrence of anaplasmosis (Niaz et al. 2021, Selim et al. 2022, Naeem et al. 2023).

Stained blood smears are commonly used to determine *Anaplasma* in the blood cells of infected animals. Blood smear microscopy is considered a gold standard technique but this requires high expertise for differential diagnosis from other blood-borne pathogens (Silaghi et al. 2017). Multiple diagnostic approaches have been employed for the identification of anaplasmosis, including PCR (Li et al. 2015), RFLP-PCR (Noaman et al. 2010), Nested PCR (Altay et al. 2022), RLB (Kolo et al. 2016), and sequencing (Altay et al. 2022). Different researchers have been utilized various genes for the molecular categorization of *A. capra*, including major surface protein 4 (Yang et al. 2017), *groEL* (Yang et al. 2016), and *gltA* (Li et al. 2015, Altay et al. 2024), and *16S rRNA* (Li et al. 2015; Ishaq et al. 2022). The geography, climate and vegetation of Pakistan facilitate the existence and spread of ticks. Based on a disease control standpoint, the detection of novel zoonotic isolates of tick-borne diseases is essential. Only two reports from Pakistan documented *A. capra* in domestic small ruminants and a wild animal (mouflon sheep) that vary with respect to difference in sample size and study locations. Consequently, the current study intended to conduct molecular surveillance and phylogenetic investigations on *A. capra* among domestic small ruminants in three agro-climatically distinct regions of Pakistan.

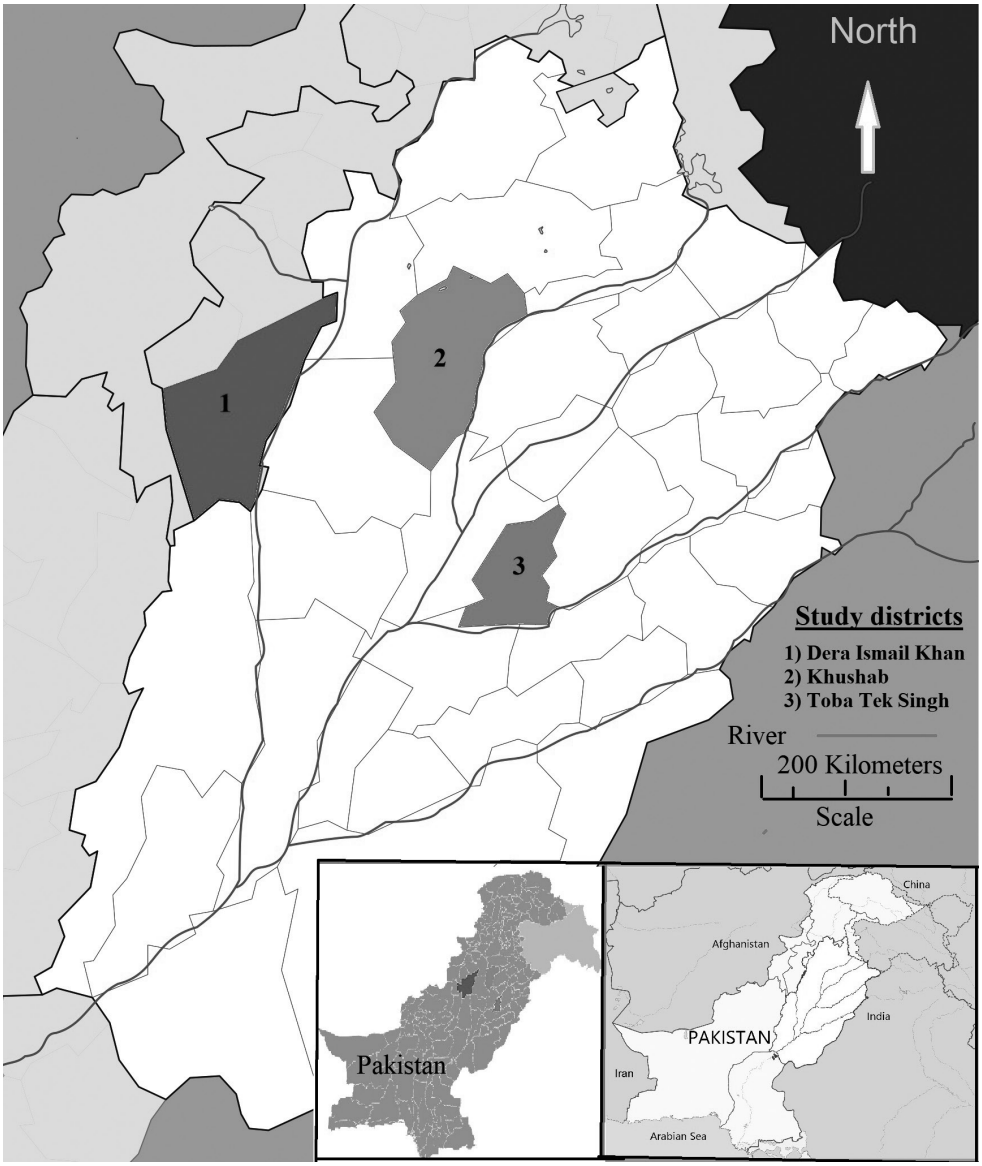

Fig. 1. Map of the study districts.

Materials and Methods

Study design

This study was conducted in the Dera Ismail Khan, Toba Tek Singh, and Khushab districts of Pakistan, from January 2023 to May 2024. These districts have distinct agro-climatic conditions and animal husbandry practices. Toba Tek Singh lies between latitude 30°24′ and longitude 70°44′ and 71°50′. The area consists of a semicircular sandbar between the Indus and Chenab rivers. Khushab is located at a latitude of 32°17′48″ N and a longitude of 72°21′9″ E. The elevation varies between 615 feet above sea level at Jauharabad to 4,992 feet above sea level in the Sakesar region. The Khushab district comprises of agricultural lowland plains, hills and lakes. Dera Ismail (D.I.) Khan district is located in the south of Khyber Pakhtunkhwa (KP), between 31°15°N and 32°30° N and 70°00° E and 71°E, on the western bank of the Indus River (Fig. 1).

A total of 600 blood samples were gathered from 300 goats and 300 sheep using a multistage sampling approach. Two Tehsils were randomly designated from each district, and within each Tehsil, ten union councils were nominated. Ten samples were collected from each union council, ensuring equal representation of sheep (n=200) and goats (n=200) from each district. The sample size was determined based on 50% prevalence at a 95% confidence level (Thrusfield et al. 2018).

Evaluation of risk factors

To assess epidemiological risk variables, a pre-tested questionnaire was administered to obtain information,

Table 1. Representative isolates selected for phylogenetic analysis mentioned along with accession numbers, animal sources and length of sequences.

Sr. No.	Accession no.	Animal	Geographic location	Sequence length
1	OR643666	Goat	Toba Tek Sing	284
2	OR643320	Goat	Toba Tek Sing	323
3	OR726314	Goat	Khushab	231
4	OR726315	Sheep	Dera Ismail Khan	231
5	OR643667	Sheep	Khushab	298

with consent, during sample collection from each farmer regarding host, management and production factors including area, age, specie, gender, feeding system, tick infestation, management types, cracks in the walls, health status, use of acaricides, animal living area, housing type, grooming practice and removal of manure. The questionnaire, containing closed-ended questions was completed immediately after the blood sample from each animal.

DNA extraction

Genomic DNA was purified from blood samples using a Gene JET Whole Blood Genomic DNA Purification Mini Kit (Thermo Fisher Scientific). Post-extraction, the DNA was frozen at -20°C pending polymerase chain reaction (PCR).

PCR

The extracted DNA was amplified targeting the 345 bp fragment of the *16S rRNA* gene of *Anaplasma* using specific primers: forward (5'-GGTACCYACAG AAGAAGTCC-3') and reverse (5'-TAGCACTCATC GTTTACAGC-3'), as described by Saleem et al. (2018). Each PCR reaction had a final volume of 25 µl, comprising 14 µl of GeneDirex Blue Master Mix (catalog No. SM213-0250), 1 µl of each primer, 4 µl of template DNA, and 5 µl of nuclease-free water. The reaction consisted of an initial denaturation at 95°C for 5 min, followed by 35 amplification cycles comprising denaturation at 95°C, annealing at 58°C, and extension at 72°C, with each step lasting 30 s. A final extension was carried out at 72°C for 10 min. Each PCR run included a positive control and a negative control (sterile distilled water). Positive control was obtained from the Department of Parasitology, University of Agriculture Faisalabad, Pakistan, isolated from goat blood. The PCR products were analyzed on a 2% agarose gel stained with GelRed. Once the gel solidified, it was placed in an electrophoresis chamber. A 100 bp DNA ladder was loaded into the first well for size reference, and 5 µl of each PCR product was loaded into the remaining wells. Electrophoresis was performed using a Bio-Rad Power Pac system at 120 V for 60 minutes. The bands were observed under UV illumination to verify that the target DNA fragment had been successfully amplified.

Sequencing and phylogeny

The positive samples (n=05) were chosen for sequencing at least one sample from each region with higher band intensity (Table 1). The obtained sequences were subjected to phylogenetic analysis using MEGA 11 software to build a likelihood phylogenetic tree with already reported *Anaplasma* isolates. Th available *16S rRNA* sequences of *A. capra* from France, Turkey, Portugal, Angola, South Korea, Iran, China, Argentina, India, Ghana and Pakistan were obtained from the GenBank database. The sequences were aligned with MUSCLE, phylogenetic relationships were established with the Maximum Likelihood approach as well as the Tamura 3-parameter model in MEGA11, supported by 500 bootstrap iterations (Tamura et al. 1992, Tamura et al. 2021).

Statistical analysis

A chi-square test was used to appraise the association between *Anaplasma* infection and disease factors. Univariate and multivariate logistic regression statistical models were used to identify potential risk factors. Statistical significance was defined as a p-value ≤ 0.05. All statistical procedures were conducted with IBM SPSS Statistics v26.

Results

Epidemiology

The findings of the study suggested that *Anaplasma* exhibited more conjunction in adults compared to juvenile specimens. Based on age, the animals were classified into two groups: under one year and above one year. The prevalence was higher in individuals older than one year (29.43%; 88/299) compared to those younger than one year (26.25%; 77/301). A non-significant correlation was seen across various age groups

Table 2. Prevalence of anaplasmosis in small ruminants in Pakistan.

Variables	Categories	Total No. of animals tested	No. of Positive animals	Prevalence (%)	X^2	p-value
Age	≤ 1 year	301	77	26.25	0.76	0.38
	> 1 year	299	88	29.43		
Species	Sheep	274	68	24.82	2.28	0.13
	Goats	326	99	30.37		
Gender	Male	214	59	27.57	0.11	0.92
	Female	386	108	27.97		
Feeding System	Outdoor browsing	381	115	30.18	2.87	0.09
	Indoor feeding	219	52	23.74		
Housing type	Wood-bricks	394	120	30.45	3.93	0.04
	Concrete-metallic	206	47	18.07		
Tick infestation	Heavy	193	66	34.19	6.91	0.03
	Moderate	162	45	27.77		
	Low	245	56	22.85		
Use of acaricide	Yes	240	56	23.33	4.03	0.04
	No	360	111	30.82		
Management type	Intensive	242	77	31.81	3.21	0.07
	Semi-intensive	358	90	25.13		
Cracks in walls	Yes	297	94	28.20	4.26	0.03
	No	303	73	24.09		
Health status	Healthy	226	58	25.66	0.85	0.36
	Emaciated	374	109	29.44		
Animal living area	Open	357	86	24.08	6.15	0.01
	Congested	243	81	33.33		
Grooming practice	Yes	292	64	21.91	9.91	0.00
	No	308	103	33.44		
Removal of manure	Less frequently	289	88	30.44	1.90	0.16
	Frequently	311	79	25.40		

(X^2=0.758, df=1, p>0.05). The gender-specific frequency of the disease exhibited a non-significant correlation (X^2=0.11, df=1, p>0.05), suggesting a greater prevalence in females (27.97%; 108/386) than males (24.48%; 59/214), regardless of the host type. The data obtained on the basis of acaricide use showed a higher prevalence (30.82%; 111/360) of anaplasmosis in animals that were not subjected to any acaricide compared to animals with acaricidal treatment (23.33%; 56/240). A significant statistical difference (p<0.05, X^2=4.03, df=1) was observed using chi-square analysis between both categories.

The small ruminants were evaluated for tick-infestation to determine the impact of ticks on disease severity. Tick load was divided into three classes: heavy, moderate, and low. The maximum occurrence was observed in animals with a heavy tick load (34.19%). While the infectivity of the disease was lower in animals that had a moderate tick infestation (27.77%). Animals with a lower tick infestation showed the least prevalence (22.85%) with a statistically significant association (p<0.05, X^2=6.91, df=2) (Table 2). Frequency of anaplasmosis in small ruminants was analyzed on the basis of housing type. The animals that were kept in wood-brick housing showed a higher positivity of disease 30.45% [120/394] while those who were kept in concrete-metallic housing (18.07% [47/206]). A statistically significant difference was found among both classes (p<0.05, X^2=3.93, df=1) mentioned in Table 2.

Animals raised in as area with cracked walls in their premises showed a higher incidence of disease (31.64%; 94/297) than those where no cracks were present in animal housing (24.09%; 73/303). A statistically significant difference (p=0.03, X^2=4.26, df=1) was found between both classes (Table 2). Small ruminants kept in open areas showed a relatively lower prevalence (24.08%; 86/357) compared to those that were being raised in congested areas (33.33%; 81/243). A statisti-

Table 3. Univariate assessment of risk factors linked with the molecular occurrence of anaplasmosis in small ruminants in Pakistan.

Univariate analysis								
Variables	β	S.E.	Wald	df	p	OR	95% C.L. for EXP (β)	
							Lower	Upper
Area	-.462	.123	14.047	1	.000	.630	.494	.802
Age	-.114	.199	.326	1	.568	.892	.604	1.319
Specie	-.328	.200	2.682	1	.101	.720	.487	1.067
Gender	.333	.269	1.537	1	.215	1.395	.824	2.361
Housing type	-.271	.230	1.397	1	.237	.762	.486	1.196
Tick load	.160	.136	1.391	1	.238	1.174	.899	1.532
Acaricidal use	-.629	.258	5.952	1	.015	.533	.322	.884
Management types	.306	.206	2.219	1	.136	1.358	.908	2.032
Feeding system	-.413	.292	2.000	1	.157	.661	.373	1.173
Cracks in walls	1.013	.242	17.476	1	.000	2.755	1.713	4.430
Health status	-.306	.220	1.948	1	.163	.736	.479	1.132
Animal living area	-.563	.220	6.547	1	.011	.570	.370	.877
Grooming practice	.598	.197	9.186	1	.002	1.819	1.235	2.679
Removal of manure	.426	.219	3.784	1	.050	1.531	.997	2.351
Constant	1.689	.887	3.622	1	.057	5.412		

* β – regression coefficient, S.E. – standard error, * df – degrees of freedom, OR – odds ratio, C.L. – confidence level.

Table 4. Assessment of risk factors linked with the molecular occurrence of anaplasmosis in small ruminants in Pakistan.

Variables	β	S.E.	Wald	df	p	OR	95% C.L. for EXP(β)	
							Lower	Upper
Management types (semi-intensive)	.300	.189	2.517	1	.113	1.350	.932	1.956
Cracks in walls (yes)	.806	.226	12.748	1	.000	2.240	1.439	3.487
Grooming practice (No)	.584	.190	9.414	1	.002	1.793	1.235	2.604
Removal of manure (less frequently)	.294	.188	2.425	1	.119	1.341	.927	1.941
Constant	.502	.226	4.958	1	.026	1.653		

* β – regression coefficient, S.E. – standard error, * df – degrees of freedom, OR – odds ratio, C.L. – confidence level.

cally significant difference ($p<0.05$, $X^2=6.15$, df=1) was found between both categories. Small ruminants who were regularly groomed showed a lower incidence of the disease (21.91%; 64/292) than those who were not subjected to grooming practice (33.44%; 103/308). A statistically significant difference ($p<0.01$, $X^2=9.91$, df=1) was observed between both categories (Table 2).

According to regression analysis, variables having a p-value ≤ 0.05 and an odds ratio (OR) ≥ 1 were considered as a significant risk factor. Univariate analysis revealed a strong statistical relationship with grooming practice ($p<0.01$, OR=1.819, CI=1.235-2.679), cracks in walls ($p<0.001$, OR=2.755, CI=1.713-4.430), and removal of manure ($p<0.050$, OR=1.531, CI=0.997-2.351) (Table 3). Nonetheless, multivariate logistic regression expressed cracks in walls ($p<0.001$, OR=2.240, CI=1.439-3.487) and grooming practice ($p<0.01$, OR=1.793, CI=1.235-2.604) as the significant risk factors (Table 4).

Sequencing and phylogenetic insights

The obtained sequences were identified as *A. capra.* We successfully obtained Genbank accession numbers OR643667 and OR643820 from goats and OR726314, OR726415, and OR643666 from sheep. The phylogeny of the *16S rRNA* gene, identified two separate clusters with 99-100% identity with other genotypes from diverse geographic regions. The phylogenetic tree indicated two major clusters. Cluster-I consisted of isolates from ticks (*Rhipicephalus microplus, Rhipicephalus sanguineus*), Persian onager, ticks, sheep, goats, humans, cattle, Gaddi sheep and ticks from China, Iran, Pakistan, Turkey, Angola, India and Portugal. The genotypes of *A. capra* isolated from goats and sheep during the current study OR643820 and OR643666, were grouped in cluster II belonging to sheep, cattle, water deer, and water buffalo from Pakistan, Ghana, France, South Korea, China, and Turkey. While *A. capra* geno-

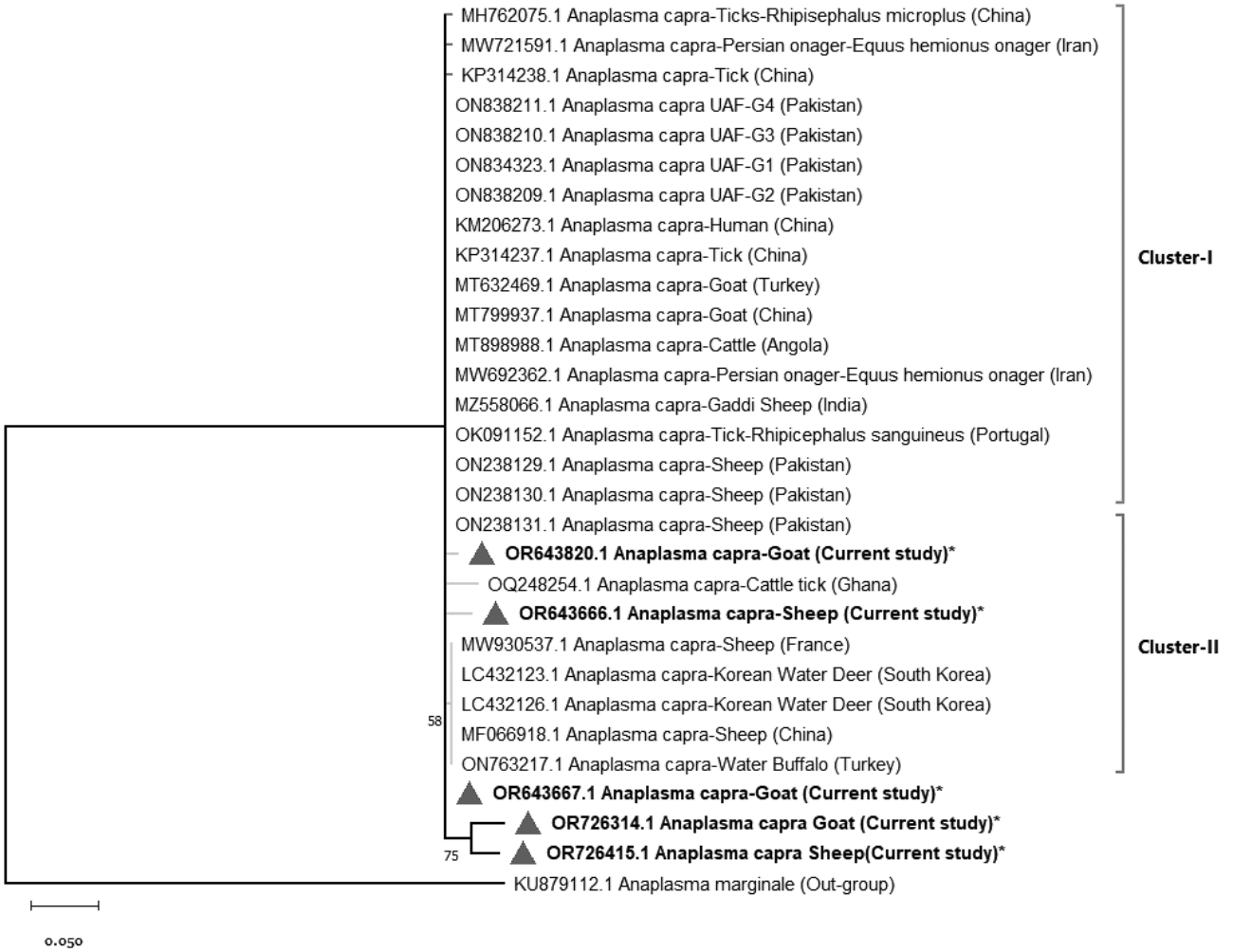

Fig. 2. Phylogenetic tree of *Anaplasma capra* isolated from sheep and goats in Pakistan. The current isolates are presented with red triangle, bold font with asterisks.

types from goats (OR643667, OR726314) and sheep (OR726415) formed individual clusters (Fig. 2).

Discussion

Climate change and global warming are facilitating the expansion of ectoparasites into new territories, hence endangering previously unexposed hosts to numerous tick-borne diseases. In Pakistan, just two studies have been conducted on *A. capra* on domestic small ruminants and Mouflon sheep (*Ovis gmelini*), these differ due to small sample size, outcomes, and small study area (Ishaq et al. 2022, Razzaq et al. 2024). The PCR results indicate an overall prevalence of 27.83% in small ruminants. Previous reports revealed comparable occurrence of anaplasmosis among sheep and goats. Likewise, the infectivity rates in goats from China (26.6%; Wang et al. 2021), Pakistan (29.52%; Ishaq et al. 2022) and French sheep (30%; Jouglin et al. 2022) are consistent with our findings. In contrast, lower positivity rates have been documented in several regions, including Chinese goats (3.4-17.7%; Guo et al. 2018, Peng et al. 2018, Zhou et al. 2024, Shin et al. 2020, Amer et al. 2019); South Korean cattle (0.3%; Miranda et al. 2021, 0.4%; Seo et al. 2019), goats (0.3%; Miranda et al. 2021), water deer (14.3%; Shin et al. 2020; 17.7%; Amer et al. 2019); Malaysian cattle (1.4%; Koh et al. 2018); Turkey (0.5%; Oguz et al. 2024); Iran – Onegar (20%; Staji et al. 2021); Pakistan – Mouflon sheep (11.43%; Ishaq et al. 2022); France – goats (13.2%; Jouglin et al. 2022), red deer (3.4%; Jouglin et al. 2019), swamp deer (14.3%; Jouglin et al. 2019); Spain – roe deer (5.8%; Remesar et al. 2022); Morocco – cattle (11.3%; Elhachimi et al. 2021); and Angola – cattle (6.12%; Barradas et al. 2021). Conversely, a substantially higher prevalence has been observed in some parts of China, where goat infection rates range from 44.6% to 59.7% (Wei et al. 2020; Lin et al. 2023). The regional ecology, tick vector dynamics, host genetic factors, and management practices contribute to variable findings.

The results indicate a higher infectivity in goats (30.37%) in comparison to sheep (24.82%); however,

these differences are not statistically significant. The findings of the current study, therefore, are comparable to the results of Arif et al. (2023) which are coherent with our results; they show that small ruminant anaplasmosis is rising. Specie difference exists among small ruminant hosts that are critical in causing the infection (Arif et al. 2023). Conversely, Shabana and coresearchers reported that sheep were infected at a higher rate compared to goats (Shabana et al. 2018). This may be related to the different study region with existence of potential vectors and genetic susceptibility of animals. Firstly, the difference in grazing behavior and habitat preference among goats and sheep is an important factor leading to infected tick vectors. Secondly, genetic susceptibility and immune response among the two species exists. Additionally, the disease infectivity may vary based on animal production practices, management, acaricide use and vector control. In brief, our findings pointed to a higher occurrence of anaplasmosis in goats than sheep though this finding was statistically non-significant.

This study showed that the frequency of anaplasmosis was higher in sheep and goats which were not treated with acaricides (30.82%) than treated (23.33%). It indicated that acaricide treatment provides protection against anaplasmosis due to likely decreased exposure to vectors. In another study Nyifi and collaborators, observed that tick control practice using acaricides can reduce the prevalence of anaplasmosis (Nyifi et al. 2023).

In our study, it was seen that animals reared in housing with cracked walls had higher disease incidence (31.64%) than those reared in an environment without cracks in the walls (24.09%). The cracks in the walls are refuge spaces for ticks, leading to increased exposure to disease vectors in conjunction with damp conditions promoting disease spread. The general literature review in veterinary and environmental health supported the notion that, in general, poor infrastructure enhances the risk of disease transmission. The pests and vectors find a hiding place in cracks and crevices (Azmat et al. 2018). Cracked walls and poor maintenance of the building might pave the way for the entry and habitation of rodents and insects, raising the possibility of disease spread.

Our study results showed that groomed animals showed a disease prevalence of 21.91% compared to 33% in poorly groomed animals. This statistically significant association suggests that grooming plays a critical role in disease management. Regular grooming assists in the early detection and removal of ectoparasites, as they are proven common vectors for many diseases (Bialke et al. 2015). Grooming also assists in maintaining skin health, which might further prevent infections that may complicate the primary diseases. Grooming considerably reduced the load of ectoparasites that acted as vectors of the pathogen, which relates directly to the reduction of disease spread (Hussain et al. 2017). Grooming should also present an opportunity to observe the skin and body closely for wounds, infections, or other systemic diseases. Studies that may indicate a low influence of grooming on disease prevalence typically focus on those conditions where other optimizing management practices apply, including nutrition and general husbandry of animals (Peng et al. 2021). Under such circumstances, the relative impact of grooming would tend to be reduced, which would indicate that grooming is part of an interconnected suite of animal health management practices, rather than a standalone measure.

Flies, mites, several insects and their life stages are completed in the feces of animals. Manure can act as a habitat and hiding place for ticks (Ganai et al. 2024). Removal of manure was identified as a risk factor for anaplasmosis in small ruminants in the current study. Mohammad and colleagues showed that less frequent manure removal was a potential risk factor for tick infestation in sheep farms in the River Nile state, Sudan (Mohammed et al. 2022). However, it is suggested that clustering may be a limiting factor in statistical analysis which should be considered in the regression model for better accuracy and reliability of analysis.

The phylogenetic study revealed unique isolates of *A. capra* grouped in two separate clusters (99-100% identity). Our isolates exhibited more homology with genotypes from Pakistan (ON238131), Ghana (OQ248252), France (MW930537), Turkey (ON763217), China (MF066918) and South Korea (LC432123, LC432126) isolated from sheep, cattle, water deer and water buffalo, and show divergence from Chinese and Iranian isolates collected from *Rhipicephalus microplus* and Persian onager (*Equus hemionus onager*), respectively. Our phylogenetic investigations were partially in line with previous research. Nonetheless, *Anaplasma* may circulate in various enzootic cycles in different regions of the world (Lin et al. 2023). Extensive monitoring of both nomadic and non-nomadic small ruminants is necessary to assess the severity of the issue, particularly concerning vector-borne hemoparasites.

Conclusions

This study indicates that anaplasmosis is widespread among small ruminants in Toba Tek Singh, Khushab, and Dera Ismail Khan districts of Pakistan. Poor grooming practices and cracks in the walls are the

major risk factors in small ruminants. The preliminary phylogenetic analysis revealed genotypes of *A. capra*. However, additional research is necessary on genetic diversity, pathogenicity, host specificity, vector competency, and transmission characteristics of this zoonotic pathogen for the efficient prevention and management of *A. capra* in Pakistan.

Conflict of Interest

For the present study the authors have declared no conflict of interests.

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Author Declarations

Ethics approval

The current study was permitted by the Directorate of Advance Studies, University of Veterinary and Animal Sciences (UVAS), Lahore (letter No. DAS/1470, dated August 24, 2023), and the Ethical Review Committee, College of Veterinary and Animal Sciences, Jhang No. CVAS/ERC-113; dated August 07, 2023. All procedures involving animals were performed in strict accordance with animal welfare and ethical guidelines. A licensed veterinarian oversaw all animal handling, and consent was acquired from the small ruminant farmers prior to sampling.

Use of generative artificial intelligence

No generative artificial intelligence tools were used.

Conflict of interest

Authors have no conflict of interest for this study.

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