Molecular Investigation and Phylogenetic Analysis of *Anaplasma spp.* and *Ehrlichia spp.* (Based on 16S rRNA gene) Isolated from Hyalomma Ticks in the Border of Iran and Pakistan

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Research Article

Keywords: Anaplasmosis, Ehrlichiose, Hard ticks, Iran, Pakistan, Tick-borne diseases

Posted Date: February 10th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-210989/v1

Abstract

Background: Anaplasmosis and Ehrlichiosis are tick-borne diseases affecting human beings and livestock in tropical and subtropical regions. Animal husbandry is the main activity of people in the border of Iran and Pakistan, where thousands of cattle cross the borders into the countries weekly.

Methods: PCR-direct sequencing of the *16S rRNA* gene was used to determine the prevalence and geographical distribution of the disease agents in *Hyalomma* hard ticks.

Results: Six *Hyalomma* species were found in the region, where *Hy.anatolicum* was the most prevalent species collected on goats, cattle, and camel. *Anaplasma* / *Ehrlichia* genomes were found in 68.3% of the specimens. *Anaplasma ovis, A.marginale,* and *E.ewingii* DNA prevalence in the infected ticks were 81.82, 9.09, and 9.09 % respectively. The DNA sequence and phylogenetic analysis of the *16S rRNA* gene confirmed the detection of these three Anaplasmosis agents, showing 99-100% identity with the strains previously reported in Genbank from different part of the world.

Conclusions: Since *A.ovis* and *A.marginale* are considered as important livestock pathogens, and *E.ewingii* is an important human pathogen, both farmers and people involved in livestock along the border of the two countries need to be made aware of the risks posed by tick infestation and the tick-borne disease.

Background

Ticks (Acari: *Ixodidae*) are considered to be the second most common vector of human diseases worldwide after mosquitoes [1], but they are the most important vectors of disease-causing pathogens in domestic and wild animals. Ticks transmit a wide variety of pathogens including viruses, bacteria and protozoa to vertebrates.

Genus of *Anaplasma* Theiler 1910, is an intracellular, Gram-negative bacterium and the representative of the order Rickettsiales classified into Anaplasmataceae family. It is a mix of emerged and emerging tick-borne pathogens that cause Anaplasmosis in humans and many mammalian species worldwide particularly in tropical and subtropical regions, including Iran [2]. Several species of *Anaplasma* have been detected in domestic animals, including *A. phagocytophilum* Foggie 1949, *A.marginale* Theiler 1910, *A. ovis* Lestoquard 1924, *A. centrale* Theiler 1911, *A. bovis* Donatien and Lestoquard 1936, and *A. platys* Dumler *et al.* 2001. *Anaplasma* species are found in a broad range of wild and domestic mammals. Ehrlichiosis, caused by *Ehrlichia* spp., and transmitted by ticks, is another tick-borne disease closely related to Anaplasmosis [3]. Some species of *Ehrlichia* have been identified as pathogens in humans. For example, *Ehrlichia (E.) chaffeensis* causes human monocytic ehrlichiosis, *E. equi, E. ewingii*, and *E. phagocytophila* causes human granulocytic ehrlichiosis, *E. sennetsu* causes a mononucleosis-like illness, and *E. canis* causes asymptomatic infection. The zoonotic nature of the human Ehrlichioses is supported by reports of natural infections with the same *Ehrlichia* species in dogs, deer, horses, and rodents [4].

Iran has various climates and each one may harbour several different tick species, which is responsible for the difference in the epidemiology of Anaplasmosis/Ehrlichiosis in different regions. The Sistan and Baluchistan province in the southeast corner of Iran has a long border with Pakistan and Afghanistan where infectious diseases do not respect international boundaries. Animal husbandry is one of the main activities of people in the province. Every week thousands of livestock including sheep, goats, cows, and buffaloes cross the borders into the countries. It is suggested about one million animals are traded or trafficked yearly to Iran [5].

Nested polymerase chain reaction (PCR) has been displayed to be a highly sensitive and specific test for the diagnosis of Anaplasmosis/Ehrlichiosis [6]. Among different markers, the *16S rRNA* gene is one of the common markers used for bacterial genotyping [3].

Despite the identification of *Anaplasma*/*Ehrlichia* species in livestock based on the molecular assays, less study has been performed in their vectors. There have been only a few studies on the detection of tick Anaplasmosis/Ehrlichiosis infections in Iran, which reported *Anaplasma*/*Ehrlichia* infection in ticks in the north and other parts of Iran [7–9].

Ixodid ticks play an important role in maintaining *Anaplasma*/*Ehrlichia* species in nature. It has been reported that *Ixodes* Latreille, 1795, *Rhipicephalus* Koch, 1844, *Dermacentor* Koch, 1844, and *Amblyomma* Koch, 1844 genera are the main vectors of *Anaplasma*/*Ehrlichia* bacteria in different regions of the world [10]. Although it was reported that *Hyalomma* ticks are proven vectors of *A. marginale* [11], *Hyalomma* spp., are known as the main vector of Crimean-Congo hemorrhagic fever [12] and there is little research on the anaplasmosis/ehrlichiosis and *Hyalomma* spp., and whether they are the probable vectors of *Anaplasma*/*Ehrlichia* spp., is still unproven, thus necessitating this study. In this study, we identified six different *Hyalomma* species of tick infesting on various domestic animals and further investigated *Anaplasma/Ehrlichia* infection status in *Hyalomma* ticks from Sistan and Baluchistan of Iran.

Methods

Sample collection and tick identification

The regions investigated include three districts of Sib & Suran County (Hiduj district) with the geographical coordinates 27°00′02″N 62°07′01″E, Sarbaz County (Pishin district) with the geographical coordinates 30°35′5.31N 66°59′41.19″E and Chabahar County (Negour district) with the geographical coordinates 25°23′20.84″ N 61°8′18.96″ E, which are located in the southeast of Iran border Pakistan (Fig. 1). The collection of ticks was performed in three randomly selected major husbandry farms in each district between November 2017 and late March 2018 when adult ticks are most active in the region. Totally, 1020 samples were randomly collected from goats, sheep, cattle, and camels. Tick collection was arbitrarily conducted based on the availability of domestic animals for 15 minutes per animal, but efforts were made to obtain a widespread representative sample within the different animal species included in the study. All ticks were transferred to vials and labelled according to their geographical origin and the

animals from which they were obtained. The collected ticks were subsequently transferred to the Entomology Laboratory in the School of Public Health at the Tehran University of Medical Sciences and were identified to species level based on morphological characteristics and the method of Estrada-Pena [13].

Dna Extraction

After species identification, the ticks were sterilized by immersion in 70% alcohol and washed in distilled water and dried on filter paper in a laminar-flow hood then stored at − 80°C until the DNA extraction. The DNA extraction was done using the G-spin Genomic DNA Extraction Kit (iNtRON Biotechnology, South Korea) and carried out according to the manufacturer instructions by grinding individual ticks in an Eppendorf microtube after isolated tick incubation in the liquid nitrogen tank. Afterward, the extracted DNA was suspended in sterile distilled water and was then stored at − 20°C prior to molecular investigation.

Molecular detection of Anaplasma **/**Ehrlichia **spp.**,

In this study, we followed the method of Li *et al* [14] to differentiate species of *Anaplasma* genera based upon genetic analyses of *16S rRNA*. The *Anaplasma***/***Ehrlichia* spp., *16S rRNA* gene was amplified using the nested PCR protocol and the species-specific primers already designed by Rar *et al* [15]. The forward and reverse primers for outer reactions were Ehr1 (5’ -GAA CGAA CGC TGG CGG CAA GC-3’) and Ehr2 (5’- AGT A(T/C)C G(A/G)A CCA GAT AGC CGC-5’) and for inner reactions were Ehr3 (5’- TGC ATA GGA ATC TAC CTA GTA G- 3’) and Ehr4 (5’- CTA GGA ATT CCG CTA TCC TCT- 3’). PCR reactions were performed in 25µL reaction mixture containing 12.5µL of the Hot Start Taq 2X Master Mix, 1µL of each of the forward and reverse primers, 2µL of DNA template and 7.5µL of nuclease-free H_2O to bring the volume to 25µL. PCR reactions were performed in a DNA thermocycler (Eppendorf, Germany) and PCR condition was done to 15 min at 95°C for initial denaturation step, 60 s at 94°C in each cycle for denaturing step, 60 s at 57°C for annealing and 60 s at 72°C for extension step followed by 35 cycles and then a final extension for 10min at 72° C. 2µL of the products of the first round of PCR was used as the template for the second round of PCR, which was carried out under the same conditions and reaction mixture as the first round except that were used as the primers [15].

To assess the presence of specific bands for *Anaplasma* spp., PCR amplification was electrophoresed in 1.5% agarose gel and the size of each PCR product was estimated using a 100 base pair (bp) ladder run on the same gel as the marker and then visualized under a UV transilluminator. Two negative controls including double distilled water and DNA template of non-infected tick and positive control (*Anaplasma* DNA) were included in each PCR assay.

Dna Sequencing And Phylogenetic Analysis

The positive PCR products were purified and bidirectional DNA sequencing was performed using the same inner PCR primers used for nested PCR amplification. The acquired sequences in this study were edited and assembled using Chromas and Bioedit software to construct consensus sequences and analysed using blast in NCBI (Nucleotide collection) database (https://www.ncbi.nlm.nih.gov/). The consensuses of confident sequences were aligned with other *Anaplasma* corresponding sequences available in Genbank using multiple-sequence alignments available in CLUSTAL Omega (https://www.ebi.ac.uk/Tools/msa/clustalo). Also, available gene sequences of *Ehrlichia ewingii*, and *Spiroplasma* sp., as outgroup, were obtained from Gen bank and combined with the *Anaplasm* sequences for phylogenetic analysis. All DNA sequences used for alignment were cut to get a consistent region (470 bp). Phylogenetic and molecular evolutionary analyses were conducted with MEGA 7 software [16]. For phylogenetic analysis, three representative sequences of *A. ovis*, one representative sequence of *A.marginale*, and one representative sequence of *E. ewingii*, obtained from this study were combined with a subset of available representative sequences of all *Anaplasma* spp., and *E. ewingii*. Details of the sequences used for this study have been shown in Table 1. The data were aligned and the Maximum likelihood method was employed to construct a phylogenetic tree. The same program was utilized to evaluate the stability of the obtained tree through bootstrap analysis with 1000 replicates.

Table 1
Details of the bacterial species used for phylogenetic analysis in this study.

Bacterial species	Origin	Gen bank ID number	References
A. *bovis*	China	MG869525	[35]
	Russia	KC484563	Direct submission
	Iran-East Azarbaijan	MH538097	Direct submission
	Iran-Borderline of Iran-Afghanistan	KM056396	Direct submission
	Iran-Ardabil	KF766097	[23]
	Iran-North Khorasan	KM517580	Direct submission
	Iran-Mazandaran	JF514506	[7]
	Iran-Hamedan	MF979832	Direct submission
	Iran- Kohgiluyeh & Boyer-Ahmad	MF979850	Direct submission
	Iran-Saveh	MG018481	Direct submission
	Iran-Golestan	KX760106	Direct submission
	Iran- Sarbaz	MK310475	This study
	Iran-Sib and Suran	MK310472	This study
	Iran- Chabahar	MH480603	This study
	Iran- Chabahar	MK310471	This study
A. bovis	Iran-Mazandaran	KP017262	Direct submission
A. *marginale*	Australia	AF414874.1	[36]
	Iran-Sarbaz	MK310488	This study
	Iran-Zabol	MK016525	Direct submission
	Iran-Khozestan	MG757665	Direct submission
	Brazil	CP023731	Direct submission
	China	MF289480	Direct submission
	Thiland	KT264188	Direct submission
	Uganda	KU686794	Direct submission
A. platys	Iran-Zabol	MK016523	Direct submission

Bacterial species	Origin	Gen bank ID number	References
A. phagocytophilum	South Korea	MF787270	Direct submission
A. odocoilei	USA	KT870132	Direct submission
A. centrale	Iran-Mazandaran	JF514509	Direct submission
	Japan	AB588977	Direct submission
A. capra [14]	China	KY242456	Direct submission
E. ewingii	Iran-Sib and Suran USA Tajikistan	MK310490	This study
	China	U96436	[37]
		KM995821	Direct submission Direct submission
		MH879869	
Spiroplasma chrysopicola [38]	USA	AY189127	[39]

Results

Ticks species and abundance

Hard tick specimens were collected in rural areas of Chabahar, Sarbaz and Sib & Suran districts situated in the southeast of Iran and were tested for the presence of *Anaplasma* by screening nested PCR assays with specific primers against the *16S rRNA* gene of the bacteria. A total of 1020 *Hyalomma* ticks were collected in the study areas where *Hy. anatolicum* was the most prevalent species in all three districts. These ticks belonged to six species including *Hy. anatolicum* Koch, 1844 (*n* = 462, 228 from cattle and 234 from goat), *Hy. asiaticum* Schulze & Schlottke, 1930 (*n* = 143, 87 from camel, 25 from goat, and 31 from cattle), *Hy. marginatum* Koch, 1844 (*n* = 203, 134 from cattle, 66 from goat and 3 from camel), *Hy. dromedarii* Koch, 1844 (*n* = 188, 46 from goat and 142 from camel), *Hy. schulzei* Olenev, 1931 (*n* = 17, 17 from goat), and *Hy. detricum* Schulze, 1919 (*n* = 6, 6 from camel) (Table 2).

Table 2
Details of tick specimens collected from animals in the study area.

Location	Tick species	No of ticks on animal			Subtotal	Total
		Cattle	Goat	Camel		
Chabahar	*Hy.marginatum*	41	36	0	77	312
	Hy.anatolicum	134	59	0	193	
	Hy.asiaticum	17	25	0	42	
Sarbaz	*Hy.marginatum*	19	13	0	32	322
	Hy.anatolicum	43	132	0	175	
	Hy.dromedarii	0	46	52	98	
	Hy.schulzei	0	17	0	17	
Sib and Suran	*Hy.asiaticum*	14	0	87	101	386
	H.dromedarii	0	0	90	90	
	Hy.detricum	0	0	6	6	
	Hy.marginatum	75	17	3	95	
	Hy.anatolicum	51	43	0	94	
Total		394	388	238	1020	1020

Anaplasma / Ehrlichia **infection in ticks**

Using broad-spectrum EHR primers, 256 out of 1020 collected ticks (25%) were tested for the presence of *Anaplasma*'s or related species *16S rRNA* gene. The result of PCR assays revealed the presence of *Anaplasma/Ehrlichia* genomes in 68.3% (175 out of 256) of the selected specimens. The species, number and prevalence of *Anaplasma/Ehrlichia* in *Hyalomma* spp., ticks at each collection site are shown in Table 3. The rate of *Anaplasma* spp., and *E.ewingii* infections was similar (67.8%-69.2%) in *Hy. anatolicum, Hy. dromedarii, Hy. asiaticum*, and *Hy. marginatum* specimens. This rate, however, was higher in *Hy. detricum* (2 out of 2, 100%) and lower in *Hy. schulzei* (2 out of 5, 40%) than the other four species. A sub set of positive PCR specimens against *Anaplasma/Ehrlichia* genome in ticks were sequenced and the consensus sequences were deposited in Gen bank. Details of the *Anaplasma/Ehrlichia* spp., positive samples are listed in Table 4.

Table 3
Details of *Anaplasma* / *Ehrlichia* infection in different *Hyalomma* species collected from Sistan and Baluchistan Province, southeast corner of Iran, 2016–2017.

Tick species	No. of collected samples	No. of specimen tested (%)	*Anaplasma* positive (%)	No. of specimens sequenced
Hy. anatolicum	462	115 (24.1)	78(67.8)	7
Hy. asiaticum	143	35(24.4)	24(68.5)	3
Hy. dromedarii	188	47(25)	32(68.1)	4
Hy. marginatum	204	52(25.4)	36(69.2)	4
Hy. detricum	6	2(33.3)	2(100)	2
Hy. schulzei	17	5(29.4)	2(40)	2
Total	1020	256(25.1)	175(68.3)	22

Table 4
Details of infected ticks to *Anaplasma* spp., / *Ehrlichia ewingii* in three districts of Sistan and Baluchistan Province, southeast corner of Iran, 2016–2017.

Tick species	Tick sex	Host	Location	Infection	Genbank ID number
Hy. anatolicum	Female	Cattle	Chabahar	*A.ovis*	MK310471
Hy. anatolicum	Female	Cattle	Sib and Suran	*A.ovis*	MK310472
Hy. anatolicum	Female	Cattle	Sib and Suran	*A.ovis*	MK310473
Hy. anatolicum	Male	Goat	Sib and Suran	*A.ovis*	MK310474
Hy. anatolicum	Female	Goat	Sarbaz	*A.ovis*	MK310475
Hy. anatolicum	Female	Goat	Sarbaz	*A.ovis*	MK310476
Hy. asiaticum	Female	Cattle	Sib and Suran	*A.ovis*	MK310477
Hy. asiaticum	Male	Goat	Sib and Suran	*A.ovis*	MK310478
Hy. asiaticum	Female	Cattle	Chabahar	*A.ovis*	MK310479
Hy .dromedarii	Female	Goat	Sarbaz	*A.ovis*	MK310480
Hy. dromedarii	Male	Camel	Sarbaz	*A.ovis*	MK310481
Hy. marginatum	Male	Cattle	Sib and Suran	*A.ovis*	MK310482
Hy. marginatum	Male	Goat	Chabahar	*A.ovis*	MK310483
Hy. marginatum	Female	Cattle	Chabahar	*A.ovis*	MK310484
Hy. detricum	Male	Camel	Sib and Suran	*A.ovis*	MK310485
Hy. detricum	Male	Camel	Sib and Suran	*A.ovis*	MK310486
Hy.schulzei	Female	Goat	Sarbaz	*A.marginale*	MK310487
Hy. schulzei	Male	Goat	Sarbaz	*A.marginale*	MK310488
Hy. anatolicum	Male	Cattle	Chabahar	*A.ovis*	MK310489
Hy. dromedarii	Female	Camel	Sib and Suran	*E.ewingii*	MK310490
Hy. dromedarii	Male	Camel	Sib and Suran	*E.ewingii*	MK310491
Hy. marginatum	Female	Cattle	Chabahar	*A.ovis*	MH480603

Sequence And Phylogenetic Analysis

Analysis of the sequence data showed that *A. ovis* was the most prevalent (18 out of 22, 81.82%) *Anaplasma* species in the study area. All of the strains of *A. ovis* isolated in this study were identical to each other and to the other Iranian strains and to the strains from China (Accession number: MG869525) and Russia (Accession number: KC484563). In addition to *A. ovis*, two *A. marginale* isolates (9.09%) and two *E. ewingii* isolates (9.09%) were found in the selected ticks. Sequences of *A. marginale* in this study were identical to their counterparts from USA, Tajikistan, and China. Similarly, the isolated strains of *E. ewingii* obtained in this study were identical with the isolates from Australia, USA, Brazil, Thailand, Iran, China, and Uganda. The sequence similarities between the isolated strains of *A. marginale* and or *E. ewingii* with the available data in Gen bank were more than 99–100%.

The phylogenetic analysis of *Anaplasma/ Erhrlichia* species was performed using the sequences obtained in this study in combination with the available data retrieved from Genbank. The bacterial species were clustered in four different clades including I) *A. ovis*, II) *A. marginale*, III) *A. platys-A. phagocytophilum-A. odocoilei*, IV) *A. centrale-A. capra* (Fig. 2). Interestingly, all *E. ewingii* isolates were associated with the branches of clade III. This analysis showed no clear geographical pattern or further association with host among the *A. ovis*, *An. marginale*, or *E. ewingii* isolates.

Discussion

This is the first comprehensive study of *Hyalomma* ticks attached to domestic animals and their associated *Anaplasma/ Ehrlichia* species conducted on the border of Iran and Pakistan, southeast corner of Iran. The results show that there are six infesting *Hyalomma* spp., ticks and that there are at least three Anaplasmosis agents that can be transmitted through a tick bite.

These infestations may cause considerable blood losses from livestock and can also transmit severe tick-borne diseases in the region. These diseases impose massive losses on the livestock sector including reducing the production of meat, milk, wool, and skin in the south corner of Iran. Results of this study is generally in accordance with observations on hard ticks and pathogens from animals in Pakistan neighbouring country and other parts of Iran, while there are some distinctive results in this study [7, 17, 22].

In this study, *Hy. anatolicum*-infested animals were found to be the most common and have the widest geographical range. This three-host tick species was previously reported as the most prevalent hard tick from different parts of Pakistan including Baluchistan of Pakistan the closest area to Baluchistan of Iran, and most parts of Iran [7, 8, 17, 18, 19, 20].

The present study provides new information about the risks of high diverse *Hyalomma* infestation of domestic animals in south-eastern Iran. This situation may address the animal traffic from neighbouring countries, which may lead to more frequent encounters with these tick species. The ticks in this study were feeding on the animals at the time of collection and were, therefore, potentially transmitting any *Anaplasma* spp., to the animals while feeding. However, the possibility that these ticks play a significant role in Anaplasmosis transmission to domestic animals where it is endemic requires further investigation.

Hyalomma spp., ticks in this region carried the *Anaplasma* and *Ehrlichia* agents, including *A. ovis, A. marginale*, and *E. ewingii*. These *Anaplasmataceae* pathogens were previously detected using molecular methods in hard ticks in several regions in Iran [17, 19, 20, 21]. The prevalence of *Hyalomma* spp., with *Ehrlichia / Anaplasma* spp., DNA in this study was 68%, which is higher than the rate of infection in the previous reports from other parts of the country. The prevalence of infection was reported as 4.6% [17], 25% [22], 43.84% [23], and 55.5% [9]. The different results regarding the tick infection rate in the study region could be explained by the different environmental factors, collection time, the sampling method, the tick community structure.

The result of this study and the above literature showed that different species of *Hyamlomma* could be one of the primary carriers and reservoirs for *Anaplasma / Ehrlichia* spp., in the country. In addition to *Hyalomma* spp., ticks, other hard ticks including *Rhipicephalous bursa, R. sanguineous, Dermacentor marginaus, Haemaphysalis erinacei, Ixodes ricinus* [7, 8, 17, 23] are reported as vectors of different tick-borne bacteria of the family Anaplasmataceae. However, in other parts of the world, the most important vector of anaplasmosis belongs to different species of *Ixodes* genus; for example, *Ixodes ricinus* in Europe, *I. persulatus* in Eastern Europe and Asia, and *I. scapularis* in North America [24].

In this study a high prevalence of the anaplasmataceae species (81.82%) was of *A. ovis* DNA. This bacterium was isolated from five tick species of *Hy. anatolicum* (31.8%), *Hy. asiaticum* (13.6%), *Hy. marginatum* (18.2%), *Hy. dromedarii* (0.9%), and *Hy. detricum* (0.9%) which were collected from cattle, goat, and camel. *Anaplasma marginale* and *E. ewingii* were found only from *Hy. schulzi* and *Hy. dromedarii* collected on goats and camels respectively. Both *A. ovis* and *A. marginale* are important livestock pathogens whereas *E. ewingii* is an important human pathogen. *Ehrlichia ewingii* mainly infects granulocytes, triggering granulocytic Ehrlichiosis in dog and human [4, 34]. Granulocytic Ehrlichiosis in humans has been described in immunosuppressed as well as immunocompetent patients, causing headache, fever, myalgia, vomiting, nausea, acute renal failure, thrombocytopenia, leukopenia and increased liver enzyme activities [25–27]. *Anaplasma ovis* is less pathogenic than other *Anaplasma* species, has got worldwide distribution, and is responsible mostly for small ruminant anaplasmosis with a low ranking fever [28, 29]. However, it may be an important disease agent for sheep and goat [30, 31]. Fever, anorexia, fatigue, milk reduction and abortion with a low death rate are the common clinical marks of *A. ovis* in infected animals [32]. *Anaplasma marginale* is known as the most important rickettsia disease in cattle. The common clinical signs of the disease are progressive haemolytic anaemia, decrease milk production, abortions, and death. In addition to cattle, other animals including water buffalo, and wild mammals like deer can be infected [33].

The present study revealed circulation of three species of Anaplasmatacea along the border of Iran and Pakistan. This finding is in agreement with the results of researchers on the border of Iran and Afghanistan as well as Pakistan who reported different tick-borne pathogens including *Ehrlichia* and *Anaplasma* in ticks [8, 18]. In Pakistan, researchers reported *A. marginale, A. centrale, A. ovis, A. platys*-like organism, *E. minasensis*, and two uncharacterized species: *Ehrlichia* sp., Multan and *Anaplasma* spp., (BL099-6).

In the current study we did not find *A. phagocytophilum* or other human granulocytic ehrlichiosis (HGE) agent in the tick specimens. Using more sensitive methods such as real-time PCR (RT-PCR) against ticks or the animal blood of tick hosts might reveal better picture of anaplasmosis agent in the region. *Anaplasma phagocytophilum* can infect various animals including goats and cows, and can be transmitted to humans by a bite from an infected tick [4].

Conclusions

In general, farmers and people who are involved in livestock farming along the border of Iran and Pakistan need to be made aware of the risks posed by tick infestation and the tick-borne disease. Pathogens carried by ticks can infect both animals and humans and monitoring of ticks and the pathogens they carry provides insight into the occurrence and spread of zoonotic diseases. Veterinarians in the region should keep these risks in mind and educate people regarding the risks as well as developing optimal approaches for tick protection protocols that maximize people's agreement.

Declarations

Acknowledgements

The authors are grateful to thank Farough Askani for his assistance in the field.

Funding: This work has supported by Tehran University of Medical Sciences, Iran, grant number 29005.

Authors' contributions

NC performed the whole study and writing the manuscript, FK accomplished phylogenetic analysis, MK helped in molecular analysis of data, JN was a major contribution in designing and sample collection, and MAO was the guarantor and analysed and interpreted the data, was the major contributor in the editing of the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

All data generated or analysed during this study are included in this published article. The sequence data are available at NCBI database.

Ethics approval and consent to participate

All procedures were performed in accordance with the terms of the Iran Animals (Scientific Procedures) Act Project License and were approved by the Tehran University of Medical Sciences Ethical Review Committee, reference number: IR.TUMS.SPH.REC.1395.926. Due to social concerns, consent to participate was obtained verbally from the owners of the animals used in this study which was approved by the committee.

Consent for publication

“Not applicable”

Competing interests

The authors declare that they have no competing interests with the publication of the manuscript or an institution or product that is mentioned in the manuscript and/or is important to the outcome of the study presented. Authors also disclose conflict of interest with products that compete with those mentioned in their manuscript.

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Figures

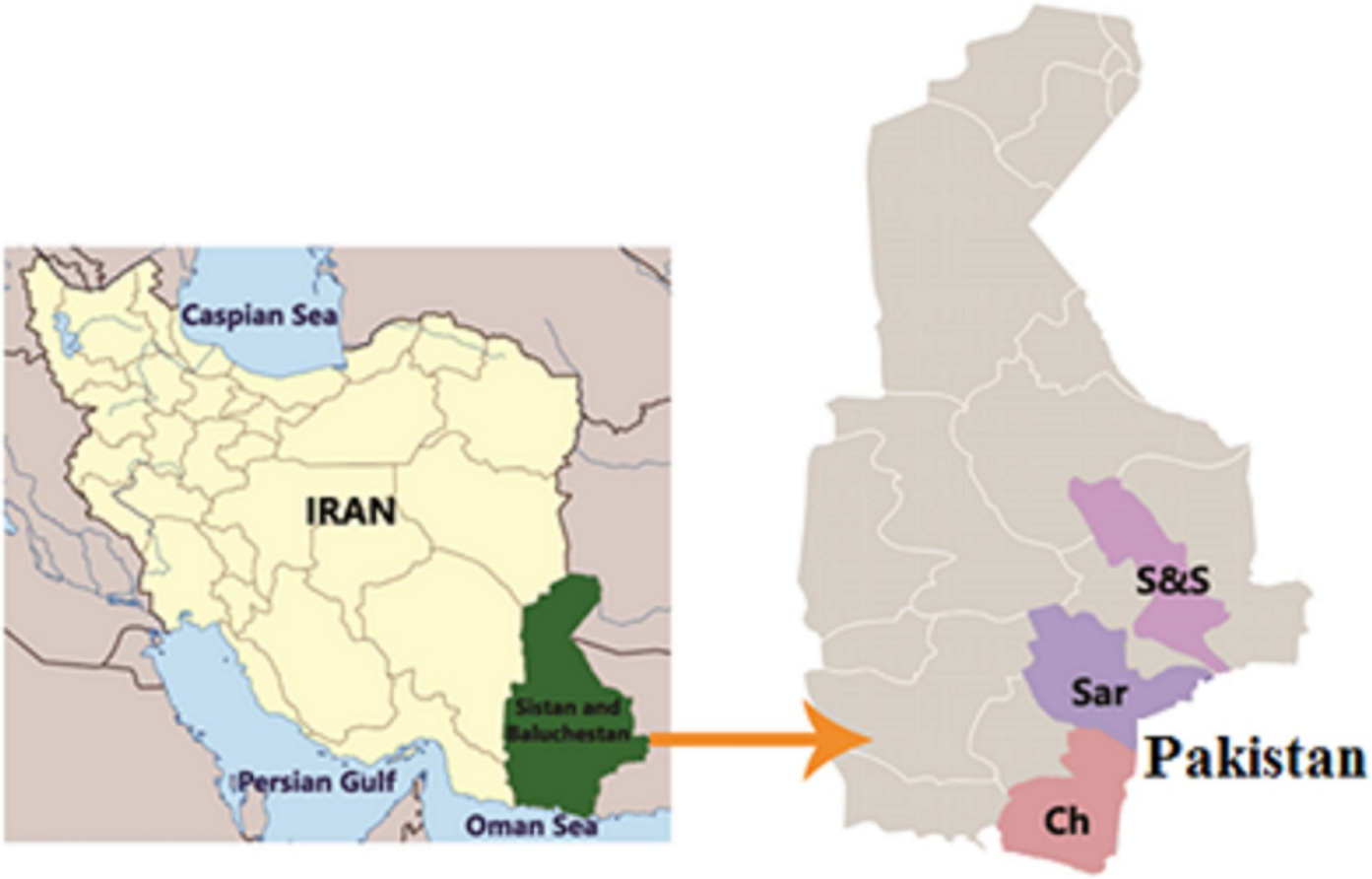

Figure 1

Map showing the locations where ticks were collected in Sistan and Baluchistan. Ch: Chabahar, Sar: Sarbaz, S & S: Sib and Suran. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Phylogenetic relationships inferred from 470 bp of 16S rRNA genes of Anaplasma/Ehrlichia species detected in this study and other available data in Genbank. The representative sequences obtained in this study are marked with black circles. The tree was computed by maximum likelihood (MEGA7.0 software). Bootstrap values are shown on nodes.

Supplementary Files

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