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ORIGINAL ARTICLE

Molecular epidemiology of Crimean–Congo hemorrhagic fever virus detected from ticks of one humped camels (*Camelus dromedarius*) population in northeastern Iran

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Abstract A comprehensive study was conducted on camel ticks to assess the epidemiological aspects of the infection in camels. From May 2012 to January 2013, 11 cities and towns from the Khorasan provinces, northeastern Iran, were randomly selected as a "cluster" and at least 14 camels were sampled from each cluster. A total of 200 camels were examined in this study, reverse transcriptase polymerase chain reaction was used for the detection of the Crimean–Congo hemorrhagic fever virus (CCHFV) genome. Tick infestation was observed in 171 of the 200 camels, 480 *ixodid* ticks were collected, and one genus was identified as *Hyalomma*. Four species were reported to be the major tick species infesting camels. Among these, *Hyalomma dromedarii* was the most predominant tick species (90.7 %), followed by *H. anatolicum* (6 %), *H. marginatum* (2.9 %), and *H. asiaticum* (0.4 %). The genome of the CCHFV was detected in 49 (10.2 %) of the 480 ticks. The CCHFV RNA was detected in two of the four tick species, and the viral genome was detected from tick samples in three South Khorasan cities. The positivity rate of ticks was as follows: Boshroyeh, 25 out of 480 (5.2 %); Birjand, 17 out of 480 (3.5 %); and Nehbandan, 7 out of 480 (1.5 %). We recommend the use of acaricides to prevent disease transmission to humans and to reduce the tick population in camels. Care should be taken by abattoir workers and by those who work closely with camels.

Keywords Camel · CCHF · Iran · Khorasan · RT-PCR

Introduction

Crimean–Congo hemorrhagic fever (CCHF) is a tick-borne hemorrhagic fever with a case-fatality rate of approximately 40 %, but it can range from 20 to 80 % in humans (Chinikar et al. 2010). The CCHFV genome has been isolated from at least 31 different tick species in the *Ixodidae* (hard ticks) and *Argasidae* (soft ticks). *Hyalomma* spp. ticks are considered most important in CCHF epidemiology; the virus was also isolated from ticks of other genera (i.e., *Rhipicephalus*, *Boophilus*, *Dermacentor*, *Haemaphysalis*, and *Ixodes* spp.) (Saijo et al. 2002; Tahmasebi et al. 2010). CCHF is a zoonotic viral disease that is asymptomatic in infected livestock, but is a serious threat to humans (Chinikar et al. 2010). The disease is caused by the CCHFV, a segmented negative-stranded RNA virus belonging to the family *Bunyaviridae*, genus *Nairovirus* (Drosten et al. 2002). The disease is one of widely distributed viral hemorrhagic fever occurring in Africa, the middle East, Asia, and some parts of Europe (Chinikar et al. 2010). Humans are infected with CCHFV either through infected tick bites, which maintain a life-long infection and are competent reservoirs, or by direct contact with virus-contaminated tissues or blood (Bishop 1996;

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Mehravaran et al. 2013). An infected tick remains infected through its life and transmits the infection to large vertebrates (Athar et al. 2003). The virus is transovarially transmitted among ticks; consequently, ticks function as CCHFV reservoirs (Hoogstraal 1979). Livestock play a role in the amplification of the virus because animals become viremic for 7 days (Hoogstraal 1979). Khorasan is a region in Iran that is particularly susceptible to the emergence of CCHF. The conditions that increase susceptibility to CCHF within the region include the presence of camel ticks across Khorasan, the importation of camels from endemic neighboring countries, and common pastures with endemic countries in the boundary area of Khorasan. This study was conducted to evaluate the presence and the state of CCHFV in ticks found on camels in the Khorasan provinces.

Materials and methods

Study area

The study was conducted in three provinces: North Khorasan, South Khorasan, and Razavi Khorasan. These provinces are located at 55° 17′–61° 15′ E and 30° 24′–38° 17′ N in northeastern Iran (Fig. 1). North Khorasan is a mountainous region with a temperate cold weather, Khorasan Razavi is a semi-desert region with mild weather, and South Khorasan is a semi-desert region experiencing arid conditions. The average annual rainfall is approximately 300–400 mm in the northern areas and 150 mm in the southern areas.

Sampling

From May 2012 to January 2013, 11 cities and towns among the Khorasan provinces were randomly selected (North Khorasan, South Khorasan, Razavi Khorasan) as a "cluster" and at least 14 camels were sampled from each cluster. Two or three ticks were collected from each camel and placed in separate sterile tubes that were labeled with the date of collection, animal number, gender, age, and area. The sampled ticks were brought to the laboratory and identified using a stereomicroscope according to general identification keys (Kaiser and Hoogstraal 1963; Walker et al. 2003; Apanaskevich and Filippova 2007; Estrada-Pena et al. 2013). Subsequently, the samples were pooled according to the same area, gender, and species of ticks and were immediately transmitted to the Arboviruses and Viral Hemorrhagic Fevers Laboratory (National Ref. Lab), Pasteur Institute of Iran, where they were stored at −70 °C until use.

Molecular detection

Ticks were individually washed twice by PBS (PBS, pH 7.4) and crushed with a mortar and pestle in 200–300 µl of PBS. Total RNA was extracted using an RNeasy mini kit (QIAGEN, Cat No. 2215716) according to the manufacturer's instructions. The extracted viral RNA was stored at −70 °C until use. For the RT-PCR, a master mix was prepared as follows: 28 µl of RNase free water, 10 µl of buffer (5 × conc.), 2 µl of dNTP mixture, 2 µl of enzyme mixture containing reverse transcriptase and Taq DNA polymerase enzymes, 1 µl of primer F2 (5′-TGGACACCT TCACAAACTC-3′), 1 µl of Primer R3 (5′-GACAATTCC CTACACC-3′), 1 µl of RNase inhibitor and 5 µl of extracted viral RNA as template. The F2 and R3 primers amplify a 536 bp fragment inside the S-segment of the CCHFV genome. The thermal cycling program for the RT-PCR, included 30 min at 50 °C for reverse transcription reaction (cDNA synthesis); 15 min at 95 °C for activation of Hot Star Taq DNA polymerase and inactivation of reverse transcriptase, followed by 35 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 45 s, and a final extension at 72 °C for 5 min. For gel-based RT-PCR product analysis, 5 µl of the PCR products was mixed with 1 µl loading buffer (6 × conc.). Then, the mixture was load in agarose gel 1.5 %, and visualized with ethidium bromide (Chinikar et al. 2004; Durden et al. 1993; Chinikar et al. 2010; Duh et al. 2008).

Statistical analysis

Descriptive statistics (i.e., frequencies, percentages, and prevalences) were used to summarize the CCHF data. Location of the noted research is shown on the GIS map (Fig. 1).

Results

Of the 200 camels examined, tick infestation was observed in 171 camels and 480 *ixodid* ticks (133 females and 347 males) were collected from different regions in the Khorasan provinces (Table 1).

One genus was identified as *Hyalomma* and four species were reported as the major tick species infesting camels in these areas. Among these, *H. dromedarii* was the most predominant tick species (90.7 %), followed by *H. anatolicum* (6 %), *H. marginatum* (2.9 %), and *H. asiaticum* (0.4 %), (Table 2).

The results of RT-PCR amplification of the S segment of the CCHFV genome using RNA extracted from each tick showed a PCR band of 536 bp (Fig. 2). The CCHFV genome was found in 49 (10.2 %) of 480 ticks and three

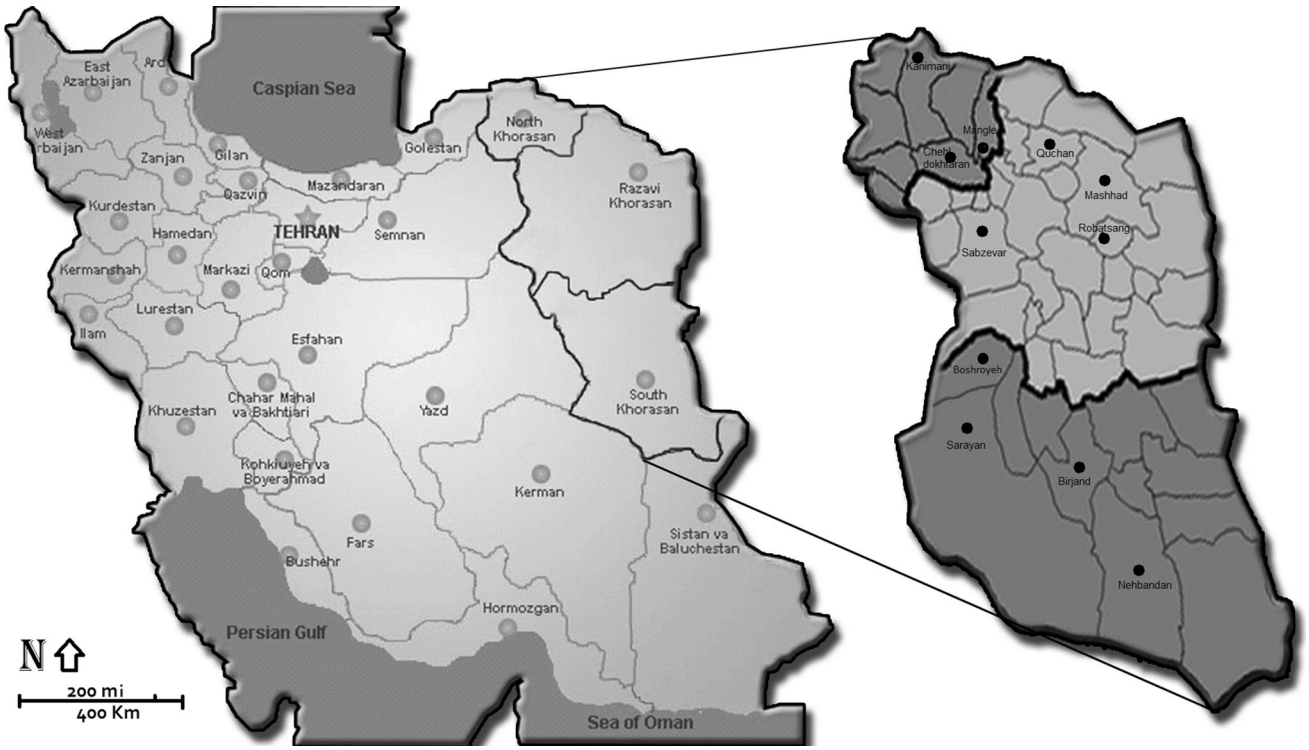

Fig. 1 Khorasan (North, Razavi, South), the study areas are shown on Iran's map

Table 1 Positive rates, number, and gender of ticks collected from different regions in the northeastern Iran

Area	Males	Females	Total	CCHF positive
Nehbandan	27	9	36	7
Sarayan	28	23	51	0
Birjand	41	7	48	17
Kanimani	26	24	50	0
Boshroyeh	52	7	59	25
Robatsang	32	0	32	0
Quchan	28	13	41	0
Sabzevar	18	13	31	0
Mashhad	31	17	48	0
Chehl dokhtaran	28	6	34	0
Mangale	36	14	50	0
Total	347	133	480	49

Table 2 Positives rates, frequency, and gender of ticks infesting camels in different areas of Khorasan

Tick spp	Male	Female	Total	CCHF positive
H. dromedarii	307 (70.6 %)	128 (29.4 %)	435 (90.7 %)	42 (8.75 %)
H. marginatum	10 (71.4 %)	4 (28.6 %)	14 (2.9 %)	0 (0 %)
H. anatolicum	29 (100 %)	0 (0 %)	29 (6 %)	7 (1.45 %)
H. asiaticum	1 (50 %)	1 (50 %)	2 (0.4 %)	0 (0 %)
Total	347 (72.2 %)	133 (27.8 %)	480 (100 %)	49 (10.2 %)

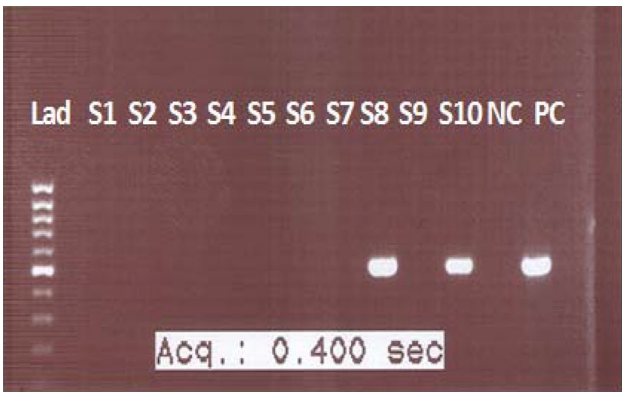

Fig. 2 Amplification of the S segment of the CCHFV genome using RT-PCR, in tick samples from the Khorasan province. *PC* positive control, *NC* negative control, *S1–10* samples S1, S2, S3, S4, S5, S6, S7 and S9 are negative, S8 and S10 are positive

(6 %) of 50 pools. All CCHF-positive ticks were male. The CCHF RNA was detected in two out of four tick species. Of the infected ticks, 42 (8.7 %) belonged to *H. dromedarii* and seven (1.5 %) belonged to *H. anatolicum* (Table 2; Fig. 2). The viral genome was detected in tick samples from three cities of South Khorasan. The positivity rate, according to pool for each city, was as follows: Birjand, one pool (2 %); Boshroyeh, one pool (2 %); and Nehbandan, one pool (2 %), whereas the positive tick numbers were as follows: Boshroyeh, 25 of 480 (5.2 %); Birjand, 17 of 480 (3.5 %); and Nehbandan, 7 of 480 (1.5 %) (Table 1).

Discussion

In this study, *H. dromedarii* was identified as the most dominant species infecting camel ticks; this is in agreement with the results obtained by Salim Abadi et al. (2010) in the Yazd province, Iran, Alwaer et al. (2004) in Libya, Lawal et al. (2007) in Nigeria, and Maha et al. (2010) in Sudan. The data obtained provide evidence for the presence of CCHFV in camels in the Khorasan province. CCHFV infection was detected in 49 (10.2 %) of 480 tick samples, which is greater than that previously detected by Salim Abadi (3.79 %) in the Yazd province of Iran (Salim Abadi et al. 2011).

It was difficult to interpret why all the positive CCHFV ticks were male, but this may be a result of more male ticks being present than female ticks (347/133), or this disparity may be due to the fact that the blood feeding course of male ticks is much longer than that of the female. All positive samples were obtained from *Hyalomma* sp. *Hyalomma* ticks are the primary vectors for the widespread transmission of CCHFV throughout Europe, Asia, the Middle East, and Africa (Ergonul 2006; Whitehouse 2004; Swanepoel et al. 1987). Although *Hyalomma* ticks are considered to be the most important vector and reservoir for the CCHF virus, the virus has also been reported in other genera of ticks (Tahmasebi et al. 2010; Mehravaran et al. 2013).

The CCHFV genome was detected in two of four tick species that were collected (*H. dromedarii,* 8.7 %; and *H. anatolicum,* 1.5 %) and this finding is similar to that of Salim Abadi et al. (2011). This result may suggest that *H. dromedarii* and *H. anatolicum* function as the primary vectors and reservoirs for CCHFV in camels in the north-eastern Iran.

Telmadarraiy et al. (2010), detected the CCHFV genome in *Rhipicephalus bursa* in one of three ticks that were sampled from camels; but in our study, we failed to discover the said genus. *H. dromedarii* is distributed throughout the world wherever camels exist (Hoogstraal 1956; Nazifi et al. 2011), and *H. anatolicum* was reported to be widely distributed throughout Iran (Nabian et al. 2007; Rahbari et al. 2007). This species transmits *Theileria annulata*, *Babesia equi*, *Babesia caballi*, *Anaplasma marginale*, *Trypanosoma theileri*, and at least five *Arboviruses*, and is an important vector of CCHFV in humans (Nabian et al. 2007; Rahbari et al. 2007).

The results from several studies suggest that the rate of infectivity of CCHF is variable and is driven by the weather and geographical diversity, the presence of different tick hosts, and different tick species. In our present study, we observed the CCHFV genome only in camel ticks from South Khorasan (especially in Birjand, Boshroyeh, and Nehbandan). This province has a unique geographical location because it borders Afghanistan on the east, the cities of Kerman and Yazd in the west, the Sistan-va-Baluchistan Province of Iran in the south, and the Khorasan Razavi Province of Iran in the north. Since 2000, the disease has been demonstrated to infect 23 out of 31 provinces in Iran: Sistan-va-Baluchistan (with 283 confirmed cases), Isfahan (with 44 confirmed cases), Fars (with 26 confirmed cases), Tehran (with 17 confirmed cases), and Khorasan (with 12 confirmed cases) had the highest prevalence of CCHF infections (Chinikar et al. 2012b).

Notably, the Sistan-va-Baluchistan province (south of South Khorasan) has not just had the highest number of CCHFV cases, but CCHF infection has been observed in this area since 2000 (Chinikar et al. 2012a). Sistan-va-Baluchistan was identified as the most CCHFV-infected province in Iran since 2000, as it shares a border with two CCHF-endemic countries—Pakistan and Afghanistan (Chinikar et al. 2010). The unique location of the South Khorasan province, which is connected in the north, south, and east to heavily infected or endemic areas of CCHF, may explain why it is the only CCHFV-positive area included in the study.

The present study indicates that people with a high-CCHF-risk occupation, including shepherds, farmers, veterinarians, and other individuals who are in close contact with ticks, should ensure appropriate precautions to avoid exposure to infected ticks, viremic animals, or contaminated camel blood or tissues. Application of commercially available insect repellents and the use of clothing impregnated with permethrin can render some protection against tick bites (Salim Abadi et al. 2011; Tahmasebi et al. 2010). Preventing camel grazing in boundary areas and the routine application of acaricidal to camels are other means of controlling ticks and the further transmission of CCHFV from neighboring countries. In addition, surveys of human and animal populations in these regions are recommended to gain an improved understanding of the distribution and epidemiology of the virus in these provinces. Our current study indicates that CCHF should be regarded as a serious health problem, subject to proper consideration by health centers in this province and neighboring regions, in order to develop strategies to decrease the tick population and to alert high-risk individuals.

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