Emergent spotted fever group *Rickettsiae* infections among hard ticks in Islamic Republic of Iran

Mohammad Bagher Ghavami¹, Zohreh Alibabaei¹, Mohammad Reza Jamavar² and Behrooz Taghiloo¹

¹Department of Medical Entomology and Vector Control, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Islamic Republic of Iran (Correspondence to MB Ghavami: Ghavami@zums.ac.ir). ²Department of Communicable Diseases, South Khorasan Health Center, Birjand, Islamic Republic of Iran.

Abstract

Background: Tick-borne rickettsioses have become a health concern worldwide following the increasing incidence in recent decades. However, there is limited information about these diseases in Islamic Republic of Iran.

Aim: This cross-sectional study was conducted to estimate the *Rickettsia* infection among ixodid ticks collected from cattle, sheep and goats in Islamic Republic of Iran.

Methods: The DNA of ixodid ticks collected from cattle, sheep and goats in 54 villages of Zanjan Province, Islamic Republic of Iran, were collected and analysed using a spectrophotometer. Rickettsial-positive samples were screened by targeting the htrA gene and fragments of gltA gene were analysed. The variables were analysed using descriptive statistics and the χ^2 test was used to compare the variables.

Results: A total of 528 ticks were tested. Overall, *Rickettsia* infection rate was 6.44%. Nine of the 12 tick species were infected. Rickettsial positive rates in *Hyalomma marginatum* and *Dermacentor marginatus* were 21.33% and 12.77%, respectively. *R. aeschlimannii*, the predominant rickettsia, was detected only in *Hy. marginatum*. *R. raoultii*, *R. sibirica* and *R. slovaca* comprised about half of the positive ticks and were recovered from more than one tick species.

Conclusion: Considering the discovery of infected ticks in the Islamic Republic of Iran, there is a need to establish a tick control programme in the country, paying attention to populations at high-risk.

Keywords: Rickettsia, spotted fever, rickettsioses, gltA gene, htrA gene, Ixodid ticks, tick-borne disease, Iran

Citation: Ghavami MB, Alibabaei Z, Jamavar M, Taghiloo B. Emergent spotted fever group rickettsiae infections among hard ticks in Islamic Republic of Iran. East Mediterr Health J. 2024;30(2):145–155. https://doi.org/10.26719/emhj.24.030.

Received: 22/01/23; Accepted: 31/10/23

Copyright © Authors 2024; Licensee: World Health Organization. EMHJ is an open access journal. This paper is available under the Creative Commons Attribution Non-Commercial ShareAlike 3.0 IGO licence (CC BY-NC-SA 3.0 IGO; https://creativecommons.org/licenses/by-nc-sa/3.0/igo).

Background

Spotted fever group (SFG) rickettsioses, caused by a neglected group of bacteria belonging to the genus *Rickettsia*, are recognized as new and emerging vectorborne diseases with a worldwide distribution (1). These infections are mainly transmitted by hard ticks and maintained in nature in cycles involving tick vectors that act as reservoirs. Variations of ecological factors alter the status of human contact with infected ticks and change the transmission map of these diseases (2).

Historically, *Rickettsia rickettsii*, *R. sibirica*, *R. japonica*, *R. conorii* and *R. australis* were considered to be the traditional SFG rickettsiae (3). More recently, application of molecular markers, such as 17-kDa lipoprotein outer membrane antigen (htrA), citrate synthase (gltA) and rickettsial outer membrane proteins A and B (OmpA and OmpB) genes in the diagnosis of rickettsioses, and variations of ecological factors have changed the epidemiological map of the world's SFG rickettsioses in the last decades (3,4).

Likewise, the Middle East region has undergone drastic environmental change, and several rickettsial diseases have been reported (5,6). Serological surveys have confirmed the presence of *R. conorii* in this region

since ancient times (3), and *R. aeschlimannii*, *R. slovaca*, *R. sibirica* and *R. raoultii* have lately been reported in the Mediterranean Basin and Transcaucasia (7–9).

R. hoogstraalii, R. monacensis and *R. helvetica* have been reported in Turkey (10). Despite records of numerous rickettsiae in Turkey, there is limited evidence of *Rickettsia* infection in the neighbouring areas of the country, particularly in northwestern Islamic Republic of Iran (3). Recent cases of *R. conorii israelensis* and other rickettsial infection of *Hyalomma asiaticum* have been reported in southern Islamic Republic of Iran (11). Surveys of tick pool samples have documented the presence of *R. slovaca, R. massiliae, R. sibirica, R. raoultii* and *R. aeschlimannii* in the north and northwestern Islamic Republic of Iran (12–14).

The northwest of Islamic Republic of Iran, the gateway of Eurasia and a link between the continents, is an important area for vector and reservoir hard ticks. This region acts as a bridge between Africa, Europe and Asia for migratory birds and has proper geographical conditions for breeding migratory birds. This natural condition favours many tick infestations in both animals and humans.

SFG rickettsiosis is a mandatory notifiable disease in northwest Islamic Republic of Iran (15); however, due to under-notification and misdiagnosis of cases, the real incidence of the disease remains obscure. Identifying rickettsial infection of hard ticks in the northwest of the country may help explain the prevalence of SFG rickettsioses in this area.

Because of the clinical importance of SFG rickettsioses, the presence of large populations of ixodid ticks and their potential for infection with rickettsial pathogens underscore the need to characterize these agents in northwest Islamic Republic of Iran. Therefore, we investigated rickettsial infections in hard ticks collected from cattle, sheep and goats in the Zanjan Province of the country.

Methodology

Study area, sampling and identification of ticks

This cross-sectional study was conducted in 54 villages of Zanjan Province where residents keep domestic animals (sheep, cattle and goats), from April to November 2019 and in 2020. The villages are located on the path of the Mediterranean, West Asian, East African and Central Asian migratory bird flyways (https://www.eaaflyway. net/the-flyway/). These villages comprise lowlands, plains and highlands with altitudes of <1000, 1000–2000, and >2000 metres above sea level, respectively (Figure 1).

The animals' owners verbally approved the collection of ticks. Adult hard ticks were collected from the ear, neck, armpit, chest, abdomen and crissum of randomly selected animals. Feeding ticks (randomly 1–4 ticks per animal) were hand-picked using tweezers and gloves, transferred to 1.8 ml cryovial tubes and stored at -20°C until further examination. Collected ticks were identified morphologically at the species level using standard taxonomic keys (16,17).

DNA extraction and evaluation of DNA quantity and quality

The collected ticks were soaked in 70% ethanol, rinsed with sterile distilled water and dried on sterile filter paper. Individual ticks were pulverized in liquid nitrogen using a sterile mortar and pestle. Fine powder of each sample was suspended in 500 μ l of lysing buffer [100 mM of Tris-HCl (pH 8.0), 0.5 mM of NaCl, 10 mM of EDTA and 1% W/V SDS], and genomic DNA (gDNA) extracted as described previously (18).

DNA samples were analysed by a spectrophotometer (NanoDrop, Thermo Fisher Scientific). Low-quality samples of <8 ng/ μ l were excluded from the study.

PCR amplification and identification of Rickettsia species

The 17kDF (5'-GCTCTTGCAACTTCTATGTT-3') and 17kDR (5'-CATTGTTCGTCAGGTTGGCG-3') primers were used for the initial detection of *Rickettsia* in total DNA. These primers recognize htrA gene, encoding 17-kDa outer membrane lipoprotein (4). Rickettsia-positive samples were further characterized using GLTF (5'-ATCCTATGGCTATTATGCTTGC-3') and GLTR (5'-TACATAACCGGTGTAAAGCTGT-3') primers as described previously (18,19).

These primers were modified based on the gltA gene of R. *conorii* (accession MK304547). Each 25 μ l total volume of PCR reaction contained 3 μ l of gDNA (25–50



ng/ μ l), 10 pM of each primer, 12.5 μ l of 2× Mix Master Red (Amplicon, Denmark) and 7.5 μ l of nuclease-free water. All amplifications were completed using the following thermal profile: initial denaturation at 95°C for 5 minutes, followed by 30 cycles consisting of denaturation at 95°C for 30 seconds, annealing at 58°C for 50 seconds, and extension at 72°C for 1 minute.

A final extension cycle at 72°C for 5 minutes was performed, and reactions were cooled at 4°C. For each reaction, a negative control of ddH₂O was used. PCR products were resolved by electrophoresis on a 1.5% agarose gel, run for 40 minutes at 80 V, visualized under a UV light and analysed with the 100-bp DNA ladder. PCR products were sequenced bidirectionally by Macrogen (Seoul, Republic of Korea) using the forward and reverse specific primers.

Data analysis

Variables were analysed using descriptive statistics presented as frequencies and percentages. The 95% confidence interval was used to statistically estimate proportions. The chi-squared test was used to compare variables. Calculated differences among variables were considered statistically significant for P<0.05.

The obtained sequences were checked and assembled using Chromas (https://technelysium.com. au/wp/ chromas/) and BioEdit 7.0 (https://bioedit.software. informer.com/7.0/). Each sequence was analysed using BLASTN (https://blast. ncbi.nlm.nih.gov/Blast.cgi) with gltA and htrA reference sequences available in the GenBank for sequence homology.

Sequence trace files were analysed with MEGAX (https://www.megasoftware.net/downloads/dload_ win_gui), DNASTAR 6 (http://www.ub.edu/dnasp/ downloadTv6.html), PopART Qt4.8.4 (http://popart.otago. ac.nz) and T-Coffee (https://tcoffee.org/Projects/tcoffee/ index.html) software for constructing phylogenetic trees and identifying haplotype groups.

Phylogenetic trees of studied groups were constructed using Neighbor-Joining and Maximum Likelihood statistical methods using the Kimura 2-parameter model under 500 bootstrap replicates. The sequences of *R. prowazekii* were included as an out-group.

Results

A total of 638 adult hard ticks were collected from 363 sheep, 167 cattle and 47 goats. Of the identified ticks, 528 ticks were tested for *Rickettsia* infection. Most of these samples were collected from sheep and cattle (*P*<0.001). The frequency of collected ticks from the plains areas was significantly higher than collected ticks from both lowlands and highlands (*P*<0.001). The abundance of ticks from infested animals related to topological zones is summarized in Table 1. Overall, 4 genera and 12 species of hard ticks were identified. *Hy anatolicum* and *Hy. marginatum* ticks were the most common species, comprising 22.92% and 14.20% of the samples, respectively (P<0.001).

Overall, Rickettsia infection was detected in 34 (6.44%) of 528 tested samples. The abundance of Rickettsia in studied ticks is summarized in Table 1. *Hy. marginatum, Dermacentor marginatus, Haemaphysalis sulcata* and *Rhipicephalus bursa* had the highest infection rates (21.33%, 12.77%, 9.52% and 9.30%, respectively).

Ticks collected from goats were free of rickettsial infection, as were all ticks collected from plains areas. Rickettsial infection was highest (19.18%) in tested ticks from lowlands, and lowest (3.70%) in ticks from the highlands (P<0.001). Rickettsia infection in ticks collected from sheep was higher than those collected from cattle (P<0.001).

Sequencing of htrA gene amplicons showed a ~370bp DNA fragment size, and their multiple alignments exhibited 3 rickettsial groups that were similar to *R. raoultii/sibirica, R. slovaca* and *R. aeschlimannii* (Figure 2). Fragments of the gltA gene recovered from 34 positive ticks yielded an 842-bp size and shared more than 99.8% identity with the reference species of *R. aeschlimannii, R. raoultii, R. sibirica* and *R. slovaca*.

Table 2 shows identified species of *Rickettsia* infecting hard ticks in the studied area. The nucleotide sequences of the gltA gene samples were deposited in the GenBank under the accession MT293336–MT293352, MW117933–MW117936 and MW219588–MW219600.

BLASTN sequence analysis of *Rickettsia* fragments in 16 positive *Hy. marginatum* ticks showed 99% nucleotide identity with the gltA gene of the *R. aeschlimannii* reference strain (accession KU961540). Six ticks of *De. marginatus* contained the DNA of *Rickettsia*, in which 99% nucleotide identity was presented with the gltA gene of reference *R. sibirica* (accession KM288711 and HM050296) and *R. raoultii* (accession MK304547) in 4 and 2 samples, respectively. Of 6 positive *Rh. bursa* ticks, *Rickettsia* DNA shared 99% identity with the gltA gene of reference *R. raoultii* (accession MK304547) in 4 ticks and *R. sibirica* (accession KM288711) in 2 ticks.

Two samples each of *Hy. anatolicum*, *Hy. detritum*, *Hy. dromedarii* and *Ha. sulcata* ticks were rickettsial positive. *Rickettsia* detected in the *Hy. anatolicum* samples shared 99% identity with *R. slovaca* (accession MN581989). *Rickettsia* obtained from the *Hy. detritum* and *Hy. dromedarii* specimens showed 98% identity with *R. sibirica* (accession HM050296).

Rickettsia recovered in the *Ha. sulcata* samples shared 99% identity with *R. raoultii* (accession MK304547). Based on phylogenetic analysis, rickettsial samples assembled in 2 monophyletic groups: *R. aeschlimannii*, the most evolved taxon, *R. raoultii*, its closest taxon, matched in one group; and italics and *R. slovaca* formed the sister group of the mentioned samples (Figure 3).

R. aeschlimannii, the predominant Rickettsia, was detected only in Hy. marginatum. Other species, R. raoultii,

Table 1 Tick collection and	l associated Ricketts	ia infection in study are	as of Zanjan Provin	ce, Islamic Republic of	Iran			
Tick species		Study areas			Host animals		Total collected ticks	Total Rickettsia infection
	Lowlands	Plains	Highlands	Cattle	Sheep	Goat	N(%; 95% CI)	N(%, 95% CI)
Dermacentor marginatus	12	24	11	5	39	3	47 (8.90; 6.61-11.61)	6 (12.77; 4.83-25.74)
Hyalomma aegyptium	4	3	Ω	7	4	1	12 (2.27; 1.18-3.94)	0 (0.00)
Hy. anatolicum	37	59	25	58	62	1	121 (22.92; 19.40-26.74)	2 (1.65; 0.20-5.84)
Hy. asiaticum	10	25	18	17	36	0	53 (10.04; 7.61-12.92)	0 (0.00)
Hy. detritum	12	6	21	0	42	0	42 (7.95; 5.79-10.60)	2 (4.74; 0.58-16.16)
Hy. dromedarii	11	16	19	37	7	7	46 (8.71; 6.45-11.45)	2 (4.35; 0.53-14.84)
Hy. marginatum	33	23	19	22	52	1	75 (14.20; 11.34-17.48)	16 (21.33; 12.71-32.32)
Hy. scupense	5	4	ŝ	12	0	0	12 (2.27; 1.18-3.94)	0 (0.00)
Hy. schulzei	7	9	ŝ	9	0	5	11 (2.08; 1.04-3.70)	0 (0.00)
Haemaphysalis sulcata	9	5	10	0	21	0	21 (3.98;2.48-6.02)	2 (9.52, 1.18-30.38)
Rhipicephalus annulatus	5	25	15	37	8	0	45 (8.52; 6.28-11.24)	0 (0.00)
Rh. bursa	6	21	13	18	25	0	43 (8.14; 5.96-10.81)	4 (9.30; 2.59-22.14)
Total N (%, 95% CI)	146 (27.65; 23.85- 31.68)	220 (41.67; 37.42-46.00)	162 (30.68; 26.77- 34.81)	219 (41.48; 37.24-45.81)	291 (55.11; 50.76- 59.41)	18 (3.41; 2.03- 5.33)	528 (100)	34 (6.44; 4.50-8.88)
Total Rickettsia Infection N(%; 95% CI)	28 (19.18; 13.14-26.51)	0 (0.00)	6 (3.70; 1.37-7.89)	4 (1.83; 0.50-4.61)	30 (10.31; 7.06-14.36)	0 (00.00)		

R. sibirica and *R. slovaca*, comprised about half of the rickettsial positive ticks. These *Rickettsiae* species infected more than one tick species (see Table 2).

Phylogenetic analysis of *R. aeschlimannii* gltA sequences identified 4 haplotypes: HI, HII, HIII and HIV, with the ratios of 44%, 25%, 18.5% and 12.5%, respectively (see Figure 4). Samples of the dominant haplotype (HI) and haplotype HIII were similar to the specimens from Egypt, Zambia and Senegal, as well as Mongolia, China and Kazakhstan.

HII and HIV were matched with the samples from Cyprus, Lebanon, Italy, France and Russia (Figure 5). Three types of *R. raoultii* matched the Chinese and Russian samples. Each of the *R. slovaca* and *R. sibirica* specimens was monotype.

The samples of *R. slovaca* were related to French and Slovakian specimens, and *R. sibirica* samples were linked to specimens from China and Mongolia, as well as Senegal (Figure 6). The fragments of *R. sibirica* we studied were completely conserved, and nucleotide analysis could not identify its current subspecies.

Discussion

This study revealed 4 *Rickettsia* species including *Dermacentor, Hyalomma, Haemaphysalis* and *Rhipicephalus* ticks from domestic animals in northwestern Islamic Republic of Iran. Despite the fact that this region is the main global route of migratory birds, to our knowledge, there has been no comprehensive study on the prevalence of *Rickettsia* species in this area.

While the tick collection method applied in this study has been used previously (12,20,21), the survey of engorged ticks is a limitation that may cause interference in the results. However, the presence of infective ticks and evidence of population exposure to these ticks may have flawed the extent of subclinical and acute forms of SFG rickettsioses in the study areas.

Our findings revealed the presence of rickettsial infected ticks in both lowlands and highlands, but not in the plains. Acaricides have been a commonly used to control ticks in the study areas for several years. However, technical, operational, and financial problems have caused heterogeneity in these areas. The absence of rickettsial infection in the ticks in the plains areas may be related to the geographical isolation of livestock, and complete coverage with acaricides.

Table 2 Rickettsia species infecting hard ticks from Zanjan Province, Islamic Republic of Iran									
Tick species	Rickettsia infecting ticks								
	R. aeschlimannii	R. raoultii	R. sibirica	R. slovaca	Total N(%)				
Dermacentor marginatus	0	2	0	4	6 (17.65)				
Hyalomma anatolicum	0	0	0	2	2 (5.88)				
Hy. detritum	0	0	2	0	2 (5.88)				
Hy. dromedarii	0	0	2	0	2 (5.88)				
Hy. marginatum	16	0	0	0	16 (47.05)				
Haemaphysalis sulcata	0	2	0	0	2 (5.88)				
Rhipicephalus bursa	0	2	2	0	4 (11.77)				
Total N(%)	16 (47.05)	6 (17.65)	6 (17.65)	6 (17.65)	34 (100)				

Figure 2 Multiple alignment of nucleotide sequences of Rickettsia htrA gene fragments in studied samples against reference Rickettsia species



Note: Accession number of reference strains is indicated in parentheses.

¹¹¹ 743-ZW.IR" (MT2833337) - R. aeschlimannii Ta7ZNJR* (MW219590) annii Taroom* (MW117936, .H. M. 1933391 sia (KU961540) R. aeschlim R. aeschij 2* (MT293342) f, des IR* (MW219591) c\$ R* (MT293343) 93345 ZNIR* (MW117935) R. conorii israelensis R98007S 594) 1ZNIR* (MW117934) R. sibirica s MW117933) MT293336) L'anne 14. 24 Mar. Mar. 2013 R 112ZN - R. raouthi Ta taZNAR+ (MT 203303) 🦵 R. raoultii Ta9ZN-JR* (MW219592) - R. raoultii TA1-ZN-IR* (MT293350) Ŗ R. raoultii 293347 .spina tatut IL BEERIN. TA2-ZN IR* (MT293351) (MW119589)

Figure 3 Phylogenetic tree constructed with partial sequences of gltA gene of Rickettsia species generated in this study

Note: The evolutionary history was inferred using the Maximum Likelihood method and Tamura-Nei model. The sequence of the reference Rickettsia species is indicated in red box.

H II (4)

Figure 4 TCS network of Rickettsia aecshlimannii gltA gene haplotypes based on all polymorphic sites

H I (7) H IV (2) H III (3)

Note: Each dash represents one single nucleotide difference between two neighbouring haplotypes. The number of each haplotype is denoted in parentheses.



Figure 5 Phylogenetic tree based on the sequences of PCR amplicons of the gltA gene of *Rickettsia aecshlimannii* detected in *Hyalomma marginatum* with the Maximum Likelihood (a) and Neighbor-Joining (b) methods

Note: Percentages indicate bootstrap values based on 1000 replicates. The reference sequences in this study are represented in red box.

This study showed the high infection rate of R. aeschlimannii in *Hy. marginatum* in a restricted lowland region, the countryside of Abbar City in Tarom County. It has long been known that Hy. marginatum is the main vector of *R. aeschlimannii* (22), and high prevalence of this pathogen has previously been reported (23,24).

Because the high tick infection rate increases the risk of *rickettsioses*, the localization of infected Hy. marginatum ticks in Tarom County of Zanjan Province should be considered in future investigations. This phenomenon may have arisen from the introduction of infected vector ticks by migratory birds and/or vertebrate hosts to this region. Consequently, further studies are recommended to elucidate such a likelihood.

Our findings indicate that 3 emergent Rickettsia species – *R. raoultii*, *R. sibirica and R. slovaca* – account for about half of the rickettsial-positive ticks in northwest Islamic Republic of Iran. *R. raoultii* has been detected in a wide range of tick genera (3,20,21,25) and human cases of this pathogen have been reported in Eurasia (3,28).

Recently, in the taxonomic revision of *Rickettsiae*, *R. raoultii* was identified as a subspecies of *R. conorii* (26);



Figure 6 Phylogenetic tree constructed with partial sequences of gltA gene of Rickettsia raoultii, R. slovaca, and R. sibirica generated in this study

Note: The evolutionary history was inferred using the Maximum Likelihood method and Tamura-Nei model. The sequence of the reference Rickettsia species is represented in the red box

however, this nomenclature is invalid (27). Recognition of *R. slovaca* in *De. marginatus* is in line with previous studies (28,29). As far as we know, this is the first report of *R. slovaca* presence in *Hy. anatolicum*. Detection of *R. raoultii* versus *Ha. sulcata* and *Rh. bursa*, and *R. sibirica* versus *Hy. spp* and *Rh. bursa* need to be confirmed.

The presence of *R*. *sibirica* in *Hy*. *detritum*, *Hy*. *dromedarii* and Rh. bursa ticks indicates the emergence of these species in the Islamic Republic of Iran. The low infection rate of this pathogen is related to its high pathogenicity in the vector ticks (3,30).

Two subspecies, namely *R. sibirica sibirica* and *R. sibirica mongolitimonae*, have been recognized for this *Rickettsia*. Cases of *R. sibirica* sibirica have been reported from North Asia in *Dermacentor* ticks (*31,32*). R. sibirica mongolitimonae infections have occurred in a wide region of southern Europe, the Middle East, North and Southern Africa, and Central Asia. This *Rickettsia* has been isolated from different tick species (*3,8*).

The presence of such emerging species as well as other *Rickettsia* species in tick infestations strongly indicates the need for increased surveillance of SFG rickettsioses,

development of diagnosis and treatment facilities, and vector management programmes in this area. Physicians need information on the occurrence of these diseases and related clinical symptoms to enhance their awareness and enable them to request for laboratory diagnostic confirmation if required. More efficient monitoring of these pathogens at local, national and international levels is imperative.

This study showed a relatively high level of rickettsial infection in *Hy. marginatum* and *De. marginatus* ticks. These ticks are commonly found in the Middle East and are passively transmitted over long distances by passerine migratory birds (33). The potential role of these ticks in the transmission of SFG rickettsioses should be taken into account in future investigations.

On behalf of the Iranian Ministry of Health and Medical Education and with the cooperation of the Ministry of Agriculture Jihad and Iran Veterinary Organization, public health officers should prepare health brochures that promote health culture and inform the population about potential emerging threats associated with these ticks.

Conclusion

Further investigation is needed to determine the potential role of *De. marginatus* and *Hy. marginatum* ticks in the transmission of SFG rickettsioses. Provision of health knowledge on emerging threats of infected ticks, surveillance of SFG rickettsioses, and development of tick control programmes are recommended to improve community health.

Funding: This study was approved by the Ethics Committee in Biomedical Research (ZUMS.REC.1397.155) and supported by the research project A-18-84-16 offered by Vice-Chancellor for Research and Technology of Zanjan University of Medical Sciences in Zanjan, Islamic Republic of Iran.

Competing interests: None declared.

Émergence d'infections à *Rickettsia* du groupe de la fièvre pourprée chez les tiques dures en République islamique d'Iran

Résumé

Contexte : Les rickettsioses transmises par les tiques sont devenues un problème de santé dans le monde entier suite à leur incidence croissante au cours des dernières décennies. Cependant, les informations concernant ces maladies sont limitées en République islamique d'Iran.

Objectif : La présente étude transversale a été menée afin d'évaluer l'infection à *Rickettsia* chez les tiques ixodidées prélevées sur les bovins, les ovins et les caprins en République islamique d'Iran.

Méthodes : L'ADN de ces tiques, prélevées dans 54 villages de la province de Zanjan (République islamique d'Iran) a été recueilli et analysé à l'aide d'un spectrophotomètre. Les échantillons positifs pour les rickettsies ont été sélectionnés en ciblant le gène *htrA* et des fragments du gène *gltA* ont été analysés. Les variables ont été étudiées à l'aide de statistiques descriptives et le test $\chi 2$ a été utilisé afin de procéder à leur comparaison.

Résultats : Au total, 528 tiques ont été testées. Dans l'ensemble, le taux d'infection à *Rickettsia* était de 6,44 %. Neuf des 12 espèces de tiques étaient infectées. Les taux positifs de rickettsies chez les tiques *Hyalomma marginatum* et les tiques *Dermacentor marginatus* étaient de 21,33 % et 12,77 % respectivement. *R. aeschlimannii*, la rickettsie prédominante, n'a été détectée que chez les tiques *Hy. marginatum*. *R. raoultii*, *R. sibirica* et *R. slovaca* se retrouvaient sur près de la moitié des tiques positives et ont été isolées sur de plusieurs espèces de tiques.

Conclusion : Compte tenu de la découverte de tiques infectées en République islamique d'Iran, il est nécessaire de mettre en place un programme de lutte contre ces dernières, en accordant une attention particulière aux populations à haut risque.

عدوى مجموعة الحُمَّى المُبَقَّعة المستجدة بالرِّيكِتْسِيَّة في القُراد الصلب بجمهورية إيران الإسلامية

محمد باقر غاوامي، زهرة علي باباي، محمد رضا جامافار، بهروز تاجيلوو

الخلاصة

الخلفية: أصبح داء الرَّيكتْسيَّات المَنْقول بالقُراد يُمثَّل قلقًا صحيًّا في جميع أنحاء العالم بعد تزايد معدلات الإصابة به في العقود الأخيرة. لكن لا توجد سوى معلومات محدودةَ عَن هَذه الأمراض في جمهورية إيران الإسلامية.

الأهداف: هدفت هذه الدراسة المقطعية الى تقدير نسبة العدوى بالرِّيكِتْسِيَّة في القراد اللَّبُود الذي جُمِع من الأبقار والأغنام والماعز في جمهورية إيران الإسلامية.

طرق البحث: نُجمع الحمض النووي للقراد اللَّبُود المأخوذ من الأبقار والأغنام والماعز في 54 قرية من محافظة زنجان، بجمهورية إيران الإسلامية، وحُلَّل بمقْياس الطيف الضَّوئِيّ. وفُحصت العينات الإيجابية المصابة بالرِّيكتْسيَّة من خلال استهداف الجين htrA وأجزاء من الجين glta. وحُلَّلت المتغيراتَ باستخدام الإحصاءات الوَصفية، واستُخدم اختبار X² للمقارنةَ بينَ المتغيرات.

التتائج: جرى اختبار 528 قُرَادَة. وبشكل عام، بلغ معدل العدوى بالرِّيكتْسيَّة 6,44٪. وأُصيبت تسعة من 12 نوعًا من القُراد. وبلغت معدلات الإصابة الإيجابية بالرِّيكتْسيَّة في القُراد من النوعين الزُّجاجي العَيْن الهامشيَّ وناخس الجلْد الهامشي 11,33 و12,77٪ على التوالي. وكُشف عن الرِّيكتْسيَّة الأشليمناي الأكثر شيوعًا فقط في القراد من النوع الزُّجاجي العَيْن الهامَشِي. بينَهَا أُصيبَ نحو نصف القُراد بالرِّيكتْسيَّة راولتي والرَّيكتْسيَّة سيبيريكا والرِّيكِتْسيَّة سلوفاكا، إذ وُجِدَت في أكثر من نوع من أنواع القُراد.

الاستنتاجات: في ضوء اكتشاف القُراد المصاب في جمهورية إيران الإسلامية، هناك حاجة إلى إنشاء برنامج لمكافحة القُراد في البلاد، *و*إيلاء الاهتهام للفئات السكانية الأكثر عرضة لخطر الإصابة به.

References

- 1. Blanton LS, Walker DH. Rickettsia rickettsii and Other Spotted Fever Group Rickettsiae (Rocky Mountain Spotted Fever and Other Spotted Fevers), in: Bennett JE, Dolin R, Blaser MJ (Eds): Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases, ninth edition, Elsevier, Philadelphia, 2020:2349-2357.
- 2. Estrada-Peña A, de la Fuente J, Latapia T, Ortega C. The Impact of Climate Trends on a Tick Affecting Public Health: A Retrospective Modeling Approach for Hyalomma marginatum (Ixodidae). PLoS One. 2015;10(5):e0125760. DOI: 10.1371/journal. pone.0125760.
- 3. Parola P, Paddock CD, Socolovschi C, Socolovschi C, Labruna MB, Mediannikov O et al. Update on tick-borne rickettsioses around the world: a geographic approach. Clin Microbiol Rev. 2013;26(4): 657–702. DOI: 10.1128/CMR.00032-13.
- 4. Eldin C, Parola P. Update on Tick-Borne Bacterial Diseases in Travelers. Curr Infect Dis Rep. 2018;20(7):1–9. DOI: 10.1007/s11908-018-0624-y.
- 5. Satjanadumrong J, Robinson MT, Hughes T, Blacksell SD. Distribution and Ecological Drivers of Spotted Fever Group Rickettsia in Asia. Ecohealth. 2019;16(4):611-626. DOI: 10.1007/s10393-019-01409-3
- 6. Spernovasilis N, Markaki I, Papadakis M, Mazonakis N, Ierodiakonou D. Mediterranean Spotted Fever: Current Knowledge and Recent Advances. Trop Med Infect Dis. 2021;6(4):172. DOI: 10.3390/tropicalmed6040172.
- 7. Keskin A, Bursali A. Detection of Rickettsia aeschlimannii and Rickettsia sibirica mongolitimonae in Hyalomma marginatum (Acari: Ixodidae) ticks from Turkey. Acarologia. 2016;56(4):533–536. DOI:10.1051/acarologia/20164140.
- 8. Ereqat S, Nasereddin A, Al-Jawabreh A, Azmi K, Harrus S, Mumcuoglu K et al. Molecular Detection and Identification of Spotted Fever Group Rickettsiae in Ticks Collected from the West Bank, Palestinian Territories. PLoS Negl Trop Dis. 2016;15;10(1):e0004348. DOI: 10.1371/journal.pntd.0004348.
- 9. Turebekov N, Abdiyeva K, Yegemberdiyeva R, Dmitrovsky A, Yeraliyeva L, Shapiyeva Z et al. Prevalence of Rickettsia species in ticks including identification of unknown species in two regions in Kazakhstan. Parasit Vectors. 2019;12(1):1–6. DOI: 10.1186/ s13071-019-3440-9.
- 10. Gargili A, Palomar AM, Midilli K, Portillo A, Kar S, Oteo JA. Rickettsia species in ticks removed from humans in Istanbul, Turkey. Vector Borne Zoonotic Dis. 2012;12(11):938–941. DOI: 10.1089/vbz.2012.0996.
- 11. Hosseini-Chegeni A, Tavakoli M, Telmadarraiy Z, Faghihi F. Molecular Detection of Spotted Fever Group Rickettsia (Rickettsia ales: Rickettsiaceae) in Ticks of Iran. Arch Razi Inst. 2020;75(3):317–325. DOI: 10.22092/ari.2019.125746.1317.
- 12. Farrokhnia M, Ghalejoogh ZY, Rohani M, Ghasemi A, Esmaeili S, Mostafavi E. Cases of Mediterranean spotted fever in southeast of Iran. Iran J Microbio. 2020;12(3):256–260. PMCID: PMC7340602.
- 13. Nadim A, Khanjani M, Hosseini-Chegeni A, Telmadarraiy Z. Identity and microbial agents related to Dermacentor marginatus Sulzer (Acari: Ixodidae) with a new record of Rickettsia slovaca (Rickettsiales: Rickettsiaceae) in Iran. Syst Appl Acarol. 2021;26(2):367–378. DOI:10.11158/saa.26.2.4.
- 14. Ghasemi A, Latifian M, Esmaeili S, Naddaf SR, Mostafavi E. Molecular surveillance for Rickettsia spp. and Bartonella spp. in ticks from Northern Iran. Plos One. 2022;17(12):e0278579. DOI: 10.1371/journal.pone.0278579.
- 15. Khamesipour F, Dida GO. Anyona DN, Razavi SM, Rakhshandehroo E. Tick-borne zoonoses in the Order Rickettsiales and Legionellales in Iran: A systematic review. PLoS Negl Trop Dis. 2018;11;12(9):e0006722. DOI: 10.1371/journal.pntd.0006722.
- 16. Estrada-Peña A, Bouattour A, Camicas J-L, Walker AR. Ticks of Domestic Animals in the Mediterranean Region. A Guide to Identification of Species. International Consortium on ticks and tick borne diseases (ICTTD-2). INCO-DEV Programme, European Union. 2004.
- 17. Hosseini-Chegeni A, Tavakoli M, Telmadarraiy Z. The updated list of ticks (Acari: Ixodidae & Argasidae) occurring in Iran with a key to the identification of species. Syst Appl Acarol. 2019;24(11):2133–2166. DOI:10.11158/saa.24.11.8.
- 18. Ghavami MB, Mirzadeh H, Mohammadi J, Fazaeli A. Molecular survey of ITS1 spacer and Rickettsia infection in human flea, Pulex irritans. Parasitol Res. 2018;117(5):1433–1442. DOI: 10.1007/s00436-018-5768-z.
- 19. Biernat B, Stańczak J, Michalik J, Sikora B, Cieniuch S. Rickettsia helvetica and R. monacensis infections in immature Ixodes ricinus ticks derived from sylvatic passerine birds in west-central Poland. Parasitol Res. 2016;115(9):3469–3477. DOI: 10.1007/ s00436-016-5110-6.
- 20. Cicculli V, Oscar M, Casabianca F, Villechenaud N, Charrel R, de Lamballerie X et al. Molecular Detection of Spotted-Fever Group Rickettsiae in Ticks Collected from Domestic and Wild Animals in Corsica, France. Pathogens. 2019;8(3):138. DOI: 10.3390/ pathogens8030138.
- 21. Guo WP, Wang YH, Lu Q, Xu G, Luo Y, Ni X et al. Molecular detection of spotted fever group rickettsiae in hard ticks, northern China. Transbound Emerg Dis. 2019;66(4):1587–1596. DOI: 10.1111/tbed.13184.
- 22. Beati L, Meskini M, Thiers B, Rickettsia aeschlimannii sp. nov., a new spotted fever group rickettsia associated with Hyalomma marginatum ticks. Int J Syst Bacteriol. 1997;47(2):548–554. DOI: 10.1099/00207713-47-2-548.
- 23. Shpynov S, Rudakov N, Tohkov Y, Matushchenko A, Tarasevich I, Raoult D et al. Detection of Rickettsia aeschlimannii in Hyalomma marginatum ticks in western Russia. Clin Microbiol Infect. 2009;15:315–316. DOI: 10.1111/j.1469-0691.2008.02256.x.
- 24. Fernández-Soto P, Encinas-Grandes A, Pérez-Sánchez R. Rickettsia aeschlimannii in Spain: molecular evidence in Hyalomma marginatum and five other tick species that feed on humans. Emerg Infect Dis. 2003;9(7):889–890. DOI: 10.3201/eid0907.030077.

- 25. Igolkina Y, Krasnova E, Rar V, Savelieva M, Epikhina T, Tikunov A et al. Detection of causative agents of tick-borne rickettsioses in Western Siberia, Russia: identification of Rickettsia raoultii and Rickettsia sibirica DNA in clinical samples. Clin Microbiol Infect. 2018;24(2):199.e9–199.e12. DOI: 10.1016/j.cmi.2017.06.003.
- 26. Hördt A, López MG, Meier-Kolthoff JP, Schleuning M, Weinhold LM, Tindall BJ et al. Analysis of 1,000+ Type-Strain Genomes Substantially Improves Taxonomic Classification of Alphaproteobacteria. Front Microbiol. 2020;11:468. DOI: 10.3389/ fmicb.2020.00468.
- 27. Oren A, Garrity GM. List of new names and new combinations that have appeared in effective publications outside of the IJSEM and are submitted for valid publication. Int J Syst Evol Microbiol. 2020;70(11):5596–5601. DOI: 10.1099/ijsem.0.004484.
- 28. Selmi M, Ballardini M, Salvato L, Ricci E. Rickettsia spp. in Dermacentor marginatus ticks: analysis of the host-vector-pathogen interactions in a northern Mediterranean area. Exp Appl Acarol. 2017;72(1):79–91. DOI: 10.1007/S10493-017-0132-z.
- 29. Pluta S, Tewald F, Hartelt K, Oehme R, Kimmig P, Mackenstedt U. Rickettsia slovaca in Dermacentor marginatus ticks, Germany. Emerg Infec Dis. 2009;15(12):2077–2078. DOI: 10.3201/eid1512.090843.
- 30. Toledo A, Olmeda AS, Escudero R, Jado I, Valcárcel F, Casado-Nistal MA et al. Tick-borne zoonotic bacteria in ticks collected from central Spain. Am J Trop Med Hyg. 2009;81(1):67–74. PMID: 19556569.
- 31. Rudakov N, Shpynov S, Fournier P-E, Raoult D. Ecology and molecular epidemiology of tick-borne rickettsioses and anaplasmoses with natural foci in Russia and Kazakhstan. Ann NY Acad Sci. 2006;1078(1):299–304. DOI: 10.1196/annals.1374.009.
- 32. Cao W-C, Zhan L, De Vlas SJ, Wen B-H, Yang H, Richardus JH et al. Molecular detection of spotted fever group Rickettsia in Dermacentor silvarum from a forest area of northeastern China. J Med Entomol. 2008;45(4):741–744. DOI: 10.1603/0022-2585(2008)45[741:mdosfg]2.0.co;2.
- 33. Chitimia-Dobler L, Schaper S, Riess R, Bitterwolf K, Frangoulidis D, Bestehorn M et al. Imported Hyalomma ticks in Germany in 2018. Parasites Vectors. 2019;12(1):1–9. DOI: 10.1186/s13071-019-3380-4.