Archives of Razi Institute, Vol. 75, No. 3 (2020) 317-325

DOI: 10.22092/ARI.2019.125746.1317

Original Article

Molecular Detection of Spotted Fever Group Rickettsia (Rickettsiales: Rickettsiaceae) in Ticks of Iran

Hosseini-Chegeni, A. [1, 2], Tavakoli, M. [2], Telmadarraiy [3], Z., Faghihi, F. [4, 5] *

1. Department of Plant Protection, Faculty of Agriculture, Lorestan University, Khorramabad, Iran
2. Agricultural Research, Education and Extension Organization (AREEO), Lorestan Agricultural and Natural Resources Research Center, Khorramabad, Iran
3. Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
4. Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran
5. Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran

Received 28 March 2019; Accepted 27 July 2019
Corresponding Author: faezefaghihi@yahoo.com

ABSTRACT

Ticks are reservoir hosts of pathogenic *Rickettsia* in humans and domestic animals. Most pathogenic *Rickettsia* species belong to the spotted fever group (SFG). The present study aimed to determine the tick species infected with *Rickettsia* based on the genus-specific 23S ribosomal ribonucleic acid (rRNA), 16S rRNA, and *citrate synthase* (*gltA*) gene fragments. A total of 61 tick specimens were selected for molecular assay and 12 samples for sequencing. Phylogenetic analysis was conducted using neighbor-joining and Bayesian inference methods. *Argas persicus*, *Haemaphysalis sulcata*, *Ha. inermis*, and *Hyalomma asiaticum* were infected by spotted fever *Rickettsia*. The SFG is the main group of *Rickettsia* that can be detected in the three genera of ticks from Iran.

Keywords: *Rickettsia*, Ticks, Spotted fever group, Phylogenetic tree, Iran

Détection Moléculaire du Groupe des Fièvres Boutonneuses Rickettsia (Rickettsiales: Rickettsiaceae) chez les Tiques d'Iran

Résumé: Les tiques sont des hôtes réservoirs de Rickettsies pathogènes chez les humains et les animaux domestiques. La plupart des espèces de Rickettsia pathogènes appartiennent au groupe des fièvres boutonneuses (GFB). La présente étude visait à déterminer les espèces de tiques infectées par Rickettsia en se basant sur les fragments du genre spécifique de l'acide ribonucléique ribosomique 23S (ARNr), de l'ARNr 16S et du citrate synthase (gltA). Un total de 61 tiques a été sélectionné pour l'analyse moléculaire et 12 échantillons pour le séquençage. L'analyse phylogénétique a été menée en utilisant des méthodes de neighbor-joining et d'inférence Bayésienne. *Argas persicus*, *Haemaphysalis sulcata*, *Ha. inermis* et *Hyalomma asiaticum* sont avérés être infectés par la fièvre boutonneuse Rickettsia. Les Rickettsia sont les principaux GFB détectés dans les trois genres de tiques d'Iran.

Mots-clés: *Rickettsia*, tiques, Groupes des fièvres boutonneuses, Arbre phylogénétique, Iran

INTRODUCTION

Ticks (Ixodida order) harbor many symbiotic microorganisms, including some *Rickettsia* species (Benson et al., 2004; Heise et al., 2010; Noh et al., 2017). As intracellular bacteria, *Rickettsiae* are symbionts in the broad sense with an intimate but not necessarily beneficial relationship with their arthropod hosts that may be opportunistic or pathogenic under various conditions, affecting vector competency (Perlman et al., 2006; Telford and Parola, 2007). According to molecular phylogenetics, the term *Rickettsia* is currently applied to arthropod-borne bacteria belonging to the genus *Rickettsia* within the family Rickettsiaceae in the order of the Rickettsiales. This genus is currently made of 24 recognized species and contains several dozens of as-yet uncharacterized strains (Fournier and Raoult, 2007). *Rickettsia* species are obligate intracellular bacteria that infect various vertebrate hosts, including humans (Noh et al., 2017). They are the most frequent organisms detected within the bacterial community of ticks (Moreno et al., 2006). Most human pathogenic *Rickettsiae* belong to the spotted fever group (SFG), including *Rickettsia rickettsii*, *Rickettsia akari*, *Rickettsia conorii*, *Rickettsia parkeri*, *Rickettsia sibirica*, *Rickettsia australis*, *Rickettsia japonica*, *Rickettsia slovaca*, and *Rickettsia africae* (Braig et al., 2009). The number of known pathogenic *Rickettsia* species has been increasing over time (Owen et al., 2006; Li et al., 2016). Within 1984-2004, nine novel *Rickettsia* species or subspecies were identified causing tick-borne rickettsioses, including six initially isolates from ticks (Raoult and Parola, 2007). Because ticks are regarded as the reservoir host of SFG *Rickettsia* (Raoult and Roux, 1997), they have been frequently examined for the presence of *Rickettsia* (Perlman et al., 2006). All tick-borne pathogenic *Rickettsia* species are transmitted by hard ticks. Some individual ticks can be concurrently infected with more than one *Rickettsia* species (Braig et al., 2009). *Rickettsiae* can be considered potential pathogens, particularly if they have been detected in the tick's salivary glands (Parola et al., 2005). When ovaries and oocytes of an adult female tick are infected, *Rickettsia* might be transovarially transmitted to at least some offspring (Raoult and Roux, 1997). The frequency of *Rickettsia* transmission through tick bite depends on tick-host specificity, abundance of the tick vector, prevalence of the infection within tick organs, and frequency of tick-human contact (Parola et al., 2005). After the revolution in bacterial taxonomy by the innovation of polymerase chain reaction (PCR), genome sequencing has become a tool in research and clinical applications (Loeffelholz and Deng, 2006; Nolte and Wittwer, 2016). The advantages of PCR include simplicity, speed, low cost, and ability to detect microorganisms without cultivation (Clay and Fuqua, 2010; Nolte and Wittwer, 2016). In addition to the importance of 23S ribosomal ribonucleic acid (rRNA) and 16S rRNA for the molecular detection of *Rickettsia*, the faster evolution of *citrate synthase* (*gltA*), a citrate synthase-encoding gene, shows that this gene is more sensitive to change than 16S rRNA (Fournier et al., 1998). To date, 50 hard and soft tick species have been recorded for the fauna of Iran (Kamali et al., 2001). In the current study, a molecular survey of *Rickettsia* agents was carried out for the first time using genus-specific 23S rRNA, 16S rRNA, and *gltA* genes for the identification of *Rickettsia* in the ticks collected from Iran.

MATERIAL AND METHODS

Study area, Collection, and Identification of Ticks. Tick specimens were collected from domestic animals in nine provinces of Iran, namely Azarbayjan-e Sharqi, Hamedan, Kerman, Kermanshah, Khorasan-e Shomali, Khuzestan, Kurdistan, Lorestan, and Semnan. Table 1 tabulates the data related to the collection of the specimens. The ticks were transported to the laboratory in a glass tube and identified at the level of species based on taxonomic keys (Estrada-Peña et al., 2004; Hosseini-Chegeni and Tavakoli, 2013; Pomerantzev, 1950) under a light stereomicroscope (SZX12-Olympus, JAPAN). Then, the tick specimens were stored at -20 °C for further examination.

Table 1. Data related to collected specimens of study

Tick species	Host/Collection place	Number of collected ticks (circa)	Number of examined individual ticks	Total number of positive tick	Target polymerase chain reaction positive and sequencing
Argas persicus	Poultry nest	150	30	20	16S and 23S
Haemaphysalis sulcata	Sheep and Goats	50	10	5	16S, 23S, and *gltA*
Haemaphysalis inermis	Sheep and Goats	A single specimen	1	1	16S
Hyalomma asiaticum	Sheep and Goats	100	20	10	16S and *gltA*

gltA: *Citrate synthase*

Polymerase Chain Reaction. Genomic deoxyribonucleic acid (gDNA) was extracted using Phenol-chloroform according to Sambrook and Russell (2001). The fragments of 16S rRNA, 23S rRNA, and *gltA* were amplified by PCR. In order to specifically and accurately amplify the target agents in ticks' bodies, six primers were newly designed, including; 16S: Fric16S (5′- CGG AGG AAA GAT TTA TCG CTG ATG -3′), Rric16S (5′- GTT TAC GGC GTG GAC TAC C -3′), 23S: Fric23S (5′- CGT GAG GGA AAG GTG AAA AG -3′), Rric23S (5′- CGC TAC CTT AGG ACC GTC -3′), *gltA*: Fric*gltA* (5′- GGY TTT ATG TCT MCT GCT TC -3′), and Rric*gltA* (5′- AGC TTC AAG TTC TAT TGC TAT TTG -3′). The PCR reactions for each gene were carried out in a thermocycler Corbett® (Australia). Touchdown temperature profile, including 5 min at 95 °C, 10 cycles (50 sec at 94 °C, 50 sec at 60-50 °C, and 1 min at 72 °C), followed by 20 cycles (50 sec at 94 °C, 50 sec at 50 °C, and 1 min at 72 °C), and final extension step (3 min at 72 °C). Each PCR reaction consisted of 12.5 µl of 2× RedMaster PCR® (Sinaclon®, Iran), 1 µl from each primer (10 pM), 4 µl of gDNA template (50-100 ng/µl), and 6.5 µl of deionized water to the final volume of 25 µl. The positive controls included the positive tick samples with successful DNA extraction, PCR amplification, and sequencing of a *Rickettsia* species. In addition, distilled water without target DNA was used as negative control.

Electrophoresis, Purification, and Sequencing. The PCR products were visualized by 1% agarose gel electrophoresis, and the selective desired bands of the different gene fragments from different tick species were purified using the GF-1 Gel DNA Recovery Kit® (Vivantis, Malaysia). Then, the purified PCR products were submitted for sequencing to Faza-Biotech® Company (Iran). Subsequently, the sequences were manually edited using FinchTV® software (version 1.4.0). Finally, all the sequences were submitted to GenBank, and accession numbers were assigned.

Phylogenetic analysis. All the sequences were aligned using SeaView software (version 4) (Gouy et al., 2010). Phylogenetic trees were constructed for 16S and *gltA* sequence data using neighbor-joining as well as 23S sequence data using Bayesian inference methods by MEGA software (version 7) and BEAST software (version 1.8.2) (Drummond et al., 2012). For this purpose, 17, 17, and 18 taxa (including sequences from the present study as well as comparable GenBank data as in- and out-group) were used for the construction of 16S, *gltA*, and 23S phylogenetic trees, respectively. The constructed clades of 16S, *gltA*, and 23S phylogenetic trees were reorganized based on 100% bootstrap support values and reasonable genetic distance differences within and between the clade members. The sequences from *Orientia tsutsugamushi* and *Wolbachia* (i.e., an endosymbiont species of *Drosophila*) were included as out-groups in both 16S and 23S phylogenetic trees in addition to *Rickettsia australis*, *R. prowazekii*, and *R. tarasevichiae* in the *gltA* phylogenetic tree.

RESULTS

Tick species, PCR, and Sequences. Totally, 301 tick specimens were collected from different parts of the study area. A total of 61 individual ticks were analyzed for *Rickettsia* agents with 36 *Rickettsia* positive tick samples (Table 1). The ticks, including *Argas persicus*,

Haemaphysalis sulcate, *Ha. inermis*, and *Hyalomma asiaticum*, were identified based on taxonomic keys from different geographical regions. Figure 1 illustrates the PCR amplification of a 590-bp fragment of 16S rRNA, 1501-bp fragment of 23S rRNA, and 903-bp fragment of *gltA* of *Rickettsia* genus in various tick samples. The 16S rRNA of *Rickettsia* was detected in *A. persicus*, *Hy. asiaticum*, *Ha. sulcata*, and *Ha. inermis* collected from Lorestan, Hamedan, Khorasan-e Shomali, and Khuzestan provinces, Iran, respectively. Moreover, 23S rRNA was detected in the *A. persicus* ticks of three different regions in Lorestan province (Aleshtar, Pol-e Dokhtar, Sepiddasht). Furthermore, *gltA* was also detected in *Hy. asiaticum* and *Ha. sulcata* from Hamedan and Khorasan-e Shomali provinces, respectively. Twelve sequences, including five 16S, five 23S, and two *gltA* sequences, were obtained after sequencing. The accession numbers were assigned in GenBank, including KY569634-41 (16S rRNA), KY784689-90 (*gltA*), and KY857839-40 (23S rRNA).

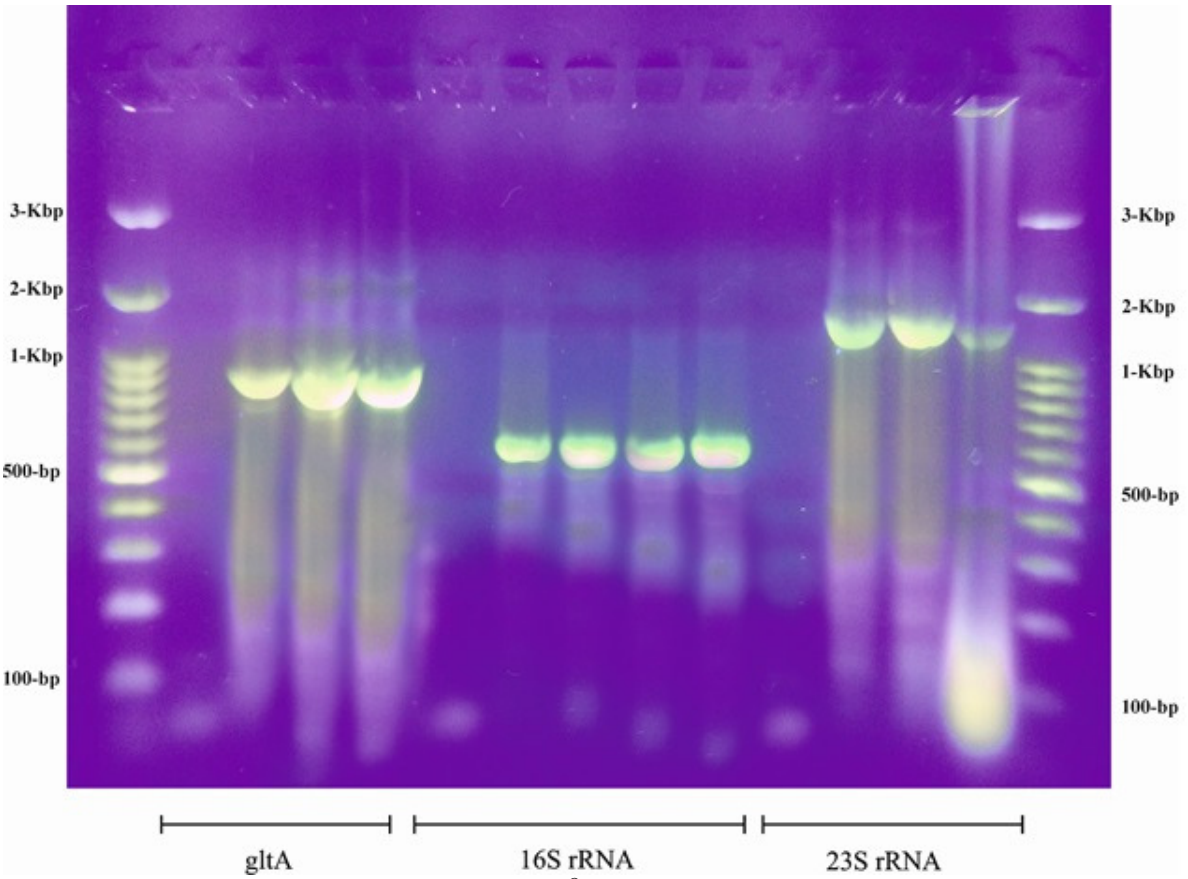

Figure 1. 1% agarose gel electrophoresis stained with Cyber Safe® showing different gene fragments of *Rickettsia* amplified in this study, including *citrate synthase* (903 bp), 16S ribosomal ribonucleic acid (rRNA) (590 bp), and 23S rRNA (1501 bp), detected from different tick species; gene samples from left to right, including negative and positive control, and then various *Rickettsia* samples isolating from ticks

16S, 23S, and *gltA* Phylogenetic Trees. Phylogenetic trees were constructed using MEGA software (version 7) and BEAST software (version 1.8.2), including in-group and out-group *Rickettsia* taxa (Figure 2). The constructed phylogeny indicated that the *Rickettsia* spp. of Iranian ticks are clustered with the GenBank data of 16S rRNA Rickettsia sequences (i.e., *R. aeschlimannii*, *R. africae*, *R. conorii*, *R.*

japonica, *R. kotlanii*, *R. massiliae*, *R. peacockii*, *R. prowazekii*, *R. raoultii*, and *R. slovaca*), 23S rRNA (i.e., *R. africae*, *R. akari*, *R. australis*, *R. conorii*, *R. felis*, *R. heilongjiangensis*, *R. japonica*, *R. massiliae*, *R.*

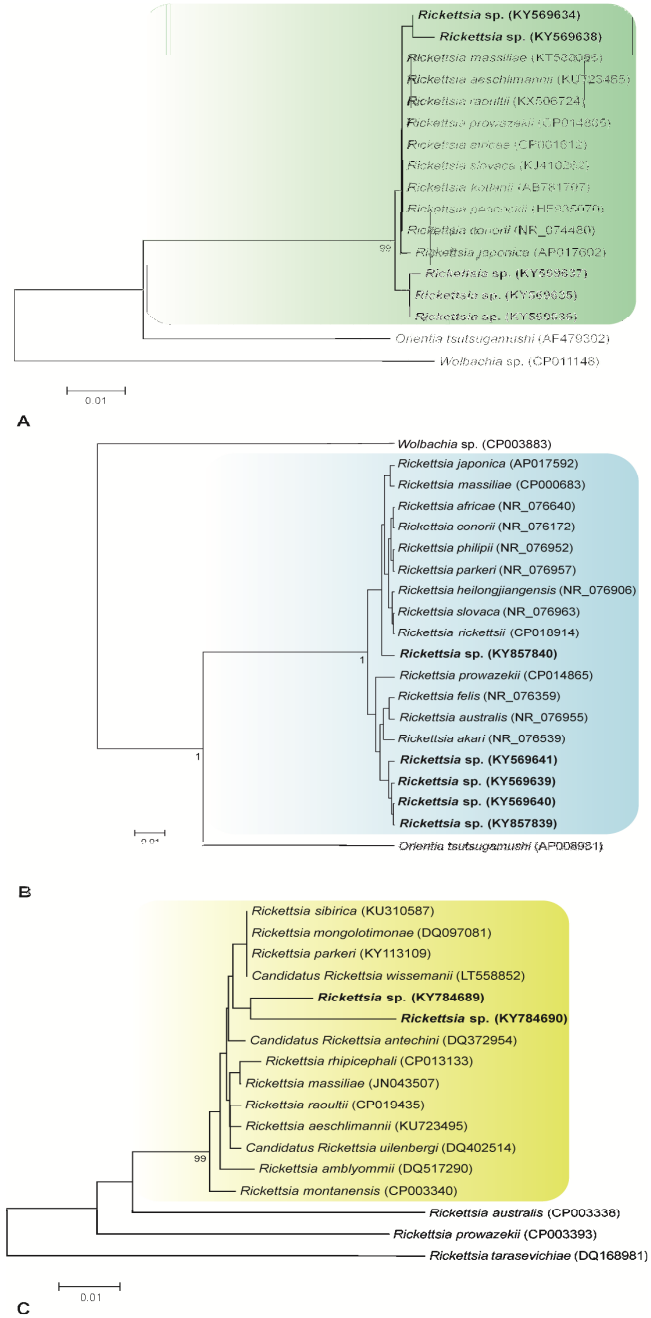

Figure 2. Phylogenetic tree of *Rickettsia* spp. inferred from 16S ribosomal ribonucleic acid (rRNA) (A), 23S rRNA (B), and *citrate synthase (gltA)* (C) sequence data constructed using neighbor-joining (A and C) as well as Bayesian inference (B) methods with 1,000 replicates; main clade in the right side of tree separated with colored rectangular shapes; taxa arranged as species name following GenBank accession number; taxon of the present study indicated as bold by genus name; nodes indicated with bootstrap (A and C) and posterior probability (B) values; *Orientia tsutsugamushi*, *Wolbachia* (i.e., an endosymbiont species of *Drosophila*) (A and B), *Rickettsia australis*, *Rickettsia prowazekii*, and *Rickettsia tarasevichiae* (C) examined as out-groups

parkeri, *R. philipii*, *R. prowazekii*, *R. rickettsia*, and *R. slovaca*), and *gltA* (i.e., *Candidatus R. antechini*, *C. R. uilenbergi*, *R. aeschlimannii*, *R. amblyommii*, *R. massiliae*, *R. mongolotimonae*, *R. montanensis*, *R. parkeri*, *R. raoultii*, *R. rhipicephali*, and *R. sibirica*). No intraspecies variation in terms of genetic distance was observed among 16S, 23S, and *gltA* sequences of *Rickettsia* clade. As much as 14% and 9% genetic distance was noticed between *Rickettsia* 16S sequences with *Wolbachia* and *Orientia tsutsugamushi*, respectively. Moreover, the genetic distance rates of 13% and 21% were observed between *Rickettsia* 23S sequences with *Orientia tsutsugamushi* and *Wolbachia*, respectively. The *Rickettsia gltA* sequence data showed 7%, 7%, and 11% of genetic distance difference with *R. australis*, *R. prowazekii*, and *R. tarasevichiae* (as out-groups), respectively. All different sequences of the *Rickettsia* clade should be considered single species according to each 16S, 23S, and *gltA* gene fragments.

DISCUSSION

The present preliminary study was designed for the determination of genus *Rickettsia* among tick species distributed in Iran for the first time. Four tick species in two families and three genera were identified which were infected with *Rickettsia* according to 16S rRNA, 23S rRNA, and *gltA* molecular evidence. To date, three *Argas*, nine *Hyalomma*, and six *Haemaphysalis* species were reported from Iran (Hosseini-Chegeni et al., 2013; Hosseini-Chegeni and Tavakoli, 2013; Hosseini-Chegeni et al., 2014; Hosseini-Chegeni et al., 2015). In the present study, rickettsial sequences were identified in *A. persicus*, *Ha. sulcata*, *Ha. inermis*, and *Hy. asiaticum* in different geographical regions of Iran. Sumrandee et al. (2016) reported a 13% prevalence of *Rickettsia* within *Amblyomma*, *Dermacentor*, *Haemaphysalis*, and *Rhipicephalus* (*Boophilus*). The bacterial diversity associated with *Dermacentor niveus* Neumann ticks in the natural environment was investigated by 16S rRNA, *gltA*, and other genes in China (Zhuang et al., 2014). The authors detected Proteobacteria (including *Rickettsia*) as a dominant microflora in the ticks collected from the field. Up to 21% infection rate of SFG *Rickettsia* in *Amblyomma* ticks was reported from Thailand (Sumrandee et al., 2014). The prevalence of *Rickettsia* infection based on the detection of the *gltA* gene varied in different species of ticks, ranging from 6-40% (Sumrandee et al., 2016). Two tick species were examined for the identification of *Rickettsia* species in a national park in Poland. The results indicated the pathogen prevalence rates of 27.5% and 42.8% for *Ixodes ricinus* (L.) and *Dermacentor reticulatus* (Fabricius), respectively. In the present study, 23S rRNA, 16S rRNA, and *gltA* genes were used to differentiate *Rickettsia* from genera *Orientia tsutsugamushi* and *Wolbachia* (i.e., an endosymbiont species of *Drosophila*) (Fam. *Rickettsiaceae*). According to Roux et al. (1997), the phylogeny inferred from the *gltA* gene is more reliable than 16S rRNA. They could discriminate two subgroups in the SFG *Rickettsia* based on the *gltA* phylogenetic tree. The 16S rRNA sequences were not useful for the taxonomy of *Rickettsia* because greater than 97% similarity exists between any two taxa (Fournier et al., 1998; Lee et al., 2003; Parola et al., 2005). Therefore, to precisely detect rickettsiae at the species level, the investigation of expression of other genes, including *gltA*, *outer membrane protein A*, *outer membrane protein B*, *120-kDa cell surface antigen 4*, and *60-kDa heat shock protein*, is recommended. *Argas persicus* is the main ectoparasite of poultry in Iran and is adapted for living inside the nest/shelter of its host (Telmadarraiy et al., 2004; Hosseini-Chegeni and Tavakoli, 2013). This tick species was positive for *Rickettsia* spp. in the current study. According to Pader et al. (2012), 57% of *A. persicus* tick pools were positive for *Rickettsia* in Ethiopia. *Argas persicus* has been reported to occasionally parasitize humans. Although *Rickettsia* pathogens have been detected in *A. persicus*, it does not mean that this species is an important vector for human rickettsial diseases (Estrada-Peña and Jongejan, 1999). *Argas persicus* was also reported as the vector of *Rickettsia*-like symbiotes (Suitor Jr and Weiss, 1961), *R. slovaca* from Armenia

(Rehacek et al., 1977), and *R. hoogstraalii* from Ethiopia (Pader et al., 2012). *Haemaphysalis sulcata* is widely distributed in Iran and is mostly detected in livestock, including goats and sheep, unlike *Ha. inermis* with more restricted distribution (Hosseini-Chegeni et al., 2014). In the present study, *Ha. sulcata* and *Ha. inermis* were positive for *Rickettsia*. Up to 26% prevalence of *Rickettsia felis*-like bacteria was reported from *Ha. sulcata* in Southern Croatia (Duh et al., 2006). Moreover, Sarih et al. (2008) detected a 77% infection prevalence of *Rickettsia* in *Ha. sulcata* in Morocco. Generally, *Haemaphysalis* ticks rarely bite humans and consequently are of little significance in the epidemiology of human pathogens (Duh et al., 2006). *Haemaphysalis inermis* was newly identified as the most important potential vector for *Rickettsia helvetica* in Hungary (Hornok et al., 2010). In other parts of the world, rickettsial agents were reported from *Haemaphysalis* ticks, namely *R. aeschlimannii* from *Ha. inermis* and *Rickettsia* endosymbiont of *Ha. sulcata* from Spain (Portillo et al., 2008) and *R. hoogstraalii* from *Ha. sulcata* (Duh et al., 2010; Tomassone et al., 2017). A limited number of studies have been carried out on the detection of *Rickettsia* in *Hyalomma* ticks. In the current study, *Rickettsia* was detected in *Hy. asiaticum,* a widely distributed tick species in Iran (Hosseini-Chegeni et al., 2013; Telmadarraiy et al., 2015). An antigenically and genotypically unique SFG *Rickettsia* was isolated from *Hy. asiaticum* in China (Yu et al., 1993). Kordová and Rehacek (1964) reported the multiplication of *Rickettsia prowazekii* in *Hy. asiaticum* and considered this species as a potential vector. According to the obtained results of the present study, SFG *Rickettsia* is the main group of *Rickettsia* that can be detected from the three genera of ticks in Iran.

The SFG *Rickettsia* is the main group of *Rickettsia* that can be detected from the three genera of ticks in Iran. It is suggested to carry out further genetic studies on other genes to determine the accurate status of *Rickettsia* species transmitted through vector ticks in Iran.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contribution

Study concept and design: Hosseini-Chegeni, A. Telmadarraiy, Z.
Acquisition of data: Hosseini-Chegeni, A., Tavakoli, M.
Analysis and interpretation of data: Hosseini-Chegeni, A.
Drafting of the manuscript: Hosseini-Chegeni, A., Faghihi, F.
Critical revision of the manuscript for important intellectual content: Faghihi, F.
Statistical analysis: Hosseini-Chegeni, A.
Administrative, technical, and material support: Tavakoli, M., Tavakoli, M., Telmadarraiy, Z.

Acknowledgment

The authors would like to express their gratitude to the villagers and ranchers living in Lorestan province who helped in tick collection for this study. The authors would also like to appreciate the director and personnel of Razi Herbal Medicines Research Center and Lorestan University of Medical Sciences for their technical assistance during this project.

References

Benson, M.J., Gawronski, J.D., Eveleigh, D.E., Benson, D.R., 2004. Intracellular symbionts and other bacteria associated with deer ticks (*Ixodes scapularis*) from Nantucket and Wellfleet, Cape Cod, Massachusetts. Appl Environ Microbiol 70, 616–620.

Braig, H.R., Turner, B.D., Perotti, M.A., 2009. Symbiotic *Rickettsia*. In: Bourtzis, K., Miller, T.A. (Eds.), Insect symbiosis, CRC Press, London UK, pp. 221–249.

Clay, K., Fuqua, C., 2010. The tick microbiome: diversity, distribution and influence of the internal microbial community for a blood-feeding disease vector. Critical needs and gaps in understanding prevention, amelioration, and resolution of Lyme and other tick-borne diseases: the short-term and long-term outcomes. Workshop report. Institute of Medicine Committee on Lyme Disease and Other Tick-Borne Diseases, Washington, DC USA, pp. 1–22.

Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol Biol Evol 29, 1969–1973.

Duh, D., Punda-Polic, V., Avsic-Zupanc, T., Bouyer, D., Walker, D.H., Popov, V.L., *et al.*, 2010. *Rickettsia hoogstraalii* sp. nov., isolated from hard-and soft-bodied ticks. Int J Syst Evol Microbiol 60, 977–984.

Duh, D., Punda-Polić, V., Trilar, T., Petrovec, M., Bradarić, N., Avšič-Županc, T., 2006. Molecular identification of *Rickettsia felis*-like bacteria in *Haemaphysalis sulcata* ticks collected from domestic animals in southern Croatia. Ann N Y Acad Sci 1078, 347–351.

Estrada-Peña, A., Bouattour, A., Camicas, J.L., Walker, A.R., 2004. Ticks of domestic animals in the Mediterranean region: a guide to identification of species, University of Zaragoza, Zaragoza Spain.

Estrada-Peña, A., Jongejan, F., 1999. Ticks feeding on humans: a review of records on human-biting ixodoidea with special reference to pathogen transmission. Exp Appl Acarol 23, 685–715.

Fournier, P.E., Raoult, D., 2007. Bacteriology, taxonomy, and phylogeny of *Rickettsia*. In: Raoult, D., Parola, P. (Eds.), Rickettsial diseases, Informa, London UK, pp. 1–13.

Fournier, P.E., Roux, V., Raoult, D., 1998. Phylogenetic analysis of spotted fever group rickettsiae by study of the outer surface protein rOmpA. Int J Syst Evol Microbiol 48, 839–849.

Gouy, M., Guindon, S., Gascuel, O., 2010. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol. Biol. Evol. 27, 221-224.

Heise, S.R., Elshahed, M.S., Little, S.E., 2010. Bacterial diversity in *Amblyomma americanum* (Acari: Ixodidae) with a focus on members of the genus *Rickettsia*. J Med Entomol 47, 258–268.

Hornok, S., Meli, M.L., Perreten, A., Farkas, R., Willi, B., Beugnet, F., *et al.*, 2010. Molecular investigation of hard ticks (Acari: Ixodidae) and fleas (Siphonaptera: Pulicidae) as potential vectors of rickettsial and mycoplasmal agents. Vet Microbiol 140, 98–104.

Hosseini-Chegeni, A., Hosseine, R., Tavakoli, M., Telmadarraiy, Z., Abdigoudarzi, M., 2013. The Iranian *Hyalomma* (Acari: Ixodidae) with a key to the identification of male species. Persian J Acarol 2, 503–529.

Hosseini-Chegeni, A., Hosseini, R., Abdigoudarzi, M., Telmadarraiy, Z., Tavakoli, M., 2015. An additional records of *Hyalomma marginatum rufipes* Koch, 1844 (Acari: Ixodidae) in southwestern and southern Iran with a molecular evidence. Iran J Anim Biosyst 11, 79–89.

Hosseini-Chegeni, A., Tavakoli, M., 2013. *Argas vespertilionis* (Ixodida: Argasidae): A parasite of Pipistrel bat in Western Iran. Persian J Acarol 2, 321–330.

Hosseini-Chegeni, A., Telmadarraiy, Z., Salimi, M., Arzamani, K., Banafshi, O., 2014. A record of *Haemaphysalis erinacei* (Acari: Ixodidae) collected from Hedgehog and an identification key for the species of *Haemaphysalis* occurring in Iran. Persian J Acarol 3, 203–215.

Kamali, K., Ostovan, H., Atamehr, A., 2001. A catalog of mites and ticks (Acari) of Iran, Islamic Azad University Scientific Publication Center, Tehran Iran.

Kordová, N., Rehacek, J., 1964. Microscopic examination of the organs of ticks infected with *Rickettsia prowazeki*. Acta Virol 8, 465–469.

Lee, J.H., Park, H.S., Jang, W.J., Koh, S.E., Kim, J.M., Shim, S.K., *et al.*, 2003. Differentiation of rickettsiae by groEL gene analysis. J Clin Microbiol 41, 2952–2960.

Li, H., Cui, X.M., Cui, N., Yang, Z.D., Hu, J.G., Fan, Y.D., *et al.*, 2016. Human infection with novel spotted fever group *Rickettsia* genotype, China, 2015. Emerg Infect Dis 22, 2153–2156.

Loeffelholz, M., Deng, H., 2006. PCR and its variations In: Tang, Y.W., Stratton, C.W. (Eds.), Advanced techniques in diagnostic microbiology, Springer, New York USA, pp. 166–184.

Moreno, C.X., Moy, F., Daniels, T.J., Godfrey, H.P., Cabello, F.C., 2006. Molecular analysis of microbial communities identified in different developmental stages of *Ixodes scapularis* ticks from Westchester and Dutchess Counties, New York. Environ Microbiol 8, 761–772.

Noh, Y., Lee, Y.S., Kim, H.C., Chong, S.T., Klein, T.A., Jiang, J., *et al.*, 2017. Molecular detection of *Rickettsia* species in ticks collected from the southwestern provinces of the Republic of Korea. Parasit Vectors 10, 20–29.

Nolte, F.S., Wittwer, C.T., 2016. Nucleic acid amplification methods overview. In: Persing, D.H., Tenover, F.C. (Eds.),

Molecular microbiology: diagnostic principles and practice, ASM Press, Washington DC USA, pp. 3–18.

Owen, H., Unsworth, N., Stenos, J., Robertson, I., Clark, P., Fenwick, S., 2006. Detection and identification of a novel spotted fever group *rickettsia* in Western Australia. Ann NY Acad Sci 1078, 197–199.

Pader, V., Buniak, J.N., Abdissa, A., Adamu, H., Tolosa, T., Gashaw, A.*, et al.*, 2012. *Candidatus Rickettsia hoogstraalii* in Ethiopian *Argas persicus* ticks. Ticks Tick Borne Dis. 3, 338–345.

Parola, P., Paddock, C.D., Raoult, D., 2005. Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. Clin Microbiol Rev 18, 719-756.

Perlman, S.J., Hunter, M.S., Zchori-Fein, E., 2006. The emerging diversity of *Rickettsia*. Proc R Soc Lond B Biol Sci 273, 2097–2106.

Pomerantzev, B.I., 1950. Fauna of USSR arachnida: Ixodid ticks (Ixodidae), Fauna SSSR, Akademii Nauk SSSR, Leningrad USSR.

Portillo, A., Santibáñez, P., Santibáñez, S., Pérez-Martínez, L., Oteo, J.A., 2008. Detection of *Rickettsia* spp. in *Haemaphysalis* ticks collected in La Rioja, Spain. Vector-Borne Zoonotic Dis 8, 653–658.

Raoult, D., Parola, P., 2007. Preface. In: Raoult, D., Parola, P. (Eds.), Rickettsial diseases, Informa, London UK.

Raoult, D., Roux, V., 1997. Rickettsioses as paradigms of new or emerging infectious diseases. Clin Microbiol Rev 10, 694–719.

Rehacek, J., Urvölgyi, J., Kovacova, E., 1977. Massive occurrence of rickettsiae of the spotted fever group in fowl tampan, *Argas persicus*, in the Armenian SSR. Acta Virol 21, 431–438.

Roux, V., Rydkina, E., Eremeeva, M., Raoult, D., 1997. Citrate synthase gene comparison, a new tool for phylogenetic analysis, and its application for the rickettsiae. Int J Syst Evol Microbiol 47, 252–261.

Sambrook, J., Russell, D.W., 2001. Molecular cloning, a laboratory manual, Cold Spring Harbor Laboratory Press, New York USA.

Sarih, M., Socolovschi, C., Boudebouch, N., Hassar, M., Raoult, D., Parola, P., 2008. Spotted fever group Rickettsiae in ticks, Morocco. Emerg Infect Dis 14, 1067–1073.

Suitor Jr, E.C., Weiss, E., 1961. Isolation of a rickettsialike microorganism (*Wolbachia persica*, n. sp.) from *Argas persicus* (Oken). J Infect Dis 108, 95–106.

Sumrandee, C., Baimai, V., Trinachartvanit, W., Ahantarig, A., 2016. Molecular detection of *Rickettsia*, *Anaplasma*, *Coxiella* and *Francisella* bacteria in ticks collected from Artiodactyla in Thailand. Ticks Tick Borne Dis 7, 678–689.

Sumrandee, C., Hirunkanokpun, S., Doornbos, K., Kitthawee, S., Baimai, V., Grubhoffer, L.*, et al.*, 2014. Molecular detection of *Rickettsia* species in *Amblyomma* ticks collected from snakes in Thailand. Ticks Tick Borne Dis 5, 632–640.

Telford, S.R., Parola, P., 2007. Arthropods and rickettsiae. In: Raoult, D., Parola, P. (Eds.), Rickettsial diseases, Informa, London UK, pp. 27–36.

Telmadarraiy, Z., Bahrami, A., Vatandoost, H., 2004. A survey on fauna of ticks in west Azerbaijan province, Iran. Iran. J Public Health 33, 65–69.

Telmadarraiy, Z., Chinikar, S., Vatandoost, H., Faghihi, F., Hosseini-Chegeni, A., 2015. Vectors of Crimean Congo Hemorrhagic Fever (CCHF) virus in Iran. J Arthropod-Borne Dis 9, 137–147.

Tomassone, L., Ceballos, L.A., Ragagli, C., Martello, E., De Sousa, R., Stella, M.C.*, et al.*, 2017. Importance of common wall lizards in the transmission dynamics of tick-borne pathogens in the northern apennine mountains, Italy. Microb Ecol 74, 961-968.

Yu, X., Jin, Y., Fan, M., Xu, G., Liu, Q., Raoult, D., 1993. Genotypic and antigenic identification of two new strains of spotted fever group rickettsiae isolated from China. J. Clin. Microbiol. 31, 83–88.

Zhuang, L., Wang, C.Y., Tong, Y.G., Tang, F., Yang, H., Liu, W.*, et al.*, 2014. Discovery of *Rickettsia* species in *Dermacentor niveus* Neumann ticks by investigating the diversity of bacterial communities. Ticks Tick Borne Dis 5, 564–568.