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Crimean-Congo hemorrhagic fever: a molecular survey on hard ticks (*Ixodidae*) in Yazd province, Iran

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ABSTRACT

Objective: To determine the rate of Crimean-Congo hemorrhagic fever virus (CCHFV) infection in hard ticks *(Ixodidae)* in Yazd province of Iran. **Methods:** A molecular survey on hard ticks *(Ixodidae)* was conducted in Yazd province during 2008 -2009. A total of 140 hard ticks (three genera and 7 species) were collected from randomly selected villages and were exanimate for presence of CCHFV reverse transcription-polymerase chain reaction (RT-PCR) method. **Results:** CCHFV genome was found in 5.71% of hard ticks. All positive ticks were from *Hyalomma* genus. Positive ticks including: *Hyalomma dromedarii, Hyalomma marginatum, Hyalomma anatolicum, Hyalomma detritum, Hyalomma asiaticum*. We were not able to find virus in in *Rhipicephalus sanguineus* and *Dermacentor marginatus*. Results exhibited that Hyalomma is the main vector in the study area. **Conclusions:** Due to the presence of virus in 24 provinces´ out of 31, we recommend the use of acaricides and repellent to prevent disease transmission among humans. Greta care should be taken by the people who are working in slaughter houses.

1. Introduction

Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne disease[1]. The virus is transmitted to humans by several methods including: ticks bite, direct contact with fresh meat or blood of infected animals[2]. Although the CCHFV has been isolated from many genera and species of hard ticks and soft ticks *(Ixodidae* and *Argasidae)* but it seems that several species of the genus *Hyalomma* (*Ixodidae* family)play more important role in transmission of disease to human[2,3]. Nosocomial outbreaks among hospital staff due to CCHF with high mortality also are another aspect of disease transmission[4-6]. The disease has a worldwide distribution and it is considered as an endemic disease in many countries of Asia, Europe, and Africa. New outbreak of this disease recorded in Kosovo, Senegal, Turkey, Bulgaria, Iran, Pakistan and Mauritania[1,7,8,9]. In Iran CCHFV was reported in 1970 and first isolated in 1978 from ticks[10,11], Afterward the case of disease was not reported properly. In 1999 an outbreak was reported from Chaharmahal and Bakhtiari province, south-west of Iran[12-14]. Finally in year 2000, CCHF was considered as an important disease and a major public health problem. The national strategy was to establish a laboratory for arboviruses and viral hemorrhagic fevers as a National Reference Laboratory in Pasteur Institute of Iran (member of National Expert Committee on Viral Hemorrhagic Fevers)[13-15]. According to the latest record CCHFV exist in 24 out of 31 provinces of Iran[9,14]. The aim of this study was to determine the rate of CCHFV infection in hard ticks (*Ixodidae*) in Yazd province of Iran.

2. Materials and methods

2.1. Study area

Yazd province (Figure 1) (31.8948°N 54.3570°E) is located in the center of Iran. This province has an area of 73 467 km^2, and according to the most recent divisions of the country, is divided into ten counties. Yazd has a climate which mostly resembles dry desert climate. Little rain along with high water evaporation, relatively low dampness, heat and great temperature changes are among the factors making this province, one of the driest parts of Iran[16]. In this study 30 villages were selected randomly and all the tick collection was carried out during year 2008-2009.

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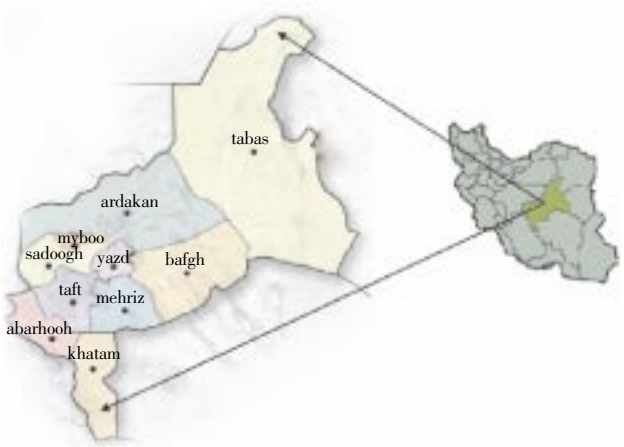

Figure 1. Study area in Yazd province, Iran.

2.2. Sample collection

Ticks collections were carried out on animal. A total of 140 hard ticks were collected from sheep, cow, goat, and camel. Collected ticks from each host were kept alive in separate labeled tube, then were transfer into the laboratory of Medical Entomology, School of Public Health, and Tehran University of Medical Sciences and were identified by morphological characteristic using a stereo- microscope based on valid identification keys[2,17]. All identified ticks were kept into micro tubes and transferred to the Arbovirus Laboratory, Pasteur institute of Iran (National Reference Laboratory of Iran) for determination of presence of CCHFV by reverse transcription-polymerase chain reaction (RT-PCR) method.

2.3. RNA Extraction and RT-PCR

In the molecular laboratory ticks were individually washed twice with PBS 1× and crushed with a mortar and pestle in 200-300 μL of PBS 1×. Total RNA was extracted from the samples using the RNA easy kit (QIAGEN, Viral RNA mini kit, GmbH, Hilden, Germany) according to the recommendations of the supplier. The RNA was dissolved in 50 mL of RNase-free water and stored at -70 ℃ until use. A master mix was prepared with QIAGEN one step RT-PCR kit (QIAGEN GmbH, Hilden, Germany) as follow: 28 μL of RNase free water (RFW), 10 μL buffer 5×, 2 μL dNTP mixed, 2 μL Reverse Transcriptase Enzyme and Taq Polymerase, 1 μL of Primer A (Forward) (5′TGGACACCTTCACAAACTC-3′) and 1 μL of Primer B (Reverse) (5′GACAAATT-CCCTACACCA-3′) and 1 μL RNase inhibitor. Forty five micro liter of master mix was added to PCR tubes and 5 μL of extracted RNA was added to the individual PCR tubes (Total volume 50 μL)[18]. The master mix typically contains all the components required for RT-PCR except the template RNA.After amplification, samples were stored either overnight at 2 to 8 ℃, or at -20 ℃ for longer-term storage. Five μL of the PCR products were mixed with 1 μL loading buffer and then electrophoresis on 1.5% agarose gels in Tris-borate EDTA buffer (TBE) were used. DNA bands were stained with ethidium bromide and were visualized on a UV transilluminator[12-14,18].

3. Results

In this study a total number of 140 hard ticks including three genera and seven species were examined for the presence of CCHFV genome. From the results it is concluded that *Hyalomma dromedarii (Hy. dromedarii)* and *Hyalomma asiaticum (Hy. asiaticum)* had the most and least frequency among *Hyalomma* genus with 56.42% (79/140) and 5.00% (7/140), respectively. While the percentage of *Hyalomma marginatum (Hy. marginatum)*, *Hyalomma anatolicum (Hy. anatolicum)*, *Hyalomma detritum (Hy. detritum)*, *Hyalomma sanguineus (Hy. sanguineus)* and *Dermacentor marginatus (D. marginatus)* were 11.43% (16/140), 8.57% (12/140), 7.14% (10/140), 10.72% (15/140) and 0.71% (1/140), respectively. Result of RT-PCR amplification of S segment of CCHFV genome using RNA extracted from each ticks showed a PCR band of 536 bp (Figure 2). After examination, the CCHFV genome was found in 5.71% of hard ticks. All positive ticks were from *Hyalomma* genus, including: *Hyalomma dromedarii, Hyalomma marginatum, Hyalomma anatolicum, Hyalomma detritum, Hyalomma asiaticum. Rhipicephalus sanguineus* and *Dermacentor marginatus* were found negative to the virus. Tick host also were found positive to the virus. CCHFV genome found in 10.71% (3/28) of ticks from cow, 3.79% (3/79) ticks from camel, 7.14% (2/28) of ticks from sheep. All ticks collected form goat were negative (Table 1).

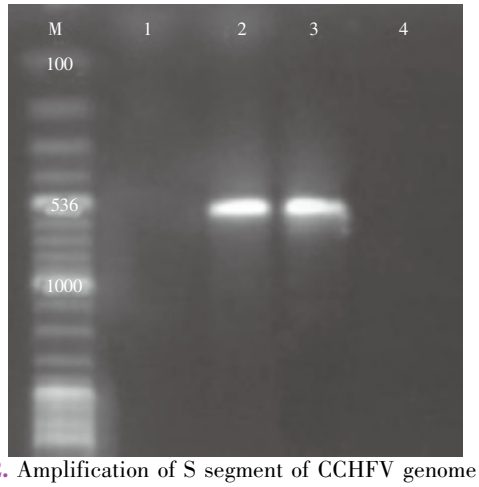

Figure 2. Amplification of S segment of CCHFV genome in ticks sample using RT-PCR in Yazd province.
Lane 1: negative control, lane 2: positive control (536 bp), lane 3: RT-PCR positive sample from infected tick (536 bp) and lane 4 negative sample. M:100 bp size marker.

4. Discussion

Molecular study revealed that CCHF genome were found in 5.71% of collected ticks and all positive ticks were from *Hyalomma* genus. Also this genus was the most frequency in the study area[19]. In others study which was conducted in Iran for determination of presence of CCHFV by RT-PCR, it was found that *Hyalomma* genus was one of the important genus that were infected to CCHFV[9,13,20,21]. Although other genera of hard ticks (*Ixodidae*) can be infected by CCHFV or transmitted it, it seems that *Hyalomma* genus play an important role as main vector of CCHFV in Iran[14]. In west Azerbaijan of Iran, Telmadarraiy *et al* reported that *Rhipicephalus* and *Dermacentor* were infected to CCHFV[21]. In our study we were not able to found the virus in these ticks. Ticks which were collected form goats were not positive. A molecular study in the Ardabil province during 2004-2005 showed CCHFV genome in 40% of ticks from goats and just *Rhipicephalus bursa* was collected from goat. Also our study revealed that positive ticks were from cow

Table 1
Result of molecular detection of CCHFV in ticks by RT-PCR.

Species	Cow		Camel		Sheep		Goat	
	No. of examined	No. of positive	No. of examined	No. of positive	No. of examined	No. of positive	No. of examined	No. of positive
Hy. dromedarii	7	1	71	2	1	0	–	–
Hy . marginatum	4	0	5	0	7	1	–	–
R. sanguineus	–	–	–	–	12	0	3	0
Hy.anatolicum	6	0	3	1	2	0	1	0
Hy . detritum	4	1	–	–	6	1	–	–
Hy . asiaticum	7	1	–	–	–	–	–	–
D. marginatus	–	–	–	–	–	–	1	–
Total	28	3	79	3	28	2	5	0

(10.71%), camel (3.79%), and sheep (7.14%), In the study of Ardebil buffalo was also found positive[9]. In our study *Hyalomma genus* play an important role for transmission of CCHFV and the main vector for CCHF. Therefore using of acaricides for killing the ticks or some repellent that give protection against tick bites are two applicable methods to decrease the risk of tick borne diseases. According to the several studies rate of infectivity to CCHF is diverse and it depends on weather and geographical diversity, different hosts for ticks and different species of ticks[21]. For control of hard ticks which are more prevalent in autumn and spring dipping method could be an appropriate measure for this purpose[22] also using tick pheromones can offer effective alternatives and safe methods for tick control[23].

Conflict of interest statement

We declare that we have no conflict of interest.

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