Vector prevalence and detection of Crimean-Congo haemorrhagic fever virus in Golestan Province, Iran

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ABSTRACT

Background & objectives: Crimean-Congo haemorrhagic fever virus (CCHFV) causes severe disease with fatality rate of 30%. The virus is transmitted to humans through the bite of an infected tick, direct contact with the products of infected livestock as well as nosocomially. The disease occurs sporadically throughout many of African, Asian and European countries. Different species of ticks serve either as vector or reservoir for CCHFV. This study was aimed to determine the prevalence of CCHFV in hard ticks (Ixodidae) in the Golestan Province of Iran.

Methods: A molecular survey was conducted on hard ticks (Ixodidae) isolated from six counties in Golestan Province, north of Iran during 2014–15. The ticks were identified using morphological characteristics and presence of CCHFV RNA was detected using RT-PCR.

Results: Data revealed the presence of CCHFV in 5.3% of the ticks selected for screening. The infected ticks belonged to Hyalomma dromedarii, Hy. anatolicum, Hy. marginatum and Rhipicephalus sanguineus species.

Interpretation & conclusion: The study demonstrated that Hyalomma ticks are the main vectors of CCHFV in Golestan Province. Thus, preventive strategies such as using acaricides and repellents in order to avoid contact with Hyalomma ticks are proposed.

Key words CCHFV; Golestan Province; Iran; Ixodidae tick

INTRODUCTION

Crimean-Congo haemorrhagic fever (CCHF), is an acute, viral, zoonotic disease with haemorrhagic manifestations and considerable mortality in humans. The CCHF virus (CCHFV) (Bunyaviridae family) has a worldwide distribution and outbreak reports have shown a sporadic resurgence¹–².

CCHF is a tick-borne disease³. The virus can be transmitted to humans through the bite of infected ticks and direct contact with fresh meat or blood of infected livestock⁴. Moreover, nosocomial transmissions of CCHF among health care workers is considered as a serious problem having high mortality rates during outbreaks⁵–⁶.

The CCHF virus has been isolated from numerous tick species, including 28 Ixodidae and two Argasidae spp. Argasid ticks do not play important roles in the geographical spread of the virus, as the CCHF virus could not replicate in their adult or nymph stages. Rise in temperature may accelerate tick abundance, especially in spring and summer, which can increase the risk of CCHFV transmission to human⁷–⁸.

CCHF is considered as an endemic disease in many countries of Asia, Europe, and Africa. Recent outbreaks of CCHFV have been reported in Kosovo, Senegal, Turkey, Bulgaria, Iran, Pakistan and Mauritania⁹–¹⁰. The main hosts for CCHFV are wild and domestic livestock, and birds. Sheep, goats and cattle develop high titres of virus in blood with no illness indication. Humans are usually infected with CCHF virus through a tick bite or close contact with viral-contaminated tissues, blood of the domestic animals, or nosocomially¹¹. The first report of CCHF virus in Iran dates back to 1970. In 1999, a CCHF outbreak was reported in Chaharmahal and Bakhtiari Provinces, south-
west of Iran. According to the latest reports, CCHFV exists in 27 of 31 Provinces in Iran. The epidemiological data on CCHF human cases in Golestan Province indicate tick bite history and majority was in close contact with livestock; and a vector screening to reveal the transmission cycles of CCHF virus in this area is missing. Thus, the present study was undertaken to determine the CCHFV prevalence in hard ticks (Ixodidae) by RT-PCR in the Golestan Province, northern Iran.

**MATERIAL & METHODS**

**Study area and sampling**

Golestan Province (36.83°N, 54.44°E) is located in the northeast of Iran at the southeastern coastline of the Caspian Sea. It has a population of 1.6 million (2006), an area of 20,380 km² and is one of the most densely populated provinces in Iran (Fig. 1).

Sample collection sites were selected based on positive human CCHF cases (approximately 1 to 10 CCHF human cases were detected annually since 2000) and ecological parameters in Golestan Province. Accordingly, among the three different ecosystems (coastline, mountain and forest), two counties from each were selected for sample collection to cover different areas. These included: (i) Kalaleh and Gonbad-e Kavus counties (representing the forest ecosystem), (ii) Azadshahr and Ramian (representing the mountain ecosystem) and (iii) Torkaman and Bandar-e Gaz (representing the coastline ecosystem).

Based on earlier investigations, an appropriate formula was selected to determine the sample size. Of the 2,054,500 existing livestock in Golestan province, 1,412,000 are sheep, 433,500 are cows and 209,000 are goats. A total of 1798 livestock including 866 sheep, 311 cows, 483 goats and 138 camels were investigated for tick collection during spring and summer seasons of 2014 and 2015.

**RNA extraction and CCHFV genome detection**

Ticks collected from each host were kept alive in separate labeled tubes, and then transferred to the laboratory of Medical Entomology, School of Public Health, Tehran University of Medical Sciences for identification by morphological characteristics using a stereomicroscope on the basis of valid identification keys. Identified ticks were kept in micro tubes and transferred to the Arboviruses and Viral Haemorrhagic Fevers Laboratory at the Pasteur Institute of Iran (National Reference Laboratory) for virus analysis by reverse transcription-polymerase chain reaction (RT-PCR) method.

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 RNeasy kit (QIAGEN, Viral RNA mini kit, GmbH, Hilden, Germany) according to the manufacturer’s protocol. The RNA was dissolved in 50 ml of RNase-free water and stored at −70°C until usage. A master mix was prepared with QIAGEN one step RT-PCR kit (QIAGEN GmbH, Hilden, Germany) and then designed primers: forward (5′TGGACACCTTCACAAACTC-3′) and reverse (5′GACAAATTCCCTACACCA-3′), were added. PCR products were visualized by electrophoresis technique in 1.5% agarose gels.

**RESULTS**

Out of the 1798 investigated livestock (993 females and 805 males), 783 (43.5%) animals were found infested with ticks. Regarding livestock infestation rate in ecosystems, 46% livestock (279/607) in forest areas, 45% livestock (254/562) in mountain areas, and 39% livestock (250/629) in coastline areas were infested with ticks. The highest tick infestation rate in livestock was observed in Gonbad-e Kavus County (52%) and lowest rate was observed in Torkaman County (33%). The highest number of ticks was found in Gonbad-e Kavus (406 ticks), whilst the lowest number was found in Ramian County (185 ticks) (Table 1).

A total of 1650 ticks were collected from sheep (572 ticks, 34%), goats (525 ticks, 32%), camels (162 ticks, 10%) and cattle (391 ticks, 24%). According to ecosystems, 417 (25%) ticks were collected in mountain areas, 662 (40%) in forest areas and 571 (35%) in coastline...
areas (Table 1). Determination of tick species revealed high frequencies for *Rhipicephalus* (n = 1132, 69%), *Hyalomma* genus (n = 502, 30%), and *Boophilus* (n = 16, 1%) (Table 2). In terms of seasonal activities, results showed that the highest number of livestock carrying ticks were found in the spring (n = 340, 43.42%) and the lowest in the autumn (n = 97, 12.38%). The ticks were collected most abundantly during the summer months (43%), followed by spring (37%), winter (10%) and autumn (9%) (Table 3).

**CCHFV prevalence in the tick populations**

Among 1650 collected ticks, 130 tick specimens (Hy. *dromedarii* (57), *Rhipicephalus* (67) and *Boophilus* (6)) were selected to screen for CCHFV RNA. The presence of CCHFV RNA was detected in 7 of the 130 (5.3%) selected ticks; out of which six belonged to *Hyalomma* genus (*Hy. dromedarii* (2/130, 1.6%), *Hy. anatolicum* (3/130, 2.3%), *Hy. marginatum* (1/130, 0.7%)) and one belonged to *Rhipicephalus* genus (*Rh. sanguineus* (1/130, 0.7%). None of the species of *Hy. asiaticum*, *Hy. detritum*, *Hy. excavatum*, *Rh. bursa*, *Rh. turanicus* and *B. annulatus* were positive for CCHFV (Table 4).

**DISCUSSION**

As ticks play a significant role in CCHFV transmission cycle, their screening is important for vector surveillance and control studies. In the current study, 10 different tick species were identified from the selected livestock. About 5.3% of Ixodidae ticks (*Hyalomma* and *Rhipicephalus* genus) were positive for CCHFV RNA in the study area. The infection rate was 1.6% for *Hy. dromedarii* (2/130), 2.3% for *Hy. anatolicum* (3/130), and 0.7% for *Hy. marginatum* and *Rh. sanguineus* (1/130) each.
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