Original Article

Molecular Assay on Detection of Crimean Congo Hemorrhagic Fever (CCHF) Virus in Ixodid Ticks Collected from Livestock in Slaughterhouse from South of Iran

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Abstract

Background: Ticks are vectors of a wide variety of pathogens that can be transmitted to humans, and tick-borne diseases are a significant public health issue worldwide. The present study was carried out on the hard tick infestation of livestock transported to Rafsanjan slaughter house in the southeast of Iran.

Methods: A cross-sectional survey was carried out biweekly from April to September 2016 to determine tick infestation of the meat-producing animals. All the livestock included in our study were thoroughly inspected for the presence of hard ticks on different parts of their bodies.

Results: A total of 258 hard ticks were collected from the body of livestock hosts. The ticks that were sampled were classified into two genera and five species: *Hyalomma marginatum*, *Hy. anatolicum*, *Hy. asiaticum*, *Hy. dromedarii*, and *Rhipicephalus sanguineus*. *Hyalomma dromedarii* was the most abundant species in the study area. More than 50 percent of the sampled ticks were collected from the body of camels brought to the slaughter house however molecular analysis showed no Crimean Congo Hemorrhagic Fever (CCHF) virus infection in tick specimens. The Sex ratio of the sampled hard ticks shows that female tick infestation was more common among the study livestock.

Conclusion: Due to the crucial role of hard ticks in the transmission of different pathogens to humans, additional investigations are necessary to determine the risk of consumption of infested meat-producing animals in the study area.

Keywords: Ticks; Livestock; Abattoir; Rafsanjan; Iran

Introduction

Deply (1, 2) introduced hard Ticks (Ixodidae) and two genera of these arthropods (*Hyalomma* and *Haemaphysalis*) for the first time in Iran. Filipova et al. (3) reported the collection of 642 ixodid ticks from small-size mammals, mainly rodents, in Iran. Later, Hoogstral

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and Valdez (4) investigated and explained the medical and veterinary implications of Ixodoidea ticks from wild sheep and goats in Iran. During the past quarter-century, many studies were conducted on fauna, biology, and geographical distribution of both soft and hard ticks in different parts of Iran (5-13). In a study conducted by Nabian et al. (7) 1720 tick specimens belonging to fourteen tick species were collected from cattle, sheep, and goats in different localities of Caspian Sea areas in the north of Iran. Seven species of hard ticks were similarly sampled from cattle and sheep in the southeast part of Iran (5). The distribution of ticks and tick infestation rate of sheep in the West of Iran was studied by Nasiri et al. (8) who identified 864 hard ticks, which were classified into two genera and five species. In a survey conducted in the eastern part of Iran on species diversity and distribution of ticks in sheep, goats, cattle, and camels, 469 adult ticks belonging to 9 species were collected (6). Hard tick infestation of Livestock and seasonal population dynamics of these ticks were investigated in the central part of Iran, and 583 hard ticks belonging to three genera and seven species were identified (12). Due to the crucial role of ticks in the transmission of pathogens such as the Crimean Congo Hemorrhagic fever virus (CCHFV) to humans, they are considered as a significant problem in public health. Crimean Congo Hemorrhagic Fever Virus CCHFV has been isolated from at least 31 species of ticks (14-16). Crimean Congo Hemorrhagic Fever Virus CCHFV was also isolated from many genera and species of hard ticks and soft ticks in a study conducted on the distribution of ticks. Hard ticks of the genus *Hyalomma* were identified as numerically dominant hard tick vectors for the transmission of CCHFV in different climatic zones of the country (14, 16). Crimean Congo Hemorrhagic Fever is currently present in most areas of Iran (17). The aim of the present study was to identify species composition of hard ticks (Acari: Ixodidae) parasitizing livestock transported to Rafsanjan abattoir for the purpose of meat production and to investigate CCHFV infection rate in collected ticks.

Materials and Methods

Sample Collection

In the present study, a cross-sectional study was carried out biweekly from April to September 2016 in Rafsanjan City of Kerman Province. The cities that livestock brought to Rafsanjan's abattoir have been shown in figure 1 using Arc-GIS10.2 software (Redlands, CA). Animals included in our study were livestock considered as hosts of hard ticks, including cattle, sheep, and camels, which were brought to Rafsanjan's abattoir for the purpose of meat production. All the livestock included in our study were surveyed for the presence of hard ticks on their body. Tick specimens collected from each host were kept alive in separate labeled holding tubes, and the information related to each tube was recorded. Using the key identification guide (18), all collected hard ticks were identified according to their morphological characteristics until the species determination.

Molecular analysis of Crimean Congo Hemorrhagic Fever (CCHF)

Tick specimens were homogenized individually in PBS using the mortar and pestle. Total RNA extraction was purified by the use of the RNeasy mini kit (QIAGEN) according to the manufacturer's kit and stored at -70 °C freezer until needed for CCHFV detection. We performed RT-PCR for a 536bp fragment in S segment of CCHFV as previously described (19). Briefly, RT-PCR reaction was carried out in a final 50µl volume with 10µl of 5x QIAGEN OneStep RT-PCR Buffer, 2µl of dNTP mixture (containing 10mM of each dNTP), 2µl of QIAGEN OneStep RT-PCR Enzyme Mix, 0.2µM of primer F2 (5'-TGGACACCTT CACAAACTC-3), 0.2µM of primer R3 (5'-GACAATTCCCTACACC-3'), 1µl of QIAGEN RNase Inhibitor (4 units/µl), and 5µl of extracted RNA as a template. The RT-PCR con-

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ditions began with 30min at 50 °C for reverse transcription reaction step and then 15min at 95 °C for initial denaturation, hot start activation and inactivation of reverse transcriptase enzyme followed by 40 cycles of 95 °C for 30s, 50 °C for 30s, 72 °C for 45s, and a final extension at 72 °C for 10min. Polymerase Chain Reaction (PCR) products were analyzed into 1.5% W/V electrophorese gel agarose.

Results

During the study period, a total of 258 hard ticks were collected from different livestock hosts, including cattle, sheep, and camel. The number and type of hard tick species collected from each livestock have been summarized in Table 1. The sampled ticks were classified into two genera, including *Hyalomma* and *Rhipicephalus*. These two genera of ticks were further classified into five species based on their morphological characteristics. The tick species comprised of *Hyalomma marginatum*, *Hy. anatolicum*, *Hy. asiaticum*, *Hy. dromedarii* and *Rhipicephalus sanguineus* (Table 1). *Hyalomma dromedarii* was the most abundant species among the collected ticks, followed by *Hy. marginatum*, as seen in Table 1. *Rhipicephalus sanguineus* was the least abundant tick species collected from the livestock in the study area. Between the different livestock hosts, the occurrence of hard ticks was more common on the body of camels; more than 50 percent (N= 137) of all collected samples were related to this host (Table 1). The sex ratio of the collected hard ticks shows that female tick infestation (60%) was more prevalent among the study animals. After a thorough inspection, ticks were collected from different body parts of the livestock. The rump region of sheep, under the tail region of cattle and camels, were the most favorable location for tick attachment (Table 2). Molecular assay results indicated no CCHFV infection in collected ticks.

Table 1. The Frequency of collected ticks in different hosts in Slaughterhouse from Rafsanjan City, Kerman Province, Iran, 2016

Species	Hosts			Total	
	Cattle	Camel	Sheep	Number	Percentage
Hy. marginatum	19	31	38	88	34.11
Hy. dromedarii	0	97	0	97	37.60
Rh. sanguineus	0	0	10	10	3.88
Hy. anatolicum	9	4	24	37	14.34
Hy. asiaticum	3	5	18	26	10.07
Total	31	137	90	258	100

Table 2. Distribution of ticks on different body parts of the animals' body in Slaughterhouse from Rafsanjan City, Kerman Province Iran, 2016

Hosts	Body parts			
	Under Tail/Rump	Belly	Thigh	Neck
Cattle	27	4	0	0
Camel	118	9	8	2
Sheep	52	23	15	0
Total	197	36	23	2

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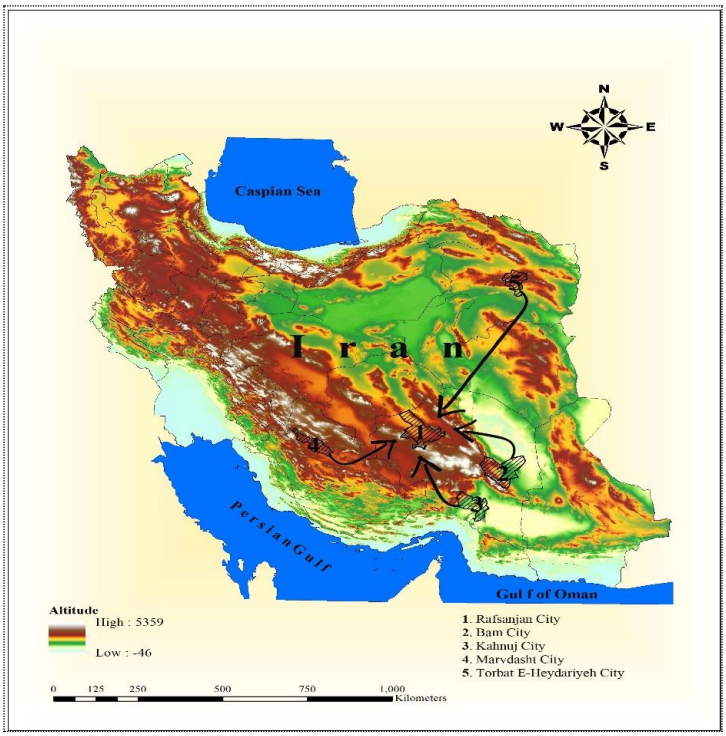

Fig. 1. Map of Iran, sites of livestock brought to Rafsanjan's abattoir in, Kerman Province, 2016

Discussion

A total of 258 hard ticks from the different livestock hosts were collected and identified. *Hyalomma dromedarii* was the most abundant among the tick species collected from study animals. On the other hand, the presence of ticks on the body of camels was more common compared with the other livestock hosts. The results of the present study also show that the rump region of sheep and under the tail region of cattle and camel are the most favorable regions for tick attachment. In our study, two genera of Ixodid ticks were identified and further classified into five species. *Hyalomma dromedarii* and *Hy. marginatum* belonging to the genus *Hyalomma* was the most numerically dominant among sampled ticks. Similar results were observed in other studies conducted in different parts of Iran (5-8, 11, 12, 20-22). The results of the present study also indicate that the adults of *Hy. dromedarii*, which were the most abundant tick species among all sampled hard ticks, prefer camels belonging to the genus *Camelus dromedarius* (Mammalia: Camelidae) for feeding. This finding is consistent with that of other studies that investigated the tick infestation rate of camels, but in some other studies, other species of hard ticks were collected from camels (6, 12, 22-26). In addition to camels, *Hy. dromedarii* has also been collected from sheep and cattle in some other studies (6, 8, 11, 12). Five tick species belonging to two genera (*Hyalomma* and *Rhipicephalus*) were identified in our study, including *Hy. marginatum*, *Hy. anatolicum*, *Hy. asiaticum*, and *R. sanguineus*. *Rh. Sanguineus*, which was the least abundant species among all collected ticks, mainly infested sheep. Similar to the results of other studies, it seems that *Rh. sanguineus* is one of the most common tick species that parasitize sheep and goats (5-7, 11, 27, 28). In some other studies that surveyed hard ticks which infested domestic ruminants, some other genera of hard ticks that were not found in our study were reported. For instance,

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in 2009, a study on tick infestation rate of sheep conducted in Abdanan County (a west area in Iran) identified *Heamaphysalis sulcate*, which belongs to the family Ixodidae, and other species of hard ticks which infested sheep (8). Hosseini-Vasoukolaei et al. (28) reported that four genera of Ixodidae (hard ticks), including *Ixodes ricinus*, *Boophilus annulatus*, *H. punctate* and *H. numidiana* infested domestic ruminants in Ghaemshahr County (north of Iran), but these species were not found in the present study. Even so, we did not expect to find *I. ricinus* in our study area due to its habitat and bio-ecology requirements, based on the results of previous studies which mentioned that the distribution of *I. ricinus* is just limited to the Caspian Sea region in the northern part of Iran (7, 9, 28, 29). In the present study, we found that ticks prefer to attach to the rump and under tail (perineum) regions, compared with other body parts, and this finding is in agreement with the results of some other studies. The preferred site of attachment of ticks has been associated with infestation season, and it seems that during warm months of the year, hard ticks prefer perineum region for attachment (5, 28).

Conclusion

In conclusion, regardless of no evidence of CCHFV infection in this study, due to the crucial role of hard ticks in the transmission of different pathogens to humans, more investigations in the study area are needed for identification of tick pathogens in order to prevent important diseases that can be transmitted to humans by ticks.

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