Research Paper

Evaluation of CCHF Infection in Hard Ticks in Razavi Khorasan Province, Iran

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ABSTRACT

Background: Crimean-Congo hemorrhagic fever (CCHF) is a potentially fatal tick-borne viral disease. Hard ticks are both carriers and reservoirs of the CCHF virus. In this regard, the present study was done to investigate the CCHF viral infection in collected ticks from livestock in Gonabad City (Southwest of Razavi Khorasan Province) in Eastern Iran.

Methods: This descriptive study was performed in rural areas of Gonabad City in 2018. The forceps sampling method collected hard ticks from livestock (goats, sheep, and cattle). The ticks were identified based on a valid taxonomic key; finally, the CCHF viral infection was evaluated using the RT-PCR technique.

Results: Between April and October 2018, 100 ticks were collected from 13 rural areas of Gonabad. The frequency of ticks collected from goats, sheep, and cows was 6.4%, 3.7%, and 89.9%, respectively. Also, 90% of ticks were Hyalomma (*Hyalomma anatolicum excavatum* (n=9), *Hyalomma lusitanicum* (n=59), *Hyalomma marginatum* (n=4), *Hyalomma anatolicuman* (n=18)) and the remaining 10% were *Rhipicephalus sanguine*. Overally, CCHF infection was observed in 14% of the ticks (*Hy. excavatum*, *Hy. lusitanicum*, and *Hy. anatolicumand Rhipicephalus sanguineus*).

Conclusion: Hyalomma species is the main vector of the CCHF virus. Due to the high abundance of hard ticks in nature and the livestock environment, special care is required in the villages. Also, due to the presence of more scattered ticks in the northern half of the country, comprehensive studies that cover a wide geographical area and cover a larger sample size are necessary.

Keywords: Hard tick, Crimean Congo hemorrhagic fevers, Hyalomma, Iran

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1. Introduction

Crimean Congo hemorrhagic fever (CCHF) is a zoonotic viral disease that is endemic in many countries in Africa, Asia, and Europe [1]. CCHF is a serious public health threat. The World Health Organization (WHO) classified it as a potential epidemic-prone emerging disease [2]. CCHF virus can be transmitted to humans mainly via the bite of infected ticks or direct contact with the blood/ tissue/ fluids of the infected animal [3]. CCHF infections in humans usually give rise to severe, acute hemorrhagic fever with a mortality rate of 10-50% [4].

CCHF virus belongs to the genus Orthonairovirus of Nairoviridae family [5]. Hard ticks play a key role in the circulation of the virus in nature, serving as both the reservoir and carrier of the CCHF virus [6]. The CCHF endemicity is directly associated with the geographical distribution of the reservoir ticks. So far, the CCHF virus nucleic acid has been identified in around 31 species of ticks, including Hyalomma, Ixodes, Amblyoma, Boophilus, and Rhipicephalus [6]. Hyalomma species are the main vector of CCHF virus [7]. Worldwide, diagnostic methods for CCHF include virus isolation, serology, and molecular techniques. Real-time PCR (RT-PCR) is a very sensitive and specialized laboratory method of DNA sampling that can identify the virus by improving the viral genome sequence [8].

CCHF is endemic in most countries in the Middle East, including Iran, Afghanistan, and Pakistan, and Hyalomma is the main vector CCHF virus (CCHFV) [9]. Khurshid et al. in 2015 [10] reported that the nucleotide diversity of strains in the Asia-1 clade is likely to be approximately 4%, while the subclade divergence is approximately 0-1%. Also, 33 of the 39 viruses grouped in subclass A with previously reported viruses from the regions, such as U75677 from Pakistan and GU456727 from Iran [10]. In Iran, the disease was first identified in 1999 [11]. Eastern provinces of Iran, such as Sistan and Baluchestan and Razavi Khorasan are the most important CCHF foci [7]. Gonabad City in the Southwest of Razavi Khorasan Province is located in proximity to Afghanistan (one of the sources of illegal transport of livestock to Iran) and the majority of people living in rural areas of this city are involved in animal husbandry. Consequently, Gonabad can be a hot spot for the disease. Recently, CCHF cases have been reported among animal herders in Gonabad. However, to the best of our knowledge, there is no information addressing the rate of CCHF virus infection in ticks in this area. The present study, therefore, was designed to evaluate CCHFV infection in hard ticks collected from livestock in rural areas of Gonabad.

2. Methods

Study location

Gonabad, with an area of 5902 square kilometers, is located at 58 degrees and 41 minutes of geographic longitude and 34 degrees and 21 minutes of latitude, and in the eastern part of the Razavi Khorasan Province, Iran (Figure 1). In general, this city is in the arid and semi-arid region in the vicinity of desert areas. Most residents of the rural areas of Gonabad are shepherds.

Figure 1. Geographic location of Gonabad City in Iran and sampling places

Tick sampling

A total of 800 animals, including 200 cattle, 100 goats, and 500 sheep from 13 villages were investigated for tick infestation from May to October 2018 (Figure 1). Using personal protective equipment, ticks were collected by forceps. The live ticks were placed in sterile falcons and transferred to the entomology laboratory to identify their genus/ species according to Walker Diagnostic Key [12]. A total of 100 ticks were referred to the Department of Arboviruses and Viral Hemorrhagic Fevers (National Reference Laboratory) at the Pasteur Institute of Iran for CCHFV infection analysis.

Molecular assay

Ticks were homogenized by Tissue Lyser II (Qiagen). Viral RNA was then extracted from the ticks' homogenates using High Pure Viral RNA Kit (Roche). All the procedures were performed according to the manufacturer's protocols [13]. Extracted RNAs were stored at -70˚C until further analyses.

Detection of CCHF virus RNA was carried out by a homemade SYBR Green one-step real-time reverse transcriptase PCR assay with the qPCR BIO SyGreen Mix Lo-ROX kit (Biosystems).

Statistical analysis

Statistical analysis was carried out using SPSS software, version 20. The chi-square test was employed to analyze the correlation between categorical variables.

3. Results

A total of 100 hard ticks (50% male and 50% female) were collected from the studied livestock in 13 villages (Figure 1). These ticks belonged to two genera, including Hyalomma (90%) and Rhipicephalus (10%). Hyalomma species were as follows: *Hy. lusitanicum* (59%), *Hy. excavatum* (9%), *Hy .anatolicum* (18%), and *Hy. marginatum* (4%). The only Rhipicephalus species was *Rh. sanguineus* (10%) (Table 1).

Fourteen out of 100 ticks (14%) were CCHF-positive according to the RT-PCR test. Infected ticks included *Hy. lusitanicum* (57.1%, n=8), *Hy. anatolicum* (21.4%, n=3), *Hy. Excavatum* (7.1%, n=1) and *Rh. sanguineus* (14.3%, n=2). No statistically significant association was found between tick species and CCHFV infection (P=0.79) (Table 1).

Similarly, there was no statistically significant association between the ticks' sex and CCHF viral infection (P=0.58) as the infection rate was the same in both sexes (Table 1).

As shown in Table 2, the majority of infected ticks (57.1%, n=8) were collected in spring (May and June); however, no significant association was observed between the month of sampling and CCHFV infection (P=0.13) (Table 2).

In terms of the tick host species, out of 14 infected ticks, nine (62.3%), four (28.6%), and one (7.1%) infected ticks were collected from cattle, sheep, and goats, respectively. There was no significant correlation between host species and tick infection (P=0.54) (Table 3).

Table 1. Frequency of collected hard ticks according to their sex and the presence of CCHFV infection

Tick Genus	Results			Sex						Total	p
				Female			Male				
	Posit.	Neg.	P	Posit.	Neg.	Total	Posit.	Neg.	Total		
Hy. lusitanicum	8	51	0.79	3	26	29	5	26	31	59	0.58
Hy. excavatum	0	4		1	4	5	-	3	3	9	
Hy. anatolicum	3	15		3	6	9	-	9	9	18	
Hy. marginatum	1	8		-	2	2	-	2	2	4	
Rh. sanguineus	2	8		-	5	5	2	3	5	10	
Total	14	86		7	43	50	7	43	50	100	

Posit.: Positive; Neg.: Negative.

Table 2. Frequency of collected hard ticks according to the month of sampling and the rresence of CCHF infection (P= 0.13)

Tick	Month and Results of RT-PCR												Total
	Positive						Negative						
	May	Jun	Jul	Aug	Sep	Total	May	Jun	Jul	Aug	Sep	Total	
Hy. lusitanicum	4	3	-	-	1	8	12	17		19	3	51	59
Hy. excavatum	-	1	-	-	-	1	2	1	5			8	9
Hy. anatolicum	-	-	3	-	-	3	2	1	3	9		15	18
Hy. marginatum	-	-		-	-			4				4	4
Rh. sanguineus	-	-	1	1	-	2			3	5		8	10
Total	4	4	4	1	1	14	16	23	11	33	3	86	100

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4. Discussion

In this study, 90% of sampled hard ticks belonged to the Hyalomma genus, which is the most important CCHF virus vector [14]. Khorasan is one of the most CCHF frequent provinces of Iran. This is due to the prosperity of the livestock industry and the transit of livestock from the southeastern regions of the country or the illegal import of livestock from the neighboring countries, namely Afghanistan. *Hy. lusitanicum*, which accounted for 50% of the sampled ticks, closely resembles the *Hy. anatolicum excavatum*. It is abundant in areas with a high population of rabbits and cattle.

The reported *Hy. marginatum* tick in this study is more prevalent in Southeast Europe, the Mediterranean area, and the Middle East. These ticks are in the larval and nymph stages of foreign parasites of small mammals, such as mice and birds. The mature stage of these ticks can be found in domestic animals, such as cattle and sheep.

Due to the positive tendency of these ticks to human blood, people in close contact with the animal or those working in livestock farms may be bitten by this arthropod. Most of the ticks of this species have been sampled from Hamedan Province [7] but in studies, abundance in Khorasan Razavi and Khuzestan Provinces has been addressed. Other reports have addressed Kermanshah, Ilam, East Azerbaijan, South Khorasan, Tehran, and Yazd. The ticks were positive for the transmission of the CCHFV. This species has been sampled more from cattle and sheep, which is consistent with our study [7]. The CCHF disease is observed in late spring and summer when the population of ticks increased with the highest abundance in Khuzestan. Due to the similarity of this species with *Hy. asiaticum* and *Hy. excavatum*, it is usually difficult to differentiate them. However, in 2012, Hosseini et al. proposed the length of the lateral groove of the scotoma as a diagnostic trait among *Hy. anatolicum* species [13].

Table 3. Frequency of collected hard ticks according to their relevant host and the presence of CCHFV infection (P= 0.54)

Tick	Tick Positive				Tick Negative				Total
	Goat	Cow	Sheep	Total	Goat	Cow	Sheep	Total	
Hy. lusitanicum		5	4	9		33	18	51	59
Hy. excavatum		1		1		4	4	8	9
Hy. anatolicum		3		3		6	9	15	18
Hy. marginatum							4	4	4
Rh. sanguineus	1			1	2	2	4	8	10
Total	1	9	4	14	2	45	39	86	100

JRH

As in rural areas, humans are often bitten by ticks, and because human antibodies to the CCHFV are low [15], the low incidence of the disease indicates the higher presence of the ticks in these study areas. Ticks do not want to feed on human blood and prefer their animal host. In rural areas, contamination of the animal may not be transmitted to humans upon compliance with the correct principles of slaughter, packaging, and storage of the meat. It also seems that the blood-eating phase in both males and females increases the likelihood of transmission of the infection, and sex does not have a direct impact on the transmission of the virus from ticks attached to livestock. Further understanding of ticks in nature requires additional studies and sampling from different geographical areas.

Regarding the month of infection, as the ticks were separated from the animal, the tick may have been on the animal for a long time and the exact time of infection of the animal from the tick or vice versa is not clear. Therefore, it is not possible to accurately assess the infection duration.

One of the reasons for more contamination in ticks isolated from cattle can be the high volume of livestock with cows or easier maintenance of this animal or its organized smuggling. Moreover, due to the lack of wool as a barrier to blood-eating, cows are better hosts for ticks [16]. Furthermore, if the cattle were smuggled from the neighboring countries, the ticks may have been on the cattle from the beginning and originated from that country, which requires further investigation.

Ripisphalus tick has two species in Iran: Sanguineus and Bursa. In 2015, Telmadarraiy et al. identified Sanguineus as a carrier of CCHF, which is consistent with our study (20% of ticks were positive) [17].

The species is widely distributed in the Mediterranean area, Central America, Europe, Africa, and Asia, with reports of human infection due to mite bites in some parts of the continents. This mite is a three-host species and feeds more from dogs, but it might bite by chance [18].

In studies conducted in the Khorasan Province, 3.8% of the sampled ticks were infected with the CCHFV, among which *Hy. marginatum* was observed [4]. In a report in 2010, Albayrak et al. expressed that the infection rates of *Hy. marginatum*, *Hy. anatolicum*, and *Hy. excavatum* ticks were 8.4%, 3.12%, and zero, respectively.

In our study, the prevalence of infection of these ticks was zero, 3%, and 1%, respectively [5].

In a study by Telmadarraiy et al. in Ardabil, in terms of infection, nine species of mites sampled were the most infected: sheep (41.9%), cattle (30%), and goats (33.3%), respectively. In general, 27% of ticks were infected, but in our study, cattle (8%) and sheep (3%) were the most infected, respectively, and 14% of ticks were infected [17].

In a study conducted in Kurdistan, Iran in 2007 by Fakorziba et al., 414 hard ticks were collected, 70% of which were from the Hyalomma genus, of which only 5.6% of ticks were positive by RT-PCR method. Positive ticks were mostly sampled from cows. Most sampled mites were *Hy. anatolicum* and *Hy. marginatum* (37% and 27%, respectively) [18].

In their report published in 2007 on ticks in Hamedan, Tahmasebi et al. sampled 328 hard ticks from 70 villages and from 30 to 20 sheep. All parts of the sheep, especially the ears, neck, tail, and perineum, were examined. Five species of mites were sampled, including *Hy. detritum* (89.6%), *Hy. anatolicum* (3.4%), and *R. sanguineus* (6.1%). The highest infection in ticks was related to *Hy. detritum* (16.32%), *Hy. anatolicum* (18.18%), *R. sanguineus* (55%), respectively [19].

In another study conducted in South Khorasan in 2012-2013, 200 camels from three cities (Boshroyeh, Birjand, and Nehbandan) were examined, and 171 camels were infected by ticks. Also, 480 hard ticks were collected, and *Hy. deromedarii* had the highest abundance (90.7%), followed by *Hy anatolicum* (6%), *Hy. marginatum* (2.9%), and *Hy. asiaticum* (0.4%). The infection rate was 10.2% as determined by RT-PCR. Tick infection of the cities of this province decreased in the following order: Boshroyeh, (5.2%), Birjand (3.5%), and Nehbandan (1.5%), [20]. The results from several studies show that the rate of infectivity of CCHF is different and depends on the weather and geographical diversity, the frequency of different tick hosts, and different tick species [21].

In Sistan and Baluchestan Province in Southeastern Iran, the CCHFV was isolated from 4.5% of the sampled ticks [22]. In Yazd Province, located in the center of Iran, as in our study, Hyalomma ticks were reported to be the main carriers of the CCHFV [23].

In conclusion, Hyalomma species is the main vector of the CCHFV. Due to the abundance of hard ticks in nature and the livestock environment, special care is required in the villages with a high abundance of such hard ticks. Also, regarding higher scattered mites in the northern half of the country, more comprehensive studies covering a wide geographical area and a larger sample size are necessary.

5. Conclusion

Hyalomma species is the main vector of the CCHFV. Due to the abundance of hard ticks in nature and the livestock environment, special care is required in the villages with a high abundance of such hard ticks. Also, regarding higher scattered ticks in the northern half of Iran, comprehensive studies covering a wide geographical area and a larger sample size are necessary.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Research Committee of Gonabad University of Medical Sciences (Code: IR.GMU.REC.1396.72).

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Authors' contributions

Data collection and writing the original draft: Hamed Ramezani Awal Riabi and Reza Ahmadi; Laboratory investigations, review and editing: Mahsa Tavakoli Rad, Mehdi Fazlalipour and Sahar Khakifirouz.

Conflict of interest

The authors declared no conflict of interest.

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