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No Detection of Crimean-Congo Hemorrhagic Fever Virus in Hard Ticks (Ixodidae) from a Highly Endemic Area in Southeast Iran

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ABSTRACT

Introduction: Crimean-Congo Hemorrhagic Fever (CCHF) is a zoonotic viral infection transmitted mainly via CCHF virus-infected ticks between vertebrate hosts. The disease occurs in almost all provinces of Iran. This study investigated the CCHFV infection in hard ticks collected from livestock in the Sistan region of Sistan and Baluchestan Province, southeast of Iran. **Methods:** In this study, ticks were collected from 220 livestock, including 150 sheep, 50 goats, 20 cows in five counties of Sistan Province (Zabol, Zehak, Hirmand, Nimruz, and Hamun). The ticks were identified under a stereomicroscope according to valid morphological keys. A reverse transcription-polymerase chain reaction (RT-PCR) method was used to detect the CCHFV genome via amplifying the S segment. **Results:** Among 100 selected ticks, RT-PCR revealed no CCHFV infection. **Conclusion:** Although no ticks were positive for CCHFV, it should be recalled that Sistan and Baluchestan province is among the highly endemic CCHF foci. As a result, further investigation and larger sample sizes are required to confirm our outcome. According to the hypothesis that direct contact with viremic livestock is more significant than tick bites in the viral transmission, more serological and molecular screening should be performed on high-risk individuals, e.g., slaughterhouse staff, ranchers, farmers, and veterinarians in the Sistan region.

INTRODUCTION

Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne negative-sense RNA virus belonging to the *Orthonairovirus* genus of the family *Nairoviridae*. Crimean-Congo hemorrhagic fever infection is characterized by headaches, high fever, chills, back and joint pain, vomiting, red eyes, petechiae, and bleeding with human fatality rates ranging from 10% to 50%. Transmission to humans occurs through ticks bite, contact with blood or tissues of viremic patients or livestock [1]. Ticks, especially the genus *Hyalomma*, are the leading vectors and reservoirs of CCHFV [2]. Among ticks, CCHFV is transmitted by co-feeding and passed on transovarially to the next generation and transstadially to the next life stages [3,4]. The virus stays with infected ticks throughout their lifespan and can be easily transmitted to other ticks and vertebrates [5]. Amplifying hosts such as various vertebrates are asymptomatic during CCHFV infection, whereas humans present clinical manifestations. Diagnostic methods for CCHFV include virus isolation, serology, and molecular-based techniques. Real-time PCR (RT-PCR), a highly sensitive and specific DNA-based assay, can identify the virus via amplifying viral genome sequences [6].

The disease was first identified in the Crimean Peninsula of Russia in the 1940s; today, it is reported from many parts of the world, including Africa, the Middle East, Europe, and Asia [7,8]. This disease was first mentioned in the book "Zakhireye Khwarazmshahi" by Al-Jurjani around 1110 AC. In Iran, CCHFV infection was first reported in 1970 and isolated in 1978 for the first time from ticks [9]. Later, outbreaks were reported from

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southwest Iran in 1999, and in 2000, CCHF was defined as a significant public health problem in Iran [2, 9].

As the name suggests, the Sistan and Baluchestan Province in the southeast of Iran comprises Sistan and Baluchestan regions. Sistan is located north of the province and includes five counties, i.e., Zabol, Zehak, Hirmand, Hamun, and Nimruz. Livestock plays a vital role in people's lives in Sistan. According to the Statistical Center of Iran, the small and large ruminant populations in Sistan and Baluchestan were estimated at 3232547 and 174354, respectively. Furthermore, CCHF is endemic in Afghanistan, which shares borders with Sistan. Illegal livestock transport occurs between cities close to the border [10]. Finally, a hot and dry climate is very suitable for tick activity. All these facts emphasize the importance of studying the parasitic fauna of the region.

This study aimed to determine the CCHFV infection in hard ticks collected from livestock in the Sistan region of Sistan and Baluchestan province, southeast of Iran.

MATERIAL AND METHODS

Study area and sample collection. Sistan and Baluchestan Province (29.4924°N 60.8669°E) shares borders with South Khorasan Province in the north, Afghanistan, and Pakistan in the east, Kerman and Hormozgan in the west, and the Sea of Oman in the south. Sistan is located north of Sistan and Baluchestan (Fig. 1). Ticks were collected from 220 livestock, including 150 sheep, 50 goats, 20 cows in five counties of Sistan (Zabol, Zehak, Hirmand, Nimruz, and Hamun) in July 2019. A multistage random sampling method was used for sampling, i.e., one village was randomly selected from each city, and three livestock holding units were randomly selected from each village for collecting tick samples. Hard ticks were randomly collected based on the species diversity, animal hosts, and geographic location using forceps and were transferred into separate labeled vials. Specimens were kept frozen at -20 ° C after each collection. All ticks were transferred to the Department of Medical Entomology, School of Public Health, Tehran University of Medical Sciences in cool boxes and were identified under a stereomicroscope according to valid morphological keys [11]. Identified ticks were then sent to the Arboviruses and Viral Haemorrhagic Fever Laboratory (National Reference Laboratory), Pasteur Institute of Iran, for molecular detection of CCHFV.

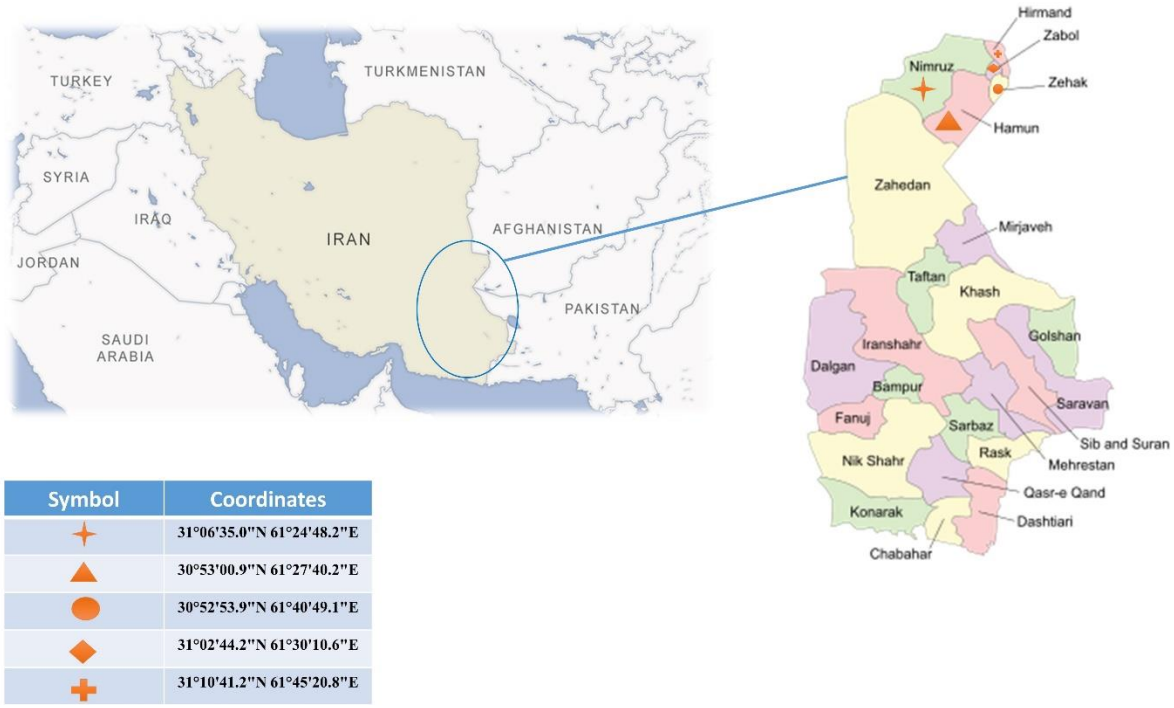

Fig. 1. The study area in the Sistan region, Iran. Sampling localities are marked with different symbols

RNA extraction and molecular detection. A one-step RT-PCR assay was used to amplify a 536-bp sequence of the S fragment in the CCHFV. Of 596 collected ticks, 100 were selected according to sex, life stage, locality, and examined for CCHFV. Ticks were individually washed twice by PBS (PBS, pH 7.4) and crushed with a mortar and pestle in 200–300 µl of PBS. According to the supplier's instructions, total RNA extraction was performed using a commercial RNeasy mini kit (QIAGEN, Cat No. 2215716) and stored at –70°C until used. A master mix with QIAGEN one-step RT-PCR kit (Cat No. 210212) included 28 µl of RNase free water, 10

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µl of buffer (5×), 2 µl of dNTP mixture (10 mM of each dNTP), 2 µl of enzyme mixture containing reverse transcriptase and *Taq* DNA polymerase enzymes, 1 µl of RNase inhibitor (4 units/µl). The final 50 µl reactions contained 1 µl (0.2 µM) of forward and reverse primes (5' TGGACACCTTCACAAACTC-3' and 5'-GACAATTCCCTACACC-3'), 5 µl template RNA, and 43 µl master mix. The amplification program included cDNA synthesis (50 °C for 30 min), denaturation (95 °C for 15 min), and 40 cycles of PCR (30 s at 95 °C for denaturation, 30 s at 50 °C for annealing, 45 s at 72 °C for extension) followed by 5 min at 72 °C for a final extension. PCR products were visualized by electrophoresis in 1.5% agarose gels [12,13].

Data analysis. The Chi-square test was used for data analysis; also 95% confidence interval for the prevalence of tick infestation was calculated using the binomial distribution. SPSS version 19 was used for statistical analysis. The significant level was considered $P < 0.05$.

RESULTS

We identified two genera, *Rhipicephalus* (40.6%) and *Hyalomma* (59.4%), comprising 239 *Rhipicephalus sanguineus* (40.1%), three *Rhipicephalus* spp. nymphs (0.5%), 283 *Hyalomma anatolicum* (47.5%), nine *Hyalomma* spp. (1.5%), and 62 *Hyalomma* nymphs (10.4%). The relationship between livestock and the prevalence of tick infestation was not statistically significant (P=0.872) (Table 1). Statistical tests also did not show any significant relationship between sampling locations and the prevalence of tick infestation (P=0.483) (Table 2).

RT-PCR results revealed no CCHFV infection among the selected 100 ticks (Fig 2, Tables 3 and 4).

Table 1. Frequency of collected ticks from livestock

Livestock	Tested (N)	Infested (N)	Prevalence (%)	*P*-value
Cow	20	12	60	
Goat	50	33	62.7	0.872
Sheep	150	94	66	
Total	220	139	63.2	-

Table 2. Frequency of collected ticks in different counties in Zabol

County	Tested (N)	Infected (N)	Prevalence (%)	*P*-value
Zabol	60	30	50	
Zehak	50	34	68	
Nimruz	40	31	77.5	0.483
Hamun	30	17	56.6	
Hirmand	40	27	67.5	
Total	220	139	63.18	-

Table 3. Species of tested ticks with RT-PCR and their hosts

Host	Tick species	No.
Cow	*Hyalomma* nymph	6
Goat	*R. sanguineus*	4
	H. anatolicum	4
	Hyalomma nymph	3
	Rhipicephalus nymph	2
Sheep	*R. sanguineus*	43
	H. anatolicum	32
	Hyalomma spp.	4
	Hyalomma nymph	2
Total		100

DISCUSSION

Crimean-Congo Hemorrhagic Fever (CCHF) is a zoonotic viral infection transmitted mainly by ticks among vertebrate hosts [14]. Most of Iran's neighboring countries, including Pakistan, Afghanistan, and Turkey, are endemic for the disease, and animal trade with these countries increases the risk of viral transmission in Iran. In Iran, CCHF occurs in provinces, with Sistan and Baluchistan, Isfahan, Fars, Tehran, Khorasan, and Khuzestan provinces comprising the highly endemic foci [15,16]. In the present study, hard ticks from different localities in five counties of the Sistan region were screened for CCHFV infection. Although Sistan is one of the main CCHF foci in Iran, the viral genome was detected in none of the ticks. Negative results may be due to 1) small sample size, 2) various animal reservoirs in the area (we did not check the ticks from some animals like camels, having the largest populations in Sistan and Baluchestan, birds, and wildlife like long-eared hedgehog found in large numbers, and 3) possibly low RNA yield due to mishandling tick specimens and the drawbacks of using cold chain during transportation.[9,14,17-18].

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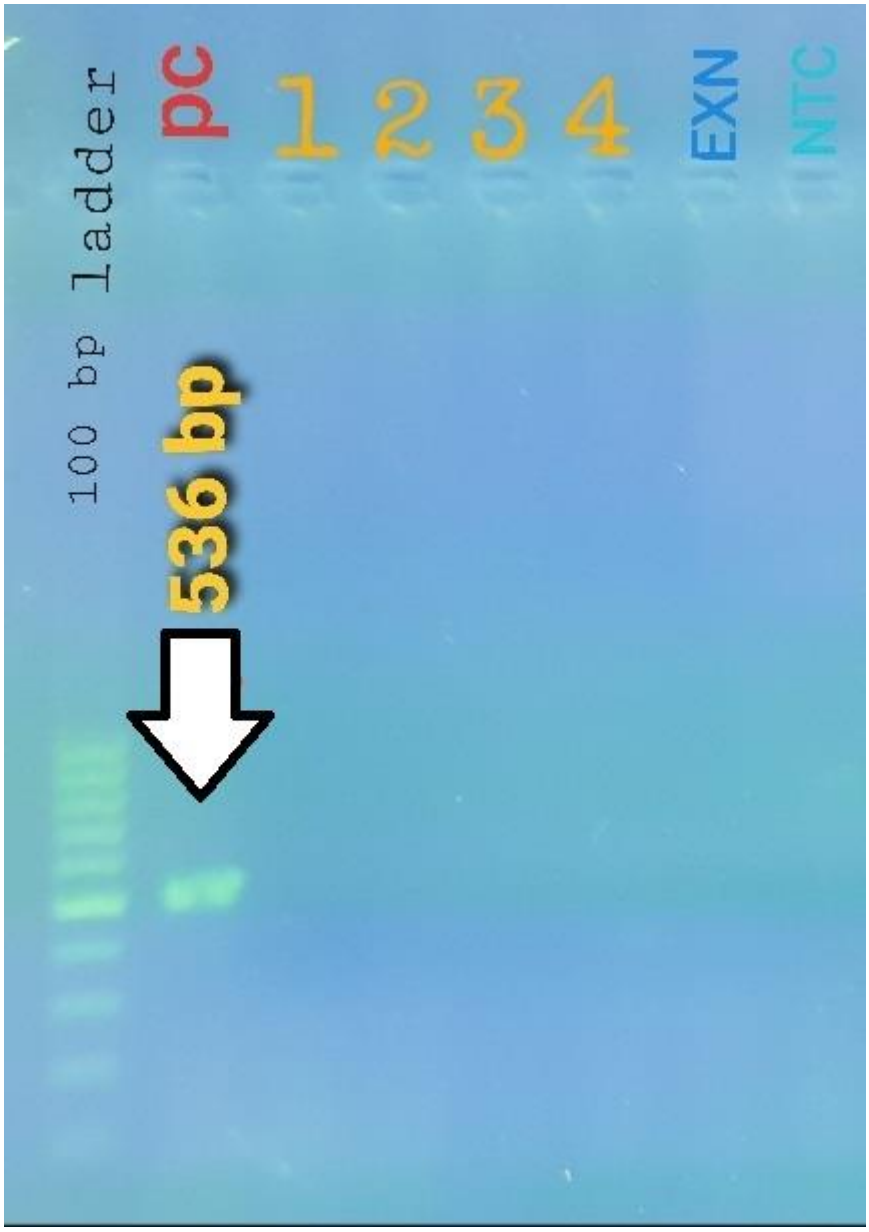

Fig. 2. Detection of CCHFV in ticks by RT-PCR. Lanes 1; 100 bp DNA ladder; lanes 2, positive control; lane3-6, ticks; lane 7, negative control; lane 8; RT-PCR negative control.

Table 4. RT-PCR results of screened ticks based on the different sampling area

County	RT-PCR Result	
	Positive (N)	Negative (N)
Zabol	-	22
Nimruz	-	17
Zehak	-	15
Hirmand	-	20
Hamun	-	26
Total	0	100

In previous studies, CCHFV infections among hard ticks in neighboring provinces of Sistan, including Kerman, Yazd, South Khorasan, and Baluchestan, were 0%, 5.7%, 35%, and 4.3%, respectively [9,17–19]. In Kerman Province, a CCHF endemic area, screening the members of the genera *Dermacentor*, *Hyalomma*, *Haemaphysalis*, and *Rhipicephalus* for CCHFV became negative for all specimens [18], similar to our study. A recent meta-analysis of tick-borne pathogens in Iran reported 4% of CCHFV among hard ticks like *Hyalomma*, *Rhipicephalus*, and *Haemaphysalis* species in Sistan and Baluchistan; the positive ticks mainly were from southern areas of Sistan and Baluchistan province [20]. In Iran, *R.*

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sanguineus, Hyalomma marginatum, *H. anatolicum*, *Hyalomma asiaticum*, *Hyalomma dromedarii* comprise most important CCHFV vectors [2]. Also, similar studies on livestock ticks in other countries have shown no or low prevalence of virus genome in ticks. In a study conducted in three areas of Sudan, none of the ticks harbored CCHF viral RNA, while 13.7% were positive for *Rickettsia* spp [21]. Tekin *et al.* (2012) also reported a low infection CCHFV rate (2%) in ticks from highly endemic regions of Turkey [22]. These findings and the result of the present study might suggest that even in some endemic areas, tick bites may not be the primary route of transmitting the virus to humans.

CCHFV infection in Iran mainly occurs due to direct contact with blood or tissues of infected livestock [20]. Moreover, tick bites seem to contribute less to viral transmission than close exposure to viremic livestock or humans in Iran [23]. These studies, alongside our results, may suggest that tick bites do not play a significant role in CCHFV transmission to humans in the Sistan region.

Further investigations with larger tick sample sizes are required to confirm our conclusion. Regarding the hypothesis that direct contact with viremic livestock plays a primary role in the viral transmission in the Sistan region, more serological and molecular screening should be considered for high-risk populations such as slaughterhouse workers, farmers, and veterinarians.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

REFERENCES

1. Sheikh AS, Sheikh AA, Sheikh NS, Asif M, Afridi F, Malik MT. Bi-annual surge of Crimean-Congo haemorrhagic fever (CCHF): a five-year experience. Int J Infect Dis. 2005; 9 (1): 37–42.
2. Telmadarraiy Z, Chinikar S, Vatandoost H, Faghihi F, Hosseini-Chegeni A. Vectors of Crimean Congo hemorrhagic fever virus in Iran. J Arthropod Borne Dis. 2015; 9 (2): 137.
3. Dohm DJ, Logan TM, Linthicum KJ, Rossi CA, Turell MJ. Transmission of Crimean-Congo hemorrhagic fever virus by Hyalomma impeltatum (Acari: Ixodidae) after experimental infection. J Med Entomol. 2014; 33 (5): 848–51.
4. Logan TM, Linthicum KJ, Bailey CL, Watts DM, Moulton JR. Experimental transmission of Crimean-Congo hemorrhagic fever virus by Hyalomma truncatum Koch. Am J Trop Med Hyg. 1989; 40 (2): 207–12.
5. Gargili A, Estrada-Peña A, Spengler JR, Lukashev A, Nuttall PA, Bente DA. The role of ticks in the maintenance and transmission of Crimean-Congo hemorrhagic fever virus: A review of published field and laboratory studies. Antiviral Res. 2017; 144: 93–119.
6. Mazzola LT, Kelly-Cirino C. Diagnostic tests for Crimean-Congo haemorrhagic fever: a widespread tickborne disease. BMJ Glob Heal. 2019; 4 (2): e001114.
7. Bente DA, Forrester NL, Watts DM, McAuley AJ, Whitehouse CA, Bray M. Crimean-Congo hemorrhagic fever: history, epidemiology, pathogenesis, clinical syndrome and genetic diversity. Antiviral Res. 2013; 100 (1): 159–89.
8. Hoogstraal H. The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe, and Africa. J Med Entomol. 1979; 15 (4): 307–417.
9. Yaser SA, Sadegh C, Zakkyeh T, Hassan V, Maryam M, Ali OM, et al. Crimean–Congo hemorrhagic fever: a molecular survey on hard ticks (Ixodidae) in Yazd Province, Iran. Asian Pac J Trop Med. 2011; 4 (1): 61–3.
10. Chinikar S, Ghiasi SM, Hewson R, Moradi M, Haeri A. Crimean-Congo hemorrhagic fever in Iran and neighboring countries. J Clin Virol. 2010; 47 (2): 110–4.
11. Walker AR. Ticks of domestic animals in Africa: a guide to identification of species. Bioscience Reports Edinburgh; 2003.
12. Farhadpour F, Telmadarraiy Z, Chinikar S, Akbarzadeh K, Moemenbellah-Fard MD, Faghihi F, et al. Molecular detection of Crimean–Congo haemorrhagic fever virus in ticks collected from infested livestock populations in a New Endemic Area, South of Iran. Trop Med Int Heal. 2016; 21 (3): 340–7.
13. Burt FJ, Swanepoel R. Molecular epidemiology of African and Asian Crimean-Congo haemorrhagic fever isolates. Epidemiol Infect. 2005; 133 (4) :659–66.
14. Telmadarraiy Z, Ghiasi SM, Moradi M, Vatandoost H, Eshraghian MR, Faghihi F, et al. A survey of Crimean-Congo haemorrhagic fever in livestock and ticks in Ardabil Province, Iran during 2004–2005. Scand J Infect Dis. 2010; 42 (2): 137–41.
15. Shafei E, Dayer MS, Telmadarraiy Z. Molecular epidemiology of Crimean-Congo hemorrhagic fever virus in ticks in northwest of Iran. J Entomol Zool Stud. 2016; 4 (5): 150–4.
16. Chinikar S, Persson S, Johansson M, Bladh L, Goya M, Houshmand B, et al. Genetic analysis of crimean-congo hemorrhagic fever virus in Iran. J Med Virol. 2004; 73 (3): 404–11.
17. Champour M, Chinikar S, Mohammadi G, Razmi G, Mostafavi E, Shah-Hosseini N, et al. Crimean-Congo hemorrhagic fever in the one-humped camel (Camelus dromedarius) in East and Northeast of Iran. J Arthropod Borne Dis. 2016; 10 (2): 168-77.
18. Khakifirouz S, Mowla SJ, Baniasadi V, Fazlalipour M, Jalali

[DOI: 10.52547/JoMMID.10.1.30]

T, Mirghiasi SM, et al. No Detection of Crimean Congo Hemorrhagic Fever (CCHF) Virus in Ticks from Kerman Province of Iran. J Med Microbiol Infect Dis. 2018; 6 (4): 108–11.

19. Mehravaran A, Moradi M, Telmadarraiy Z, Mostafavi E, Moradi AR, Khakifirouz S, et al. Molecular detection of Crimean-Congo haemorrhagic fever (CCHF) virus in ticks from southeastern Iran. Ticks Tick Borne Dis. 2013; 4 (1–2): 35–8.
20. Khoobdel M, Jafari AS, Telmadarraiy Z, Sedaghat MM, Bakhshi H. Tick-borne pathogens in Iran: A meta-analysis. Asian Pac J Trop Med 2021; 14 (11): 486-504.
21. Shuaib YA, Elhag AM-AW, Brima YA, Abdalla MA, Bakiet AO, Mohmed-Noor SE-T, et al. Ixodid tick species and two tick-borne pathogens in three areas in the Sudan. Parasitol Res. 2020; 119 (2): 385–94.
22. Tekin S, Bursali A, Mutluay N, Keskin A, Dundar E. Crimean-Congo hemorrhagic fever virus in various ixodid tick species from a highly endemic area. Vet Parasitol. 2012; 186 (3–4): 546–52.
23. Salehi-Vaziri M, Baniasadi V, Jalali T, Mirghiasi SM, Azad-Manjiri S, Zarandi R, et al. The first fatal case of Crimean-Congo hemorrhagic fever caused by the AP92-like strain of the Crimean-Congo hemorrhagic fever virus. Jpn J Infect Dis. 2016; 69 (4): 344–6.

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