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Seroprevalence of Crimean-Congo haemorrhagic fever virus in one-humped camels and its ribonucleic acid in ticks attached to one-humped camels (*Camelus dromedarius*) imported from Pakistan into Iran

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Abstract

This study was conducted to specifically investigate the seroprevalence of Crimean-Congo haemorrhagic fever virus (CCHFV) in one-humped camels, and CCHFV ribonucleic acid (RNA) in ticks attached to one-humped camels (*Camelus dromedarius*) imported from Pakistan into Iran. Crimean-Congo haemorrhagic fever is a tick-borne viral zoonotic disease caused by one of the most medically important arboviruses belonging to the genus *Orthonairovirus* in the family *Nairoviridae*. The study areas are regions near the Iran-Pakistan border in Sistan and Baluchestan province. Ticks and serum were analyzed by molecular and enzyme-linked immunosorbent assay (ELISA) methods, respectively. A cross-sectional study was conducted; data were analyzed using a statistical package for the social sciences (SPSS). Descriptive statistics were used to summarize the quantitative variables. One genus was identified as Hyalomma; four species of Hyalomma were observed. From 99 blood samples, 33 samples (33.3%) were found positive for CCHFV-specific immunoglobulin G (CCHF-IgG), and 12 out of the 99 serum samples had borderline results. Two out of the 33 positive serum samples were obtained from female camels, and 31 positive serum samples were obtained from male camels (gender difference was not statistically significant, P=0.97).

Additionally, 176 ixodid ticks were collected from different regions of the one-humped camels' bodies. All of the 176 collected ticks, which were attached to camels, were negative for CCHFV in the Reverse transcription-polymerase chain reaction (RT-PCR) result. The serum prevalence of CCHFV is significantly high among one-humped camels imported into Iran from Pakistan; therefore, it is a better alternative to import camel meat instead of tick burdened high-risk camels. Also, tick control is considered an effective procedure for CCHFV control.

Keywords: CCHFV, Dromedary camel, ELISA, Iran, RT-PCR

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Introduction

Crimean-Congo haemorrhagic fever (CCHF) is a tick-borne viral zoonotic disease caused by one of the most medically important arboviruses belonging to the genus *Orthonairovirus* in the family *Nairoviridae* (Ergönül, 2006). The disease is widespread in

various countries in Africa, Central Asia, and Eastern and Southern Europe (Al-Abria et al., 2017). Human CCHF cases have been reported in almost all provinces in Iran, especially in the southeast, near the Iran-Pakistan and Iran-Afghanistan borders (Keshtkar-Jahromi et al., 2013). To the best of our knowledge, 15 studies from 11 countries have investigated the seroepidemiology of CCHF in camels (Saidi et al., 1975; Spengler et al., 2016). In total, these studies have evaluated 5272 camels and indicated an average seropositivity rate of 13.41% (Table 1). The CCHFV is transmitted to humans by tick bite, exposure to blood or

Table 1. Seroepidemiology of CCHFV in camels of different countries: NR, not reported; AGDP, agar gel diffusion precipitation; CF, complement fixation; ELISA, enzyme-linked immunosorbent assay; IFA, immunofluorescence assay; RPHI, reverse passive haemagglutination inhibition assay

Number	Country	Sample No.	% Seropositivity	Assay
1	Pakistan	1	100	IgG ELISA
2	China	10	40	RPHI
3	Kenya	499	26	AGDP, IFA
4	Iraq	99	23.2	CF
5	Iran	99	19.1	AGDP
6	Oman	109	16	IgG ELISA
7	Niger	353	13.6	IgG ELISA
8	Sudan	3802	12	AGDP, IFA
9	Egypt	34	8.8	CF
10	Sudan	13	7.7	IgG ELISA
11	UAE	80	6.3	IgG ELISA
12	Russia	NR	1.4	AGDP
13	Iran	157	0	AGDP
14	India	3	0	CF, AGDP
15	Egypt	10	0	IgG ELISA

tissues of viremic livestock, direct contact with blood and body fluids of infected patients, and the consumption of viremic animals' tissues (Fazlalipour et al., 2016). High-risk occupations for CCHF are slaughterhouse workers, shepherds, healthcare workers, veterinarians, laboratory staff, and physicians (Altaf et al., 1998; Sharifi-Mood et al., 2014). In nature, CCHFV is maintained in an enzootic cycle between ticks and vertebrates (Spengler et al., 2016). Ticks of the genus Hyalomma are reservoirs of the CCHFV and act as the most important vector of the CCHFV (Spengler and Estrada-Peña, 2018). However, the CCHFV genome has also been detected in other genera ticks, including Rhipicephalus, Boophilus, Dermacentor, Haemaphysalis, and Ixodes spp. (Chinikar et al., 2010; Duh et al., 2008; Hoogstraal, 1979). Wild and domestic mammals are amplifying hosts for CCHFV (Gargili et al., 2017). Animals develop high titers of virus in their blood and remain viremic for about two weeks (Saidi et al., 1975); their infection is generally

asymptomatic (Mertens et al., 2013; Sun et al., 2009). Detection of viral ribonucleic acid in ticks and anti CCHFV IgG antibodies in animals' blood are used as indicators of the viral circulation in an area (Whitehouse, 2004). Since the one-humped camel is one of the leading domestic animals in the Middle East and almost always is burdened with a large number of ticks (Perveen et al., 2020), this study was conducted to specifically investigate the seroprevalence of CCHFV in one-humped camels and CCHFV RNA in ticks attached to one-humped camels and imported from Pakistan into Iran.

Materials and Methods

The study areas are regions near the Iran-Pakistan border and near Zahedan and Mirjaveh, cities in Sistan and Baluchestan province. These areas, located at 29°29'46.68"N, 60°51'46.44"E in the southeast of Iran are desert regions that experience arid conditions repeatedly; the mean temperature, relative humidity, and annual rainfall are 29°C, 16%, and 12.2 mm respectively (Figure 1).

Figure 1. Map of the study area in Iran. The sampled areas near Zahedan and Mirjaveh in Sistan and Baluchestan province are shown with asterisks on the inset map.

Insufficient vigilance makes these areas adequately suitable for legal/illegal animal importation. Consequently, the risk of CCHF and other disease transmission exists in these regions, and CCHFV can be imported from Pakistan to Iran periodically. A cross-sectional study was conducted to estimate the serum prevalence of CCHFV-specific IgG antibodies and detect the prevalence of CCHFV genomes, by using the molecular technique RT-PCR on blood and attached ticks collected from different body regions of one-humped camels that were imported from Pakistan into Iran. Between August 2015 and December 2016, at five different periods during four seasons, 320 one-humped camels were examined, and 108 blood samples were collected from 8 herds, 10-15 samples per herd, while they were being imported to Iran.

A qualified veterinarian performed the blood collection following proper physical restraint of the camels to ensure personal and animal safety. The study received ethical clearance from the Research Board of the Ferdowsi Faculty of Veterinary Medicine, Mashhad, Iran.

Sampling

As a whole, 108 blood samples were taken from the jugular vein, and 9 out of the 108 samples were not suitable for further processing due to contamination (93 male 1-4.5 years old and six female 4.5-10 years old one-humped camels). Serum was separated and kept frozen at −20 °C until used for detection of CCHFV-specific IgG antibodies. Hard ticks were collected from various parts of the camels' bodies. A total of 176 hard ticks (randomly 1-2 or more ticks from each camel) were collected. All ticks were handled with gloves and forceps with great care, using forceps to prevent any damage to the ticks. Adult live ticks were inserted into a sterile tube and kept under specific humidity and temperature in a cold box during transfer to the laboratory of Veterinary Entomology at the Ferdowsi University of Mashhad. Tick species were identified using a stereomicroscope and valid taxonomic keys coupled with expert acarologist support (Champour et al., 2014; Aslani et al., 2017; Swanepoel et al., 1985; Walker et al., 2003). Identified ticks were transferred to the department of Arboviruses

and Viral Hemorrhagic Fevers (National Reference Laboratory) where RT-PCR was used to analyze them to detect CCHF viral genomes.

Molecular detection

Ticks were individually washed twice by PBS (phosphate-buffered saline, PH 7.4) and crushed with a mortar and pestle in 200-300µl of PBS. Total RNA was extracted using a RNeasy mini kit (Qiagen, Cat No. 2215716) according to the manufacturer's instructions. The extracted viral RNA was stored at −70 °C until analysis. The RT-PCR was performed in 50 µl reaction volumes containing ten µl 5x Qiagen one-step RT-PCR buffer, two µl of dNTP mixture (10 mM), 0.6 µM of each forward (F2: 5'- TGGACACCTTCACAAACT C-3') and reverse (R3: 5'-GACAATTCCCTA CACC- 3') primers, 2 µl Qiagen one-step RT-PCR enzyme mix, 1µl of RNase inhibitor and 5µl of extracted viral RNA as a template. 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 40 cycles of 95 °C for 30 sec, 50 °C for 30 sec, 72 °C for 45 sec, and a final extension at 72 °C for 5 min (Altaf et al., 1998; Chinikar et al., 2010; Duh et al., 2008).

IgG ELISA

The ELISA plates were coated overnight at 4 °C with CCHF antigen diluted at 1:500 in PBS 1%. Then the plates were washed three times by PBST (PBS containing 0.3% tween), mouse hyperimmune ascitic fluid diluted at 1:700 in PBSTM (PBST containing 3% milk powder) was added into the plates and incubated for 1 hour at 37 °C. The washing step was repeated, and diluted serum samples in PBSTM at 1:100 were added to the plates and incubated for 1 hour at 37 °C. After washing, the peroxidase-labeled anti-camel immunoglobulin diluted in PBSTM at 1:350 was added, and the plates incubated for 1 hour at 37 °C. Then the plates were washed three times with PBST.

Finally, tetramethylbenzidine was added, and the plates incubated for 15 min at room temperature. The enzymatic reaction was stopped by the addition of sulphuric acid (4N), and the optical densities (ODs) were read by the ELISA reader (Anathos 2020) at 450 nm and 620 nm. Samples with OD≥0.2, 0.18≤OD<0.2, and OD<0.18 were considered positive, borderline, and negative, respectively (Chinikar et al., 2010; Perveen et al., 2020; Estrada-Pena et al., 2004).

Statistical analysis

Data were analyzed using the SPSS version 16.0 statistics package. Descriptive statistics (i.e., prevalence and percentage) were used to summarize the quantitative variables.

Results

From the 320 examined one-humped camels, tick infestation was observed in 108 camels (33.75%). According to the herdsmen, the other one-humped camels had been treated with anti-parasitic drugs. Finally, 176 ixodid ticks (132 males and 44 females) were collected from different regions of the one-humped camels' bodies. One genus was identified as *Hyalomma*, and four species of *Hyalomma* were observed. These species consist of *H. dromedarii*, *H. marginatum*, *H. anatolicum*, and *H. impeltatum*. The population frequency of *H. dromedarii* (147 ticks, 83.5%) was higher than the others, and *H. impeltatum* had the lowest frequency (2 ticks, 1.1%). *H marginatum* comprised about 19 ticks (10.8%), and *H. anatolicum* accounted for 8 ticks (4.6%) from the total collected tick species (Table 2).

Table 2. The frequency of tick infestation in one-humped camels, between August 2015 and December 2016 in areas around the cities of Zahedan and Mirjaveh in the Sistan and Baluchestan province of Iran

Tick spp.	Male	Female	Total
Hyalomma dromedarii	117	30	147 (83.5%)
Hyalomma marginatum	6	13	19 (10.8%)
Hyalomma anatolicum	7	1	8 (4.6%)
Hyalomma impeltatum	2	0	2 (1.1%)
Total	132 (75.0%)	44 (25.0%)	176 (100%)

From the 108 blood samples taken, nine samples were not suitable for further processing due to contamination. From the 99 remaining samples (93 male 1-4.5 years old and 6 female 4.5-10 years old), 33 samples were found positive for CCHFV-specific IgG antibodies, accounting for an overall prevalence rate of 33.3%, and 12 out of 99 serum samples had borderline results. Two out of the 33 positive serum samples were obtained from female camels (one 7 and the other 10 years old), and 31 positive serum samples were obtained from male 1-4.5 years old camels (camel gender difference was not statistically significant, P=0.97). All the 176 collected adult ticks (44 female ticks and 132 male ticks) were negative for CCHFV in the RT-PCR result.

Discussion

Monitoring CCHFV infection in livestock and ticks is an appropriate tool for assessing CCHFV circulation and risk of infection within a region (Whitehouse, 2004; Spengler et al., 2019). Since adult ticks of the genus Hyalomma, the main reservoir and vector of CCHFV, tend to feed preferentially on large vertebrates (Guilherme et al., 1996; Champour et al., 2014), the one-humped camel was selected to assess the rate of CCHFV infection. One-humped camels generally harbour many ticks (Perveen et al., 2020), and the CCHFV develops CCHFV-specific IgG antibodies in camel blood (Champour et al., 2014; Champour et al., 2016). Despite the massive importation of ticks that burden one-humped camels imported into Iran and previous reports that showed *H. dromedarii* as CCHFV reservoirs (Guilherme et al., 1996; Champour et al., 2014), reliable information showing specific risks to the one-humped camel as a direct or indirect reservoir of CCHFV is unknown. Therefore, it could be essential to find relevant scientific data as documentation (Guilherme et al., 1996; Champour et al., 2014). Areas with a warm climate, high vegetation, grasslands, bushlands, forests, and agricultural areas are suitable habitats for Hyalomma ticks and correlate with high-risk areas for CCHFV infections (Al-Abria et al., 2017; Garcia et al., 2006; Khan et al., 1997). Ecological factors in the study's districts are ideal for developing CCHFV reservoirs like the *H. dromedarii* tick. Therefore, *H. dromedarii* was the most dominant species of tick on one-humped camels, and this result is in agreement with the outcome obtained by Salimabadi et al. (2010) and Champour et al. (2013) in Iran, and Lawal in Nigeria (Lawal et al., 2007). In this study, a high seropositivity rate of 33.3% was observed among 99 imported camels from Pakistan. Apart from the 100% seroprevalence reported in camels from Pakistan (1/1) (Estrada-Pena et al., 2007) and the 40% reported from China

(4/10) (Chumakov and Smirnova, 1972), our result is the highest infection rate in camels ever reported. In Iran, three studies in 1972, 1975 and 2014 investigated the infection of the camel to the CCHF virus. Saidi et al. (1975) detected no CCHFV antibodies in 157 camels from the south and southeast of Iran. However, Chumakov and Smirnova (1972) found CCHF antibodies in 19.1% of 99 camels in Iran. In a recent study by Champour et al. (2014), a seropositivity rate of 5.29% was reported in 170 camels from Khorasan provinces in the northeast of Iran. These authors showed lesser seropositivity in Iranian camels compared to Pakistani one-humped camels. This finding suggests that imported camels from Pakistan may pose a higher risk of CCHFV infection to the high-risk population.

The possible cross-reactivity with close Nairoviruses like Dugbe virus, Nairobi sheep disease virus and Qalyub viruses should be considered in CCHF serological tests. Antibodies to other Nairoviruses may exist independently or in conjunction with CCHFV-specific antibodies (Saidi et al., 1975; Swanepoel et al., 1983). Therefore, positive results of serological assays should be interpreted as “suggestive of CCHFV seropositivity” in areas not previously identified to have CCHFV transmission (Saidi et al., 1975; Swanepoel et al., 1983; Tarif et al., 2012; Lasecka et al., 2015). However, in endemic areas like Iran and Pakistan, reports of seropositivity could be regarded as positive proof (Lasecka et al., 2015). Despite seropositive camels, we did not observe any CCHFV infected ticks in the RT-PCR result. This result contrasts with previous studies conducted by Champour in Iran and that of Jabbari in Pakistan (Champour et al., 2014; Jabbari et al., 2013) where CCHFV RNA was identified in ticks attached to one-humped camels and other domestic animals. Although it is plausible that there should be a relationship between animal seropositivity and the detection of viral genomes in infesting ticks (Saidi et al.,1975), the detection of anti-CCHFV antibodies cannot be 100% indicative of the presence of CCHFV positive ticks and vice versa. Uninfected ticks from seropositive animals and infected ticks on seronegative animals have also been reported previously (Saidi et al.,1975). In conclusion, the serum prevalence of CCHFV is significantly high among one-humped camels imported into Iran from Pakistan, so it is advised to slaughter one-humped camels in standard abattoirs in the boundary area of Iran and import camel meat instead of tick burdened high-risk one-humped camels. Also, tick control is considered an effective procedure for CCHFV control.

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Conflict of interest

The authors have no conflicts of interest to declare for this study.

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References

Al-Abria, S. S., Al Abaidanib, I., Fazlalipour, M., Mostafavi, E., Leblebicioglu, H., Pshenichnaya, N., Memish, Z. A., Hewson, R., Petersen, E., Mala, P., Nhu Nguyen, T. M., Rahman Malik, M., Formenty, P., Jeffries, R. 2017. Current status of Crimean-Congo haemorrhagic fever in the World Health Organization Eastern Mediterranean Region: issues, challenges, and future directions. *Int. J. Infect. Dis.*, 58: 82-89.

Altaf, A., Luby, S., Jamil, A., Najam, A., Aamir, Z., Khan, J., Mirza, S.,

McCormick, J., Fisher-Hoch, S. 1998. Outbreak of Crimean-Congo haemorrhagic fever in Quetta, Pakistan: contact tracing and risk assessment. *Trop. Med. Int. Health.*, 3: 878–882.

Aslani, D., Salehi-Vaziri, M., Baniasadi, V., Jalali, T., Azad-Manjiri, S., Mohammadi, T., Khakifirouz, S., Fazlalipour, M. 2017. Crimean-Congo hemorrhagic fever among children in Iran. *Arch. Virol.*, 162(3): 721-725.

Berezin, V. V., Chumakov, M. P., Stolbov, D. N., Butenko, A. M. 1971. On the problem of natural hosts of Crimean hemorrhagic fever virus in Astrakhan region. *Tr. Inst. Polio.*, 19: 210-216.

Champour, M., Chinikar, S., Mohammadi, G. R., Razmi, G. R., Mostafavi, E., Shah-Hosseini, N., Khakifirouz, S., Jalali, T. 2016. Crimean-Congo Hemorrhagic Fever in the One-Humped Camel (Camelus dromedarius) in East and Northeast of Iran. *J. Arthropod-Borne. Dis.*, 10: 168-177.

Champour, M., Chinikar, S., Mohammadi, G. R., Razmi, G. R., Shah-Hosseini, N., Khakifirouz, S., Mostafavi, E., Jalali, T. 2014. Molecular epidemiology of Crimean-Congo hemorrhagic fever virus detected from ticks of one-humped camels (Camelus dromedarius) population in northeastern Iran. *J. Parasit. Dis.*, 40(1): 110-115.

Champour, M., Mohammadi, G. R., Chinikar, S., Razmi, G. R., Mostafavi, E., Jalali, T. 2013. Frequency of hard-ticks and the influence of age and sex of camel on tick's infestation rates in one-humped camel (Camelus dromedarius) population in the northeast of Iran. *Sci. Parasitol.*, 14(2): 89-93.

Champour, M., Chinikar, S., Mohammadi, G. R., Razmi, G. R., Mostafavi, E., Shah-Hosseini, N., Khakifirouz, S., Jalali T. 2014. Seroepidemiology of Crimean-Congo hemorrhagic fever virus in one-humped camels (Camelus dromedarius) population in the northeast of Iran. *J. Vector. Borne. Dis.*, 51: 62-65.

Chinikar, S., Ghiasi, S. M., Hewson, R., Moradi, M., Haeri, A. 2010. Crimean-Congo hemorrhagic fever in Iran and neighboring countries. *J. Clin. Virol.*, 47: 110-114.

Chumakov, M., Smirnova, S. 1972. Detection of antibodies to CHF in wild and domestic animal blood sera from Iran and Africa. *Virus. Zabolev.*, 367-368.

Darwish, M., Imam, I., Omar, F., Hoogstraal, H. 1978. Results of a preliminary seroepidemiological survey for Crimean-Congo hemorrhagic fever virus in Egypt. *Acta. virol.*, 22: 77.

Duh, D., Nichol, S. T., Khristova, M. L., Saksida, A., Hafner-Bratkovic, I., Petrovec, M., Dedushaj, I., Ahmeti, S., Avsic-Zupanc, T. 2008. The complete genome sequence of a Crimean-Congo hemorrhagic fever virus isolated from an endemic region in Kosovo. *Virol. J.*, 14: 5-7.

Ergönül, O. 2006. Crimean-Congo haemorrhagic fever. *Lancet. Infect. Dis.*, 6: 203–14.

Estrada-Pena, A., Bouattour, A., Camicas, J. L., Walker, A. 2004. Ticks of domestic animals in the Mediterranean region: A guide to identification of species. University of Zaragoza, Zaragoza Spain.

Estrada-Pena, A., Zatansever, Z., Gargili, A., Aktas, M., Uzun, R., Ergonul, O., Jongejan, F. 2007. Modeling the spatial distribution of Crimean-congo hemorrhagic fever outbreaks in Turkey. *Vector. Borne. Zoonotic. Dis.*, 7: 667-678.

Fazlalipour, M., Baniasadi, V., Mirghiasi, S. M., Jalali, T., Khakifirouz, S., Azad-Manjiri, S., Mahmoodi, V., Naderi, H. R., Zarandi, R., Salehi-Vaziri, M. 2016. Crimean-Congo Hemorrhagic Fever Due to Consumption of Raw Meat: Case Reports From East-North of Iran. *Jpn. J. Infect. Dis.*, 69(3): 270-1.

Garcia, S., Chinikar, S., Coudrier, D., Billecocqa, A., Hooshmand, B., Crance, J. M., Garin, D., Bouloya, M. 2006. Evaluation of a Crimean-Congo hemorrhagic fever virus recombinant antigen expressed by Semliki Forest Suicide virus for IgM and IgG antibody detection in human and animal sera collected in Iran. *J. Clin. Virol*., 35: 154-159.

Gargili, A., Estrada-Peña, A., Spengler, J. R., Lukashev, A., Nuttall, P. A., Bente, D. A. 2017. The role of ticks in the maintenance and transmission of Crimean-Congo hemorrhagic fever virus: A review of published field and laboratory studies. *J. antiviral*., 144: 93-119.

Guilherme, J. M., Gonella-Legall, C., Legall, F., Nakoume, E., Vincent, J. 1996. Seroprevalence of five arboviruses in Zebu cattle in the Central African Republic. *Trans. R. Soc. Trop. Med. Hyg*., 90: 31-33.

Hoogstraal, H. 1979. The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe, and Africa. *J. Med. Entomol*., 15: 307-417.

Horton, K. C., Wasfy, M., Samaha, H., Abdel-Rahman, B., Safwat, S., Abdel Fadeel, M., Mohareb, E., Dueger, E. 2014. Serosurvey for zoonotic viral and bacterial pathogens among slaughtered livestock in Egypt. *Vector. Borne. Zoonotic. Dis*., 14: 633-9.

Jabbari, A., Alijanpour, E., Tabasi, S. 2013. Facts about Crimean-Congo hemorrhagic fever and the role of intensive care in treatment and outcome. *Anesth. Essays. Res*., 7: 282-285.

Keshtkar-Jahromi, M., Sajadi, M., Ansari, H., Mardani, M., Holakouie-Naieni, K. 2013. Crimean-Congo hemorrhagic fever in Iran. *Antiviral. Res*., 100: 20–28.

Khan, A. S., Maupin, G. O., Rollin, P. E., Noor, A. M., Shurie, H. H. M., Shalabi, A. G. A., Wasef, S., Haddad, Y. M. A., Sadek, R., Ijaz, K., Peters, C. J., Ksiazek, T. G. 1997. An outbreak of Crimean-Congo hemorrhagic fever in the United Arab Emirates. *Am. J. Trop. Med. Hyg*., 57: 519-525.

Lasecka, L., Bin-Tarif, A., Bridgen, A., Juleff, N., Waters, R. A., Baron, M. D. 2015. Antibodies to the Core Proteins of Nairobi Sheep Disease Virus/Ganjam Virus Reveal Details of the Distribution of the Proteins in Infected Cells and Tissues. *PloS. One*., 23,10(4): https://doi: 10.1371/journal.pone.0124966.

Lawal, M. D., Amen, I. G., Ahmed, A. 2007. Some ectoparasites of Camelus dromedarius in Soko Nigeria. *J. Entomol*., 4: 143-148.

Mertens, M., Schmidt, K., Ozkul, A., Groschup, M. H. 2013. The impact of Crimean-Congo hemorrhagic fever virus on public health. *Antiviral. Res*., 98(13): 248-260.

Morrill, J. C., Soliman, A. K., Imam, I. Z., Botros, B. A., Moussa, M. I., Watts, D. M. 1990. Serological evidence of Crimean-Congo haemorrhagic fever viral infection among camels imported into Egypt. *J. Trop. Med. Hyg*., 93: 201-204.

Perveen, N., Muzaffar, S. B., Al-Deeb, M. A. 2020. Population Dynamics of Hyalomma dromedarii on Camels in the United Arab Emirates. *Insects*., 11: 320-25.

Rodrigues, F. M., Padbidri, V. S., Ghalsasi, G. R., Gupta, N. P., Mandke, V. B., Pinto, B. D., Hoon, R. S., Bapat, M. B., Mohan, Rao C. V. 1986. Prevalence of Crimean haemorrhagic fever-Congo virus in Jammu & Kashmir State. *Indian. J. Med. Res*., 134-138.

Saidi, S., Casals, J., Faghih, M. A. 1975. Crimean hemorrhagic fever-Congo (CHF-C) virus antibodies in man, and in domestic and small mammals, in Iran. *Am. J. Trop. Med. Hyg*., 24: 353-357.

Salimabadi, Y., Telmadarriy, Z., Vatandoost, H., Chinikar, S., Oshaghi, M. A., Moradi, M., Mirabzadeh Ardakan, E., Hekmat, S., Nasiri, A. 2010. Hard ticks on domestic ruminants and their seasonal population dynamics in

Yazd Province Iran. *Iran. J. Arthropod. Borne. Dis*., 4: 66-71.

Sharifi-Mood, B., Metanat, M., Alavi-Naini, R. 2014. Prevalence of Crimean-Congo Hemorrhagic Fever Among High Risk Human Groups. *Int. J. High Risk. Behav. Addict*., 3(1): 115-20.

Spengler, J. R., Bergeron, E., Rollin, P. E. 2016. Seroepidemiological Studies of Crimean-Congo Hemorrhagic Fever Virus in Domestic and Wild Animals. *PloS. Negl. Trop. Dis*., 10(1): 1-4210.

Spengler, J. R., Bergeron, E., Spiropoulou, CF. 2019. Crimean-Congo hemorrhagic fever and expansion from endemic regions. *Curr. Opin. Virol*., 34: 70-78.

Spengler, J. R., Estrada-Peña, A., Garrison, A. R., Schmaljohn, C., Spiropoulou, C. F., Bergeron, E., Bente, D. A. 2016. A chronological review of experimental infection studies of the role of wild animals and livestock in the maintenance and transmission of Crimean-Congo hemorrhagic fever virus. *Antiviral. Res*., 135: 31–47.

Spengler, J. R., Estrada-Peña, A. 2018. Host preferences support the prominent role of Hyalomma ticks in the ecology of Crimean-Congo hemorrhagic fever. *PLoS. Negl. Trop. Dis*., 8, 12(2): e0006248.

Sun, S., Dai, X., Aishan, M., Wang, X., Meng, W., Feng, C., Zhang, F., Hang, C., Hu, Z., Zhang, Y. 2009. Epidemiology and phylogenetic analysis of Crimean-Congo hemorrhagic fever viruses in Xinjiang, China. *J. Clin. Microbiol*., 47: 2536-2543.

Swanepoel, R., Shepherd, A. J., Leman, P. A., Shepherd, S. P., Miller, G. B. 1985. A common-source outbreak of Crimean-Congo haemorrhagic fever on a dairy farm. *S. Afr. Med. J*., 68: 6357.

Swanepoel, R., Struthers, J. K., Shepherd, A. J., McGillivray, G. M., Nel, M. J., Jupp, P. G. 1983. Crimean-Congo hemorrhagic fever in South Africa. *Am. J. Trop. Med. Hyg*., 32: 1407-1415.

Tantawi, H., Shony, M., Al-Tikriti, S. K. 1981. Antibodies to Crimean-Congo haemorrhagic fever virus in domestic animals in Iraq: a seroepidemiological survey. *Int. J. Zoon*., 8: 115-120.

Tarif, A. B., Lasecka, L., Holzer, B., Baron, M. D. 2012. Ganjam virus/Nairobi sheep disease virus induces a pro-inflammatory response in infected sheep. *Vet. Res*., 43: 71.

Walker, A. R., Bouattour, A., Camicas, J. 2003. Ticks of domestic animals in Africa, a guide to identification of species. Unpublished Bioscience Report 42, Coniston Drive Edinburgh Scotland, UK, 1-221.

Whitehouse, C. A. 2004. Crimean-Congo hemorrhagic fever. *Antiviral. Res*., 64(3): 145-160.

Williams, R. J., Al-Busaidy, S., Mehta, F. R., Maupin, G. O., Wagoner, K. D., Al-Awaidy, S., Suleiman, A. J., Khan, A. S., Peters, C. J., Ksiazek, T. G. 2000. Crimean-Congo haemorrhagic fever: a seroepidemiological and tick survey in the Sultanate of Oman. *Trop. Med. Int. Health*., 5: 99-106.