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Seroepidemiology of Crimean-Congo hemorrhagic fever virus in one-humped camels (*Camelus dromedarius*) population in northeast of Iran

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Crimean-Congo haemorrhagic fever (CCHF) is one of the most widely distributed viral hemorrhagic fevers occurring in Africa, the middle East, Asia and some parts of Europe[1]. CCHFV is generally transmitted between ticks and vertebrates by bites or by contact[2]. An infected tick remains infected throughout its life and transmits the infection to large vertebrates[3]. Livestock play a role in the amplification of the virus because the animals become viremic for seven days[4]. During the last decade, Turkey, Bosnia, and especially Iran still have the most frequent outbreaks of CCHF worldwide, with continued reports in the 21st century[5]. This study was performed to evaluate the status of CCHF in the camels of Khorasan provinces.

The study was conducted in Khorasan, the largest province of Iran until it was divided into three provinces on 29 September 2004. These provinces are located at 55° 17′ to 61° 15′ E and 30° 24′ to 38° 17′ N in the north-eastern Iran (Fig. 1). North Khorasan is a mountainous region with a temperate cold weather. Khorasan Razavi is a semi-desert region with mild weather while south Khorasan is a semi-desert region experiencing arid conditions. Average annual rainfall is approximately 300–400 mm in the northern areas and 150 mm in the central and southern areas.

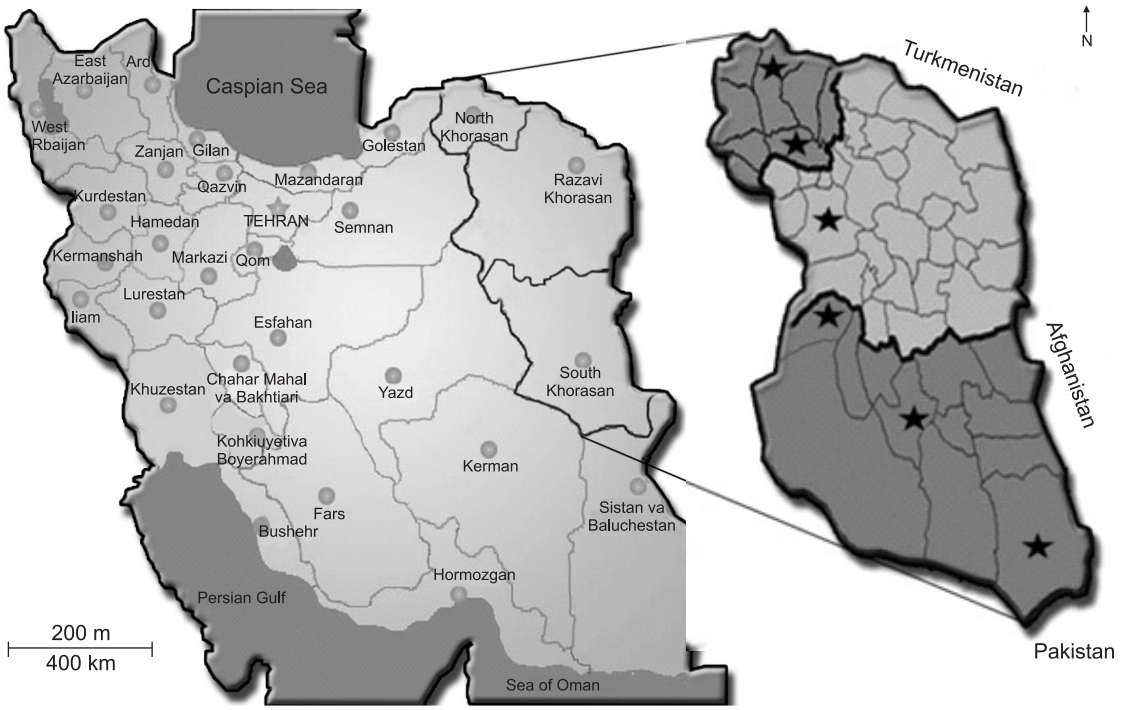

Fig. 1: Iran, center of provinces is shown with small bold circle; in study areas (North, Khorasan, Khorasan Razavi and South Khorasan), CCHF positive areas are shown with asterisks.

From May 2012 to January 2013, 11 cities and towns were selected randomly among the Khorasan provinces (North Khorasan, South Khorasan and Razavi Khorasan) as a "cluster" and at least 14 camels were sampled. From each camel 20 ml blood was collected from the jugular vein, each tube was labeled with the date of collection, animal number, sex, age, and area. Samples were immediately transported to a laboratory and centrifuged at 5000 rpm for 10 min. Serum was then separated and transferred into holding tubes and sent to the Arboviruses and Viral Haemorrhagic Fever Laboratory (National Reference Laboratory) and stored at –70°C until further analysis.

For IgG detection, the ELISA plates were coated with mouse hyperimmune ascetic fluid diluted in phosphate-buffered saline 1×PBS and incubated overnight at 4°C. The native or recombinant antigen (produced in our laboratory) diluted in PBSTM (PBS containing 0.05% Tween and 3% skim milk) was added to the plates and the plates were incubated for 3 h at 37°C and extensively washed. Serum samples diluted in PBSTM were added, and the plates were incubated for 1 h at 37°C. After washing, the peroxidase-labeled antihuman or animal immunoglobulin diluted in PBSTM was added to each well and the plates were incubated for 1 h at 37°C. The plates were then washed thrice with PBS containing 0.5% Tween (PBST). Finally, hydrogen peroxide and tetramethyl benzidine (TMB) were added and the plates were incubated for 15 min at room temperature. The enzymatic reaction was stopped by the addition of sulphuric acid (4 N) and the plates were read by ELISA reader (Anathos 2020) at 450 and 620 nm. Taken together, an IgG-positive serum was considered as positive control and a negative serum taken as negative control in the IgG ELISA[6].

Data were analyzed using IBM/SPSS software version 20.0 SPSS statistics package. Descriptive statistics (i.e. frequencies and percentages) were used to summarize the quantitative variables. Location of the study areas is shown on the GIS map (Fig. 1).

In total, sera from 170 camels were collected from different regions of three provinces. A total of 9 (5.29%) out of 170 camels were IgG-positive. Positivity rates for the provinces varied and are shown in Table 1. Eight out of nine positive samples were taken from female camels.

We found CCHF IgG antibody in camel sera in all three provinces studied (North Khorasan, South Khorasan, and Razavi Khorasan) and these results, as well as previous reports by Chinikar *et al*[7], may indicate that CCHF is endemic in these regions, or that it has emerged from neighbouring countries into these regions.

We could not ascertain why approximately all positive-IgG sera samples were taken from females (8/9), but this may be due to the presence of more females than males in the sample (136 females *vs* 34 males). This finding is not in accordance with results obtained by Saidi *et al*[8], who found that CCHF samples were negative in all 157 camels which had been studied from south or southeast Iran. But these results are similar to those of Williams *et al*[9], who found that 17(16%) of 109 camels sampled in Oman were positive for CCHF IgG antibody.

All three studied provinces (North Khorasan, South Khorasan, and Razavi Khorasan) have an unusual geographical distribution, as they border Pakistan and Afghanistan to the east, the cities of Kerman, Yazd and Semnan in the west, the province of Sistan-va-Baluchistan in the south and Turkmenistan in the north. Since 2000, it has been demonstrated that the disease has infected 27 out of 31 provinces in Iran, with Sistan-va-Baluchistan

Table 1. Positive rates, number and sex of camels sera collected from different regions in northeast of Iran

Area	No. of sera collected	No. of males	No. of females	No. of IgG positive	% positive
Nehbandan	12	1	11	2	1.175
Sarayan	13	1	12	0	0
Birjand	25	4	21	2	1.175
Kanimani	14	5	9	1	0.587
Boshroyeh	16	2	14	2	1.175
Robatsang	16	6	10	0	0
Quchan	14	3	11	0	0
Sabzevar	15	7	8	1	0.587
Mashhad	16	4	12	0	0
Chehl dokhtaran	14	0	14	1	0.587
Mangale	15	1	14	0	0
Total	170	34	136	9	5.29

(283 confirmed cases); Isfahan (with 44 confirmed cases); Fars (26 confirmed cases); Tehran (17 confirmed cases); and Khorasan (12 confirmed cases) having the highest prevalence of CCHF infections. Notably, the province of Sistan-va-Baluchistan (south of South Khorasan) has not only had the highest number of CCHF cases but CCHF infection has also been observed in this area since 2000[7]. Sistan-va-Baluchistan has been shown to be the most CCHFV-infected province in Iran since 2000, as it shares a border with two CCHF endemic countries, Pakistan and Afghanistan[10]. The location of South Khorasan, which is connected to heavily infected or endemic areas of CCHF, shares a large border area with neighbouring countries, shares common pasture with herds from CCHF-endemic areas and experiences the illegal importation of animals across these borders, that may explain why it is the most CCHF IgG-positive area included in the study.

Another important issue is the presence of disease reservoirs and vectors, such as *Hyalomma* spp, in this area. The CCHFV genome has been isolated from at least 31 different tick species in *Ixodidae* (hard ticks) and *Argasidae* (soft ticks)[11]. As mentioned by Champour *et al*[12] the most important ticks affecting camels in this region are *H. dromedarii, H. anatolicum*, *H. marginatum* and *H. asiaticum*. *Hyalomma* spp ticks are considered to be the most important in the epidemiology of CCHFV in camels in these areas[4, 12].

Six out of the eleven cities and towns studied yielded camel sera which were IgG-positive for CCHF, and it has been shown that CCHF is distributed across these three provinces. The highest prevalence of IgG antibodies was found in South Khorasan. Our results revealed a lower prevalence of seropositivity among camels (5.29%) than in other domestic animals (30%) in Iran[13]. However, due to the fact that the camels remain in herds more than other animals[12] and also because camel pasture is very widely distributed geographically, this small percentage has a significant effect on the epidemiology of CCHF. The importance of camels in the epidemiology of CCHF in Russia and Astrakhan Oblast has been previously reported[14].

Most of the countries bordering Iran, including Pakistan and Afghanistan in the east and Turkey in the west are CCHF-endemic regions. In addition to this, CCHF was first discovered in Crimea (located in the northwest of Iran)[15]. Because CCHF is a serious threat to Iran, imported animals, particularly camels, that carry a large number of ticks, should be screened and treated more carefully. This study suggests that people engaged in a high CCHFV-risk occupation in these provinces, such as shepherds, farmers, veterinarians and other persons who are in close contact with ticks, should take precautions to avoid exposure to infected ticks, viremic animals and contaminated camel blood or tissues.

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