J Med Microbiol Infect Dis, 2018, 6 (4): 108-111
DOI: 10.29252/JoMMID.6.4.108

Original Article

No Detection of Crimean Congo Hemorrhagic Fever (CCHF) Virus in Ticks from Kerman Province of Iran

Sahar Khakifirouz[1,2], Seyed Javad Mowla[2], Vahid Baniasadi[1], Mehdi Fazlalipour[1], Tahmineh Jalali[1], Seyedeh Maryam Mirghiasi[1], Mostafa Salehi-Vaziri[1,3]*

[1]Department of Arboviruses and Viral Hemorrhagic Fevers (National Ref. Lab), Pasteur Institute of Iran, Tehran, Iran; [2]Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran; [3]Research Centre for Emerging and Reemerging Infectious Disease, Pasteur Institute of Iran, Tehran, Iran

Received Mar. 03, 2019; Accepted Apr. 28, 2019

Introduction: Crimean Congo Hemorrhagic Fever (CCHF) is a fatal tick-borne viral zoonosis with a case fatality rate of 5% to 30%. CCHF has been documented as the most frequent tick-borne viral infection in Iran with more than 50 cases annually. Kerman Province in the south of Iran is one of the CCHF-endemic areas of the country, but no data on infection of ticks with this virus from this area is available. This study aimed to investigate the CCHFV infection among ticks collected from 4 different counties in this province. **Methods**: In 2011, a total of 203 hard ticks were collected from Kerman, Jiroft, Sirjan, and Kuhbanan counties in Kerman Province, southeast of Iran. Infection of ticks with CCHFV was investigated using RT-PCR targeting the small segment of the viral genome. **Results:** Out of 203 ticks, *Dermacentor* (50.24%) was the most frequent genus followed by *Hyalomma* (39.39%), *Haemaphysalis* (9.85%) and *Rhipicephalus* (0.49%). Our results showed no CCHFV infection of ticks. **Conclusion**: Our finding indicates no circulation of CCHFV in ticks from Kerman Province. However, as Kerman Province is an endemic region for CCHF, further investigations are needed to have a better understanding of the CCHFV vectors in this region. *J Med Microbiol Infec Dis, 2018, 6 (4): 108-111.*

Keywords: Crimean Congo Hemorrhagic Fever, Tick-Borne Diseases, Ixodidae, Iran.

INTRODUCTION

Crimean-Congo Hemorrhagic Fever (CCHF) is a virulent emerging tick-borne viral zoonotic disease occurring in vast geographical areas including 31 countries in Africa, Europe, Asia, and the Middle East. The causative agent, CCHF virus (CCHFV) is a three segmented negative-sense RNA virus classified in the genus *Orthobunyavirus*, family *Nairoviridae*, order *Bunyavirales* [1-3]. Infection with CCHFV can lead to a severe hemorrhagic fever in humans with a case fatality rate of 5-50%. In some outbreaks, mortality rates of up to 80% were reported [4]. In nature, the virus is perpetuated in an enzootic cycle between ticks and vertebrates. People acquire infection via the accidental infective tick bites, or through direct contact with blood or tissues of viremic animals or patients [4]. Hard ticks, especially the members of the genus *Hyalomma*, are considered as both reservoir, and vector of CCHFV. The virus can spread via transstadial, transsexual and transovarial transmission routes among ticks. Also, ticks might acquire the infection via co-feeding, *i.e.*, the transmission of the virus from infected ticks to uninfected ticks during simultaneous blood feeding on the same host [5, 6].

Iran, located in the Middle East, is one of the endemic countries for CCHF with more than 50 confirmed human cases per year [7]. CCHF has been documented in almost all provinces of the country, with highly endemic areas including Sistan and Baluchestan, Khorasan Razavi, Fars, Esfahan, and Kerman [8, 9]. Unlike human infections, not much data on tick vectors is available from Iran, and a few studies have investigated CCHFV infection among ticks. As ticks play a significant role in the circulation of the virus, the study of the tick fauna and their rate of infection with CCHFV can provide useful information on the epidemiology of CCHFV in a region. Therefore, this study aimed to investigate the potential tick vectors for CCHFV in Kerman Province, southeast of Iran.

MATERIAL AND METHODS

Study Area. During the past two decades, Kerman Province has been reported as one of the highly endemic areas for CCHFV infection. Kerman is the largest province of Iran covering an area of 182,000 km² and is 400-600 m above sea level. Located in the southeast of the country, this province shares borders with five provinces including Yazd and South Khorasan in the north, Sistan and

***Correspondence:** Mostafa Salehi-Vaziri
Department of Arboviruses and Viral Hemorrhagic Fevers (National Reference Laboratory), Pasteur Institute of Iran, No. 69, Pasteur Ave, Tehran, Iran, 1316943551.
Email: mostafavaziri1985@gmail.com
Tel/Fax: +98 (21) 64112821

Baluchestan in the east, Hormozgan in south and Fars Province in the west. The climate varies in different areas of this province. In the north of the province, the weather is dry and hot, while in the south it is mild and pleasant.

The study was conducted in four counties of Kerman Province, including Kerman, Jiroft, Sirjan, and Kuhbanan (Fig. 1).

Sample Collection. We collected ticks during June, July and August 2011, at the pick of their activity. During several visits to each county, hard ticks were randomly collected by hand from the cattle, goats, and sheep as described previously [10]. Briefly, after wearing gloves, overall and mask, the entire body of animals were searched, and ticks were collected by forceps and transferred to 2-ml Eppendorf tubes. The specimens data including locality, host species, and date of sampling were recorded on the collection tubes. The specimens were kept at -80°C until sent to the Department of Medical Entomology, School of Public Health, Teheran University of Medical Sciences. The ticks were examined under a stereomicroscope with a magnification of ×10 and identified to species based on morphological features reflected in taxonomical keys [11]. The specimens were transported to the Department of Arboviruses and Viral Hemorrhagic Fevers (National Reference Laboratory), Pasteur Institute of Iran for molecular detection of CCHFV infection.

CCHF Virus RT-PCR. The ticks were washed twice with sterile PBS and crushed individually with a mortar and pestle in 200–300 µl of PBS (pH=7.4). Total RNA was extracted by RNeasy mini kit (QIAGEN, Germany) according to the manufacturer's instructions. The extracted RNAs were stored at -70ºC until used. A CCHFV positive human serum sample was included in all extraction procedure to ensure the quality of RNA.

RT-PCR mix contained 10 µl 5x OneStep RT-PCR Buffer (QIAGEN, Germany), 400 µM dNTPs, 2 µl of enzyme mixture containing reverse transcriptase and *Taq* DNA polymerase enzymes, 0.6 µM of each primer F2 (5'-TGGACACCTTCACAAACTC-3), and R3 (5'-GAC-AATTCCCTACACC-3'), 1 µl RNase inhibitor, 5 µl extracted RNA, and RNase free distilled water to a final volume of 50 µl. The F2 and R3 primers amplify a 536 bp fragment of the small segment of the CCHFV genome. Sterile distilled water and RNA extracted from a serum sample of a confirmed CCHF patient were included in all assays as the negative and positive controls, respectively. The thermal cycling program for the RT-PCR, included an initial cycle of 30 min at 50°C for reverse transcription reaction (cDNA synthesis), followed by 15 min at 95°C for activation of Hot Star *Taq* DNA polymerase and inactivation of reverse transcriptase, and 40 cycles of 94°C for 30 s, 50°C for 30 s, 75°C for 45 s, and a final extension at 72°C for 10 min [12].

Ethical Approval. The ethical approval for this study was obtained from the Ethical Review Committee, Tarbiat Modares University, Iran, Tehran on 10 November 2015 (Reference No. 142D/1995).

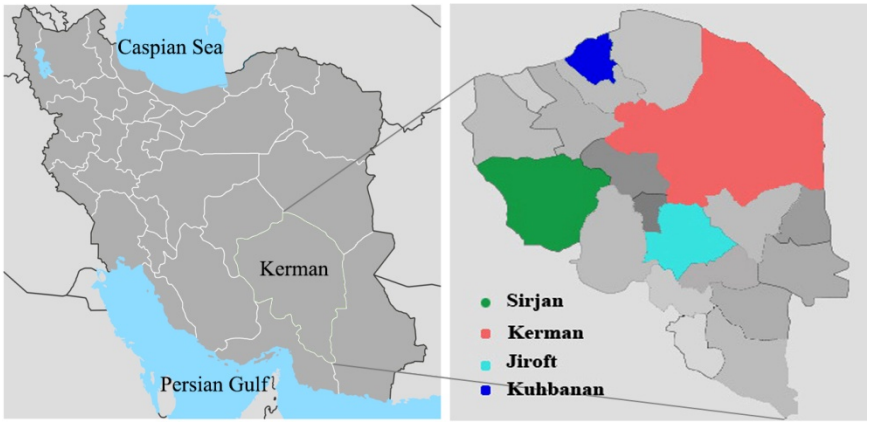

Fig. 1. The four counties in Kerman Province, Iran from which the hard ticks were collected

RESULTS

In this study, 203 hard ticks were collected from 4 different regions of Kerman Province, including 50 ticks from Kerman (from 11 goats and 39 sheep), 23 from Jiroft (from 21 goats and 2 sheep), 59 from Kuhbanan (from 1 goat and 58 sheep) and 71 from Sirjan (from 11 goats, 54 sheep and 6 cows) (Table 1).

We identified 4 genera including *Dermacentor* (50.24%), *Hyalomma* (39.39%), *Haemaphysalis* (9.85%) and *Rhipicephalus* (0.49%). *Hyalomma* genus comprised *H. marginatum* (N=51), *H. asiaticum* (N=15), *H. anatolicum* (N=12), and non-identified species of *Hyalomma* (N=2).

Hyalomma asiaticum (N=15, 65.21%), *D. marginatus* (N=30, 60%), *D. marginatus* (N=36, 61.01%) and *D. marginatus* (N=36, 50.70%) were the most common tick species in Jiroft, Kerman, Kuhbanan, and Sirjan, respectively (Table 2). The most common species collected from the cattle was *H. marginatum* (N=5, 83.33%), from sheep, was *D. marginatus* (N=102, 66.66%) and from the goat was *H. asiaticum* and *H. marginatum* (N=15, 34.09%) (Table 1). According to RT-PCR results, none of the 203 samples was positive for CCHFV infection (Fig 2).

Table 1. Frequency of tick species according to their relevant host

Tick Species	Animal Host			Total
	Cow, N (%)	Sheep, N (%)	Goat, N (%)	
D. marginatus	0	102 (66.66)	0	102
H. marginatum	5 (83.33)	31 (20.26)	15 (34.09)	51
Ha. Sulcata	0	16 (10.45)	4 (9.09)	20
H. asiaticum	0	0	15 (34.09)	15
H. anatolicum	1 (16.67)	4 (2.61)	7 (15.90)	12
***Hyalomma* spp.**	0	0	2 (4.54)	2
R. Sanguineus	0	0	1 (2.27)	1
Total	6 (100)	153 (100)	44 (100)	203

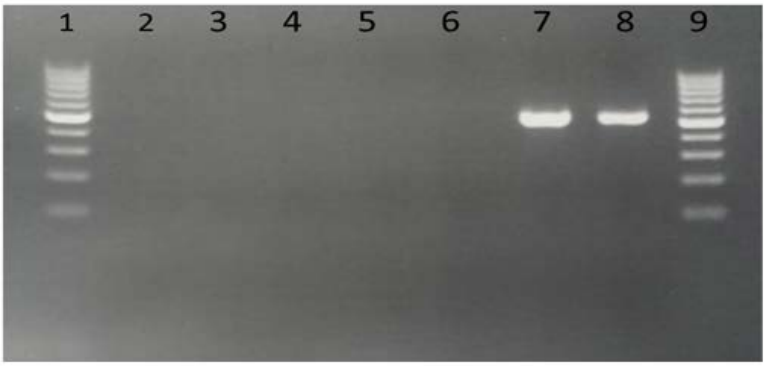

Fig. 2. CCHFV RT-PCR results. Lanes 1 and 9, 100 bp DNA ladder; Lanes 2-6, Tick specimens; Lane 7, Extraction positive control; Lane 8, RT-PCR positive control.

Table 2. Frequency of tick species in Kerman Province, Iran, based on counties and the gender

Tick Species	County				Female, N (%)	Male, N (%)
	Kerman, N (%)	Sirjan, N (%)	Jiroft, N (%)	Kuhbanan, N (%)		
D. marginatus	30 (60)	36 (50.70)	0 (0)	36 (61)	45 (22.16)	57 (28.07)
H. anatolicum	6 (12)	1 (1.40)	4 (17.40)	1 (1.70)	4 (1.97)	8 (3.94)
H. marginatum	5 (10)	31 (43.67)	0	15 (25.43)	17 (8.37)	34 (16.73)
Ha. sulcata	9 (18)	3 (4.23)	2 (8.70)	6 (10.17)	6 (2.95)	14 (6.89)
***Hyalomma* spp.**	0 (0)	0 (0)	2 (8.70)	0 (0)	2 (0.98)	0 (0)
R. sanguineus	0 (0)	0 (0)	0 (0)	1 (1.70)	1(0.49)	0 (0)
H. asiaticum	0 (0)	0 (0)	15 (65.20)	0 (0)	7 (3.44)	8 (3.94)
Total	50 (100)	71 (100)	23 (100)	59 (100)	82 (40.36)	121 (59.57)

DISCUSSION

In this study, we analyzed hard tick samples collected from Kerman Province for the presence of CCHFV infection. Although the hard tick samples were collected from various geographic areas in this province, the viral genome was detected in none of the samples. As Kerman is one of the main foci of CCHF in Iran, we expected a high CCHFV infection of hard ticks. In this study, although all steps were performed according to the standard protocols of the national reference laboratory, no CCHFV infection in ticks was observed. Our negative results can be explained by the following reasons: 1) mishandling of tick specimens during transportation might have resulted in a low yield of RNA, 2) collection of ticks from suburban areas and not from rural areas where the majority of cases occur.

Based on the previous studies, the rate of CCHFV infection among hard ticks in neighboring provinces of Kerman, including Yazd, and Sistan and Baluchestan was around 5% [13, 14]. In a study conducted from 2008 to 2009 in Yazd Province, 8 out of 140 *Hyalomma* ticks (5.7%) were positive for CCHFV infection [14]. Moreover, Mehravaran and colleagues (2011) reported almost a similar rate (4.3%) in ticks of Sistan and Baluchestan [13]. In their study, a total of 140 *Ixodidae* and *Argasidae* ticks were examined for CCHFV infection, of which 6 *Ixodidae* ticks (5 *Hyalomma* spp. and 1 *H. inermis*) were positive [13]. In contrast, the rate of CCHFV infection among ticks of northern provinces is higher [15, 16]. In 2011, in Ardabil Province, CCHFV RNA was detected in 36 out of 130 hard and soft ticks (27.7%) including *H. aegyptium* (n=1), *H. marginatum* (n=1), *H. schulzei* (n=1), *Hyalomma* spp. (n=6), *R. bursa* (n=18) and *Ornithodoros lahorensis* (n=9) [16]. Also in Hamadan, 63 out of 328 hard and soft ticks (19.2%) including *Argas reflexus, H. anatolicum, H. detritum,* and *R. Sanguineus* were positive for CCHFV [15]. Therefore, regarding the low CCHFV infection rate among ticks in southern areas compared to northern provinces, a more significant number of ticks should be checked to detect the virus.

The majority of CCHFV infection in Iran is due to direct contact with blood or tissues of viremic livestock [17]. In comparison with tick bites, CCHFV transmission

associated with close exposure to livestock is more common in eastern parts of Iran; most of the livestock imported from neighboring countries pass through these areas [8]. This data alongside our finding indicates that tick bites do not play a significant role in CCHFV transmission to human in Kerman Province.

To best of our knowledge, the present study is the first investigating CCHFV infection among ticks of Kerman Province. Although the result of the present study suggests the low risk of CCHF transmission via tick bites, further investigation with larger sample sizes are required to elucidate this hypothesis.

ACKNOWLEDGMENT

The authors express their gratitude to Dr. ZakkyehTelmadarraiy and the personnel of the department of Arboviruses and Viral Hemorrhagic Fevers (National Reference Laboratory), Pasteur Institute of Iran for their technical support. This work was funded by Tarbiat Modares University, Iran, Tehran.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

REFERENCES

1. Maes P, Alkhovsky SV, Bào Y, Beer M, Birkhead M, Briese T, et al. Taxonomy of the family Arenaviridae and the order Bunyavirales: update 2018. Arch Virol. 2018; 163 (8): 2295-310.

2. Spengler JR, Bergeron É, Spiropoulou CF. Crimean-Congo hemorrhagic fever and expansion from endemic regions. Curr Opin Virol. 2019; 34: 70-8.

3. Blair PW, Kuhn JH, Pecor DB, Apanaskevich DA, Kortepeter MG, Cardile AP, et al. An emerging biothreat: Crimean-Congo hemorrhagic fever virus in southern and western Asia. Am J Trop Med Hyg. 2019; 100 (1): 16-23.

4. Bente DA, Forrester NL, Watts DM, McAuley AJ, Whitehouse CA, Bray M. Crimean-Congo hemorrhagic fever: history, epidemiology, pathogenesis, clinical syndrome and genetic diversity. Antiv Res. 2013; 100 (1): 159-89.

5. Gargili A, Thangamani S, Bente DA. Influence of laboratory animal hosts on the life cycle of *Hyalomma marginatum* and implications for an in vivo transmission model for Crimean-Congo hemorrhagic fever virus. Front Cell Infect Microb. 2013; 3: 39.

6. Hoogstraal H. The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe, and Africa. J Med Entomol. 1979; 15 (4): 307-417.

7. Ahmadkhani M, Alesheikh AA, Khakifirouz S, Salehi-Vaziri M. Space-time epidemiology of Crimean-Congo hemorrhagic fever (CCHF) in Iran. Ticks Tick-Borne Dis. 2018; 9 (2): 207-16.

8. Mostafavi E, Haghdoost A, Khakifirouz S, Chinikar S. Spatial analysis of Crimean Congo hemorrhagic fever in Iran. Am J Trop Med Hyg. 2013; 89 (6): 1135-41.

9. Champour M, Chinikar S, Mohammadi G, Razmi G, Shah-Hosseini N, Khakifirouz S, et al. Molecular epidemiology of Crimean–Congo hemorrhagic fever virus detected from ticks of one humped camels (Camelus dromedarius) population in northeastern Iran. J Parasit Dis. 2016; 40 (1): 110-5.

10. Kayedi MH, Chinikar S, Mostafavi E, Khakifirouz S, Jalali T, Hosseini-Chegeni A, et al. Crimean–Congo hemorrhagic fever virus clade iv (Asia 1) in ticks of western Iran. J Med Entomol. 2015; 52 (5): 1144-9.

11. Estrada-Pena A, Bouattour A, Camicas J, Walker A. Ticks of domestic animals in the Mediterranean region. University of Zaragoza, Spain. 2004; 131.

12. Fazlalipour M, Baniasadi V, Mirghiasi SM, Jalali T, Khakifirouz S, Azad-Manjiri S, et al. Crimean-Congo hemorrhagic fever due to consumption of raw meat: a case reports from East-North of Iran. Jpn J Infect Dis. 2016: JJID. 2015.498.

13. Mehravaran A, Moradi M, Telmadarraiy Z, Mostafavi E, Moradi AR, Khakifirouz S, et al. Molecular detection of Crimean-Congo haemorrhagic fever (CCHF) virus in ticks from southeastern Iran. Ticks Tick-Borne Dis. 2013; 4 (1-2): 35-8.

14. Yaser SA, Sadegh C, Zakkyeh T, Hassan V, Maryam M, Ali OM, et al. Crimean–Congo hemorrhagic fever: a molecular survey on hard ticks (Ixodidae) in Yazd Province, Iran. Asian Pac J Trop Med. 2011; 4 (1): 61-3.

15. Tahmasebi F, Ghiasi SM, Mostafavi E, Moradi M, Piazak N, Mozafari A, et al. Molecular epidemiology of Crimean-Congo hemorrhagic fever virus genome isolated from ticks of Hamadan Province of Iran. J Vector Dis. 2010; 47 (4): 211-7.

16. Telmadarraiy Z, Ghiasi SM, Moradi M, Vatandoost H, Eshraghian MR, Faghihi F, et al. A survey of Crimean-Congo haemorrhagic fever in livestock and ticks in Ardabil Province, Iran during 2004–2005. Scand J Infect Dis. 2010; 42 (2): 137-41.

17. Salehi-Vaziri M, Baniasadi V, Jalali T, Mirghiasi SM, Azad-Manjiri S, Zarandi R, et al. The first fatal case of Crimean-Congo hemorrhagic fever caused by the AP92-like strain of the Crimean-Congo hemorrhagic fever virus. Jpn J Infect Dis. 2016; 69 (4): 344-6.