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Original Article

Molecular Detection of Crimean-Congo Hemorrhagic Fever Virus in Ticks in Qom Province, Iran, 2011-2012

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Abstract

Background and Aims: Crimean-Congo hemorrhagic fever (CCHF) is a zoonosis caused by a Nairovirus of the family Bunyaviridae. Infection is transmitted to humans mostly by Hyalomma ticks. This study was conducted to determine the rate of CCHFV infection in ticks in Qom province of Iran.

Materials and Methods: In this study, Reverse transcription – polymerase chain reaction (RT-PCR) was used to detect partial sequence of the CCHF small (S) genome segment in ticks.

Results: CCHFV genome was found in 7.9% of hard ticks. All positive ticks were from Hyalomma genus and Hyalomma marginatum species. We were not able to find virus in in Hy. anatolicum, Hy. schulzei, Hy. dromedarii, Rhipicephalus Sanguineus and Argas persicus.

Conclusion: Results exhibited that Hyalomma marginatum is the main vector in the study area.

Keywords: Ticks; CCHF; Qom; Iran

Introduction

Crimean-Congo hemorrhagic fever (CCHF) is a zoonosis caused by a Nairovirus of the family Bunyaviridae. CCHF is one of the most widely distributed viral hemorrhagic fevers and has been reported in Africa, the Middle East and Asia, as well as parts of Europe. There is no approved vaccine or specific treatment against CCHF virus (CCHFV) infections (1). CCHFV was reported in Iran in 1970. There was no report of clinical CCHF until 1999, when an outbreak reports from Shahr-e-Kord town ship and subsequently other outbreaks were reported in different provinces of Iran. In 2000, CCHF was recognized as a major public health problem necessitating implementation of reliable method for antibody detection (2-4). The most important source for acquisition of the virus by ticks is believed to be infected small vertebrates on which immature Hyalomma and Rhipicephalus ticks feed. Once infected, the tick remains infected through its developmental stages, and the mature tick may transmit the infection to large vertebrates, such as livestock. Domestic ruminant animals, such as cattle, sheep and goats, are viraemic (virus circulating in the bloodstream) for around one week after becoming infected (5). Humans who become infected with CCHF acquire the virus from direct contact with blood or other infected tissues from livestock during this time, or they may become infected from a tick bite (6). Clinical features usually include a rapid progression characterized by hemorrhage, myalgia and fever, with a lethality rate up to

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Table 1. HBs Ag prevalence among blood donations in the whole country (30 provinces), and also in high prevalence area (S&B) and low prevalence area (Fars) during 2001-2010.

Genus/species	Plain		Mountain		Total	
	No of Samples.	%	Num.	%	Frequency	Percent
Hyalomma dromedarii	147	19.75	93	12.5	240	32.25
Hyalomma schulzei	117	15.72	79	10.61	196	26.34
Hyalomma marginatum	41	5.51	23	3.09	64	8.61
Hyalomma anatolicum	30	4.03	20	2.68	50	6.72
Rhipicephalus sangiuneus	81	10.88	51	6.85	132	17.74
Argas persicus	42	5.64	20	2.68	62	8.34
Total	458	61.55	286	38.45	744	100

30%. Numerous studies have been conducted on the CCHFV infection since 2000 and variant virus infection was found in deferent species of ricks in Iran (2, 7-11). So far, however, no study has been done in the Qom Province in this regard. Thus, this study aimed to find the rate of CCHFV infection in tick population in the Qom Province.

Methods

Study area

Qom province is located in the central part of Iran, between 50° 06'–51° 58' E and 34° 09'–35° 11' N with an area of 11,237 km² covering 0.89% of the total land of Iran. Its provincial capital is the city of Qom (Fig. 1). Based on the most recent census in 2010, the province has a population of approximately 1,200,000 out of which 91.2% resides in urban areas and 8.8% in rural vicinities. Geographically, the province comprises mountainous areas, foothills and plains. The annual rainfall was 86.9 mm and relative humidity was ranged between 8.5% in June and 89.1% in December. The province contains 1 city, 5 counties, 9 rural districts, and 256 villages. In the Qom Province, there are 28,726 households, of which 17,559 families raise 133,650 sheep as their occupation (12).

Sample collection

Ticks collected from the mentioned sheep were kept alive in separate vials and labeled, collection points were noted. The ticks were sent to the entomology laboratory, school of Public Health and the Institute of Public Health Research, Tehran University of Medical Science for species identification. The identified ticks were pooled into micro tubes and transferred to the Arbovirus laboratory, Pasteur Institute of Iran. Eighty eight ticks (6 species) were tested for the presence of CCHF virus by reverse transcription-polymerase chain reaction (RT-PCR) method.

RNA Extraction and RT-PCR on ticks

Ticks were individually washed twice with PBS 1X and crushed with a mortar and pestle in 200-300 µL of PBS 1X. Total RNA was extracted from the samples using the RNA easy Kit (QLAGEN, Viral RNA mini kit, GmbH, Hilden, Germany) according to the recommendations of the supplier. The RNA was dissolved in 50 µl of RNase-free water and stored at -70˚ C until use. A master mix was

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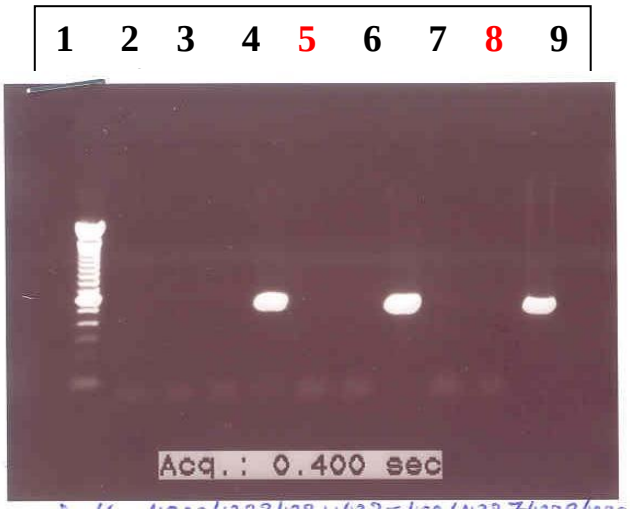

Fig. 1. A 536 bp region of the S segment of CCHF genome was amplified, directly from tick samples by RT–PCR. **Lane 1:** Marker (100 bp DNA ladder) CCHF positive control; **lane 2:** negative control; **Lane11:** CCHF positive control; **lanes 5&8:** samples from infected ticks positive; **other lanes:** samples from infected ticks negative.

prepared with QIAGEN one step RT-PCR kit (QLAGEN GmbH, Hilden, Germany) as fallow: 28 µl of RNase Free water (RFW) 10UL buffer 5x ,2µL dNTP mix,2µL Reverse Transcriptas Enzyme and Taq Polymerase, 1µl of Primer A (Forward) ("5TGGACACCTTCACAAACTC-3 ") and 1µl of Primer B (Reverse) (5"GACAAATTCCCTACACCA-3") and 1 µL RNase inhibitor. Forty five microliter of master mix was added to PCR tubes and 5 UL of extracted RNA was added to the individual PCR tubes (total volume 50 µL**)** (13). **(**The master mix typically contains all the components required for reverse transcription – polymerase chain reaction (RT-PCR) except the template RNA.

Using a molecular-based diagnostic assay--the reverse transcription-polymerase chain reaction (RT-PCR)—88 ticks were examined for CCHFV infection. Out of these, 6 were diagnosed with CCHFV infection, all of which belonging to the family Hy. Marginatum (Table 2). All these infected ticks were caught in plain areas in the spring season (Table 3).

Results

This study was carried out in 25 villages, a total of 750 sheep and 200 camels were examined for infection during four seasons, out of which 72 sheep (9.6%) and 23 camels (11.5%) were discovered to be infected. A total number of 744 ticks were collected and identified using standard identification keys, out of which 91.7% were from the family Ixodidae (Hyalomma = 73.9% and Rhipicephalus = 17/8%) and 8.3% were from the family Argasidae and the genus Argas. 38.45% of the tick sample were caught in villages located in mountainous regions, and the rest, 61.55%, were collected from rural plain areas. (Table 1).

Discussion

In this study, 744 ticks were collected from ruminants' bodies in 25 villages, with the three genera including Hyalomma (73.9%), Rhipicephalus (17.8%) and Argas (8.3%). Like many studies in Iran (14, 15, 16) which have reported the dominant genus of hard ticks to be Hyalomma, our study revealed the same finding. The dominant species of hard ticks in

Table 2. The percentage of ticks infected with CCHFV based on tick species in Qom in 2011-2012.

Genus/species	No. of Samples	No. of infected ticks	% infected
Hyalomma dromedarii	17	0	0
Hyalomma marginatum	25	6	6.81
Hyalomma anatolicum	10	0	0
Hyalomma schulze	11	0	0
Argas persicus	12	0	0
Rhipicephalus sangiuneus	13	0	0
Total	88	6	6.81

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Table 3. The percentage of ticks infected with CCHFV based on the season and topographic conditions in the Qom Province in 2011-2012.

Season / areas	Plain		Mountain		Total	
	Investigated. No (%)	Infected No (%)	Investigated. No (%)	Infected No (%)	Frequency of infected	Percent of infected
Spring	18(20.45)	6(6.81)	10(11.36)	0(0)	6	6.81
Summer	22(25)	0(0)	13(14.77)	0(0)	0	0
Autumn	12(13.64)	0(0)	7(7.95)	0(0)	0	0
Winter	4(4.54)	0(0)	2(2.27)	0(0)	0	0
Total	56(63.63)	6(6.81)	32(36.37)	0(0)	6	6.81

Qom was Hy. Dromdoreii, similar to a study in Yazd (16), while in Western Azerbaijan Hy. Anatolicum was dominant (15), in the north of the country the genus Rhipicephalus and the family, Rh. Sanguineus were dominant (17, 18). Regarding the geographical distribution of ticks in Qom, there was no significant difference between the sampled species and also between their population. More clearly, in both mountainous and plain areas, the species Hy. dromdoreii was dominant, while other species including Hy. Schulzei, Rh. Sanguineus, Hy.margintum, and Hy.anatolicum were of less dominance. Although Hyalomma sp. Ticks are considered the most important in the epidemiology of CCHF as main vectors and reservoir of the virus, the virus has been reported in other 4 genera of hard ticks (Rhipicephalus, Haemaphysalis, Dermacentor and Ixodid sp.) and also in 2 species of soft ticks (Argas persicus and Ornithodoros lahorensis) (2, 19).88 ticks from 3 different genera (Hyalomma, Rhipicephalus, Argas) were examined for CCHFV infection. Subsequently, the findings revealed that only the genus Hyalomma and the species Hy. Marginatum were positive. Other species including Hy. anatolicum, Hy. schulzei, A. persicus, Rh. sanguineus, and Hy. dromedarii were negative. In Zahedan, the genus Hyalomma was tested positive (7), and, similarly, in the Yazd Province, only the genus Hyalomma was positive, with the species involving Hyalomma dromedarii, Hyalomma marginatum, Hyalomma anatolicum, Hyalomma detritum, and Hyalomma asiaticum (8). On the other hand, the species Rhipicephalus sanguineus and Dermacentor marginatus were not found to be infected (8). Likewise, in the studies conducted in Kurdistan, the CCHFV infection test was positive solely for the genus Hyalomma whereas the other genera Haemaphysalis, Rhipicephalus, and Dermacentor were not infected (9). Concerning other species, Rhipicephalus sp. and Haemaphysalis sp. were reported to be CCHFV-infected in Hamadan for the first time in Iran (2). In the same study, in addition to the Hy. Dromedarii, Hy. Marginatum and Hy. Anatolicum, the species H. punctata, Rh. Bursa and Rh. Sanguineus were reported to be CCHFV-infected. As for the family Argasidae, the genera Ornithodoros lahorensis was tested positive in the Khorasan Province for the first time (10) and then in the Chahar-Mahal-o Bakhtiari Province (11). Although in the Qom Province the species Hy. anatolicum, Hy. schulzei, Rh. sanguineus, Hy. dromedarii tested negative for CCHFV, the conclusion can safely be drawn that all species of hard ticks in Qom can be potential carriers of CCHFV

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considering the epidemic statistics. This study conducted in Hamadan (2) infective ticks was collected in both mountainous (16%) and plain areas (5%). However, in our study, 33.3% of ticks caught in plain areas were CCHFV-infected whereas, in mountainous regions, no infected tick was found. Also, the findings of researches in other country have showed that the vectors of CCHF are many species of ticks. It depends on the distribution of ticks in every area. For instance in turkey, the virus has been reported in *R. bursa* and *H. Marginatum* (20). And in India *Hyalomma anatolicum* ticks have transmitted the CCHFV (21). As was reported, the dominant species in the Qom Province was Hy. Dromdoreii. However, Hy. Marginatum was also reported to be infective. Thus, it can be concluded this species can have a significant role in the transmission cycle of CCHF virus in the province.

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