Asian Biomedicine Vol. 10 No. 6 December 2016; 603 - 607 DOI: 10.5372/1905-7415.1006.530

Brief communication (Original)

Molecular epidemiology of Crimean–Congo hemorrhagic fever virus in ticks collected from western Iran

Taher Mehran[a], Dayer Mohammad Saaid[a], Jalali Tahmineh[b,c], Khakifirouz Sahar[b], Telmadarraiy Zakkyeh[d], Salehi-Vaziri Mostafa[b]

[a]*Department of Parasitology and Medical Entomology, Tarbiat Modares University, Tehran 14115-331, Iran*

[b]*Laboratory of Arboviruses and Viral Hemorrhagic Fevers (National Reference Laboratory), Pasteur Institute of Iran, Tehran, Iran*

[c]*Department of Biology, Shahed University, Tehran, Iran*

[d]*Department of Entomology, Tehran University of Medical Science, Tehran, Iran*

Background: Crimean–Congo hemorrhagic fever (CCHF) is a tick-borne viral disease that is endemic in Africa, southeastern Europe, and Asia. Ticks are both the reservoir and the vector of the CCHF virus (CCHFV). Determining the virus infection rate of the tick population in different geographical regions is necessary to design public health policies to prevent CCHF outbreaks.

Objectives: To determine the prevalence, seasonal activity, and the frequency of CCHFV infection of the tick population in Hamadan province of west Iran.

Methods: This cross-sectional study was conducted in 3 counties of Hamadan Province from June 2013 to May 2014. The study areas included both lowland (plains) and highland (mountains) and covered 5% of the villages where 10 herds per village of sheep and goats were randomly selected for hard tick collection.

Results: We examined 983 sheep and goats, and 881 ticks were collected and identified before being preserved for molecular tests. The collected ticks belonged to 3 genera including, *Rhipicephalus* (95.6%, n = 842), *Hyalomma* (4.1%, n = 36) and *Haemaphysalis* (0.4%, n = 4). After species identification, 100 randomly selected ticks were analyzed using reverse transcriptase–polymerase chain reaction (RT-PCR) to detect viral infection. CCHFV infection was observed in 7 collected ticks, of which 4 belonged to *R. sanguineus*, 2 belonged to *R. bursa*, and one *Hy. asiaticum*.

Conclusions: *Hyalomma* and *Rhipicephalus* ticks are the main vectors of CCHFV in Hamadan province where CCHF is focal and endemic.

Keywords: Crimean–Congo hemorrhagic fever, Hamadan, Iran, molecular epidemiology, tick-borne diseases, zoonotic viral disease

Crimean–Congo hemorrhagic fever (CCHF) is a potentially severe viral disease with a case–fatality rate of 30%–50% [1]. The etiology of the disease was first described in the Crimea in 1944 and currently is endemic in Europe, Africa, Central Asia, and Middle East [2-6]. The causative agent of the disease is the Crimean–Congo hemorrhagic fever virus (CCHFV) belonging to the genus Nairovirus of the family Bunyaviridae [7]. Because of its epidemic potential, high mortality rate, nosocomial outbreaks, and difficulties in treatment and prevention, CCHF outbreaks are considered to be a serious threat to public health. Iran is recognized as a major focus of CCHF in the Middle East where the disease was reported from different Iranian provinces including Fars, Khorasan, and Yazd provinces [1, 8-10].

The CCHF virus is transmitted to humans either by the bite of an infected tick or through direct contact with blood or tissues of viremic livestock animals or humans [11, 12]. Given that ticks are both the reservoir and the vector of the CCHFV, they play an important role in CCHF epidemiology. In nature, the CCHF virus is maintained by hard ticks (Ixodidae). Despite the notion that *Hyalomma* species are the main vectors of CCHF virus, other ticks including *Rhipicephalus*, *Haemaphysalis*, and *Dermacentor* can act as reservoirs of this virus in Iran [13-15].

Correspondence to: Mohammad Saaid Dayer, Department of Parasitology and Entomology, Tarbiat Modares University, Tehran, Iran. Tarbiat Modares University, Tehran 14115-331, Iran. E-mail: dayer@modares.ac.ir

Determining the infection rate of the tick population in different geographical regions and identification of tick fauna are critical for designing public health strategies to avoid CCHF outbreaks. Therefore, this study was designed to collect hard ticks from livestock and to detect CCHFV infection within the collected ticks in 3 counties namely Asadabad, Nahavand, and Famnin in Hamadan province, west of Iran.

Materials and methods

Study area and sampling

Hamadan province is located in the west of Iran, placed between 45°32′ and 48°E and 34°47′ and 35°1′ N. Despite its high altitude and cold climate, Hamadan is one of the main animal farming centers in Iran. Therefore, a large number of its residents are in direct contact with livestock and exposed to an increased risk of CCHFV infection.

After approval by the Ethics Committee of Tarbiat Modares University (reference No. 52/6855) observing the national guidelines on animal care, including the wellbeing of the animals during sampling, this study was conducted during June 2013 to May 2014. Sampling was performed in 5% of villages randomly selected in Asadabad, Nahavand, and Famnin counties after documented informed consent from the property/farm owners for collecting ticks from the live host animals. Provincial authority approval from the Health Center of Assadabad County under the Hamadan University of Medical Sciences (reference No. 16/41/144) was issued for the researchers to refer to local veterinary and health care offices for assistance and coordination. In total, 881 ticks were collected from plain (Asadabad and Famnin) and mountain (Nahavand) region in Hamadan province. Sample collection was conducted according to Tahmasebi et al. [14]. Collected ticks were kept alive in separate labeled vials. After species determination, 100 randomly selected hard ticks were sent to the Laboratory of Arboviruses and Viral Hemorrhagic Fevers (National Reference Laboratory) Pasteur Institute of Iran, to detect CCHFV infection.

RNA extraction

Ticks were separately washed twice with phosphate-buffered saline, pH 7.4 (PBS) and then crushed using a mortar and pestle in 200 µl of PBS. Total RNA of ticks was extracted using RNeasy plus Mini Kits (Cat. No. 74136, Qiagen, Venlo, The Netherlands) according to the manufacturer's protocol. The extracted total RNA was stored at –70°C until further analysis [16].

Reverse transcriptase–polymerase chain reaction (RT-PCR)

To amplify the genomic RNA of CCHFV, a OneStep RT-PCR Kit (Cat. No. 210212, Qiagen) was employed. All components were mixed to prepare the master mix as follows: RNase free water 28 (L), 5× Qiagen OneStep RT-PCR Buffer (10 µL), dNTP Mix (containing 10 mM of each dNTP) 2 (µL), Iran-F2 primer 5′-TGGACACCTTCACAAACTC-3′ (10 µM) 1 µL, Iran-R3 5′-GACAATTCCCTACACC-3′ primer (10 µM) 1 µL, enzyme mix (2 µL), and RNase inhibitor 0.5 µL (Cat. No. 10777-019, Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). Finally, 5 µL of template was added to the tube and the thermal-cycler program was started. The cycling parameters were as follows: reverse transcription for 30 min at 50°C, 95°C for 15 min as hot start, 35 cycles of 30 s of denaturation at 95°C, 30 s of annealing at 50°C, 45 s of elongation at 72°C, with final elongation of 5 min at 72°C.

Product analysis

We examined 5 µL of the PCR amplicons on an 1.5% agarose gel using gel loading buffer with DNA stain, (Cat. No. PCR-255-bl, Jena Bioscience, Germany) and Gene ruler 100 bp ladder (Cat. No. SM0242, Thermo Scientific) as a molecular-weight size marker.

Statistical analysis

The data were analyzed using SPSS Statistics for Windows, version 20 (IBM Corp, Armonk, NY, USA) and $P < 0.05$ was considered significant.

Results

A total of 881 hard ticks were collected from 141 sheep and goats in the present study. All ticks belonged to 3 genera including, *Rhipicephalus* (95.6%, n = 842), *Hyalomma* (4.1%, n = 36) and *Haemaphysalis* (0.4%, n = 4). The most frequently detected species was *R. sanguineus* (65.1%, n = 574), followed by *R. bursa* (29.3%, n = 258). The remaining ticks were as follows: *R. sp* (0.9%, n = 8,), *Hy. anatolicum* (1.6%, n = 14), *Hy. asiaticum* (1%, n = 9), *Hy. dromedarii* (0.68%, n = 6), *Hy. marginatum* (0.1%, n = 1), *Hy. schulzei* (n = 1, 0.1%), *Hy. Sp* (n = 5, 0.56%), and *Hae. sulcata*

(n = 3, 0.34%). The highest hard tick activity was observed during June (n = 703, 79.8%) followed by May (n = 71, 8.1%) and July (n = 68, 7.7%), whereas the lowest density of the hard ticks was seen in November (n = 3, 0.34%), October (n = 4, 45%), and August (n = 7, 0.8%). No tick was found in the study areas from December to April.

The RT-PCR amplification of the S segment of the CCHFV genome using RNA extracted from each tick showed a band of 536 bp (**Figure 1**). Molecular tests confirmed the CCHFV infection in 7% of collected ticks. Among the 7 infected ticks, 4 were female, 2 were male, and one was a nymph. The CCHFV-positive tick species belonged to *R. sanguineus* (n = 4), *R. bursa* (n = 2), and *Hy. asiaticum* (n = 1) (**Table 1**).

All infected ticks were collected from sheep. The majority of CCHFV-positive ticks were collected from male animals. The infection was most common in one-year-old sheep. Four of CCHFV-positive ticks were among those collected form highland areas, whereas the remaining 3 ticks were from lowland origin.

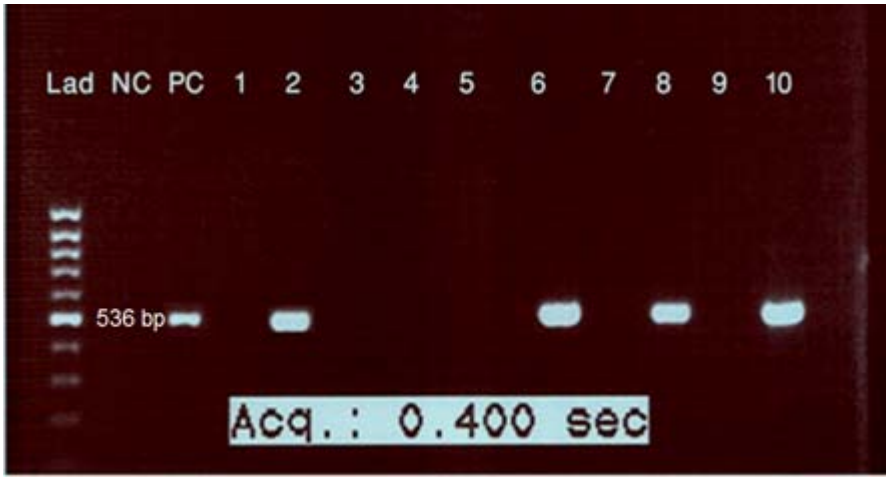

Figure 1. Agarose gel (1.5%) electrophoresis of amplified S segment DNA from the Crimean–Congo hemorrhagic fever virus genome using reverse transcriptase–polymerase chain reaction of RNA in tick samples from Hamadan province. Lad, 100 bp marker ladder; NC, negative control; PC, positive control (536 bp); lanes 2, 6, 8, and 10 positive tick samples

Table 1. Rate of the Crimean–Congo hemorrhagic fever virus infection in collected ticks

Tick species	Percent of 881 ticks	Reverse transcriptase–polymerase chain reaction positive cases (Of 7 infected ticks in 100 randomly selected)
R. sanguineus	65.1%	4
R. bursa	29.0%	2
R. sp	0.9%	0
Hy. asiaticum	1.02%	1
Hy. anatolicum	1.6%	0
Hy. dromedarii	0.1%	0
Hy. marginatum	0.1%	0
Hy. schulzei	0.1 %	0
Hy. sp	0.56 %	0
Hae. sulcata	0.34 %	0

Discussion

Ticks are important vectors of several infections affecting animals and humans [17]. Determination of the tick fauna and their transmitted infectious agents are essential for controlling the tick-borne diseases [18]. In the present study, we investigated prevalence of CCHFV in hard ticks in Asadabad, Nahavand, and Famnin counties of Hamadan Province, western Iran

We found CCHFV infection in 7% of tested ticks, which is a lower rate than obtained by similar earlier studies conducted in Hamadan province. Moradi et al. [19] reported in Bahar (central part of Hamadan Province) the rate of CCHFV infection was 11.3% among collected ticks. Another study conducted in the same province showed an even higher CCHFV infection rate at 19.3% [14]. However, the reported rates of CCHFV infection in ticks showed variation in time and geographically so that the infection rate was 28% in Ardabil province (northwest of Iran) [18], 6.6% in Ilam province (southwest of Iran) [9], and 5.7% in Yazd province (center of Iran) [20]. The variation of CCHFV infection has been attributed to weather and geographical diversity, animal hosts of ticks, and ecological requirements of various tick species [21, 20]. Comparing the CCHFV infection rates reported earlier from Hamadan and Ardabil provinces with those more recently obtained from Ilam, and Yazd provinces, one may conclude that the rate of infected ticks in Iran has decreased since 2009.

Although *Hyalomma* ticks are the major vector and reservoir for CCHFV in many parts of the world including Iran, the virus has been isolated from other genera of ticks [22]. In the present study, CCHFV infection was detected both in *Rhipicephalus* and *Hyalomma* ticks. This finding is consistent with that reported by Moradi et al. [19] who identified infected ticks to belong to *Rhipicephalus* and *Hyalomma* genera. Similarly, Tahmasebi et al. [14] showed that CCHFV infected ticks from Hamadan province were *Rhipicephalus* and *Hyalomma* ticks. Our findings suggest that *Hyalomma*, and *Rhipicephalus* ticks are the primary vectors of CCHFV in Hamadan province. The data from this study may contribute to developing control strategies for hard ticks and tick-borne diseases such as CCHF in Hamadan province as one endemic focus of CCHF infection in Iran.

Acknowledgment

This study was funded by our institutional research board.

Conflict of interest statement

The authors declare that there is no conflict of interest in this research.

References

1. Chinikar S, Ghiasi SM, Hewson R, Moradi M, Haeri A. Crimean-Congo hemorrhagic fever in Iran and neighboring countries. J Clin Virol. 2010; 47:110-4.
2. Begum F, Wisseman CL Jr, Casals J. Tick-borne viruses of West Pakistan. IV. Viruses similar to or identical with, Crimean hemorrhagic fever (Congo-Semunya), Wad Medani and Pak Argas 461 isolated from ticks of the Changa Manga Forest, Lahore District, and of Hunza, Gilgit Agency, W. Pakistan. Am J Epidemiol. 1970; 92:197-202.
3. Burney MI, Ghafoor A, Saleen M, Webb PA, Casals J. Nosocomial outbreak of viral hemorrhagic fever caused by Crimean Hemorrhagic fever-Congo virus in Pakistan, January 1976. Am J Trop Med Hyg. 1980; 29:941-7.
4. Schwarz TF, Nsanze H, Ameen AM. Clinical features of Crimean-Congo haemorrhagic fever in the United Arab Emirates. Infection. 1997; 25:364-7.
5. Sheikh AS, Sheikh AA, Sheikh NS, Rafi-U-Shan, Asif M, Afridi F, et al. Bi-annual surge of Crimean-Congo haemorrhagic fever (CCHF): a five-year experience. Int J Infect Dis. 2005; 9:37-42.
6. Mardani M, Rahnavardi M, Rajaeinejad M, Naini KH, Chinikar S, Pourmalek F, et al. Crimean-Congo hemorrhagic fever among health care workers in Iran: a seroprevalence study in two endemic regions. Am J Trop Med Hyg. 2007; 76:443-5.
7. Bente DA, Forrester NL, Watts DM, McAuley AJ, Whitehouse CA, Bray M. Crimean-Congo hemorrhagic fever: History, epidemiology, pathogenesis, clinical syndrome and genetic diversity. Antiviral Res. 2013; 100:159-89.
8. Chinikar S, Mazaheri V, Mirahmadi R, Nabeth P, Saron MF, Salehi P, et al. A serological survey in suspected human patients of Crimean-Congo hemorrhagic fever in Iran by determination of IgM specific ELISA method during 2000 - 2004. Arch Iran Med. 2005; 8:52-5.
9. Sharifinia N, Rafinejad J, Hanafi-Bojd AA, Chinikar S, Piazak N, Baniardalan M, et al. Hard ticks (Ixodidae) and Crimean-Congo hemorrhagic fever virus in South West of Iran. Acta Med Iran. 2015; 53:177-81.
10. Fakoorziba M, Neghab M, Alipour H, Moemenbellah-Fard M. Tick borne Crimean-Congo haemorrhagic fever in Fars province, Southern Iran: epidemiologic characteristics and vector surveillance. Pak J Biol Sci. 2006; 9:2681-4.

11. Sargianou M, Papa A. Epidemiological and behavioral factors associated with Crimean–Congo hemorrhagic fever virus infections in humans. Expert Rev Anti Infect Ther. 2013; 11:897-908.
12. Whitehouse CA. Crimean–Congo hemorrhagic fever. Antiviral Res. 2004; 64:145-60.
13. Gergova I, Kunchev M, Kamarinchev B. Crimean-Congo hemorrhagic fever virus-tick survey in endemic areas in Bulgaria. J Med Virol. 2012; 84:608-14.
14. 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 Borne Dis. 2010; 47:211-6.
15. Telmadarraiy Z, Chinikar S, Vatandoost H, Faghihi F, Hosseini-Chegeni A. Vectors of Crimean Congo hemorrhagic fever virus in Iran. J Arthropod Borne Dis. 2015; 9:137-47.
16. 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; 40:110-5.
17. Tekin S, Bursali A, Mutluay N, Keskin A, Dundar E. Crimean-Congo hemorrhagic fever virus in various ixodid tick species from a highly endemic area. Vet Parasitol. 2012; 186:546-52.
18. 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; 42:137-41.
19. Moradi AR CS, Oshaghi MA, Vatandoost H, Houlakoui Naeini K, Zahirnia AH, Telmadarraiy Z. Molecular detection of Crimean-Congo hemorrhagic fever (CCHF) virus in Ticks (Ixodidae, Argasidae) of Hamadan province. Biochem Cell Arch. 2008; 8:119-23.
20. 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; 4:61-3.
21. Estrada-Peña A, Jameson L, Medlock J, Vatansever Z, Tishkova F. Unraveling the ecological complexities of tick-associated Crimean-Congo hemorrhagic fever virus transmission: a gap analysis for the western Palearctic. Vector Borne Zoonotic Dis. 2012; 12: 743-52.
22. Güneº T. [Crimean-Congo hemorrhagic fever.] Mikrobiyol Bul. 2006; 40:279-87. (article in Turkish)