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Molecular detection of *Rickettsia aeschlimannii*, *Candidatus* Rickettsia shennongii, *Rickettsia* sp. and *Coxiella burnetii* in ticks collected from camels

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Tick-borne bacteria of the genera *Rickettsia* and *Coxiella* cause several emerging veterinary and human infectious diseases. Ticks of the genus *Hyalomma* are medically important vectors due to their potential role in the transmission of pathogens to vertebrate hosts. There is an inadequate knowledge on tick-borne *Rickettsia* spp. and *Coxiella* spp. in ticks infesting transhumant camels in Pakistan. In this study, we conducted a molecular survey for screening of *Rickettsia* spp. and *Coxiella* spp. in ticks infesting camels. Seven hard tick species including *Hyalomma dromedarii*, *Hyalomma anatolicum*, *Hyalomma scupense*, *Hyalomma isaaci*, *Hyalomma turanicum*, *Hyalomma asiaticum*, and *Rhipicephalus sanguineus* s.l were confirmed on camels in three distinct physiographic regions of Khyber Pakhtunkhwa, Pakistan. A subset of morphologically identified ticks were subjected to molecular assays for the genetic characterization of ticks and the detection and genetic characterization of *Rickettsia* and *Coxiella* species using standard genetic markers. Ticks screened for pathogens resulted in the detection of *Rickettsia aeschlimannii* and *Candidatus* Rickettsia shennongii and *Coxiella burnetii*. The molecular analysis further reveals the presences of an undetermined *Rickettsia aeschlimannii*-like species, that is making a distinct phylogenetic clade with *R. aeschlimannii*. The detection of pathogens in camel ticks poses potential health hazards as these ticks frequently bites humans. Molecular screening of *Rickettsia* spp. and *Coxiella* spp. associated with camel ticks is a preliminary step toward the surveillance of evaluating their zoonotic threats in the region.

Keywords *Hyalomma*, Tick-borne pathogens, Transhumant, Camel, *Rickettsia*, Q-fever

Dromedary or one humped camel has an estimated population of 30 million and are most common in desert or semi desert areas from eastern Asia to northern Africa[1]. Desert areas of Pakistan in southeastern and central parts, provides flourishing grounds for the camels, thus, enlisted among top 20 countries with large camel population, having an estimated population of 1.02 million[2]. Even during dry seasons, when dairy production from other livestock like goats, sheep, and cattle becomes insufficient, camels offer a good source of meat and dairy products. Besides this, camels are playing a major role in barani agriculture (the agricultural practices that depends on rainfall) in Pakistan[3]. Among the 20 camel breeds reported from Pakistan, six breeds including Marecha, Gaddi, Ghulmani, Khader, Maya and Larri (Sindhi) are common in Khyber Pakhtunkhwa (KP). Despite genetic characterization, these camel breeds are recognized by the local camel breeders and herders[4].

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Camels are commonly infested by ticks of the genera *Hyalomma*, *Dermacentor*, *Rhipicephalus*, *Amblyomma*, *Ixodes*, *Argas*, *Ornithodoros* and *Otobius*[5,6]. *Hyalomma dromedarii* is the principal tick of camels in the Africa, Middle East and Asia[7], including Pakistan[8]. Previous surveys in Pakistan have reported that camels are mostly infested by different ticks including *H. dromedarii*, *Hyalomma scupense*, *Hyalomma anatolicum* and *Rhipicephalus microplus*[8,9].

Advent of molecular techniques and the increase of the number of tick sequences that have been annotated in GenBank, have made it possible to classify ticks using genetic traits rather than just morphology[10]. In recent years, this approach was employed for the classification and characterization of several tick taxa, that has enabled thorough investigations of the ticks' preferences for hosts and population variations[11,12]. Mitochondrial genes, in particular cytochrome oxidase (*cox 1*) are helpful as genetic markers because of their strong maternal inheritance and in some cases higher rate of evolutionary changes than nuclear genes[8,13,14].

Several tick species have the potential to parasitize camels which reduce milk and meat output[15]. Although camels are economically valuable sources and can survive in extreme weather, ticks and tick-borne diseases limit their productivity. Additionally, camel tick *Hy. dromedarii* may serve as a vector of pathogenic bacteria including *Coxiella burnetii* and spotted fever group *Rickettsia* spp. as well as protozoan like *Theileria camelensis*[5]. Molecular detection using different genetic markers are important in revealing pathogens of camel associated ticks[16]. In Pakistan few studies on ticks associated *Rickettsia* spp. and *Coxiella* spp. are reported from other hosts like goats[17], sheep[18,19], stray dogs[20], and cattle[21]. Additionally, no attention has been made to the detection of *Rickettsia* and *Coxiella* species in camel ticks, especially in KP. Therefore, the current surveillance has been aimed to target the ticks' infesting camels and their associated *Rickettsia* and *Coxiella* species in southern and central KP, a fertile region for camel production.

Materials and methods

Ethical statement

All experimental protocols of this study were ethically approved by ASRB (Advance Study and Research Board) of Faculty of Chemical and Life Sciences, Abdul Wali Khan University Mardan, Pakistan under the notification number: Dir/A&R/AWKUM/2022/10,012. Moreover,

all methods were carried out in accordance with the relevant guidelines and regulations. It has been confirmed that all methods are reported in accordance with ARRIVE guidelines (https://arriveguidelines.org). The owners of camels were informed, and approval was taken verbally, prior to the host observation for the tick's collection.

Study area

The proposed area for tick's collection was the southern and northern regions of Khyber Pakhtunkhwa (KP), comprising 9 districts: Lakki Marwat (32.3619° N 70.5452° E), Dera Ismail Khan (D.I. Khan) (31.4953° N 70.547° E), Tank (32.748° N, 70.1348° E), Karak (33.1277° N, 71.0973° E), Kohat (33.4973° N, 71.5249° E), Peshawar (34.0151° N, 71.5249° E), Nowshera (34.0105° N, 71.9876° E), Mardan (34.1986° N, 72.0404° E) and Buner (34.3943° N, 72.6151° E). The selected regions consist of three types of terrains i.e. sandy plains, hilly area and piedmont plain. Most of the area in Lakki Marwat, Karak and D.I. Khan consists of sandy plains, having low rainfall. The semi desert condition of the proposed region is the best flourishing ground for the camel, where the camels play a vital role in Barani agriculture. In piedmont plains (Peshawar, Mardan and Nowshera) and hilly regions (Kohat, Buner and west of Tank districts), camels are used for the transport in steep mountains (Fig. 1).

Ticks' collection and identification

Transhumant camels were observed for tick collection in the study regions during April 2019- September 2019. Ticks were collected from camels in the animal markets, farms, open fields, and pastures, regardless of their precise location within the proposed survey regions. Ticks were collected with the help of fine tweezer, followed by putting in tubes containing 100% ethanol. Moreover, the companion hosts of the camels living in same stable, farm or herds were surveyed for tick collection. For further analyses, ticks were stored in appropriately labeled locked microtubes containing 100% ethanol after being cleaned with distilled water and 70% ethanol.

Collected ticks were morphologically identified under the stereo microscope (SZ61, Olympus, Tokyo, Japan) using standard morpho taxonomic keys[7,22–25].

Analysis of risk associated with camel ticks

Risk variables associated with camels (age, gender, breed, management, terrain type) were examined by taking the questionnaire-based information from the owners of examined camels. The age of the host can affect the infestation of ticks; based on age three groups including calf (< 1 year), juvenile (1–5 years) and adult (> 5 years) were surveyed. The camel population was surveyed in three terrain types, which were sandy desert, piedmont plain and hilly regions. Additionally, tick infestations were surveyed in solitary and co-herded camels, as well as in different camel breeds occurring in the region. The prevalence was calculated by following the formula: number of tick infested hosts/number of total observed hosts x 100. Abundance was calculated by the mean number of ticks on each host.

DNA extraction and polymerase chain reaction (PCR)

Total 124 ticks (ten males, ten unfed females, and five unfed nymphs of each tick species infesting camels while all four male ticks of camel infested *Rh. sanguineus* sensu lato) were randomly selected and individually subjected for DNA extraction. Prior to the DNA extraction, ticks were washed with distilled water and 70% ethanol to become contaminants free. These rinsed ticks were dried on tissue paper and cut with sterile scalp blade. DNA was extracted from grinded ticks manually through standard phenol chloroform method[26] and quantified through NanoDrop (NanoQ, Optizen, South Korea).

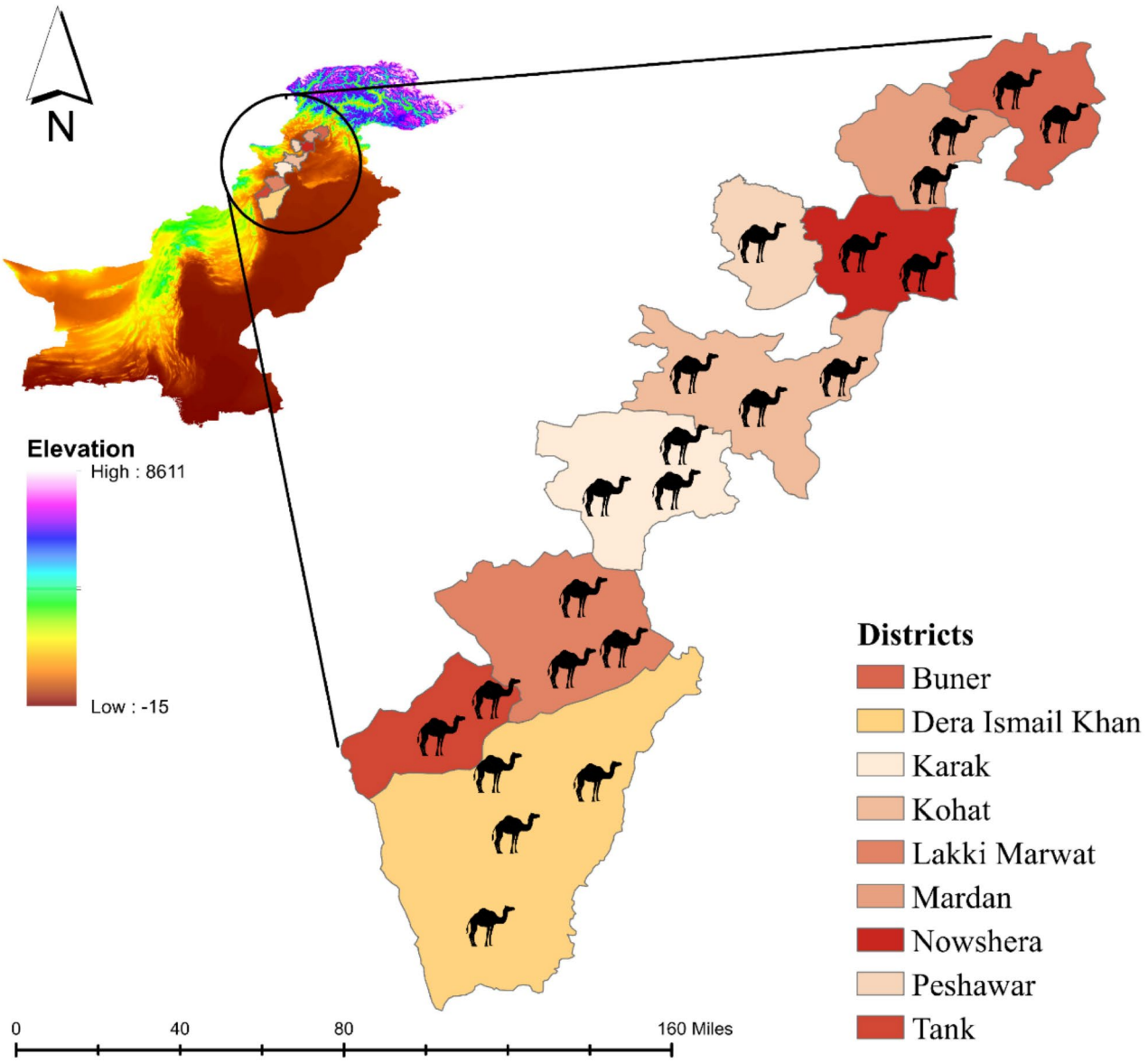

Fig. 1. Map showing the collection localities of camel infested ticks.

The whole genomic extracted DNA of ticks was subjected to conventional PCR (GE-96G, Bioer, Hangzhou, China) and screened for amplification of target genes of ticks, *Rickettsia* and *Coxiella* species. A fragment of *cox1* (*cytochrome c oxidase subunit 1*) was used for the characterization of ticks, *gltA* (*citrate synthetase*), *ompA* (*outer membrane protein A*) and *ompB* (*outer membrane protein B*) partial genes were used for the detection and genetic characterization of *Rickettsia* spp., while *groEL* (*chaperonin* protein) partial fragment was used for the detection and genetic characterization of *Coxiella brunetti*. Tested primers sequences and annealing temperatures are mentioned in [Table 1]. The PCR reaction mixture was prepared in 25 µL containing 1 µL (10µM) of each forward and reverse primer, 2 µL (50 ng) of genomic DNA, 8.5 µL of 'nuclease free water' and 12.5 µL DreamTaq green 2x PCR MasterMix (Thermo Fisher Scientific, Waltham, MA, US). In each PCR, *Hyalomma kumari cox1* and *Rickettsia massiliae* DNA were used as positive control for ticks and bacterial pathogens, respectively. The 'nuclease free water' was used as a negative control.

A 2% agarose gel was used for running the PCR amplified products, the target bands were visualized in gel documentation system (BioDoc-It® Imaging System, UVP, LLC, Upland, USA). The positive PCR products were purified and cleaned using standard DNA purification Kit (GeneClean® II kit, Qbiogene, Illkirch-Graffenstaden, France).

Sequencing and phylogenetic analysis

For molecular analysis, 9 positive *cox1* sample of each tick species (3 males, 3 unfed females and 3 unfed nymphs) except *Rh. sanguineus* s.l (it was found only in district Buner), and all amplified samples of pathogens were

Organism	Gene	Primer sequences (5′-3′)	PCR conditions; Initial denaturation, X-cycles (denaturation, annealing, extension), final extension	Amplicon size	References
Tick	*cox1*	F-GGAACAATATATTTAATTTTTGG R-ATCTATCCCTACTGTAAATATATG	95 °C for 5 min, 40 cycles (95 °C for 30 s, **55**°C for 1 min,72 °C for 1 min), 72 °C for 5 min	850 bps	[13]
Rickettsia	*gltA*	F: GCAAGTATCGGTGAGGATGTAAT R: GCTTCCTTAAAATTCAATAAATCAGG	95 °C for 3 min, 40 cycles (95 °C for 15 s, **48**°C for 30 s, 72 °C for 30 s), 72 °C for 7 min.	401 bps	[27]
	ompA	F-ATGGCGAATATTTCTCCAAAA R-GTTCCGTTAATGGCAGCATCT	95 °C for 3 min, 35 cycles (95 °C for 20 s, **55**°C for 30 s, 63 °C for 1 min), 72 °C for 7 min	532 bps	[28]
	ompB	F-CCGCAGGGTTGGTAACTGC R-CCTTTTAGATTACCGCCTAA	95 °C for 3 min, 40 cycles (95 °C for 30 s, **50**°C for 30 s, 68 °C for 1.5 min), 68 °C for 7 min	862bps	[29]
Coxiella	*groEL*	$^{+}$F-TTGAAAAYATGGGCGCKCAAATGGT $^{+}$R-CGRTCRCCAAARCCAGGTGC	95 °C for 3 min, 30 cycles (95 °C for 30 s, **56**°C for 30 s,72 °C for 1.5 min), 72 °C for 7 min	655 bps	[30]
		$^{++}$F-GAAGTGGCTTCGCRTACWTCAGACG $^{++}$R-CCAAARCCAGGTGCTTTYAC		619 bps	

Table 1. Primers employed for the amplification of targeted sequences of ticks and associated *Rickettsia* and *Coxiella* species. $^{+}$ First run of nested PCR, $^{++}$ second run of nested PCR.

sequenced bi-directionally (Macrogen, South Korea) through Sanger sequencing[31]. For phylogenetic analysis of ticks and pathogens, obtained sequences were assembled and trimmed to eliminate the poor nucleotide regions using SeqMan V. 5 (DNASTAR, USA). Trimmed sequences were subjected to BLAST (Basic Local Alignment Search Tool) at NCBI (National Center for Biotechnological Information) to download identical sequences for corresponding ticks and pathogens. Downloaded and obtained sequences along with appropriate outgroups were aligned using ClustalW multiple alignment in BioEdit V.7.0.5[32]. Aligned sequences were used to construct phylogenetic tree through Tamura-Nei model and Maximum-Likelihood statistical method[33] keeping bootstrap value 1000 in MEGA-X[34].

Results

Epidemiology of ticks

Ticks survey of transhumant camels in nine districts resulted into total collection of 598 ticks, wherein females were most widespread counting 299 (50%), followed by males 183 (30.60%) and nymphs 116 (19.39%). The morphologically identified ticks were assigned into six species of the genus *Hyalomma* and one species of the genus *Rhipicephalus*. *Hyalomma dromedarii* was the most common tick (290, 48.49%) infesting camels throughout the region. *Hyalomma anatolicum* (107, 17.89%) was the second most prevalent tick collected in all districts except Buner. The third most outnumbered tick was *Hyalomma scupense* (71, 11.87%), this tick species was reported from most of study districts except Buner, Mardan and Kohat. *Hyalomma isaaci* was the fourth dominant species infesting camels counting for 56 (9.36%) ticks. This species was only limited to three districts including Lakki Marwat, Kohat and Mardan. *Hyalomma turanicum* (39, 6.52%) was the least diverse species in terms of distribution, only limited to two districts of southern KP comprising Lakki Marwat and Kohat. *Hyalomma asiaticum* was also the least diverse species in terms of distribution counting 29 (5.18%) ticks, only limited to northern Khyber Pakhtunkhwa, reported from two districts including Mardan and Buner. The least reported species in comparison of both numbers and distribution was *Rhipicephalus sanguineus* s.l counting for 4 (0.67%) ticks. This species was observed only in Buner district [Table 2].

Ticks on the companion hosts

Overall, 173 companion animals (65.04%) of camels out of 266 observed hosts sharing same herd or shed were tick infested. The prevalence of tick infestation on companion/co-herded hosts was; cattle (48/74, 64.86%), goats (36/51, 70.58%), sheep (58/91, 63.73%), and dogs (31/50, 62%). *Hyalomma dromedarii*, *Haemaphysalis montgomeryi*, *Rhipicephalus turanicus*, *Hy. anatolicum* and *Rh. sanguineus* s.l were collected from these companion hosts. Detailed information of these companion hosts and associated ticks are shown in [Table 3]. Ticks of the companion hosts were not included in further analysis pursuing the target objectives.

Associated risk variables with camel ticks

Five different variables that may represent the associated risks to the collected camel ticks were analyzed. These variables included host gender, age, bread, management and terrain type. Both genders were infested by all tick species except *Rh. sanguineus* s.l which was only collected from males (Fig. 2B). Host management can affect tick diversity and infestation rate of hosts, co-herded camels were mostly infested by ticks compared to solitary kept camels (Fig. 2E and F). Camel population and distribution is dependent on the terrain type; sandy desert is the most favorable terrain type. Six different camel breeds were examined for the tick collection which are Marecha, Gaddi, Ghulmani, Khader, Maya and Larri (Sindhi). Comprehensive data on risks associated with camel ticks has been presented in figure (Fig. 2) and supplementary table [Table S1].

Detection and prevalence of pathogens in camel ticks

Out of 124, 34 tested ticks including 23 *Hy. dromedarii*, six *Hy. anatolicum*, two *Hy. scupense* and three *Hy. asiaticum* were positive for pathogens, while the screening of *Hy. isaaci*, *Hy. turanicum* and *Rh. sanguineus* s.l ticks resulted in unsuccessful detection of any pathogen. *Rickettsia aeschlimannii* was detected in eight *Hy. dromedarii* and two *Hy. scupense* ticks, whereas undetermined *R. aeschlimannii*-like was detected in a single *Hy. anatolicum* tick based on the *ompB* amplification. *Candidatus* Rickettsia shennongii was detected in seven *Hy. dromedarii* and three *Hy. anatolicum* ticks. *Coxiella burnetii* was detected in eight *Hy. dromedarii*, two *Hy. anatolicum* and three *Hy. asiaticum* ticks (Fig. 3).

Molecular and phylogenetic analysis of camel ticks

Partial *cox 1* fragments were successfully amplified for the selected ticks, which revealed the presence of six *Hyalomma* species i.e. *Hy. dromedarii*, *Hy. anatolicum*, *Hy. scupense*, *Hy. asiaticum*, *Hy. isaaci*, *Hy. turanicum* and one *Rhipicephalus* species i.e. *Rhipicephalus sanguineus*. The *cox 1* of *Hy. dromedarii* (PP716460) fragment showed 100% identity with same species from Pakistan (ON529118) and 99.61–99.87% with the same species from Cameroon (OK576092), Tunisia (MT040954) and Egypt (KU323789). The obtained *Hy. anatolicum cox 1* (PP716458) sequence revealed high identity (99.07–99.87%) with same species from India (OL799138), Pakistan (ON528934), China (KF583577), Turkey (MW546283) and Egypt (OK340836). Sequence analysis of *Hy. scupense* (PP716462) fragment showed 100% identity with same species from Pakistan (ON529973) and 99.72% with the same species from France (KX000638), China (KF583581) and Turkey (MW546282). The *cox1* (PP729297) of *Hy. asiaticum* fragment showed 99.58% with the same species from Turkey (MW546281), China (OM368315, KF583578) and Kazakhstan (KU364332), while *cox1* of the *Hy. isaaci* (PP716461) revealed 100% identity with same species from Pakistan (ON529271) and 99.43–99.62% with the same species from Sri Lanka (KU130605) and Pakistan (KU130604). The *Hy. turanicum cox 1* (PP716472) showed 100% identity with same species from Iraq (KM235709), Saudi Arabia (MH094478) and 99.82% identity with the same species from Iraq (KU130646). Sequence analysis of *Rhipicephalus sanguineus* (PP716478) fragment showed 100% identity

Locality	Hosts (camel)	*Hy. dromedarii*			*Hy. anatolicum*			*Hy. scupense*			*Hy. isaaci*			*Hy. turanicum*			*Hy. asiaticum*			*Rh. sanguineus* s.l		
	CI/CE (percentage)	M	F	N	M	F	N	M	F	N	M	F	N	M	F	N	M	F	N	M	F	N
Dera Ismail Khan	17/20 (85%)	12	23	7	5	9	2	4	6	3	0	0	0	0	0	0	0	0	0	0	0	0
Lakki Marwat	31/34 (91.17%)	17	38	12	8	13	5	6	11	4	5	7	2	6	12	3	0	0	0	0	0	0
Tank	14/16 (87.5%)	9	18	8	3	8	2	3	5	2	0	0	0	0	0	0	0	0	0	0	0	0
Karak	15/18 (83.34%)	11	16	5	2	7	4	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0
Kohat	13/17 (76.47%)	8	15	7	4	3	0	0	0	0	12	14	8	5	8	5	0	0	0	0	0	0
Peshawar	9/12 (75%)	6	11	3	3	5	3	4	4	2	0	0	0	0	0	0	0	0	0	0	0	0
Nowshera	10/14 (71.42%)	8	13	5	2	6	2	5	5	3	0	0	0	0	0	0	0	0	0	0	0	0
Mardan	11/15 (73.34%)	7	9	4	4	3	4	0	0	0	3	4	1	0	0	0	4	6	1	0	0	0
Buner	8/13 (61.53%)	5	8	5	0	0	0	0	0	0	0	0	0	0	0	0	7	9	4	4	0	0
Total	128/159	83	151	56	31	54	22	23	34	14	20	25	11	11	20	8	11	15	5	4	0	0
Grand total	128/159 (80.50%)	290 (48.49%)			107 (17.89%)			71 (11.87%)			56 (9.36%)			39 (6.52%)			31 (5.18%)			4 (0.67%)		

Table 2. District wise prevalence of camel infesting ticks in Khyber Pakhtunkhwa. *CI: Camel infested, CE: Camel examined, M: Male, F: unfed female, N: unfed nymph.

	Host	HI/HE* (percentage)	*Hy. dromedarii*	*Ha. montgomeryi*	*Rh. turanicus*	*Hy. anatolicum*	*Rh. sanguineus* s.l
Dera Ismail Khan	Dogs	4/7 (57.14%)	0	0	3	0	9
	Cattle	10/14 (71.42%)	4	0	0	16	0
	Total	14/21 (66.67%)	4	0	3	16	9
Lakki Marwat	Sheep	11/16 (68.75%)	3	0	8	9	0
	Dogs	9/13 (69.23%)	2	0	4	0	12
	Cattle	13/19 (68.42%)	5	0	0	21	0
	Total	33/48 (68.75%)	10	0	12	30	12
Tank	Sheep	7/12 (58.34%)	4	0	9	0	0
	Dogs	6/8 (75%)	0	0	5	0	11
	Cattle	9/13 (69.23%)	6	0	0	15	0
	Total	22/33 (66.66%)	10	0	14	15	11
	Sheep	2/5 (40%)	3	0	6	0	0
Karak	Dogs	3/5 (60%)	0	0	4	0	9
	Cattle	7/12 (58.34%)	0	0	0	17	0
	Total	12/22 (54.55%)	3	0	10	17	9
Kohat	Sheep	4/8 (50%)	0	0	7	5	4
	Cattle	5/9 (55.56%)	0	0	2	0	14
	Total	9/17(52.94%)	0	0	9	5	18
Peshawar	Dogs	3/7 (42.85%)	0	0	5	0	7
	Cattle	4/7 (57.14%)	4	0	0	10	0
	Total	7/14 (50%)	4	0	5	10	7
Nowshera	Sheep	15/22 (68.18%)	4	11	0	0	7
	Dogs	6/10 (60%)	0	0	7	0	12
	Total	21/32 (65.62%)	4	11	7	0	19
Mardan	Goat	17/25 (68%)	0	13	4	0	9
	Sheep	12/17 (70.59%)	0	11	4	0	0
	Total	29/42 (69.05%)	0	24	8	0	9
Buner	Goat	19/26 (73.07%)	0	18	0	0	0
	Sheep	7/11 (63.64%)	0	8	0	0	7
	Total	26/37 (70.27%)	0	26	0	0	7
	Grant total		35	61	68	93	101

Table 3. Ticks on the companion hosts in different localities of the surveyed regions. *HI: Hosts infested, HE: Hosts examined.

with same species from Pakistan (ON530888) and 99.67% with the same species from India (MZ424730) and Iran (KT313112, KT313113). Phylogenetically all obtained sequences were clustered with their corresponding species (Fig. 4). Data regarding the identities of each sequence is provided in supplementary file S2.

Phylogenetic analysis of *Rickettsia*

Two *Rickettsia* spp. comprising *Rickettsia aeschlimannii* and *Ca*. R. shennongii were detected based on the amplification of *gltA*, *ompA* and *ompB* fragments in ticks. Obtained *gltA* sequence of *R. aeschlimannii* (PP726672) showed 100% identity with same species detected in *Hyalomma marginatum* from Mongolia (MH267736), Russia (KU961540, DQ235776) and Kazakhstan (MW922554), *Hy. asiaticum* from Kazakhstan (MW922557), *Hyalomma lusitanicum* from Spain (OK205217), *Hyalomma rufipes* and *Hyalomma truncatum* from Senegal (HM050283, HM050276). The obtained *gltA* sequence shared 99.71% identity with *R. aeschlimannii* detected in *Pediculus humanus* from Mali (KY937992) and human blood from Kenya (KX227762). Obtained *gltA* sequence of *Ca*. R. shennongii (PP726669) showed 100% identity with same species detected in *Rhipicephalus haemaphysaloides* from China (OL856117) and *Rh. sanguineus* s.l from Pakistan (OQ627013). Phylogenetically, the obtained *gltA* sequences formed cluster with corresponding species (Fig. 5).

Obtained *ompA* (PP726673) sequence of *R. aeschlimannii* showed 100% identity with same species detected in *Hyalomma lusitanicum* and *Rhipicephalus pusillus* from Spain (Ok205206, Ok205027), *Rh. microplus* from France (MH797772) and Kenya (KX227779), environmental tick from Italy (JN944634), and Zambia (LC565682). Obtained *ompA* sequence shared 99.78% identity with *R. aeschlimannii* detected in *Hy. marginatum* from Turkey (OM127994) and Lithuania (MT973816). Obtained *ompA* sequence of *Ca*. R. shennongii (PP726670) showed 100% identity with same species detected in *Rh. sanguineus* s.l from Pakistan (OQ632789). The *ompA* sequence of *Ca*. R. shennongii showed 99.81% identity with same species detected in *Rh. haemaphysaloides* (OL856103) and *Rh. sanguineus* (OL856104) from China. Phylogenetically, obtained *ompA* sequences clustered with corresponding species (Fig. 6).

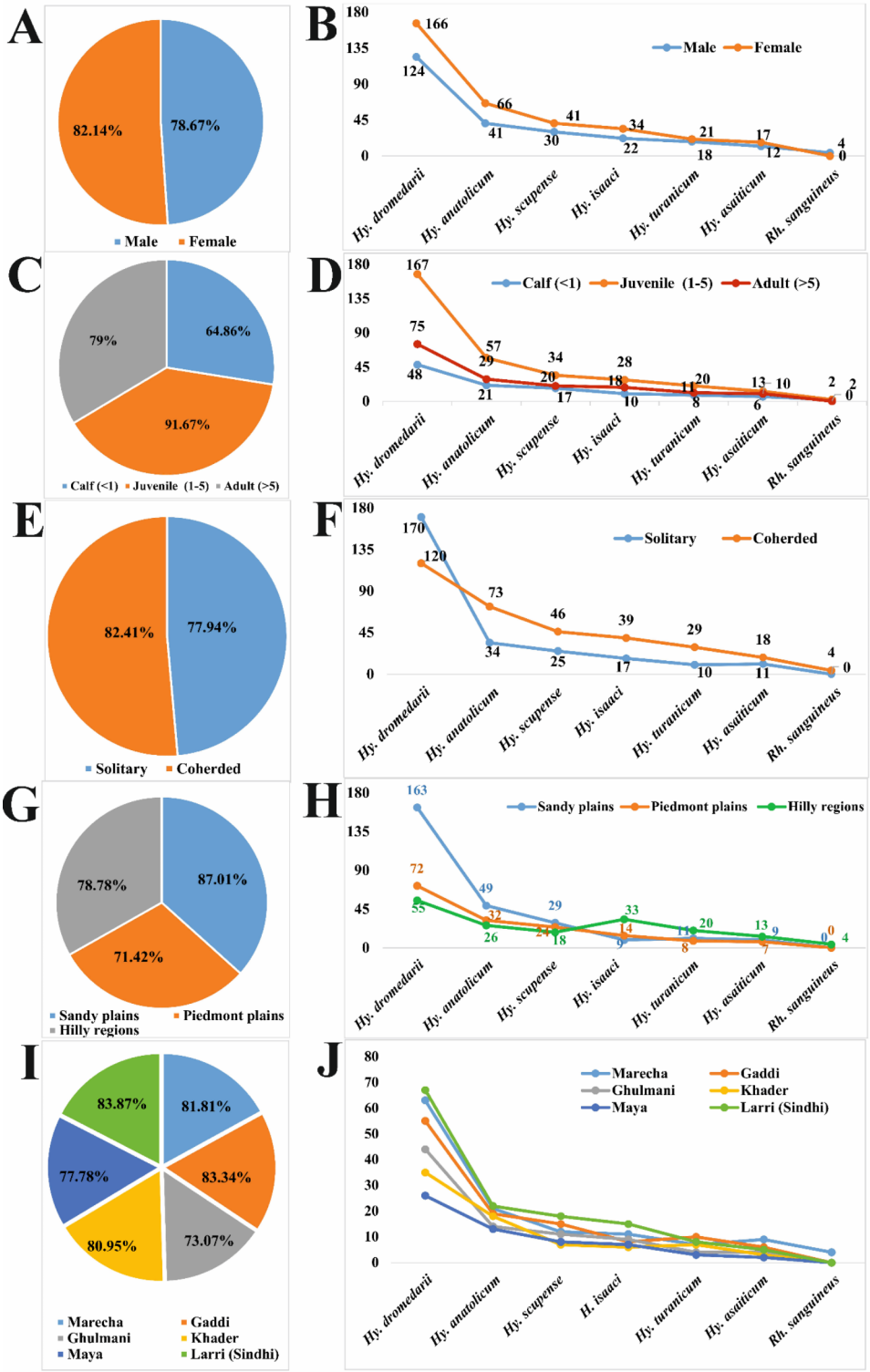

Fig. 2. Associated variable risks regarding camels with tick infestations **A**: gender-wise camel data, **B**: gender-wise tick collection, **C**: age-wise camel data, **D**: age-wise tick collection, **E**: management-wise camel data, **F**: management wise tick collection, **G**: camel data in various terrain type, **H**: tick collection in various terrain type, **I**: breeds-wise camel data, **J**: breeds-wise tick collection.

Obtained *ompB* (PP726674) sequence of *R. aeschlimannii* showed 100% identity with same species detected in *Hy. marginatum* from Portugal (LC229608) and Russia (KU961544), *Dermacentor marginatus* from Kazakhstan (MW9430414), *Hyalomma lusitanicum* from Italy (MH532261), and *Amblyomma tigrinum* from Bolivia (GQ180863). Obtained *ompB* sequence shared 99.46% identity with *R. aeschlimannii* detected in *Hy.*

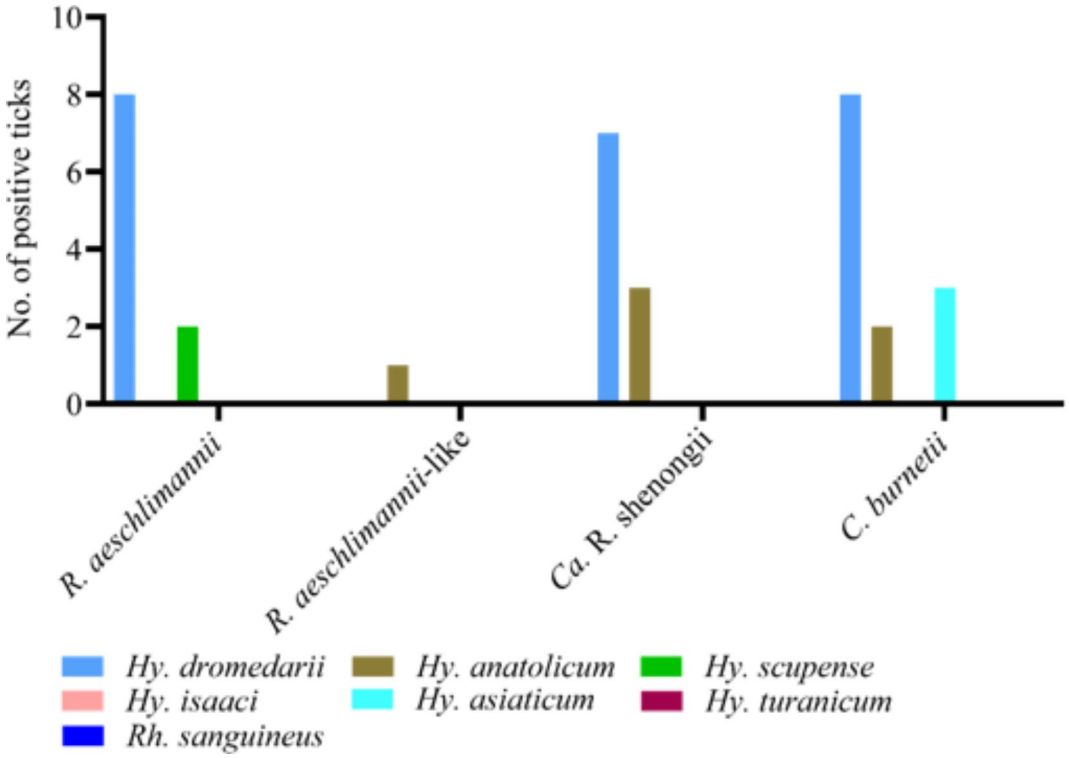

Fig. 3. Prevalence of *Rickettsia* spp. and *Coxiella* spp. in camel infested ticks.

marginatum from Morocco (AF123705). Obtained *ompB* sequence of *Ca*. R. shennongii (PP726671) showed 100% identity with same species detected in *Rh. sanguineus* from Pakistan (OQ632792). The *Ca*. R. shennongii *ompA* sequence showed 99.87% identity with same species detected in *Rh. haemaphysaloides* from China (ON015826). Phylogenetically, the obtained *ompB* sequence clustered with corresponding species (Fig. 3). Another undetermined *Rickettsia* sp. (*R. aeschlimannii*-like) was detected based on successful amplification of *ompB* sequence in *Hy. anatolicum*. This *ompB* (PP726675) sequence showed 98.16% identity with *R. aeschlimannii* sequences detected in *Hy. asiaticum* from China (MF098413) and *Hy. truncatum* from Kenya (HM050278). In *ompB* based phylogenetic tree, this sequence has formed a distinct clade with *R. aeschlimannii* group (Fig. 7). Data regarding the identities of each sequence is provided in supplementary file S2.

Phylogenetic analysis of *Coxiella burnetii*

Coxiella burnetii was detected in ticks based on the amplification of *groEL* fragments. Obtained *groEL* (PP726676) fragment of the *C. burnetii* showed 100% maximum identity with same species detected in *Homo sapiens* from Japan (AP019759) and Romania (CP103435), *Bothricroton concolor* from Australia (CP032542), Placenta of goats from Netherlands (CP014555), *Dermacentor andersoni* from Russia (CP040059), and *Dermacentor reticulatus* from Slovakia (MG860513). Obtained *groEL* sequence shared 99.80% identity with *C. burnetii* detected in *Haemaphysalis flava* from China (ON455116). Phylogenetically, the obtained *groEL* sequence clustered with corresponding species (Fig. 8). Data regarding the identities of each sequence is provided in supplementary file S2.

Discussion

The semi deserted climatic conditions of southern KP suit the breeding of camel husbandry. However, camel associated ticks and tick-borne diseases limit the growth of this sector, affecting the socio economy of people in the region. Herein, we for the first time reported the diversity of camel ticks, and their associated *Rickettsia* and *Coxiella* spp. in the region. Among the identified tick species, camels infested by *Hy. isaaci* and *Hy. turanicum* were reported for the first time in Pakistan. Moreover, rickettsial DNA of *Ca.* R. shennongii, *R. aeschlimannii*, an undetermined *Rickettsia* sp. (*R. aeschlimannii*-like) and *C. burnetii* were detected in these ticks, which are major zoonotic concerns[14,18,35]. Information on molecular diversity and distribution of camel infesting ticks and their associated pathogens will be useful in understanding disease surveillance.

Seven tick species observed parasitizing camels during this study were *Hy. anatolicum*, *Hy. asiaticum*, *Hy. dromedarii*, *Hy. isaaci*, *Hy. scupense*, *Hy. turanicum* and *Rh. sanguineus* s.l. Tick co-occurrence may potentially facilitate pathogens transmission between different hosts and may contribute to their survival in the environment[18,36–38]. Camels are the principal hosts of *Hyalomma dromedarii*[7], however co-herding with other host species may lead to the infestation of diversified tick species[37,39]. Cattle, goats, sheep, horses and dogs were the observed companion hosts, which were infested by *Hy. dromedarii*, *Hy. anatolicum*, *Ha. montgomeryi*, *Rh. turanicus* and *Rh. sanguineus* s.l, during previous reports in this region[8,18,20,40]. Ticks of the co-herded hosts

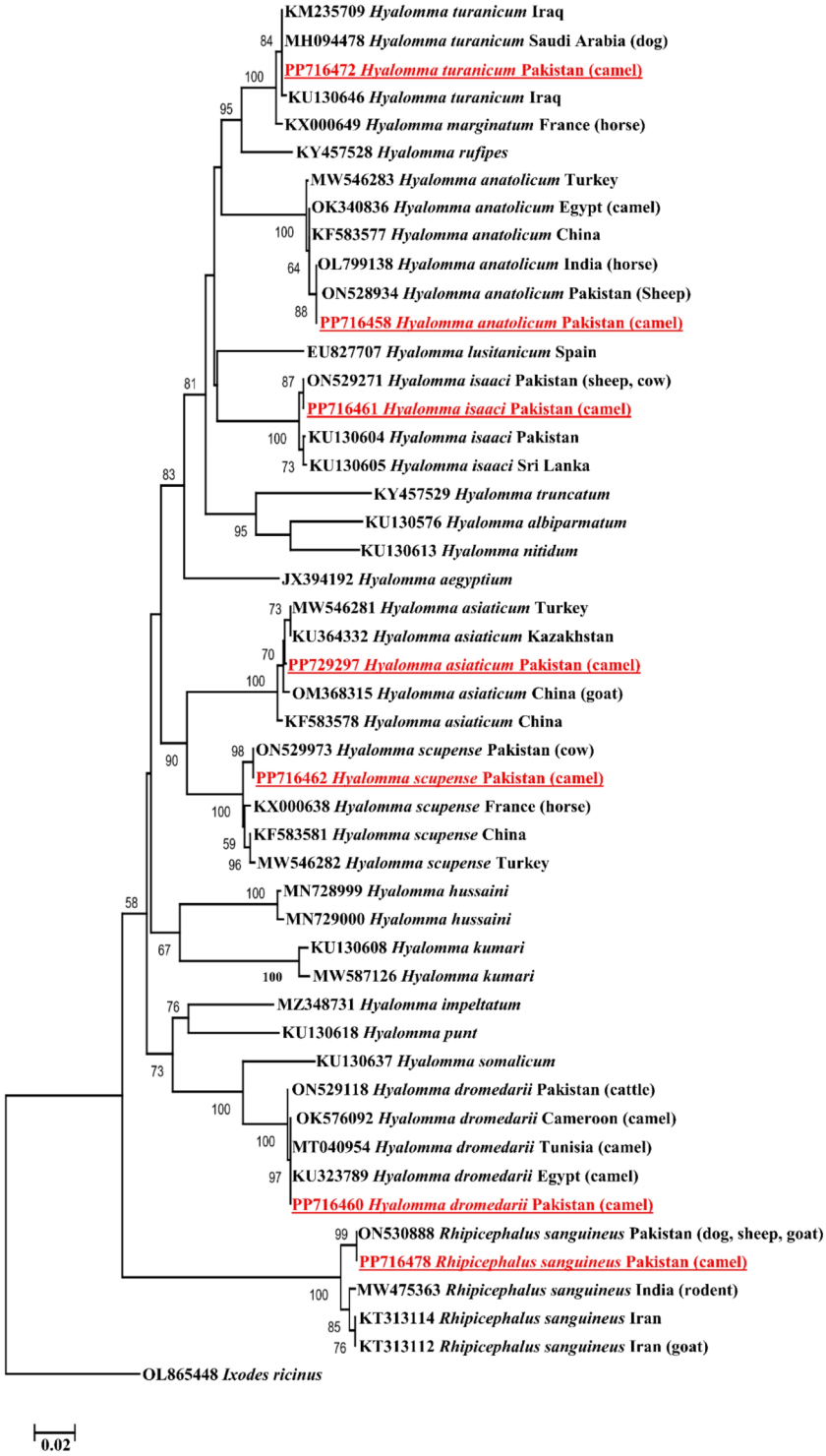

Fig. 4. Phylogenetic tree based on nucleotide sequences of *cox 1* of *Hyalomma* spp. Ixodes ricinus sequence was taken as an outgroup. Following 1000 bootstrapping values, phylogenetic tree support > 55% bootstrap levels in each node. The present study sequences (PP716458, PP716460, PP716461, PP716462, PP716472, PP716478, PP729297) are indicated by an underline and red color fonts.

were exempted from further analysis as previously these ticks had been screened for different pathogens[8,12,38,41]. *Hyalomma* ticks have wide host range, the reports regarding their association with camel have been confirmed globally including Saudi Arabia[42], Egypt[43], Israel[36], Kenya[39], Pakistan[44], Iraq, United Arab Emirates, Kazakhstan and Iran[7].

Fig. 5. Phylogenetic trees based on nucleotide sequences of *gltA* sequences of *Rickettsia* spp. *Rickettsia canadensis* sequence was taken as an outgroup. Following 1000 bootstrapping values, phylogenetic tree support > 55% bootstrap levels in each node. The present study sequences (PP726669, PP726672) are indicated by an underline and red color fonts.

There is a high incidence of zoonotic diseases in KP due to the suitable environment for tick reproduction[19,20,40]. In this study, *R. aeschlimannii* and *Ca*. R. shennongii were detected in camel infested *Hy. dromedarii*, *Hy. anatolicum* and *Hy. scupense*. The presence of *Rickettsia* in various *Hyalomma* spp. has been reported from different regions including Morocco[45], Israel[36], Italy[46], Germany[47], Tunisia[48], Algeria[49] and Pakistan[20,50], which indicates the global distribution of *Rickettsia* in various ticks infesting diverse hosts. Camels in Pakistan are kept close to ranches and herds of other companion hosts like goats, cattle, and sheep, thus the presence of *Rickettsia* in camel ticks my pose zoonotic threats. In these areas, healthcare providers should consider spotted fever group rickettsiosis (SFGR) in patients diagnosed with infections of unidentified origin and clinical symptoms comparable to rickettsiosis. An undetermined *Rickettsia* sp. of the *R. aeschlimannii* group has been detected in camel tick, through the amplification of *ompB* partial fragments, although its amplification of *gltA* and *ompA* was unsuccessful, failure in amplification of rickettsial genes has been previously reported in several cases[18,51].

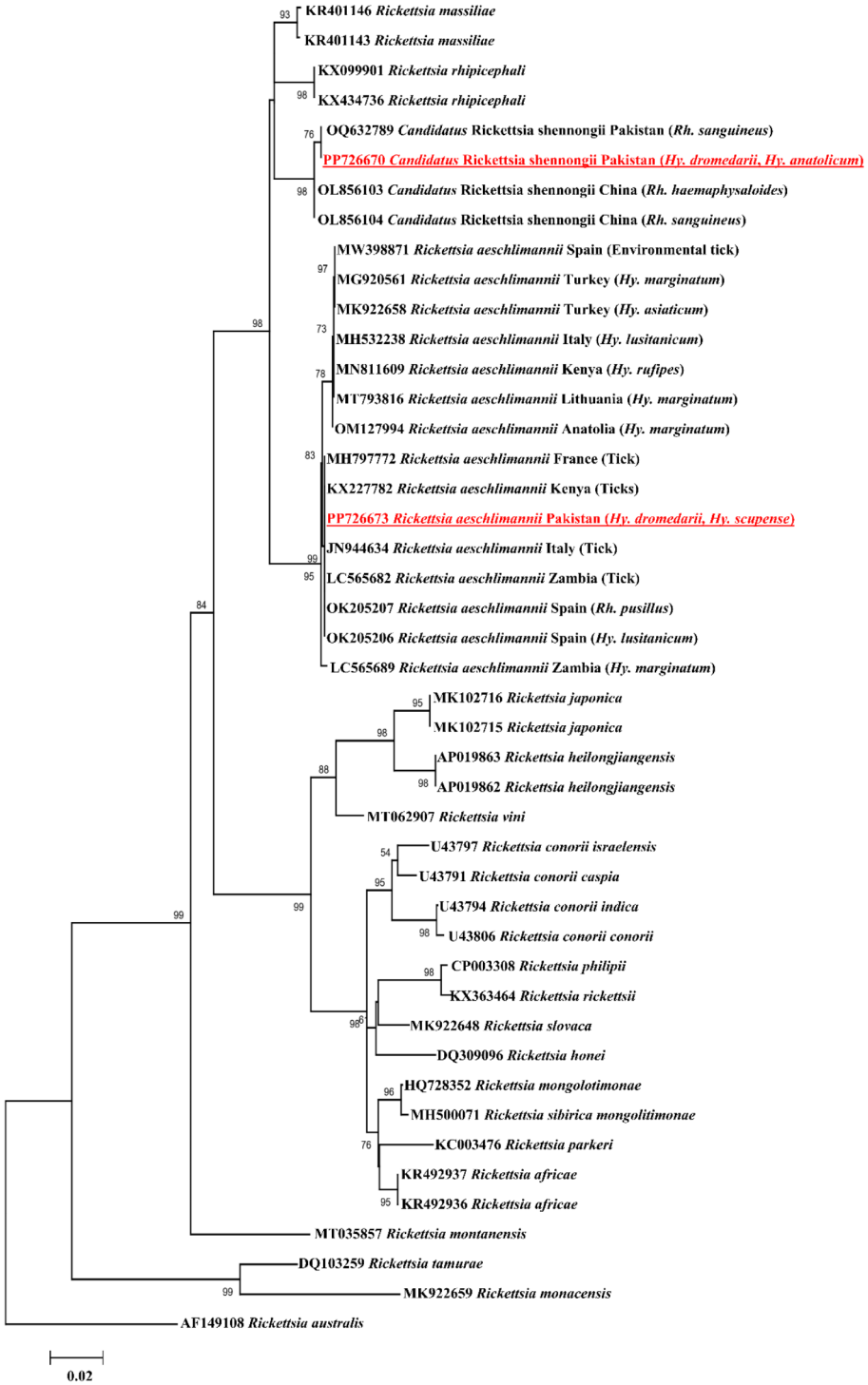

Fig. 6. Phylogenetic tree based on nucleotide sequences of *ompA* partial gene of *Rickettsia* spp. *Rickettsia australis* sequence has been taken as an outgroup. Following 1000 bootstrapping values, phylogenetic tree support > 55% bootstrap levels in each node. The present study sequences (PP726670, PP726673) are indicated by underline and red color fonts.

This indicates the occurrence of an unexplored fauna of tick-borne *Rickettsia* in the region which needs further research.

Coxiella burnetii has been detected in humans and animals in several regions. Livestock is one of the significant reservoirs of this pathogen[52]. The tick's potential role as vector has been suggested through the detection of

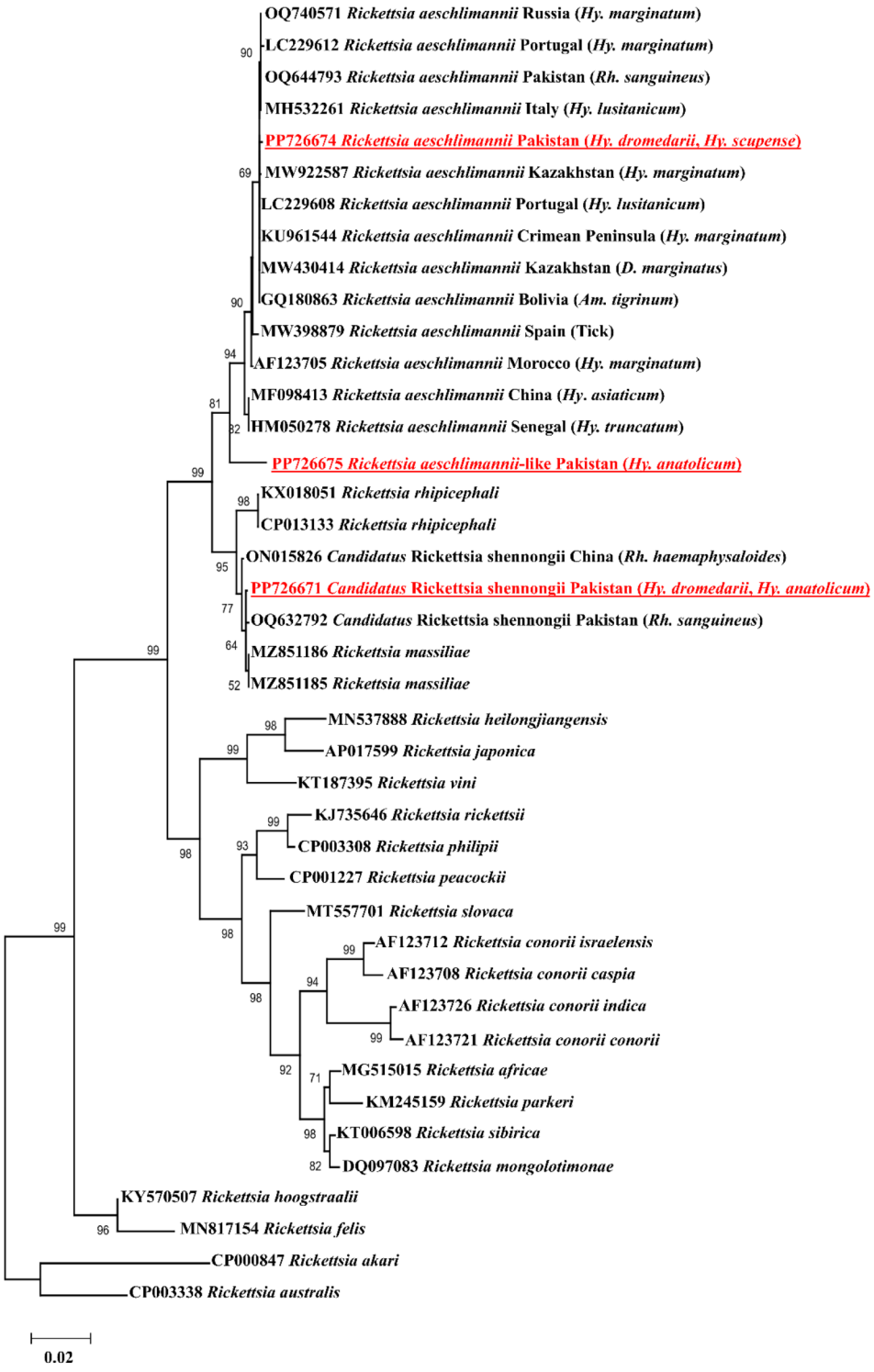

Fig. 7. Phylogenetic tree based on nucleotide sequences of *ompB* of *Rickettsia* spp. *Rickettsia australis* and *Rickettsia akari* sequences have been taken as an outgroup. Following 1000 bootstrapping values, phylogenetic tree support > 55% bootstrap levels in each node. The present study sequences (PP726671, PP726674, PP726675) are indicated by underline and red color fonts.

C. burnetii in various tick species. Numerous species of ticks, notably *Haemaphysalis cornupunctata* and *Hy. anatolicum* in Pakistan, have been found to carry this bacterium[21]. The current investigation led to the detection of *C. burnetii* in *Hy. anatolicum*, *Hy. dromedarii*, and for the first time in *Hy. asiaticum* in Pakistan. The presence of this bacteria in tick infesting camels suggests their widespread distribution in the region.

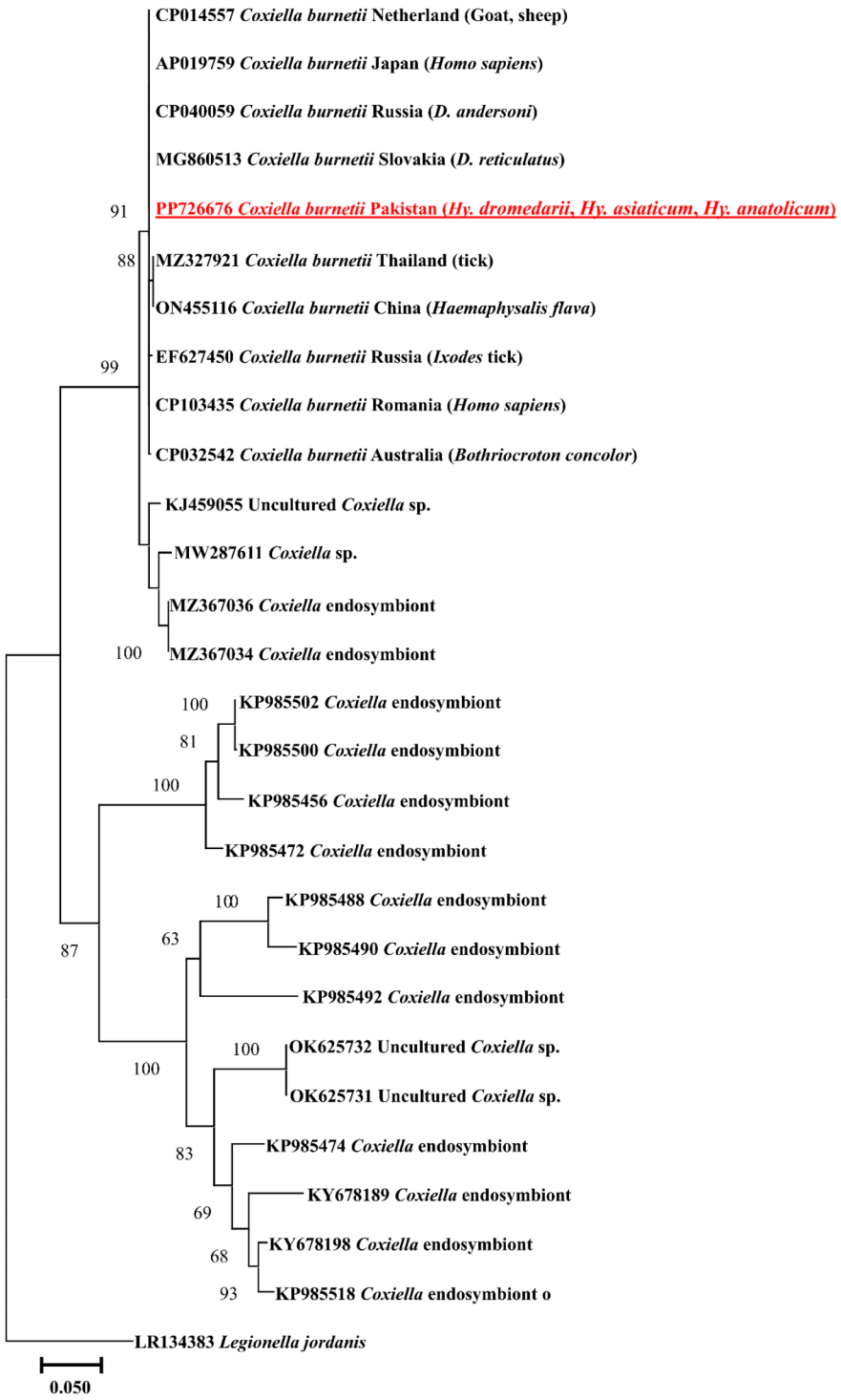

Fig. 8. Phylogenetic tree based on nucleotide sequences of *groEL* partial gene of *Coxiella* spp. *Legionella jordanis* sequence was taken as an outgroup. Following 1000 bootstrapping values, phylogenetic tree support > 55% bootstrap levels in each node. The present study sequence (PP726676) is indicated by an underline and red color fonts.

Camel infested ticks are the confirmed vectors of various disease-causing agents, that might pose risks to camel herders, people associated with camel dairy (milk sellers and buyers), meat (butchers and flesh eaters), and leather. The processing of a few ticks for the molecular detection of selected two tick-borne pathogens based on specific genetic markers is the limitation of this study. The insights obtained from this work will be valuable in future surveillance and prevention of these infectious agents.

Conclusion

Camel husbandry has been threatened by ticks and their associated *Rickettsia* and *Coxiella* species. The current study revealed the identification and phylogenetic analysis of seven hard tick species infesting camels in the selected regions. Moreover, this study reports the detection of four pathogens including *Ca.* R. shennongii, *R. aeschlimannii*, an undetermined *Rickettsia* sp. (*R. aeschlimannii*-like) and *C. burnetii* in camel ticks. *Candidatus* R. shennongii has been detected for the first time in camel ticks. Molecular screening of pathogens associated with camel ticks indicates the possible zoonotic transmission from ticks to humans due to their frequent contact and sharing of habitats. To safeguard the lives of camel herders and their camels from zoonotic threats, comprehensive molecular surveillance regarding the epidemiological status of different tick-borne pathogens is needed in the regions.

Data availability

Sequence data that support the findings of this study have been deposited in the NCBI.

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References

1. Zhu, S., Zimmerman, D. & Deem, S. L. A Review of Zoonotic Pathogens of Dromedary Camels. *EcoHealth* 16, 356–377; (2019). https://doi.org/10.1007/s10393-019-01413-7
2. Faye, B., Chaibou, M. & Vias, G. Integrated impact of climate change and socioeconomic development on the evolution of camel farming systems. *Br. J. Environ. Clim. Change.* **2**, 227–244. https://doi.org/10.9734/BJECC/2012/1548 (2012).
3. Ahmed, T. & Kanwal, R. Biochemical characteristics of lactic acid producing bacteria and preparation of camel milk cheese by using starter culture. *Pak Vet. J.* **24**, 87–91 (2004).
4. Sabahat, S. et al. SNP discovery and population structure analysis in Lassi and Marecha camel breeds using a genotyping by sequencing method. *Anim. Genet.* **51**, 620–623. https://doi.org/10.1111/age.12953 (2020).
5. Perveen, N., Muzaffar, S. B. & Al-Deeb, M. A. Ticks and tick-borne diseases of livestock in the Middle East and North Africa: a review. *Insects.* **12**, 83. https://doi.org/10.3390/insects12010083 (2021).
6. El-Alfy, E. S. et al. Tick-borne pathogens in camels: a systematic review and meta-analysis of the prevalence in dromedaries. *Ticks Tick. Borne Dis.* **15**, 102268. https://doi.org/10.1016/j.ttbdis.2023.102268 (2024).
7. Apanaskevich, D. A., Schuster, A. L. & Horak, I. G. The genus Hyalomma: VII. Redescription of all parasitic stages of H. (Euhyalomma) dromedarii and H. (E.) schulzei (Acari: Ixodidae). *J. Med. Entomol.* **45**, 817–831. https://doi.org/10.1093/jmedent/45.5.817 (2008).
8. Alam, S. et al. Spatio-temporal patterns of Ticks and Molecular Survey of Anaplasma marginale, with notes on their phylogeny. *Microorganisms*. **10**, 1663. https://doi.org/10.3390/microorganisms10081663 (2022).
9. Yasmeen, N. et al. Distribution, prevalence and diversity of ticks (Acari: Ixodidae) infesting one-humped camels from Southwest Punjab, Pakistan. *Vet. Parasitol. Reg. Stud. Rep.* **37**, 100825. https://doi.org/10.1016/j.vprsr.2022.100825 (2022).
10. Sanches, G. S. et al. Molecular, biological, and morphometric comparisons between different geographical populations of Rhipicephalus sanguineus Sensu lato (Acari: Ixodidae). *Vet. Parasitol.* **215**, 78–87. https://doi.org/10.1016/j.vetpar.2015.11.007 (2016).
11. Kanduma, E. G. et al. Multi-locus genotyping reveals absence of genetic structure in field populations of the brown ear tick (*Rhipicephalus appendiculatus*) in Kenya. *Ticks Tick. Borne Dis.* **7**, 26–35. https://doi.org/10.1016/j.ttbdis.2015.08.001 (2016).
12. Ahmad, I. et al. Description of male, redescription of female, Host Record, and phylogenetic position of Haemaphysalis danieli. *Pathogens*. **11**, 1495. https://doi.org/10.3390/pathogens11121495 (2022).
13. Chitimia, L. et al. Genetic characterization of ticks from southwestern Romania by sequences of mitochondrial cox1 and nad5 genes. *Exp. Appl. Acarol.* **52**, 305–311. https://doi.org/10.1007/s10493-010-9365-9 (2010).
14. Aiman, O., Ullah, S., Chitimia-Dobler, L., Nijhof, A. M. & Ali, A. First report of *Nosomma monstrosum* ticks infesting Asian water buffaloes (*Bubalus bubalis*) in Pakistan. *Ticks Tick. Borne Dis.* **13**, 101899. https://doi.org/10.1016/j.ttbdis.2022.101899 (2022).
15. El Tigani-Asil, E. T. A. et al. Molecular Investigation on Tick-Borne Hemoparasites and *Coxiella burnetii* in Dromedary Camels (*Camelus dromedarius*) in Al Dhafra Region of Abu Dhabi, UAE. *Animals* 11, 666; (2021). https://doi.org/10.3390/ani11030666
16. Cabezas-Cruz, A. et al. Handling the microbial complexity associated to ticks. *Ticks tick-borne Pathogens*. **1**https://doi.org/10.5772/intechopen.80511 (2018). (IntechOpen.
17. Ahmad, I. et al. First molecular-based confirmation of *Dermacentor marginatus* and associated *Rickettsia raoultii* and *Anaplasma marginale* in the Hindu Kush Mountain range. *Animals*. **13**, 3686. https://doi.org/10.3390/ani13233686 (2023).
18. Khan, S. M. et al. Phylogenetic position of *Haemaphysalis kashmirensis* and *Haemaphysalis cornupunctata*, with notes on Rickettsia spp. *Genes*. **14**, 360. https://doi.org/10.3390/genes14020360 (2023).
19. Ullah, S. et al. First Report of *Rickettsia conorii* in *Hyalomma kumari* Ticks. *Animals*. **13**, 1488. https://doi.org/10.3390/ani13091488 (2023).
20. Ali, A. et al. First report on tick-borne pathogens detected in ticks infesting stray dogs near butcher shops. *Front. Vet. Sci.* **10**, 1246871. https://doi.org/10.3389/fvets.2023.1246871 (2023).
21. Ali, A. et al. Molecular detection of *Coxiella* spp. in ticks (Ixodidae and Argasidae) infesting domestic and wild animals: with notes on the epidemiology of tick-borne Coxiella burnetii in Asia. *Front. Microbiol.* **14**, 1229950. https://doi.org/10.3389/fmicb.2023.1229950 (2023).
22. Apanaskevich, D. A. Differentiation of closely related species Hyalomma anatolicum and H. excavatum (Acari: Ixodidae) based on a study of all life cycle stages, throughout entire geographical range. *Parazitologiia*. **37**, 259–280 (2003).
23. Apanaskevich, D. A. & Horak, I. G. The genus *Hyalomma* Koch, 1844: V. Re-evaluation of the taxonomic rank of taxa comprising the *H.* (*Euhyalomma*) *marginatum* Koch complex of species (Acari: Ixodidae) with redescription of all parasitic stages and notes on biology. *Int. J. Acarol.* **34**, 13–42. https://doi.org/10.1080/01647950808683704 (2008).
24. Apanaskevich, D. A. & Horak, I. G. The genus Hyalomma. XI. Redescription of all parasitic stages of H. (Euhyalomma) asiaticum (Acari: Ixodidae) and notes on its biology. *Exp. Appl. Acarol.* **52**, 207–220. https://doi.org/10.1007/s10493-010-9361-0 (2010).

25. Apanaskevich, D. A., Filippova, N. A. & Horak, I. G. The genus *Hyalomma* Koch, 1844. X. Redescription of all parasitic stages of *H.* (*Euhyalomma*) *scupense* Schulze, 1919 (=H. detritum Schulze) (Acari: Ixodidae) and notes on its biology. *Folia Parasitol.* **57**, 69–78. https://doi.org/10.14411/fp.2010.009 (2010).
26. Sambrook, J., Fritsch, E. F. & Maniatis, T. *Molecular Cloning: A Laboratory Manual (No. Ed. 2)* (Cold Spring Harbor Laboratory Press, 1989).
27. Labruna, M. B. et al. *Rickettsia* species infecting *Amblyomma cooperi* ticks from an area in the state of Sao Paulo, Brazil, where Brazilian spotted fever is endemic. *J. Clin. Microbiol.* **42**, 90–98. https://doi.org/10.1128/JCM.42.1.90-98.2004 (2004).
28. Regnery, R. L., Spruill, C. L. & Plikaytis, B. Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. *J. Bacteriol.* **173**, 1576–1589. https://doi.org/10.1128/jb.173.5.1576-1589.1991 (1991).
29. Roux, V. & Raoult, D. Phylogenetic analysis of members of the genus Rickettsia using the gene encoding the outer-membrane protein rOmpB (ompB). *Int. J. Syst. Evol. Microbiol.* **50**, 1449–1455. https://doi.org/10.1099/00207713-50-4-1449 (2000).
30. Duron, O., Jourdain, E. & McCoy, K. D. Diversity and global distribution of the Coxiella intracellular bacterium in seabird ticks. *Ticks Tick. Borne Dis.* **5**, 557–563. https://doi.org/10.1016/j.ttbdis.2014.04.003 (2014).
31. Sanger, F., Nicklen, S. & Coulson, A. R. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* 74, 5463–5467; (1977). https://doi.org/10.1073/pnas.74.12.5463
32. Hall, T., Biosciences, I. & Carlsbad, C. J. G.B.B. BioEdit: an important software for molecular biology. *GERF Bull. Biosci.* **2**, 60–61 (2011).
33. Tamura, K., Stecher, G. & Kumar, S. MEGA11: molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol.* **38**, 3022–3027. https://doi.org/10.1093/molbev/msab120 (2021).
34. Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **35**, 1547–1549. https://doi.org/10.1093/molbev/msy096 (2018).
35. Ali, A., Baby, B. & Vijayan, R. From Desert to Medicine: a review of Camel Genomics and Therapeutic products. *Front. Genet.* **10**, 17. https://doi.org/10.3389/fgene.2019.00017 (2019).
36. Kleinerman, G. et al. Molecular detection of *Rickettsia africae*, *Rickettsia aeschlimannii*, and *Rickettsia sibirica mongolitimonae* in camels and *Hyalomma* spp. ticks from Israel. *Vector Borne Zoonotic Dis.* **13**, 851–856. https://doi.org/10.1089/vbz.2013.1330 (2013).
37. Kamani, J., Baneth, G., Apanaskevich, D. A., Mumcuoglu, K. Y. & Harrus, S. Molecular detection of *Rickettsia aeschlimannii* in *Hyalomma* spp. ticks from camels (*Camelus dromedarius*) in Nigeria, West Africa. *Med. Vet. Entomol.* **29**, 205–209. https://doi.org/10.1111/mve.12094 (2015).
38. Khan, Z. et al. Molecular survey and genetic characterization of Anaplasma marginale in ticks collected from livestock hosts in Pakistan. *Animals*. **12**, 1708. https://doi.org/10.3390/ani12131708 (2022).
39. Getange, D. et al. Ticks and tick-borne pathogens associated with dromedary camels (*Camelus dromedarius*) in northern Kenya. *Microorganisms*. **9**, 1414. https://doi.org/10.3390/microorganisms9071414 (2021).
40. Ali, A., Numan, M., Ullah, S., Khan, M. & Kamran, K. Genetic characterization of *Haemaphysalis* (*Rhipistoma*) *indica* and *Haemaphysalis* (*Segalia*) *montgomeryi* ticks (Ixodoidea: Ixodidae). *Ticks Tick. Borne Dis.* **14**, 102105. https://doi.org/10.1016/j.ttbdis.2022.102105 (2023).
41. Ali, A. et al. Risk factors associated with tick infestations on equids in Khyber Pakhtunkhwa, Pakistan, with notes on *Rickettsia massiliae* detection. *Parasit. Vectors*. **14**, 363. https://doi.org/10.1186/s13071-021-04836-w (2021).
42. Alanazi, A. D. et al. Ticks and associated pathogens in camels (*Camelus dromedarius*) from Riyadh Province, Saudi Arabia. *Parasit. Vectors*. **13**, 1–9. https://doi.org/10.1186/s13071-020-3973-y (2020).
43. Okely, M., Chen, Z., Anan, R. & Gad-Allah, S. Updated checklist of the hard ticks (Acari: Ixodidae) of Egypt, with notes of livestock host and tick-borne pathogens. *Syst. Appl. Acarol.* **27**, 811–838. https://doi.org/10.11158/saa.27.5.1 (2022).
44. Hussain, N. et al. Prevalence of different tick species on livestock and associated equines and canine from different agro-ecological zones of Pakistan. *Front. Vet. Sci.* **9**, 1089999. https://doi.org/10.3389/fvets.2022.1089999 (2023).
45. Beati, L., Meskini, M., Thiers, B. & Raoult, D. *Rickettsia aeschlimannii* sp. nov., a new spotted fever group rickettsia associated with *Hyalomma marginatum* ticks. *Int. J. Syst. Evol. Microbiol.* **47**, 548–554. https://doi.org/10.1099/00207713-47-2-548 (1997).
46. Chisu, V. et al. Detection of *Rickettsia hoogstraalii*, *Rickettsia helvetica*, *Rickettsia massiliae*, *Rickettsia slovaca* and *Rickettsia aeschlimannii* in ticks from Sardinia, Italy. *Ticks Tick. Borne Dis.* **8**, 347–352. https://doi.org/10.1016/j.ttbdis.2016.12.007 (2017).
47. Rumer, L. et al. *Rickettsia aeschlimannii* in *Hyalomma marginatum* ticks, Germany. *Emerg. Infect. Dis.* **17**, 325. https://doi.org/10.3201/eid1702.100308 (2011).
48. Demoncheaux, J. P. et al. First detection of *Rickettsia aeschlimannii* in *Hyalomma dromedarii* ticks from Tunisia. *Ticks Tick. Borne Dis.* **3**, 398–402. https://doi.org/10.1016/j.ttbdis.2012.10.003 (2012).
49. Bitam, I., Kernif, T., Harrat, Z., Parola, P. & Raoult, D. First detection of *Rickettsia aeschlimannii* in *Hyalomma aegyptium* from Algeria. *Clin. Microbiol. Infect.* **15**, 253–254. https://doi.org/10.1111/j.1469-0691.2008.02274.x (2009).
50. Majid, A. et al. First report of spotted fever group *Rickettsia aeschlimannii* in *Hyalomma turanicum*, *Haemaphysalis bispinosa*, and *Haemaphysalis montgomeryi* infesting domestic animals: updates on the epidemiology of tick-borne Rickettsia aeschlimannii. *Front. Microbiol.* **14**, 1283814. https://doi.org/10.3389/fmicb.2023.1283814 (2023).
51. Ogata, H. et al. Genome sequence of *Rickettsia bellii* illuminates the role of amoebae in gene exchanges between intracellular pathogens. *PLoS Genet.* **2**, 76. https://doi.org/10.1371/journal.pgen.0020076 (2006).
52. Eldin, C. et al. From Q fever to *Coxiella burnetii* infection: a paradigm change. *Clin. Microbiol. Rev.* **30**, 115–190. https://doi.org/10.1128/CMR.00045-16 (2017).

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Author contributions

A.A., (Abid Ali) and S.U., designed the study. A.A., S.U., H.T. M.K. T.T. A.A (A. Alouffi), M.M.A, and I.d.S.V.J., performed the experimental designing of the study, collected the ticks, carried out molecular experiments and phylogenetic analyses. A.A., S.U., I.d.S.V.J., H.T. M.K. T.T. A.A (A. Alouffi), M.M.A, H.T and M.K contributed to writing, editing and revision of the manuscript. All authors agreed to the published version of the manuscript.

Declarations

Competing interests

The authors declare that the current study was conducted in the absence of any financial or commercial

support that could made any Conflict of interest.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-73663-7.

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