Archives of Razi Institute, Vol. 75, No. 1 (2020) 39-46

DOI: 10.22092/ARI.2019.122532.1259

Original Article

Molecular Detection of *Anaplasma marginale* and *Anaplasma ovis* (Rickettsiales: *Anaplasmataceae*) in Ixodid Tick Species in Iran

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Received 14 October 2018; Accepted 20 February 2019
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ABSTRACT

The present study was conducted as the first molecular detection of *Anaplasma* species in tick samples based on the sequencing of *major surface proteins 4* (*msp4*) gene fragments in different parts of Iran. A total of 130 tick specimens were collected from Hormozgan, Lorestan, and Guilan, Iran, within 2015 to 2017. *Hyalomma asiaticum*, *Hyalomma dromedarii*, *Rhipicephalus sanguineus*, and *Rhipicephalus* (*Boophilus*) species were identified in different geographical regions. An amplicon of 464-bp *msp4* of *Anaplasma* was amplified using polymerase chain reaction in various tick species. Three sequences, including one *Anaplasma marginale* from *R.* (*Boophilus*) species and two *Anaplasma ovis* from *Rhipicephalus sanguineus*, were obtained after sequencing. It is concluded that bovine and ovine anaplasmosis agents are present in tick samples in Iran. The use of the gene families of six major surface proteins for the detection of various Anaplasma species is recommended.

Keywords: *Anaplasma*, Phylogenetic tree, *msp4*, Iran, Tick

Détection Moléculaire des Espèces *Anaplasma marginale* et *Anaplasma ovis* (Rickettsiales: Anaplasmataceae) Chez les Tiques Ixodidés en Iran

Résumé: Le but de cette étude était la mise en place d'une méthode de détection moléculaire basée sur le séquençage du gène *msp4* des espèces d'*Anaplasma* dans des échantillons de tiques collectés dans différentes régions d'Iran. Au total, 130 échantillons de tiques ont été collectés dans les provinces de Hormozgan, Lorestan et Guilan de 2015 à 2017. *Hyalomma asiaticum*, *H. dromedarii*, *Rhipicephalus sanguineus* et *R. (Boophilus)* sp. ont été identifiés dans différentes régions géographiques. Un amplicon de msp4 de 464 pb d'*Anaplasma* de diverses espèces a été amplifié par PCR. Trois séquences, dont une appartenant à *Anaplasma marginale* de *R. (Boophilus)* sp. et deux à *A. ovis* de *Rhipicephalus sanguineus* ont été obtenus après séquençage. Ces résultats

montrent que les anaplasmoses bovines et ovines peuvent potentiellement être transmises par des tiques en Iran. Cette étude suggère également que l'utilisation des familles de gènes de six protéines de surface majeures est efficace dans la détection de diverses espèces d'*Anaplasma*.
Mots-clés: *Anaplasma*, Arbre phylogénétique, *msp4*, Iran, Tique

INTRODUCTION

Anaplasma marginale (*A. marginale*) (Rickettsiales: Anaplasmataceae), the type species of the genus *Anaplasma*, is the causative agent of bovine anaplasmosis, which is widely distributed in tropical and subtropical regions around the world (Rar and Golovljova, 2011). *Anaplasma ovis* (*A. ovis*) (Rickettsiales: Anaplasmataceae), the agent of ovine anaplasmosis, may cause mild to severe diseases in sheep, deer, and goats; however, cattle are not infected with *A. ovis* (Aubry and Geale, 2011). *A. marginale* and *A. ovis* are intraerythrocytic pathogens infecting various domestic and wild ruminants (Birtles, 2012).*A. ovis* and *A. marginale*, which are closely related, can be mechanically transmitted by blood-contaminated mouthparts of biting flies and mosquitoes. They can also be biologically transmitted by ticks, which is more efficient, compared to mechanical transmission (Kocan et al., 2010). *Boophilus* tick is able to transmit *A. marginale* through the experimental transmission of infection to cattle (Futse et al., 2003). The transmission of *A. ovis* to humans is yet to be clearly understood (Chochlakis et al., 2010). Approximately 20 species of ticks have been reported capable of transmitting *A. marginale*, namely *Boophilus decoloratus* and *Rhipicephalus simus* in Africa, *Boophilus microplus* in Australia, and *Dermacentor* species in the United States (de la Fuente et al., 2001; Kocan, 2001). *A. marginale* and *A. ovis* infections can be persistent in cattle, goats, and tick hosts, both of which serve as reservoirs (Brayton, 2012). Fatal bovine anaplasmosis is a major problem in endemic countries due to *A. marginale* (Hornok et al., 2012). Despite mild clinical symptoms of *A. ovis* infection, it should be considered an important constraint of livestock production due to the high prevalence of *A. ovis* in such countries (Renneker et al., 2013). Six major surface proteins (MSPs) have been identified on *A. marginale* related to cattle and ticks (de la Fuente et al., 2001). The *major surface proteins 4* (*msp4*) sequences have been reported as acceptable genetic markers for inferring the phylogeographic patterns of *A. marginale* on a broad geographic scale (Kocan et al., 2002). Genetic heterogeneity has been shown among the geographical strains of *A. marginale* explained by tick vector and tick pathogen interactions (Waner et al., 2010). *A. marginale* and *A. ovis* have been reported in the blood samples of small ruminants in Iran with cattle and sheep as reservoir hosts of *A. marginale* (Noaman et al., 2009; Yousefi et al., 2017). To date, no studies have focused on detecting a specific polymerase chain reaction (PCR) and sequencing of *Anaplasma* species within tick specimens. The present study was designed to determine the presence of *Anaplasma* species in various tick species based on PCR amplifying and sequencing of *msp4* gene fragments in different parts of Iran.

MATERIAL AND METHODS

Collection and identification of ticks. A total of 130 tick specimens were collected from Hormozgan, Lorestan, and Guilan, Iran, within 2015 to 2017 (Table 1). The tick specimens were collected by forceps from the hosts. Live specimens were carefully transported to the Laboratory of Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences. The ticks were identified based on species level under a

light stereomicroscope (SZX12-Olympus®, Japan) following the identification key of Hosseini-Chegeni et al. (2019). Then, 20 tick specimens were selected from different areas for molecular assays.

Polymerase Chain Reaction. Genomic DNA was extracted using cetyltrimethylammonium bromide according to Doyle and Doyle (1987). A fragment of *msp4* was amplified through PCR. In order to specifically and accurately amplify the target agent in ticks, the primers were newly designed by the authors of the present study, including, F*msp4*: 5′- GTY ARR GGC TAY GRC AAG AG -3′ and R*msp4*: 5′- AGT RAA CTG GTA GCT WAT YCC A -3′. The PCR reactions for each gene were carried out in a thermocycler (MyCycler™ Thermal Cycler, Bio-Rad®, USA). The touchdown temperature profile included 5 min at 95 °C, 10X (50 sec at 94 °C, 50 sec at 60-50 °C, and 1 min at 72 °C), followed by 20X (50 sec at 95 °C, 50 sec at 50 °C, and 1 min at 72 °C), and a final extension step (3 min at 72 °C). Each PCR reaction consisted of 12.5 µl RedMaster PCR® (Sinaclon®, Iran) 2X, 1 µl of each primer (10 pM), 4 µl genomic DNA template (50-100 ng/µl), and 6.5 µl distilled water to the final volume of 25 µl. The DNA extracted from PCR positive tick samples with successful sequencing of *A. marginale* and *A. ovis* was used as a positive control. In addition, distilled water was utilized instead of target DNA in all PCR reactions as a negative control.

Electrophoresis, Purification, and Sequencing. The PCR products were visualized by 1% agarose gel electrophoresis, and the selective desired bands of different gene fragments from different tick species were purified using GeneAll Expin™ Combo GP Kit (South Korea). Then, 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). The corrected sequences were compared with the submitted sequences in GenBank using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Finally, all sequences were submitted to GenBank, and accession numbers were assigned.

Phylogenetic analysis. All sequences were aligned using SeaView software (version 4) (Gouy et al., 2010), and genetic distances among the sequences were calculated using maximum composite likelihood in MEGA software (version 7) (Kumar et al., 2016).

Table 1. Collected data related to tick species in present study

Species	Life stage	Location	Host	Collected specimens (n)	Polymerase chain reaction *Anaplasma* positive (n)	Sequencing (acc. no)
Hyalomma asiaticum	Nymph	Lorestan[1]	Persian jird	20	5 (pool)	-
Hyalomma asiaticum	Nymph	Lorestan[2]	Persian jird	15	5 (pool)	-
Hyalomma asiaticum	Male	Lorestan[3]	Sheep	10	1	-
Rhipicephalus sanguineus	Female	Lorestan[3]	Sheep	5	1	-
Rhipicephalus sanguineus	Male	Lorestan[4]	Goat	10	1	*Anaplasma ovis* (MH017205)
Rhipicephalus sanguineus	Male	Lorestan[5]	Unknown	10	1	*Anaplasma ovis* (MH017206)
Hyalomma dromedarii	Female	Hormozgan[6]	Camel	30	1	Failed
Rhipicephalus (*Boophilus*) species	Female	Guilan[7]	Caspian red deer	30	1	*Anaplasma marginale* (MH017204)

[1]Khorramabad-Kuhdasht road, Zamzam village; [2]Khorramabad-Tehran road, Lorestan University of Medical Sciences; [3]Selseleh county, Qalayi rural district, Chamgorgali village; [4]Khorramabad-Aleshtar road, Kakareza village; [5]Aligudarz county, Besharat district; [6]Qeshm island, [7]Talesh county, Lisar protected area

Phylogenetic tree were constructed for *msp4* sequence data using Bayesian inference methods by BEAST software (version 2.4.8) (Bouckaert et al., 2014). For this purpose, the data of 37 *Anaplasma msp4* sequence, including the sequences from the present study, as well as the sequences from GenBank data, were used to construct an *msp4* phylogenetic tree. The constructed clades of the phylogenetic tree were reorganized based on 100% support of posterior probability values and reasonable genetic distance differences within and between the clade members. The sequence of *Anaplasma phagocytophilum* (EU857672) was included as an outgroup in the phylogenetic tree.

RESULTS

Tick species, polymerase chain reaction, and sequences. In the present study, *Hyalomma asiaticum*, *Hyalomma dromedarii*, *Rhipicephalus sanguineus*, and *Rhipicephalus* (*Boophilus*) species were identified in different geographical regions. The collected data related to ticks in this study are summarized in Error! Reference source not found.. An amplicon of 464-bp *msp4* was amplified in tick species. Three sequences, including one *A. marginale* from *Rhipicephalus* (*Boophilus*) species and two *A. ovis* from *Rhipicephalus sanguineus*, were obtained after sequencing. The accession numbers of MH017204 (*A. marginale*) and MH017205-6 (*A. ovis*) were assigned in GenBank.

Major surface protein 4 phylogenetic tree. Phylogenetic trees were constructed using BEAST software, including ingroup and outgroup *Anaplasma* taxa (Figure 1). The *msp4* constructed phylogeny indicating two main clades includes *A. marginale* and *A. ovis* with a 10% genetic distance difference observed between the clades. No intraspecies variation in terms of genetic distance was noticed within the members of the two clades. No additional *msp4* sequence of *Anaplasma* taxa was observed in GenBank. The *msp4* phylogenetic tree generated from the sequence data consistently supported the monophyly of *A. marginale* and *A. ovis* clades. The phylogenetic tree may be observed as a fully resolved tree with high posterior probability values and dichotomous topology (Figure 1).

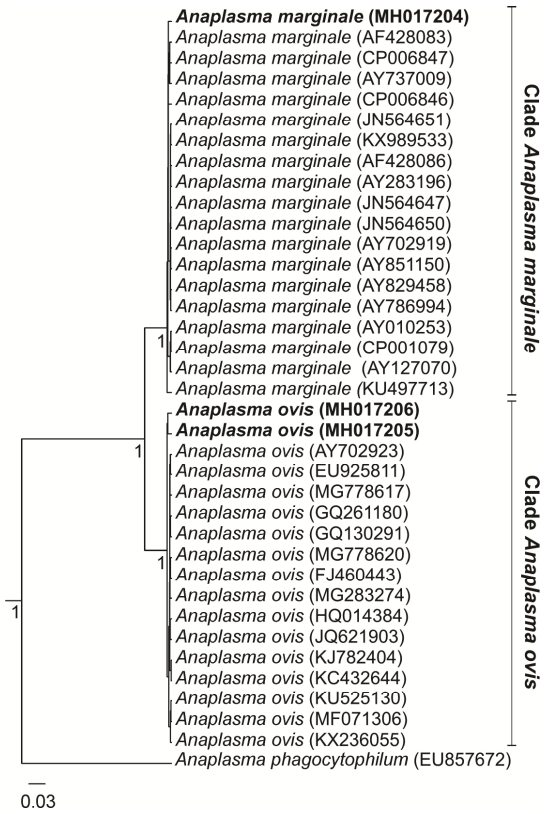

Figure 1. Phylogenetic tree generated based on *major surface proteins 4* sequence data of *Anaplasma* species in this study and similar sequences from GenBank database constructed using Bayesian inference method; main *Anaplasma* clades separated in right side of phylogenetic tree; taxa of present study are bold and defined with a name and GenBank accession number; posterior probability values inserted in place of nodes; branch lengths proportional to evolutionary changes; *Anaplasma phagocytophilum* included as outgroup.

DISCUSSION

In the present study, two economically important pathogen agents, namely *A. marginale* and *A. ovis*, were detected in tick samples collected from Iran. Most studies have mainly emphasized animal hosts, such as the ruminants in various provinces of Iran (Spitalska et al., 2005; Ahmadi et al., 2009; Noaman et al., 2009; Hosseine-Vasoukolaei et al., 2010; Jalali et al., 2013; Yousefi et al., 2017). In northern and northeastern Iran,

out of 193 blood samples, 123 samples were *A. ovis* positive as confirmed by restriction fragment length polymorphism on *msp4* gene fragment (Ahmadi et al., 2009). Similar to the present study, a study was conducted in Khuzestan, Iran, where *Anaplasma marginale* and *A. ovis* were simultaneously detected in 50% of *Anaplasma* infected blood samples (Jalali et al., 2013). In a study carried out by Yousefi et al. (2017), 27.5% of sheep and goats were positive for *A. ovis* and *A. marginale*. According to 16S ribosomal ribonucleic acid (rRNA), *A. ovis* was reported in human and sheep samples in Mazandaran, Iran, (Hosseine-Vasoukolaei et al., 2010), as well as the sheep samples in Fars, Iran (Spitalska et al., 2005). Moreover, positive cases of *A. marginale* were observed in 75 *Anaplasma* suspected blood samples in Isfahan, Iran (Noaman et al., 2009). In the present study, *Hyalomma asiaticum*, *Hyalomma dromedarii*, *Rhipicephalus sanguineus*, and *Rhipicephalus* (*Boophilus*) species were identified in different geographical regions. *Rhipicephalus* (*Boophilus*) species were identified in northern Iran on Caspian red deer (*Cervus elaphus maral*). The ticks were collected on the dead marals. In addition, these specimens were positive for a *Brucella*-like bacterium (Hosseini-Chegeni et al., 2017). The range of maral includes the Caspian provinces of northern Iran, Crimea, Turkey, and Caucasus (Kiabi et al., 2004). *Hyalomma asiaticum* and *Hyalomma dromedarii* are the most important ectoparasites of domestic animals in Iran (Hosseini-Chegeni et al., 2013). *Rhipicephalus sanguineus* is the most prevalent vector species distributed all over Iran (Telmadarraiy et al., 2015). In the present study, three sequences, including one *A. marginale* from *Rhipicephalus* (*Boophilus*) species and two *A. ovis* from *Rhipicephalus sanguineus*, were obtained after sequencing. The occurrence of anaplasmosis due to *A. marginale* and *A. ovis* has been reported in different countries adjacent to Iran, namely Turkey (Bilgic et al., 2017), Pakistan (Jabbar et al., 2015), Russia (Vasilevich et al., 2017), and Saudi Arabia (Al-Khalifa et al., 2009). In other countries, *A. ovis* was more prevalent (40.5%) than other *Anaplasma* species in the northwest of China (Yang et al., 2015), Ethiopia (5.1%) (Abdela et al., 2018), and co-occurrence of *A. ovis* with different pathogens in tick species in Serbia (Tomanović et al., 2013). Two similar isolates of *A. marginale* from cattle and *Rhipicephalus* tick vector were detected in China (Cui et al., 2018). Five tick species, namely *Dermacentor marginatus*, *Haemaphysalis punctata*, *Ixodes ricinus*, *Ixodes ventalloi*, and *Rhipicephalus sanguineus* (s.l.) in Portugal, were positive for *A. marginale* (Antunes et al., 2016). The partial 16S rRNA was used for the detection of *A. ovis* in tick species of Iran (Hosseini-Vasoukolaei et al., 2014). After the BLAST analysis of 16S rRNA sequences of this study [Acc. Nos.: JF514503-12], it was observed that this is not a reliable gene sequence regarding the occurrence of specific *Anaplasma* species. Studies conducted on 16S rRNA of *Anaplasma* species in tick vector encountered similar weaknesses. In the present study, *msp4* phylogeny indicating two main clades were *A. marginale* and *A. ovis* with a 10% genetic distance difference observed between the clades. The *msp4* fully resolved the tree, generated from the sequence data, and consistently supported the monophyly of *A. marginale* and *A. ovis* clades. The msp4 may conduce to study genetic diversity in *Anaplasma* species, especially *A. marginale* strains in certain regions (de la Fuente et al., 2005). Phylogeny derived from *A. ovis msp4* may vary among geographic regions, as well as in sheep and deer hosts, yet less than the variation observed between *A. marginale* strains (de la Fuente et al., 2007).

The present study was carried out as the first molecular detection of *Anaplasma* species in tick samples based on the sequencing of *msp4* gene fragments in different parts of Iran. It is concluded that bovine and ovine anaplasmosis are present in tick samples in Iran; therefore, it is necessary to develop suitable strategies to detect and breakdown the natural cycle of disease with the emphasis on tick control. Moreover, it is required to perform further studies to

determine the presence of *Anaplasma* agent in the salivary gland and other organs of ticks to determine the vectorial capacity of each tick vector. It is recommended to use the gene families of other MSPs, such as *msp1*, *msp2*, and *msp5*, in different tick species, especially *Ixodes ricinus*, for the detection of *Anaplasma phagocytophilum*.

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.
Analysis and interpretation of data: Hosseini-Chegeni, A.
Drafting of the manuscript: Hosseini-Chegeni, A., Faghihi, F., Telmadarraiy, Z.
Critical revision of the manuscript for important intellectual content: Faghihi, F.,
Statistical analysis: Hosseini-Chegeni , A.
Administrative, technical, and material support: Hosseini-Chegeni , A., Tavakoli, M., Goudarzi, G. H., Telmadarraiy, Z., Sharifdini, M., Faghihi, F., Ghanbari, M.K.

Acknowledgment

The authors would like to acknowledge the contribution of Lorestani villagers to this ongoing study. Had it not been for their kind support and generosity, the authors would have not been able to collect the data of the present study. The authors would also like to extend their gratitude and appreciation to the director and personnel of Razi Herbal Medicines Research Center and Lorestan University of Medical Sciences for their technical support.

References

Abdela, N., Ibrahim, N., Begna, F., 2018. Prevalence, risk factors and vectors identification of bovine anaplasmosis and babesiosis in and around Jimma town, Southwestern Ethiopia. Acta Trop 177, 9-18.

Ahmadi, H.M., Khaki, Z., Rahbari, S., Kazemi, B., Bandehpour, M., 2009. Molecular identification of anaplasmosis in goats using a new PCR-RFLP method. Iran J Vet Res 10, 367-372.

Al-Khalifa, M., Hussein, H., Diab, F., Khalil, G., 2009. Blood parasites of livestock in certain Regions in Saudi Arabia. Saudi J Biol Sci 16, 63-67.

Antunes, S., Ferrolho, J., Domingues, N., Santos, A., Santos-Silva, M., Domingos, A., 2016. Anaplasma marginale and *Theileria annulata* in questing ticks from Portugal. Exp Appl Acarol 70, 79-88.

Aubry, P., Geale, D., 2011. A review of bovine anaplasmosis. Transbound Emerg Dis 58, 1-30.

Bilgic, H.B., Bakırcı, S., Kose, O., Unlu, A.H., Hacılarlıoglu, S., Eren, H., *et al.*, 2017. Prevalence of tick-borne haemoparasites in small ruminants in Turkey and diagnostic sensitivity of single-PCR and RLB. Parasite0 Vector 10, 211.

Birtles, R., 2012. Rickettsiales infections. In: Gavier-Widén, D., Duff, J.P., Meredith, A. (Eds.), Infectious diseases of wild mammals and birds in Europe, Blackwell, Oxford UK, pp. 363-371.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., *et al.*, 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. PLOS Comput. Biol 10, e1003537.

Brayton, K.A., 2012. Persistence and antigenic variation. In: Azad, A.F., Palmer, G.H. (Eds.), Intracellular pathogens II: Rickettsiales, American Society of Microbiology, Herndon, Virginia, US, pp. 366-390.

Chochlakis, D., Ioannou, I., Tselentis, Y., Psaroulaki, A., 2010. Human anaplasmosis and *Anaplasma ovis* variant. Emerg Infect Dis 16, 1031-1032.

Cui, Y., Wang, X., Zhang, Y., Yan, Y., Dong, H., Jian, F., *et al.*, 2018. First confirmed report of outbreak of theileriosis/anaplasmosis in a cattle farm in Henan, China. Acta Trop 177, 207-210.

de la Fuente, J., Atkinson, M.W., Naranjo, V., de Mera, I.G.F., Mangold, A.J., Keating, K.A., *et al.*, 2007. Sequence analysis of the *msp4* gene of *Anaplasma ovis* strains. Vet Microbiol 119, 375-381.

de la Fuente, J., Lew, A., Lutz, H., Meli, M.L., Hofmann-Lehmann, R., Shkap, V., *et al.*, 2005. Genetic diversity of *Anaplasma* species major surface proteins and implications

for anaplasmosis serodiagnosis and vaccine development. Anim Health Res Rev 6, 75-89.

de la Fuente, J., Van Den Bussche, R.A., Kocan, K.M., 2001. Molecular phylogeny and biogeography of North American isolates of *Anaplasma marginale* (Rickettsiaceae: Ehrlichieae). Vet Parasitol 97, 65-76.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19, 11-15.

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.

Futse, J.E., Ueti, M.W., Knowles, D.P., Palmer, G.H., 2003. Transmission of *Anaplasma marginale* by *Boophilus microplus*: retention of vector competence in the absence of vector-pathogen interaction. J Clin Microbiol 41, 3829-3834.

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.

Hornok, S., Micsutka, A., De Mera, I.F., Meli, M., Gönczi, E., Tánczos, B., *et al.*, 2012. Fatal bovine anaplasmosis in a herd with new genotypes of *Anaplasma marginale*, *Anaplasma ovis* and concurrent haemoplasmosis. Res Vet Sci 92, 30-35.

Hosseine-Vasoukolaei, N., Telmadarraiy, Z., Vatandoost, H., Yaghoobi Ershadi, M.R., Hosseine Vasoukolaei, M., Oshaghi, M.A., 2010. Survey of tick species parasiting domestic ruminants in Ghaemshahr county, Mazandaran province, Iran. Asian pac J Trop Med, 3, 804-806.

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 Acarol2, 503–529.

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

Hosseini-Vasoukolaei, N., Oshaghi, M.A., Shayan, P., Vatandoost, H., Babamahmoudi, F., Yaghoobi-Ershadi, M.R., *et al.*, 2014. *Anaplasma* infection in ticks, livestock and human in Ghaemshahr, Mazandaran province, Iran. J Arthropod-Borne Dis 8, 204-211.

Jabbar, A., Abbas, T., Saddiqi, H.A., Qamar, M.F., Gasser, R.B., 2015. Tick-borne diseases of bovines in Pakistan: major scope for future research and improved control. Parasite Vector 8, 283.

Jalali, S., Khaki, Z., Kazemi, B., Bandehpour, M., Rahbari, S., Razi Jalali, M., *et al.*, 2013. Molecular detection and identification of *Anaplasma* species in sheep from Ahvaz, Iran. Iran J Vet Res 14, 50-56.

Kiabi, B.H., Ali Ghaemi, R., Jahanshahi, M., Sassani, A., 2004. Population status, biology and ecology of the Maral, *Cervus elaphus maral*, in Golestan National Park, Iran. Zool Middle East 33, 125-138.

Kocan, K.M., 2001. Anaplasmosis. In: Service, M.W. (Ed.), Encyclopedia of arthropod-transmitted infections of man and domesticated animals, CABI Publishing, New York USA, pp. 28-33.

Kocan, K.M., de la Fuente, J., Blouin, E.F., Coetzee, J.F., Ewing, S., 2010. The natural history of *Anaplasma marginale*. Vet Parasitol 167, 95-107.

Kocan, K.M., De La Fuente, J., Blouin, E.F., Garcia-Garcia, J.C., 2002. Adaptations of the tick-borne pathogen, *Anaplasma marginale*, for survival in cattle and ticks. Exp Appl Acarol 28, 9-25.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33, 1870–1874.

Noaman, V., Shayan, P., Amininia, N., 2009. Molecular diagnostic of *Anaplasma marginale* in carrier cattle. Iran J Parasitol 4, 26-33.

Rar, V., Golovljova, I., 2011. *Anaplasma*, *Ehrlichia*, and "*Candidatus Neoehrlichia*" bacteria: pathogenicity, biodiversity, and molecular genetic characteristics, a review. Infect Genet Evol 11, 1842-1861.

Renneker, S., Abdo, J., Salih, D., Karagenç, T., Bilgiç, H., Torina, A., *et al.*, 2013. Can *Anaplasma ovis* in small ruminants be neglected any longer? Transbound Emerg Dis 60, 105-112.

Spitalska, E., Namavari, M.M., H., H.-M., Shad-del, F., Amrabadi, O.R., Sparagano, O.A.E., 2005. Molecular surveillance of tick-borne diseases in Iranian small ruminants. Small Rum Res 57, 245-248.

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.

Vasilevich, F., Kovalchuk, S., Babiy, A., Arkhipov, A., Arkhipova, A., Glazko, T., *et al.*, 2017. Molecular identification of isolates *Anaplasma marginale* found in

blood of cattle on the territory of Moscow region. Russian J Parasitol 40, 179-182 [In Russian].

Waner, T., Mahan, S., Kelly, P., Harrus, S., 2010. Rickettsiales. In: Gyles, C.L., Prescott, J.F., Songer, J.G., Thoen, C.O. (Eds.), Pathogenesis of bacterial infections in animals, Blackwell, Iowa USA, pp. 589-621.

Yang, J., Li, Y., Liu, Z., Liu, J., Niu, Q., Ren, Q., *et al.*, 2015. Molecular detection and characterization of *Anaplasma* spp. in sheep and cattle from Xinjiang, northwest China. Parasite Vector 8, 108.

Yousefi, A., Rahbari, S., Shayan, P., Sadeghi-dehkordi, Z., Bahonar, A., 2017. Molecular detection of *Anaplasma marginale* and *Anaplasma ovis* in sheep and goat in west highland pasture of Iran. Asian Pac J Trop Biomed 7, 455-459.