



Research Paper

Crimean Congo Hemorrhagic Fever Virus in Ticks Isolated from Ruminants in Jahrom, Fars Province, Southern Iran



Saman Rastgoo¹, Gholamreza Shokoohi¹, Hooshmand Ghorbaani Barnaaji¹, Ahmad Abolghazi^{2*}

1. Department of Medical Parasitology, School of Medicine, Jahrom University of Medical Sciences, Jahrom, Iran.

2. Department of Medical Parasitology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.



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ABSTRACT

Background: Crimean Congo hemorrhagic fever (CCHF) is an emerging tick-borne viral zoonotic disease that is endemic in Iran. The CCHF virus is spread by an RNA virus of the genus *Orthonairovirus*, the family *Nairoviridae*. CCHF has been identified as the most frequent viral hemorrhagic fever in Iran. The route of CCHF transmission to humans is through infected tick bites, contact with infected livestock, infected blood or tissues of humans.

Objectives: The aim of this study was to investigate the infection of livestock with different species of ticks and also to determine the presence of CCHF virus in the ticks in Jahrom township.

Materials & Methods: A total of 200 ticks were collected from Jahrom township, Fars Province, Southern Iran. To detect virus in infected ticks, total RNA was extracted using the RNeasy Mini Kit Qiagen and subjected to reverse transcriptase-polymerase chain reaction.

Results: Of all the ticks tested, twelve (6%) were positive for the virus, 124 (62%) belonged to the species *Rhipicephalus sanguineus*, 37 (18.5%) belonged to *Hyalomma anatolicum*, 23 (11.5%) belonged to *Hyalomma marginatus*, 7 (3.5%) belonged to *Hyalomma asiaticum*, 4 (2%) belonged to *Hyalomma dromedarii*, and 5 (2.5%) belonged to *Rhipicephalus bursa*. Of the 200 ticks collected in the area, 117 (58.5%) were male and 83 (41.5%) were female.

Conclusion: In this study, for the first time, we analyzed tick samples collected from Jahrom township for the presence of CCHF virus. The findings of our study might be valuable for designing preventive measures regarding CCHF in Fars province, although further studies are required.

Keywords: Crimean Congo hemorrhagic fever, Reverse transcriptase PCR, Tick

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* Corresponding Author:

Ahmad Abolghazi, PhD.

Address: Department of Medical Parasitology, Hamadan University of Medical Sciences, Hamadan, Iran.

Tel: +98 (910) 4968651

E-mail: ahmadabolghazi@gmail.com

1. Introduction

In veterinary sciences, ticks are classified as the most important ectoparasites as domestic animals [1]. In the world, about 80% of cows are infected with ticks. In developing countries ticks are one of the reason for reduction of the effective factors in milk and meat of domestic animals and disease transmission in the world [2, 3]. All over the world ticks cause economic losses among livestock and health damage in human, including Iran [4, 5]. In Iran some species such as *Rhipicephalus sanguineus*, *Hyalomma marginatum*, *H. asiaticum*, *H. anatolicum* and, *H. dromedarii* were known as the most frequent. Research has shown that both families Argasidae (soft ticks) and Ixodidae (hard ticks) are found in Fars province, that in this region *Hyalomma* spp. has reported as the highest frequent. Ticks are a good vectors for the transition of bacteria and protozoa [6, 7]. About 10% of ticks feed on domestic animals, especially cattle, sheep and goats, and due to their ability to transmit the infective agents of human and animal diseases are of significant health importance [8, 9]. Ticks transmit a group of viral hemorrhagic fevers. Crimean-Congo hemorrhagic fever (CCHF) or Central Asian hemorrhagic fever that is a viral disease of acute human-animal hemorrhagic fever caused by a virus of the family Nairoviridae a single-stranded RNAs of the genus Orthonairovirus [10, 11]. The most important carrier and reservoir of this virus in nature is a tick of the family Ixodidae (hard ticks) of the genus *Hyalomma* [12]. This disease has one of the widest geographical distributions among known viruses transmitted by ticks. CCHF has been reported from more than 30 countries in the world. Also, in Iran has been reported. The study carried out by Chinikar et al. in 2010 for estimating the prevalence of CCHF in Iran in 23 provinces showed that Fars province has the third highest frequency of serum contamination by Crimean Congo Hemorrhagic Fever Virus [13]. Contact with infected blood, secretions and tissues of infected animals, as well as human contact with infected humans is one of the most common ways of transmitting the infection to humans [14]. This study was conducted in Jahrom township to investigate the infection of livestock (goats, sheep, cattle and camels) with different species of ticks and also to determine the presence of CCHF virus in the ticks of this region.

2. Materials and Methods

The current study was performed in Jahrom, Fars Province. Jahrom is located at South West of Iran. For this survey, 200 ticks were collected from camel, cattle,

sheep and goats in 2019. Adult ticks were collected from the skin of various organs of the animals, including the skin ears, head and neck, under the tail and genital area, groin, and the surface of the hair and skin. Then the ticks were transferred to numbered glass tubes. The characteristic was recorded and transferred to the medical parasitology laboratory of Jahrom University of Medical Sciences. Ticks were identified to species according to identification standard keys [15]. Ticks were stored at -80°C until testing for the presence of viral RNA and the positive control sample was prepared from the laboratory of Arboviruses and Viral Hemorrhagic Fevers, Pasteur Institute, the national reference lab of Iran. The first step was extract fragments of the virus genome. For this purpose, the ticks are washed in the buffer, crushed and homogenized. Then, using RNA extraction kit from QIAGEN Germany, RNeasy plus mini kit made in Germany was used. Finally, the RNA was extracted and transferred to a freezer at -80°C . Then, for all samples, transcriptase-polymerase chain reaction (RT-PCR) was performed with instructions and primers described by Albayrak et al. [16]. After that all samples were analyzed by gel agarose 1.5% electrophoresis. Finally, the results were analyzed using SPSS software, version 21.

3. Results

From the total ticks, 124 (62%) belonged to the species *Rhipicephalus sanguineus*, 37 (18.5%) belonged to *Hyalomma anatolicum*, 23 (11.5%) belonged to *Hyalomma marginatus*, 7 (3.5%) belonged to *Hyalomma asiaticum*, 4 (2%) belonged to *Hyalomma dromedarii* and 5 (2.5%) belonged to *Rhipicephalus bursa*. Of the 200 ticks collected in the area, 117 (58.5%) were male and 83 (41.5%) were female. The frequency of tick species caught was calculated separately by the host (Figure 1).

Out of a total of 200 ticks collected in the four seasons of spring, summer, autumn and winter in 2019, the highest number of ticks 156 (78%) was caught in the spring (Figure 2).

Out of 200 ticks examined for CCHF virus, 12 (6%) were positive. The findings based on the tick species and the host are shown in Figure 3. All positive samples have been a specific band about of 536 bp (Figure 4).

4. Discussion

Crimean Congo hemorrhagic fever is one of the most important viral diseases transmitted by arthropods [15-17]. Iran is one of the countries that are endemic in terms of the presence of this virus. According to the research-

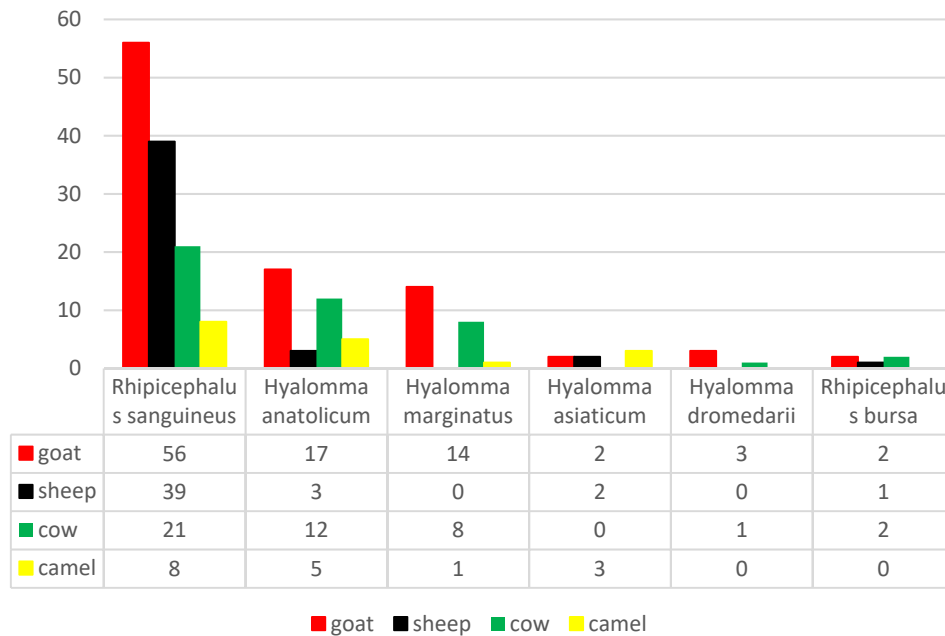


Figure 1. The frequency of ticks according to the host in Jahrom, South of Iran



ers' findings, climatic conditions and hosts play an important role in the transmission of the CCHF virus in the natural cycle and epidemiology of arthropod-borne diseases such as CCHF [18]. Studies conducted in different provinces of the country to determine the infection rate of hard and soft ticks with CCHF virus indicate that the distribution pattern and the infection rate in various animals are significantly different [19, 20]. Studies have shown that ticks have a high ability to infest different vertebrates, including mammals, reptiles, and birds, and between the different hosts, the tick species and their frequency were different. Therefore, the variety in the host may cause differences in the amount

of infestation and tick species [21]. In previous studies conducted in different parts of the country, the prevalence of this disease has been different. In Sistan and Baluchestan, Yazd, Mervdasht, Darrehshahr, Abdanan, Kermanshah, Lorestan, and Kashan, which was almost similar to the results of this study, which was about 6% [22-29]. Maybe one of the reasons for this similarity depends on the similarity of climatic conditions in these areas, which have hot and dry climates. According to previous studies on ticks in different parts of the country to find the genome of the CCHF virus, the highest percentage of infection is related to the study in Ardabil Province in 2008 with 27.7% and the lowest percentage

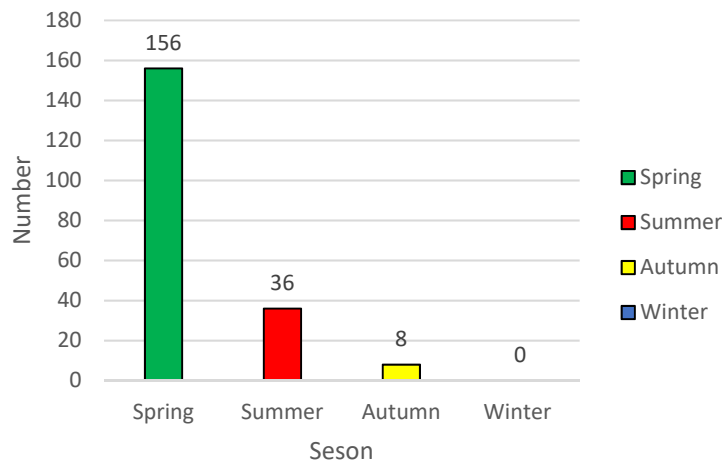


Figure 2. Seasonal variation of collecting ticks in Jahrom, South of Iran



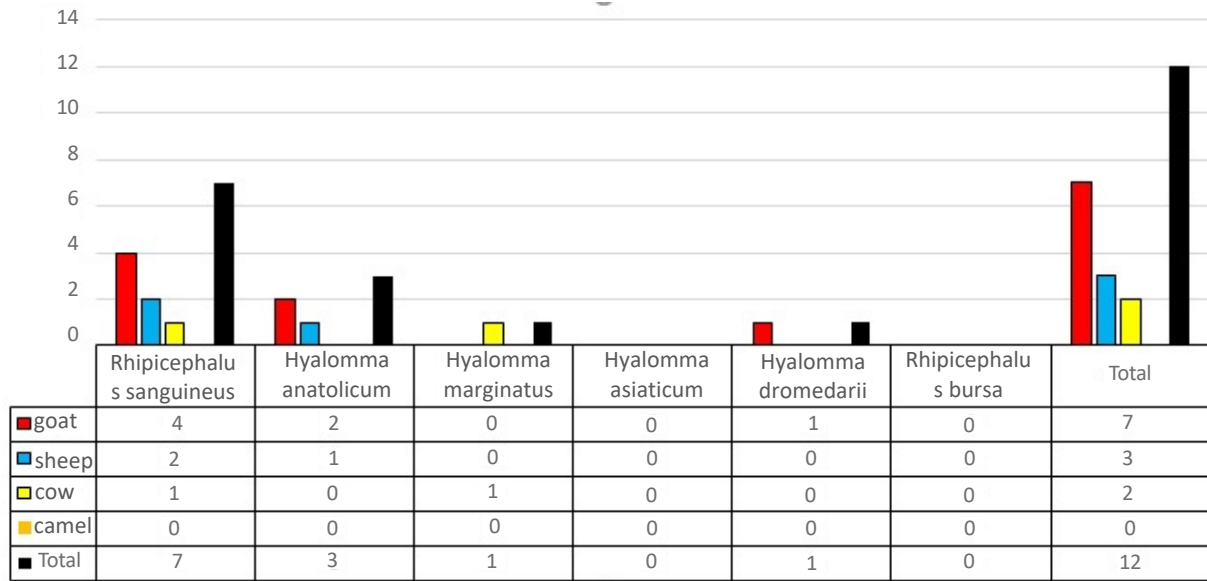


Figure 3. The frequency of ticks with CCHF virus according to the host in Jahrom, south of Iran



of infection is related to the study in Kashan in 2013 with 1.29% [29-33]. Based on past studies of ticks in various hosts such as cattle, goats, and sheep, the incidence of tick infestations differed. During the Sanandaj study, the researchers reported only five positive samples, four of them were in cattle and only one in goats and no positive sheep samples were reported. In another study in Yazd, researchers reported the highest frequency of cattle contamination (about 10%), while all samples were negative in ticks isolated from goats [21]. However, in this study, the most significant infection was associated with iso-

lated ticks in goats, and no positive cases were reported in ticks isolated in camels. It may suggest that in any region, the abundance of domestic animals and the tendency of ticks to feed on their blood are important factors in the positivity of ticks. Studies have shown that, in addition to climatic conditions and geographical diversity, the presence of multiple hosts and species of ticks is effective in the prevalence of CCHF in each region [28]. For various reasons, including the high mortality of this disease, climatic diversity in each region, diversity of hosts and diversity of carriers, it seems very important

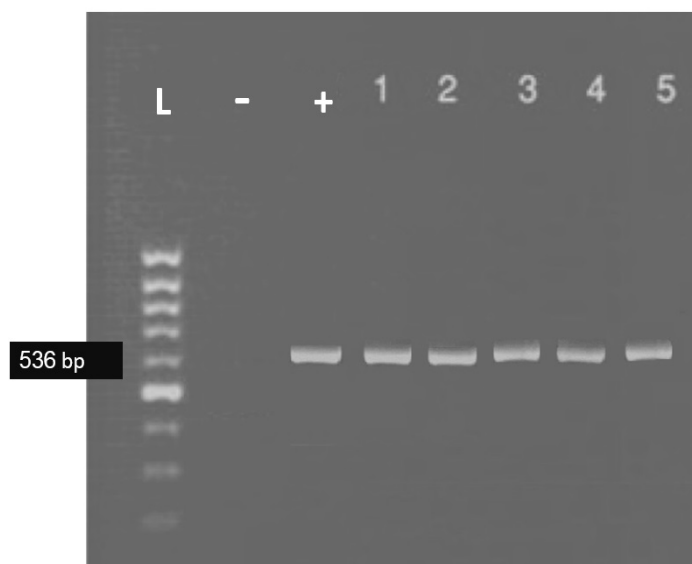


Figure 4. Electrophoresis of positive samples isolates of ticks in Jahrom, Iran. Lane -: Negative control, Lane +: Positive control, Lane 1-5: Samples of current study, Lane L: 100 bp ladder marker (CinnaGen Co.)



to study the prevalence in each region. It should be noted that the sharp band 400bp in the ladder may be due to the agarose gel not being well mixed, excessive reaction of the fluorescent material with nucleic acid or other reasons. Lack of sequencing and phylogenetic analysis should be a study limitation and recommended for future studies.

5. Conclusion

In this study for the first time we analyzed tick samples collected from Jahrom for presence of CCHF. Although the tick samples were collected from different geographic area in this township, CCHF genome was detected in samples. Considering that most of the livestock in this region were goats and that the ticks isolated from the goats were the most infected, they should focus more attention on the prevention and elimination of ticks in domestic animals, especially goats. The finding of our study might be valuable for designing of preventive measures regarding CCHF in Fars Province although further studies are required.

Ethical Considerations

Compliance with ethical guidelines

Ethical committee has approved this study of [Jahrom University of Medical Sciences](#) (Code: IR.JUMS.REC.1398).

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Authors' contributions

Investigation, conceptualization, writing-original draft, and writing-review and editing: Saman Rastgoo; Conceptualization, methodology, writing-original draft, and writing-review and editing: Gholamreza Shokoohi; Conceptualization, methodology, writing-original draft, and writing-review and editing: Hooshmand Ghorbaani Barnaaj; Methodology, investigation, writing-original draft, writing-review and editing, and supervision: Ahmad Abolghazi.

Conflict of interest

The authors declare no conflict of interest

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