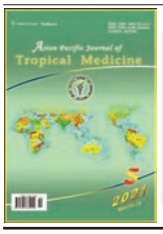

Original Article

Asian Pacific Journal of Tropical Medicine

apjtm.org

doi: 10.4103/1995-7645.315893

Impact Factor: 1.94

Circulation of *Brucellaceae*, *Anaplasma* and *Ehrlichia* spp. in borderline of Iran, Azerbaijan, and Armenia

Rahimeh Abdoli[1#], Hasan Bakhshi[1#], Sedigheh Kheirandish[2], Faezeh Faghihi[3], Asadollah Hosseini-Chegeni[4], Mohammad Ali Oshaghi[1], Zakkyeh Telmadarraiy[1,5], Mohammad Mehdi Sedaghat[1✉]

[1]*Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*

[2]*Department of Oral and Maxillofacial Pathology, School of Dentistry, Bushehr University of Medical Sciences, Bushehr, Iran*

[3]*Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran*

[4]*Department of Plant Protection, Faculty of Agriculture, Lorestan University, Khorramabad, Iran*

[5]*Rahyan Novin Danesh (RND) Private University, Sari, Mazandaran, Iran*

ABSTRACT

Objective: To estimate the infection of ticks to *Anaplasma*, *Ehrlichia*, *Babesia*, *Theileria*, and Brucellaceae using molecular methods in borderline of Iran, Azerbaijan, and Armenia.

Methods: Totally, 2 022 ticks were collected from different livestock. Then, species were diagnosed under stereomicroscope according to valid morphological keys. Tick DNA was extracted followed by PCR to detect *Anaplasma*, *Ehrlichia*, *Theileria*, *Babesia* and Brucellaceae infection in ticks.

Results: A total of 498 males [24.62% (95% *CI* 22.76%-26.57%)], 741 females [36.64% (95% *CI* 34.54%-38.79%)], 782 nymphs [38.67% (95% *CI* 36.55%-40.84%)] and 1 larva [0.04% (95% *CI* 0.00%-0.28%)] were identified. Among identified samples, we found four genera including *Hyalomma*, *Rhipicephalus*, *Haemaphysalis*, and *Dermacentor*. Molecular assay revealed that the prevalence of ticks to *Anaplasma* or *Ehrlichia*, and Brucellaceae was 22.02% (95% *CI* 16.01%-29.06%) and 15.03% (95% *CI* 9.43%-22.26%), respectively. Phylogenetic analysis showed that the identified *Anaplasma* sp. had the most similarity with *Anaplasma centrale*, *Anaplasma platys*, *Anaplasma camelii*, and *Anaplasma phagocytophilum*, submitted in GenBank. Furthermore, the detected *Ehrlichia* sp. and Brucellaceae bacterium had the most similarity with *Ehrlichia ruminantium* and *Mycoplana peli*, respectively. However, no sign of the presence of *Theileria* and *Babesia* spp. was seen in the studied samples.

Conclusions: Anaplasmosis, ehrlichiosis and brucellosis should be considered as important health threats in northwestern Iran and consistent monitoring on infection of ticks and livestock should be performed regularly.

KEYWORDS: Tick; *Anaplasma*; *Ehrlichia*; Brucellaceae; PCR

1. Introduction

Ticks are one of the most important external blood-sucking parasites of vertebrates that cause the transmission of many pathogens including arboviruses, parasites and bacteria while they suck blood. The most important diseases transmitted by ticks are spotted fever, Rocky Mountains fever, Siberia tick typhus, tularemia, Lyme disease, tick-borne relapsing fever, Crimean-Congo hemorrhagic fever, babesiosis, anaplasmosis and brucellosis[1,2]. Ticks can suck blood on mammals, birds, reptiles, and amphibians, while most of them have a different mammalian host at each stage of their lives. These arthropods become infected from an infected vertebrate host and after a while, they are able to transmit pathogens to a new host by blood-sucking activity at another stage of life and the disease cycle remains stable in the nature[3].

Many diseases are transmitted through the bite of ticks: The Brucellaceae family comprises pathogens and soil bacteria with *Brucella* as an important genus. Brucellosis is caused by different *Brucella* species which are Gram-negative rod-shaped facultative intracellular bacteria that usually persist for life[4]. Zoonosis

#These authors contributed equally to this work

✉To whom correspondence may be addressed. E-mail: Sedaghmm@tums.ac.ir

How to cite this article: Abdoli R, Bakhshi H, Kheirandish S, Faghihi F, Hosseini-Chegeni A, Oshaghi MA, et al. Circulation of *Brucellaceae*, *Anaplasma* and *Ehrlichia* spp. in borderline of Iran, Azerbaijan, and Armenia. Asian Pac J Trop Med 2021; 14(5): 223-230.

Article history: Received 6 January 2021 Revision 25 April 2021
Accepted 26 April 2021 Available online 25 May 2021

species include *Brucella* (*B.*) *abortus*, *B. canis*, *B. melitensis*, and *B. suis*. Unpasteurized milk, undercooked infected meat, close contact with animal secretions are the main routes of transmission to human; on the other hand, ticks usually transmit the disease to sheep, cattle, goats and some other animals[5]. All provinces of Iran are endemic for brucellosis, especially Hamadan, Markazi, Lorestan, Kermanshah, West Azerbaijan, South Khorasan and East Azerbaijan, imposing high economic loss due to livestock infertility and abortions, veterinary expenses and has serious threat to human health[4]. Anaplasmosis is another zoonotic disease caused by obligate intra-cellular bacteria (Family Anaplasmataceae: Order Rickettsiales), which is usually transmitted through a number of hematophagous species of ticks especially Ixodidae family. Species of veterinary importance include: *Anaplasma* (*A.*) *bovis*, *A. centrale*, *A. marginale*, *A. ovis*, *A. phagocytophilum* (zoonotic) and *A. platys*[6,7]. Anaplasmosis in livestock is characterized by some symptoms including acute fever, anorexia, weight and milk reduction and finally death[8]. *Ehrlichia* genera is also an obligate intracellular bacterium (Family Anaplasmataceae: Order Rickettsiales) that infect animals and humans[9]. Piroplasmosis caused by *Theileria* and *Babesia* species leads to major economic losses to the livestock industry especially in Asia[10]. Piroplasmosis ranges from subclinical to acute with signs including fever, anemia, severe lethargy, and circulatory shock[11,12].

Iran is endemic for many diseases including some arthropod-borne diseases. Many studies have been carried out in many provinces, while in some provinces the related investigations seem to be insufficient. One of these provinces is East Azerbaijan. The province shares common borders with the Republic of Azerbaijan and Armenia in the north. In some parts of East Azerbaijan, animal husbandry plays a major role in people's lives. In addition, the common border with other countries allows the livestock and wildlife trafficking, which plays an undeniable role in transmitting the diseases from one country to another.

This study was performed to estimate the infection of ticks with pathogens *Anaplasma*, *Ehrlichia*, *Babesia*, *Theileria* and Brucellaceae using molecular methods in Ahar and Kaleybar cities of East Azerbaijan province, north of Iran.

2. Materials and methods

2.1. Study area

East-Azerbaijan is located in northeast of Iran (38.076 6° N 46.280 0° E), bordering with Armenia, Republic of Azerbaijan in north, Ardabil Province in east, West Azerbaijan Province in west, and Zanjan Province in south (Figure 1). East-Azerbaijan covers an area of 47 830 km^2 and Sahand Mountain lying at south is the highest area (3 707 m), whereas the lower lying areas are around Ahar at north. Climate of East-Azerbaijan is cold mountainous affected by Mediterranean continental.

Ahar is located in a mountainous area (38° 28' N 47° 04'E) limited to Kaleybar from the north, Harris from the south, Varzeqan from the west and Meshkinshahr from the east. The climate is cold mountainous. This city is surrounded by important mountains such as Gheez Qalehsi (1 266 m) and Hashtsar (2 536 m) which are stretched from north to south and west. The lowest point of Ahar is Qarahsu River, which is located in the eastern parts. Kaleybar is also located in a mountainous area (38° 51'N 47° 01'E) which is bordered by the Republic of Azerbaijan, Armenia and Aras River from the north, Jolfa city from the west, Ardabil province (Moghan plain) from the east and Ahar city from the south. Its main mountain range is called Qare Dagh, which has several peaks with different heights such as Gasht Sar (2 940 m), Ghazi Bolagh (2 700 m), Shivar (2 652 m), Sarparq (2 860 m) and Zinghaloo (2 900 m). Kaleybar has a mountainous climate and the whole region is covered with forests and pastures.

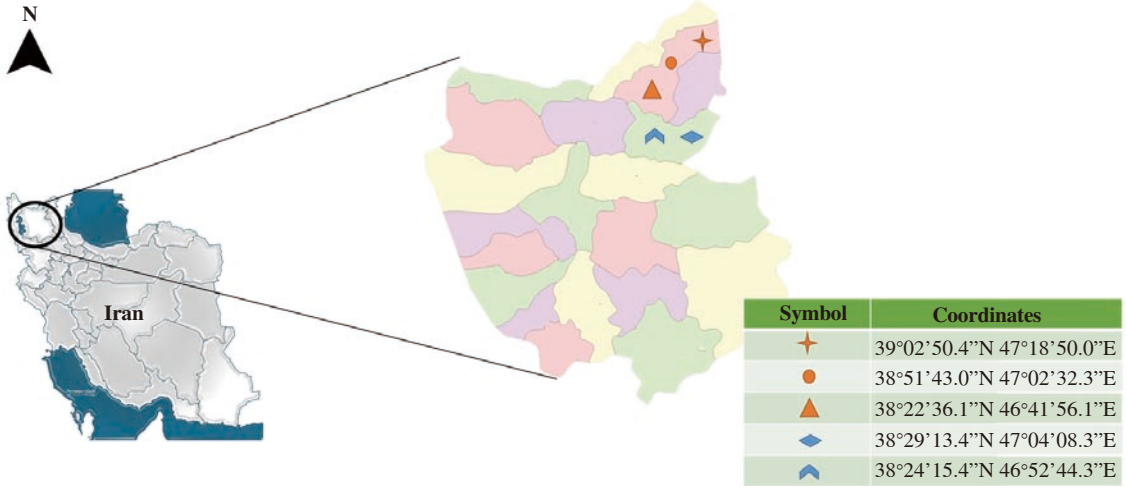

Figure 1. East Azerbaijan province is located in northwest of Iran. The sampling locations are marked with asterisks.

Table 1. Details of the primers, target gene and product size of PCR assays.

Species	Target gene	Nucleotide sequences	PCR product size	Reference
Anaplasma sp. or *Ehrlichia* sp.	*16S* rRNA	Ehr1: 5′-AAC GAA CGC TGG CGG CAA GC-3′	524 bp	[16]
		Ehr2: 5′-AGT AYC GRA CCA GAT AGC CGC-3′		
		Ehr3: 5′-TGC ATA GGA ATC TAC CTA GTA G-3′		
		Ehr4: 5′-CTA GGA ATT CCG CTA TCC TCT-3′		
Babesia sp. or *Theileria* sp.	*18S* rRNA	F1: 5′-ACC ATT TGC TAC GGA ATA ACT CAG-3′	443 bp, 449 bp	[16]
		R1: 5′-CAG GCG GAA TGT TTA ATG CG-3′		
		F2: 5′-CCA AGG ACT CAG-3		
		R2:5′-CAC CTC AGC GTC AGT AAT GG-3′		
Brucellaceae	*16S* rRNA	5'-F1: ACCATTTGCTACGGAATAACTCAG-3'	712 bp	[17]
		R1: 5'-CAGGCGGAATGTTTAATGCG-3'		
		F2: 5′-CCA AGG ACT CAG-3		
		R2: 5′-CAC CTC AGC GTC AGT AAT GG-3′		

Table 2. Prevalence of ticks in respect to sex, life stage and sampling season.

Species	Spring			Summer					Total	
	Male	Female	Total	Male	Female	Nymph	Larva	Total	No.	% (95% *CI*)
Hyalomma anatolicum	5	5	10	302	481	0	0	783	793	39.21 (37.08-41.39)
Hyalomma spp.	0	3	3	0	18	782	1	801	804	39.76 (37.62-41.93)
Hyalomma marginatum	69	64	133	31	21	0	0	52	185	9.14 (7.93-10.49)
Rhipicephalus bursa	14	12	26	26	53	0	0	79	105	5.19 (4.27-6.25)
Rhipicephalus sanguineus	23	34	57	4	12	0	0	16	73	3.61 (2.84-4.52)
Dermacentor marginatus	5	13	18	8	6	0	0	14	32	1.58 (1.09-2.23)
Hyalomma asiaticum	4	3	7	6	1	0	0	7	14	0.69 (0.38-1.16)
Haemaphysalis sulcata	0	7	7	0	5	0	0	5	12	0.59 (0.31-1.03)
Haemaphysalis punctata	1	2	3	0	0	0	0	0	3	0.14 (0.03-0.43)
Haemaphysalis concinna	0	1	1	0	0	0	0	0	1	0.04 (0.00-0.28)
Total (%)	121 (5.98)	144 (7.12)	265 (13.10)	377 (18.64)	597 (29.52)	782 (38.67)	1 (0.04)	1 757 (86.89)	2 022	100

2.2. Collection and identification of ticks

A total of 2 022 ticks were collected from different parts of East-Azerbaijan province during spring and summer, 2018 as ticks have their highest activity during these seasons. Multistage random sampling method was used and based on Cochran's formula (95% confidence level), the sample size required for each molecular assay was determined[13]. Ticks were separated from different body parts including ear, groin, tail, back and neck. Sheep, goat and cow were sampled as animals of interest. There were no specific criteria for animal selection. However, it should be noted that all the animals were apparently healthy and were mostly under 3 years old. Forceps were used with special care not to harm the ticks and animals. Ticks were then placed in appropriately labeled tubes and transferred to the Department of Medical Entomology, School of Public Health, Tehran University of Medical Sciences, Iran along with cold chain for species identification. Stereomicroscope was used to determine genus and spe cies according to valid morphological keys[14].

2.3. DNA extraction, molecular detection of pathogens and sequencing

DNA was extracted using Exgene DNA extraction kit (GeneAll, Korea) according to the manufacturer's instructions. A 20 μL reaction mixture containing 10 mm Tris-HCl (Ph 9.0), 30 mm KCl, 1.5 mm $MgCl_2$, 250 mm dNTPs, 0.5 mm each sense and antisense primers, 1 U Taq DNA polymerase and 2 μL of DNA were used for PCR. PCR thermal program was as follows: Initial denaturation at 94 ℃ for 5 min followed by 35 cycles of amplification (1 min denaturation at 94 ℃, 1 min annealing at 57 ℃ and 1 min elongation at 72 ℃[15]. A 524 bp fragment of the *16S* rRNA gene of *Anaplasma* and *Ehrlichia* was amplified by nested-PCR[16]. *18S* rRNA was targeted to amplify *Babesia* sp./*Theileria* sp. genome[16]. Finally, *16S* rRNA of Brucellaceae genome was targeted for the production of 712 bp amplicons[17]. Sets of primers and their characteristics for each PCR reaction is listed in Table 1. Genomic DNA of *Anaplasma*, *Ehrlichia*, *Babesia*, *Theileria* and *Brucella* species were obtained from the Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Iran and used as positive controls. Double distilled water was served as negative controls. The PCR analysis was performed on 1.5% agarose and UV condition. Among the positive samples of each species, the samples with a sharp band of the expected size were chosen for sequencing to confirm the accuracy of the PCR reaction. Sequencing of selected PCR products was performed by Takapouzist Company, Iran. Sequences were checked to correct any sources of error using Chromas® software. The nucleotide sequences were then blasted by BLASTN (http://www.ncbi.nlm.nih.gov/BLAST) program.

2.4. Statistical analysis

SPSS V19 (IBM, USA) was used for statistical analysis. Descriptive statistics were used to summarize the variables. Also

95% confidence interval for prevalence of tick infestation was calculated using binomial distribution (significant level less than 0.05).

3. Results

3.1. Identification and characteristics of ticks

Out of a total of 1 400 livestock (sheep, goat, buffalo and cattle), 2 022 hard ticks were collected. Four genera and 9 species were morphologically identified including: *Hyalomma* (*Hy.*) *anatolicum*: 39.21% (95% *CI* 37.08%-41.39%), *Hy. marginatum*: 9.14% (95% *CI* 7.93%-10.49%), *Hy. asiaticum*: 0.69% (95% *CI* 0.38%-1.16%), *Hy. nymph*: 38.67% (95% *CI* 36.55%-40.84%), *Hyalomma* sp.: 1.03% (95% *CI* 0.76%-1.76%), *Hy. larva*: 0.04% (95% *CI* 0.00%-0.28%), *Rhipicephalus* (*Rh.*) *bursa*: 5.19% (95% *CI* 4.27%-6.25%), *Rh. sanguineus*: 3.61% (95% *CI* 2.84%-4.52%), *Haemaphysalis* (*Hae.*) *sulcata*: 0.59% (95% *CI* 0.31%-1.03%) *Hae. concinna*: 0.04% (95% *CI* 0.00%-0.28%), *Hae. punctate*: 0.14% (95% *CI* 0.03%-0.43%) and *Dermacentor marginatus*: 1.58% (95% *CI* 1.09%-2.23%). The rate of tick collection in spring and summer was 13.10% (95% *CI* 11.66%-14.65%) and 86.89% (95% *CI* 85.35%-88.34%), respectively. A total of 498 males [24.62% (95% *CI* 22.76%-26.57%)], 741 females [36.64% (95% *CI* 34.54%-38.79%)], 782 nymphs [38.67% (95% *CI* 36.55%-40.84%)] and 1 larva [0.04% (95% *CI* 0.00%-0.28%)] were identified (Table 2). Totally, 1 666, 316, 22 and 18 ticks were collected from cattle, sheep, goats and buffalos, respectively.

3.2. Molecular detection

Table 3 summarizes the data related to tick species which were examined for detection of *Anaplasma*, *Ehrlichia*, *Babesia*, *Theileria* and Brucellaceae using nested PCR assay for detection of a 524 bp fragment of *Anaplasma*, *Ehrlichia* spp. *16S* rRNA indicated that *Hy. asiaticum*, *Hae. sulcata*, *Hy. anatolicum*, *Rh. bursa*, *D. marginatus*, *Hy. marginatum* and *Rh. sanguineus* were positive. Amplification

Table 3. Detail of No. of species examined using PCR for detection of parasites.

Species	No. of positive ticks/No. of examined ticks for PCR			Prevalence % (95% *CI*)		
	An or Eh	Br	Ba or Th	An or Eh	Br	Ba or Th
Hyalomma asiaticum	5/11	3/11	0/12	45.45 (16.7-76.62)	27.27 (6.02-60.97)	0 (0.00-26.47)
Haemaphysalis sulcata	2/5	2/5	0/4	40.00 (5.27-85.37)	40.00 (5.27-85.37)	0 (0.00-60.24)
Hyalomma anatolicum	16/53	0/25	0/59	30.18 (18.34-44.34)	0 (0.00-13.72)	0 (0.00-6.06)
Rhipicephalus bursa	5/24	4/24	0/26	20.83 (7.13-42.15)	16.66 (4.74-37.38)	0 (0.00-13.23)
Dermacentor marginatus	2/15	6/15	0/15	13.33 (1.66-40.46)	40.00 (16.34-67.71)	0 (0.00-21.80)
Hyalomma marginatum	4/32	5/25	0/38	12.50 (3.51-28.99)	20.00 (6.73-40.70)	0 (0.00-9.25)
Rhipicephalus sanguineus	3/25	0/25	0/26	12.00 (2.55-31.22)	0 (0.00-13.72)	0 (0.00-13.23)
Haemaphysalis concinna	0/1	0/1	0/2	0 (0.00-97.50)	0 (0.00-97.50)	0 (0.00-84.19)
Haemaphysalis punctata	0/2	0/2	0/1	0 (0.00-84.19)	0 (0.00-84.19)	0 (0.00-97.50)
Total	37/168	20/133	0/183	22.02 (16.01-29.06)	15.03 (9.43-22.26)	0 (0.00-2.00)

95% *CI* for prevalence of tick infestations was calculated using binomial distribution (significant level less than 0.05).

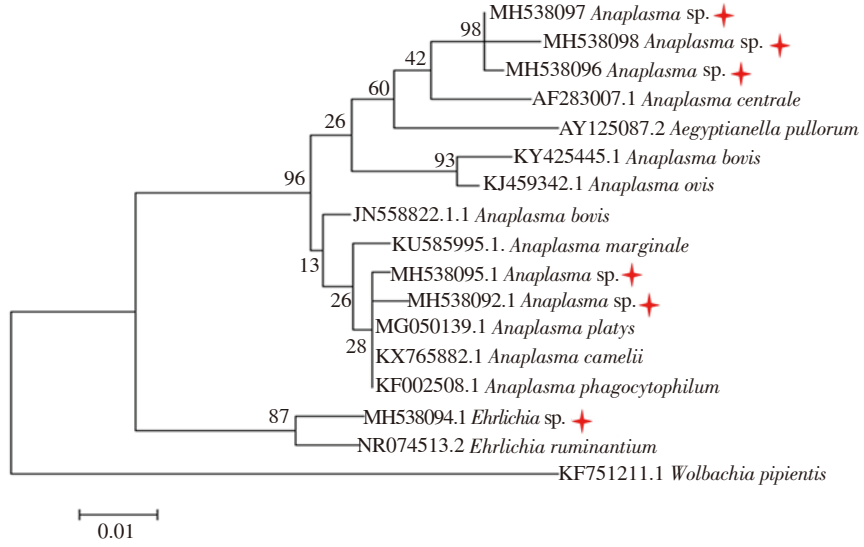

Figure 2. Evolutionary tree of *Anaplasma* and *Ehrlichia* sequences. Data was obtained from this study and similar sequences from other investigations, deposited in GenBank; *Wolbachia pipientis* included as outgroup. The scale bar indicates an evolutionary distance of 0.01 nucleotides per position in the sequence. Sequences derived from the present study are marked with asterisks. The percentage of trees in which the associated taxa clustered together is shown next to the branches.

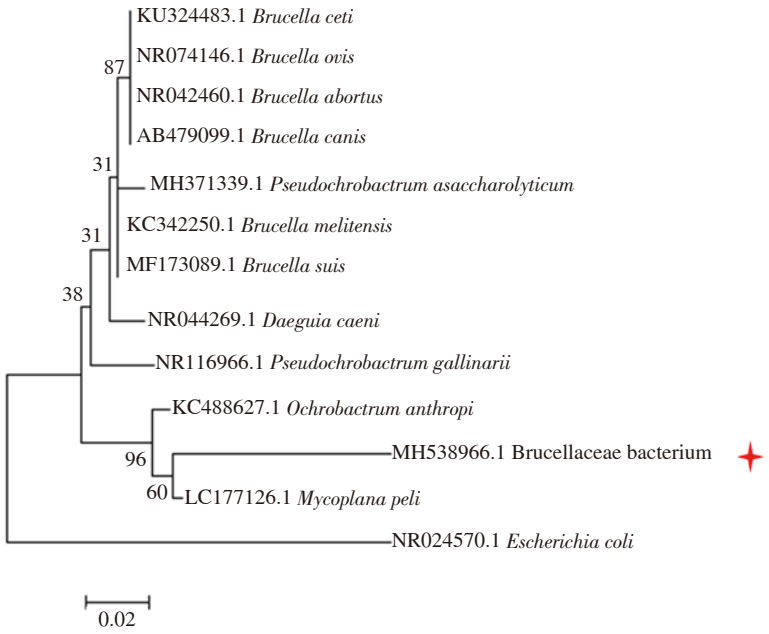

Figure 3. Evolutionary tree of Brucellaceae sequence. Data was obtained from this study and similar sequences from other investigations, deposited in GenBank; *Escherichia coli* included as outgroup. The scale bar indicates an evolutionary distance of 0.02 nucleotides per position in the sequence. The sequence derived from the present study is marked with an asterisk. The percentage of trees in which the associated taxa clustered together is shown next to the branches.

of a 712 bp fragment of *16S* rRNA from Brucellaceae genome also revealed that *Hy. asiaticum*, *Hae. sulcata*, *Rh. bursa*, *D. marginatus* and *Hy. marginatum* were positive. On the other hand, no genomic material from *Babesia* spp./*Theileria* spp. was detected in tested samples (Table 3).

3.3. Sequencing and phylogenetic analysis

A total of 6 sequences with a sharp band of the expected size were submitted and the accession numbers MH538092 (*Anaplasma* sp.), MH538094 (*Ehrlichia* sp.), MH538095 (*Anaplasma* sp.), MH538096 (*Anaplasma* sp.), MH538097 (*Anaplasma* sp.), MH538098 (*Anaplasma* sp.) and MH538966 (Brucellaceae) were assigned in GenBank. Submitted sequences were aligned using Clustal-W algorithm. The evolutionary tree of *Anaplasma* and *Ehrlichia* sequences obtained from the present study (MH538092, MH538094, MH538095, MH538096, MH538097, and MH538098) and similar sequences from other investigations, deposited in GenBank was inferred by using the maximum likelihood method with bootstrap of 1 000 replications[18,19](Figure 2). Furthermore, the evolutionary tree of Brucellaceae sequence obtained from this study (MH538966) and similar sequences from other investigations, deposited in GenBank was inferred using the maximum likelihood method with bootstrap of 1 000 replications[18,19](Figure 3).

Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach, and then selecting the topology with superior log likelihood value. Trees were drawn to scale, with branch lengths measured in the number of substitutions per site[19]. The results of phylogenetic analysis revealed that three identified *Anaplasma* sp. had the most similarity with *A. centrale* (Accession Number: AF283007.1). Two other detected *Anaplasma* sp. had the most similarity with *A. platys*, *A. camelii* and *A. phagocytophilum* (Accession Numbers MG050139.1, KX765882.1, and KF002508.1 respectively). Further to detected *Ehrlichia* sp., it has the most similarity with *Ehrlichia ruminantium* (Accession number: NR074513.2) (Figure 2). Furthermore, the detected Brucellaceae bacterium had the most similarity with *Mycoplana peli* (Accession number: LC177126.1).

4. Discussion

Ticks are considered as important vectors of pathogens which play a critical role in the survival and transmission of the pathogens that cause diseases in humans and animals[20]. In the present study, *Hy. anatolicum* was determined as the dominnat specie and *Hyalomma* as

the dominant genera. Conducted studies in north in Hamadan, West-Azerbaijan and Ardabil provinces showed that *Hyalomma* genera was reported as the predominant genus of collected ticks[21–24]. It should be also noted that in many northern provinces, *Rhipicephalus* spp. were determined as the dominant species[25–27] and in some studies, both of the genera were considered to be dominant[28]. In the current investigation, four genera were detected at the Azerbaijan-Armenia borderline in Iran, including *Hyalomma*, *Rhipicephalus*, *Dermacentor* and *Haemaphysalis*. All of these genera have been reported to be incriminated for transmission of Crimean Congo hemorrhagic fever virus in different countries including Iran[29]. The detected tick genera in the present study have also been considered important from the medical and veterinary perspective[30]. The most infected hosts in terms of tick infestation were cattle and most of ticks were collected in the summer which indi cate higher activity of ticks in hot temperature. Furthermore, 37 out of the 168 (22%) tested ticks were positive for the *Anaplasma*, *Ehrlichia* spp. including *Hy. asiaticum*, *Hae. sulcata*, *Hy. anatolicum*, *Rh. bursa*, *D. marginatus*, *Hy. marginatum* and *Rh. sanguineus*. Tajedin *et al*. reported tick infection with *Anaplasma* or *Ehrlichia* spp. across East-Azerbaijan (71%) which is higher than our result[31]. This difference can be justified due to larger sample size and host variability in the present study. Jafar-Bekloo *et al*., indicated the rate of tick infection with *Anaplasma* and *Ehrlichia* spp. using molecular assays in north of Iran: 25% of ticks were infected including: *R. sanguineus*, *R. bursa*, *Hy. marginatum* and *Hy. scupense*, which is in concordance with our study except that *Hy. scupense* was not detected in the present study[6]. In a molecular study on *Anaplasma* and *Ehrlichia* spp. identification in *Hyalomma* ticks in border line of Iran-Pakistan, it was indicated that *Hy. anatolicum* was the most prevalent specie (in line with the present study) and *Anaplasma* or *Ehrlichia* spp. were detected in 68.3% of the specimens[32]. The higher prevalence might be due to the hot and dry climate of the Iran-Pakistan border, the greater rate of traditional animal husbandry in the region and the lack of awareness of animal health principles. A *16S* rRNA gene fragment of *Anaplasma* species was identified in 26.4%, 49.5%, 58.3% and 59% of tested ticks at Afghanistan borderline, Mazandaran, Kerman and Savadkooh regions of Iran, respectively[9,15,33,34]. Further to the previous studies, it can be concluded that in most parts of Iran, *Rhipicephalus* spp. are the most infected ticks to *Anaplasma* spp. and *Ehrlichia* spp.

The role of Argasidae and Ixodidae ticks in preservation and transmission of Brucellaceae in nature and from one animal to another have already been speculated. Previously, the presence of *B. abortus* in lice of several ruminants was reported[35–37]. To the best of our knowledge, few molecular studies have been performed on the infection of ticks with Brucellaceae family in Iran and other parts of the world. A *Brucella*-like bacterium was detected in a *Boophilus* tick using PCR method. Ticks were collected from Talesh County, Guilan Province, north of Iran[17]. Molecular detection of tick-borne pathogens harbored by ticks collected from livestock in the Xinjiang Uygur Autonomous Region, China revealed that 26.2% of tested ticks were infected with *Brucella* sp.[38]. *B. melitensis* and *B. abortus* were also detected in eggs, larvae and engorged females of *D. marginatus* collected from sheep and goats from different parts of China[39]. In the present study, the Brucellaceae genome fragment was detected in *Hy. asiaticum*, *Hae. sulcata*, *Rh. bursa*, *D. marginatus* and *Hy. marginatum*. The members of the genera *Hyalomma*, *Dermacentor*, *Rhipicephalus* and *Haemaphysalis* seem to act as vectors of *Brucella* spp. in nature. However, further studies seem necessary to confirm the result.

No *Babesia* sp./*Theileria* sp. genome was found in the collected ticks which may suggest that ticks don't have a significant role in the circulation of these pathogens in the study area. Other studies from other parts of Iran, however, have shown various results in terms of tick infection with *Babesia* sp./*Theileria* sp. Molecular surveillance of *Theileria* (*T.*) *ovis*, *T. lestoquardi* and *T. annulata* infection in sheep and ixodid ticks in Razavi-Khorasan province, north east of Iran revealed that 9% of *R. turanicus* were positive for *Theileria* species[40]. Hasheminasab and colleagues reported that *Rhipicephalus* spp. and *Hy. anatolicum* had the highest infection rate with *Babesia* and *Theileria* in Dehgolan, Iran[41]. Molecular detection of *Theileria* spp. in tick vectors in Fasa and Kazeroun areas of Iran also revealed that one pool of *H. turanicus* was infected with *T. ovis*[42]. Habibi *et al*. showed 18 isolated tick DNAs (66.7%) from dogs in Shahriar County, Iran were infected with *T. annulata*[43]. Tick infection with *Theileria* and *Babesia* in Iran renges between 6.1% to 55% and 6.25% to 76%, respectively[6,44–48]. It seems that differences between the results of the present study and similar studies in other regions of Iran is due to differences in the sampling season, sample size, climate conditions, mollecular methods, livestock management and husbandry. It is recommended that anaplasmosis, ehrlichiosis as well as brucellosis should be considered important health issues in north-west of Iran and consistent monitoring on tick vectors and livestock should be performed regularly. It is also suggested to conduct the study in autumn and winter seasons on vertebrate hosts (especially domestic animals) and the people who are at risk (veterinarians and ranchers) to clarify the status of diseases more accurately. Boundaries must be carefully monitored and the transportation of animals, even wild animals, must be closely observed.

In the present study, we detected the infection of tick with *Anaplasma* sp./*Ehrlichia* sp. and Brucellaceae. It can also be concluded that in the north and north-west of the country, *Hyalomma* and *Rhipicephalus* species are the most prevalent and important species among hard ticks.

Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

Both R.A. and H.B. contributed to performing the experiments and contributed to the final version of the manuscript. S.K., F.F., A.H.C and M.A.O. analyzed the data and edited the manuscript. Both Z.T. and M.M.S. supervised the project and contributed to the final version of the manuscript. All authors reviewed the manuscript.

Refrences

[1] Boulanger N, Boyer P, Talagrand-Reboul E, Hansmann Y. Ticks and tick-borne diseases. *Med Mal Infect* 2019; **49**(2): 87-97.

[2] Madison-Antenucci S, Kramer LD, Gebhardt LL, Kauffman E. Emerging tick-borne diseases. *Clin Microbiol Rev* 2020; **33**(2): e00083-18. doi: 10.1128/CMR.00083-18.

[3] Wikel SK. Ticks and tick-borne infections: Complex ecology, agents, and host interactions. *Vet Sci* 2018; **5**(2): 60.

[4] Golshani M, Buozari S. A review of brucellosis in Iran: Epidemiology, risk factors, diagnosis, control, and prevention. *Iran Biomed J* 2017; **21**(6): 349.

[5] Christopher S, Umapathy BL, Ravikumar KL. Brucellosis: Review on the recent trends in pathogenicity and laboratory diagnosis. *J Lab Physicians* 2010; **2**(2): 55.

[6] Ghaffar A, Ijaz M, Ali A, Farooqi SH, Rehman A, Ali MM, et al. First report on molecular characterization of anaplasmosis in small ruminants in Pakistan. *J Parasitol* 2020; **106**(3): 360-368.

[7] Uminski K, Kadkhoda K, Houston BL, Lopez A, MacKenzie LJ, Lindsay R, et al. Anaplasmosis: An emerging tick-borne disease of importance in Canada. *IDCases* 2018; **14**: e00472.

[8] Kuttler KL. *Anaplasma* infections in wild and domestic ruminants: A review. *J Wildl Dis* 1984; **20**(1): 12-20.

[9] Taira M, Ando S, Kawabata H, Fujita H, Kadosaka T, Sato H, et al. Isolation and molecular detection of *Ehrlichia* species from ticks in western, central, and eastern Japan. *Ticks Tick Borne Dis* 2019; **10**(2): 344-351.

[10]Ahmed J, Yin H, Schnittger L, Jongejan F. Ticks and tick-borne diseases in Asia with special emphasis on China. *Parasitol Res* 2002; **88**(1): S51-S55.

[11]Zanet S, Trisciuoglio A, Bottero E, De Mera IGF, Gortazar C, Carpignano MG, et al. Piroplasmosis in wildlife: *Babesia* and *Theileria* affecting free-ranging ungulates and carnivores in the Italian Alps. *Parasit Vectors* 2014; **7**(1): 70.

[12]Azmi K, Ereqat S, Nasereddin A, Al-Jawabreh A, Baneth G, Abdeen Z. Molecular detection of *Theileria*, *Babesia*, and *Hepatozoon* spp. in ixodid ticks from Palestine. *Ticks Tick Borne Dis* 2016; **7**(5): 734-741.

[13]Cochran WG. *Sampling techniques*. New York: John Wiley & Sons; 2007.

[14]Walker AR. *Ticks of domestic animals in Africa: A guide to identification of species.* Edinburgh: Bioscience Reports; 2003.

[15]Ranjbar R, Anjomruz M, Enayati AA, Khoobdel M, Rafinejad A, Rafinejad J. *Anaplasma* infection in ticks in Southeastern Region of Iran. *J Arthropod Borne Dis* 2020; **14**(2). doi: https://doi.org/10.18502/jad.v14i2.3730.

[16]Rar VA, Livanova NN, Panov VV, Kozlova IV, Pukhovskaya NM, Vysochina NP, et al. Prevalence of *Anaplasma* and *Ehrlichia* species in *Ixodes persulcatus* ticks and small mammals from different regions of the Asian part of Russia. *Int J Med Microbiol* 2008; **298**: 222-230.

[17]Hosseini-Chegeni A, Tavakoli M, Telmadarraiy Z, Sedaghat MM, Faghihi F. Detection of a *Brucella*-like (Alphaproteobacteria) bacterium in *Boophilus* spp. (Acari: Ixodidae) from Iran TT. *J Med Microbiol Infect Dis* 2017; **5**(3): 66-68.

[18]Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol* 1993; **10**(3): 512-526.

[19]Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 2018; **35**(6): 1547-1549.

[20]Banafshi O, Hanafi-Bojd AA, Karimi M, Faghihi F, Beik-Mohammadi M, Gholami S, et al. Tick ectoparasites of animals in borderline of Iran-Iraq and their role on disease transmission. *J Arthropod Borne Dis* 2018; **12**(3): 252.

[21]Telmadarraiy Z, Moradi AR, Vatandoost H, Mostafavi E, Oshaghi MA, Zahirnia AH, et al. Crimean-Congo hemorrhagic fever: A seroepidemiological and molecular survey in Bahar, Hamadan province of Iran. *Asian J Anim Vet Adv* 2008; **3**(5): 321-327.

[22]Telmadarraiy Z, Ghiasi SM, Moradi M, Vatandoost H, Eshraghian MR, Faghihi F, et al. A survey of Crimean-Congo haemorrhagic fever in livestock and ticks in Ardabil Province, Iran during 2004-2005. *Scand J Infect Dis* 2010; **42**(2): 137-141.

[23]Salarilak S, Vatandoust H, Telmadarei Z, Entezar MR, Kia EB. Seasonal activity of ticks and their importance in tick-borne infectious diseases in West Azerbaijan, Iran. *Iranian J Arthropod–Borne Dis* 2008; **2**(2): 28-34.

[24]Davoudi J, Hoghooghi-Rad N, Golzar-Adabi S. Ixodid tick species infesting cows and buffaloes and their seasonality in West Azerbaijan. *Res J Parasitol* 2008; **3**(3): 98-103.

[25]Sarani M, Telmadarraiy Z, Moghaddam AS, Azam K, Sedaghat MM. Distribution of ticks (Acari: Ixodidae) infesting domestic ruminants in mountainous areas of Golestan Province, Iran. *Asian Pac J Trop Biomed* 2014; **4**: S246-S251.

[26]Razmi GR, Najarnejad V, Rashtibaf M. Determination the frequency of Ixodid ticks on the sheep in Khorasan Razavi province, Iran. *Archiv Razi*

Institute 2011; **66**(2): 129-132.

[27]Nasibeh HV, Zakkyeh T, Hassan V, Reza YEM, Morteza HV, Ali OM. Survey of tick species parasiting domestic ruminants in Ghaemshahr county, Mazandaran province, Iran. *Asian Pac J Trop Med* 2010; **3**(10): 804-806.

[28]Telmotelmodarreiy Z, Bahrami A, Vatandoust H. A survey on fauna of ticks in West Azerbaijan Province, Iran. *Iranian J Publ Health* 2004; **33**(4): 65-69.

[29]Telmadarraiy Z, Chinikar S, Vatandoost H, Faghihi F, Hosseini-Chegeni A. Vectors of Crimean Congo hemorrhagic fever virus in Iran. *J Arthropod Borne Dis* 2015; **9**(2): 137.

[30]Fatemian Z, Salehzadeh A, Sedaghat MM, Telmadarraiy Z, Hanafi-Bojd AA, Zahirnia AH. Hard tick (Acari: Ixodidae) species of livestock and their seasonal activity in Boyer-Ahmad and Dena cities of Kohgiluyeh and Boyer-Ahmad Province, Southwest of Iran. *Vet World* 2018; **11**(9): 1357.

[31]Tajedin L, Bakhshi H, Faghihi F, Telmadarraiy Z. High infection of *Anaplasma* and *Ehrlichia* spp. among tick species collected from different geographical locations of Iran. *Asian Pac J Trop Dis* 2016; **6**(10): 787-792.

[32]Oshaghi MA, Choubdar N, Karimian F, Koosha M, Nejati J. Molecular identification of *Anaplasma/Ehrlichia* spp. based on *16S* rRNA gene in *Hyalomma* ticks in border line of Iran-Pakistan. *Parasitol Res* 2009; **104**(5): 1243-1248.

[33]Hosseini-Vasoukolaei N, Oshaghi MA, Shayan P, Vatandoost H, Babamahmoudi F, Yaghoobi-Ershadi MR, et al. *Anaplasma* infection in ticks, livestock and human in Ghaemshahr, Mazandaran Province, Iran. *J Arthropod Borne Dis* 2014; **8**(2): 204.

[34]Ebrahimzade Abkooh E, Pazhoom F, Nabiyan S, Shayan P. *Anaplasma* infection in ticks infestating sheep and goat grazed in Savadkooh, Mazandaran. *The 6th International Congress of Clinical Laboratory and Clinic. Proceedings of the 2014 International Congress.* 14 February 2014. Tehran, Iran: Iran University of Medical Sciences; 2014.

[35]Cheville NF, Rogers DG, Deyoe WL, Krafsur ES, Cheville JC. Uptake and excretion of *Brucella abortus* in tissues of the face fly (*Musca autumnalis*). *Am J Vet Res* 1989; **50**(8): 1302-1306.

[36]Neglia G, Veneziano V, De Carlo E, Galiero G, Borriello G, Francillo M, et al. Detection of *Brucella abortus* DNA and RNA in different stages of development of the sucking louse *Haematopinus tuberculatus*. *BMC Vet Res* 2013; **9**(1): 236.

[37]Zheludkov MM, Tsirelson LE. Reservoirs of *Brucella* infection in nature. *Biol Bull* 2010; **37**(7): 709-715.

[38]Li Y, Wen X, Li M, Moumouni PFA, Galon EM, Guo Q, et al. Molecular detection of tick-borne pathogens harbored by ticks collected from livestock in the Xinjiang Uygur Autonomous Region, China. *Ticks Tick Borne Dis* 2020; **11**(5): 101478. doi: https://doi.org/10.1016/j.ttbdis.2020.101478.

[39]Wang Q, Zhao S, Wureli H, Xie S, Chen C, Wei Q, et al. *Brucella* melitensis and *B. abortus* in eggs, larvae and engorged females of *Dermacentor* marginatus. *Ticks Tick Borne Dis* 2018; **9**(4): 1045-1048.

[40]Razmi G, Yaghfoori S. Molecular surveillance of *Theileria ovis*, *Theileria lestoquardi* and *Theileria annulata* infection in sheep and ixodid ticks in Iran. *Onderstepoort J Vet Res* 2013; **80**(1): 1-5.

[41]Hasheminasab SS, Moradi P, Wright I. A four year epidemiological and chemotherapy survey of babesiosis and theileriosis, and tick vectors in sheep, cattle and goats in Dehgolan, Iran. *Ann Parasitol* 2018; **64**(1): 43-48.

[42]Yaghfoori S, Razmi G, Heidarpour M. Molecular detection of *Theileria* spp. in sheep and vector ticks in Fasa and Kazeroun areas, Fars Province, Iran. *Archiv Razi Institute* 2013; **68**(2): 159-164.

[43]Habibi G, Imani A, Afshari A, Bozorgi S. Detection and molecular characterization of *Babesia canis* vogeli and *Theileria annulata* in free-ranging dogs and ticks from Shahriar County, Tehran Province, Iran. *Iran J Parasitol* 2020; **15**(3): 321.

[44]Rajabi S, Esmaeilnejad B, Tavassoli M. A molecular study on *Babesia* spp. in cattle and ticks in West-Azerbaijan province, Iran. *Vet Res Forum* 2017; **8**(4): 299-306.

[45]Tavassoli M, Tabatabaei M, Mohammadi M, Esmaeilnejad B, Mohamadpour H. PCR-based detection of *Babesia* spp. infection in collected ticks from cattle in west and north-west of Iran. *J Arthropod Borne Dis* 2013; **7**(2): 132.

[46]Shayan P, Hooshmand E, Rahbari S, Nabian S. Determination of *Rhipicephalus* spp. as vectors for *Babesia ovis* in Iran. *Parasitol Res* 2007; **101**(4): 1029-1033.

[47]Seidabadi M, Razmi G, Naghibi A. Molecular detection of *Babesia* spp. in sheep and vector ticks in North Khorasan province, Iran. *Iran J Vet Med* 2014; **8**(1): 35-39.

[48]Zakkyeh T, Ali OM, Nasibeh HV, Reza YEM, Farhang B, Fatemeh M. First molecular detection of *Theileria ovis* in *Rhipicephalus sanguineus* tick in Iran. *Asian Pac J Trop Med* 2012; **5**(1): 29-32.