Hard Ticks (Ixodidae) and Crimean-Congo Hemorrhagic Fever Virus in South West of Iran

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Abstract- Ticks are vectors of some important arthropod-borne diseases in both fields of veterinary and medicine, such as Lyme, tularemia, Rocky Mountain spotted fever, and some types of encephalitis as well as Crimean Congo hemorrhagic fever (CCHF). Iran is known as one of the main foci of CCHF in west of Asia. This study was conducted in DarrehShahr County because of the development of animal husbandry in this area to detect the fauna and viral infection of the hard ticks of livestock. A cross-sectional survey was conducted during 2011-2012 with random sampling in four villages. A sample of ticks was subjected to RT-PCR method for detection of viral infection. During the study period, 592 Ixodidae ticks were collected and identified as seven species of *Hyalomma asiaticum*, *Hy. marginatum*, *Hy. anatolicum*, *Hy. dromedarii*, *Hy. detritum*, *Rhipicephalus bursa* and *Rh. sanguineus*. More than 20% of these ticks were examined to detect the genome of CCHF virus while 6.6% were positive. All species of *Hyalomma* were found to be positive. A high rate of livestock was found to be infected with hard ticks, which can act as the vectors of the CCHF disease. Regarding infection of all five *Hyalomma* species captured in this area, this genus should be considered as the main vector of CCHF. Planning control program can be performed based on the obtained data on seasonal activity of Ixodidae to prevent animal infestation as well as to reduce the risk of CCHF transmission.

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Introduction

Ticks are blood-sucking arthropods; exist around the world as obligate parasites of the vertebrates (1). They transmit some viruses, bacteria, rickettsia and protozoa to human and animals so that it is a significant issue in the livestock industry (2). The environment is changing fast, human and livestock populations are increasing and therefore, patterns of relationship between ticks, hosts and parasites will change as a result of any variation in the density of each particle of this triangle (3). Knowledge about the epidemiology of vector-borne diseases, especially tick-borne diseases, will be useful in the control strategies for those diseases (4).

Crimean Congo Hemorrhagic Fever (CCHF) is one of the most common tick-borne diseases with a mortality rate of about 30%. This disease is endemic in large areas of Africa, as well as the eastern and central parts of the Europe. In recent years, the cases of the disease are significantly increased in Albania, Kosovo, Turkey and Iran while there are reports of CCHF from Greece (5). The epidemic form of CCHF usually occurs in areas where the public health services are poor, and high mortality may occur. Furthermore, climate, environmental and agricultural changes may affect the distribution of the ticks and the disease emergence (6). In the previous decade many cases of the disease have been reported from India, Russia, Eastern Europe,

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French, Pakistan, Afghanistan, Iran, Central Asia countries, Iraq, United Arab Emirates, Greece, Portugal, and different areas of the Africa continent (7).

Studies in Iran show the first report of CCHF virus in *Alveonasus lahorensis,* a soft tick collected in the north east of Iran (8). During 1998, two epidemics of the disease occurred in the eastern neighbors of Iran and led to death in some cases (7). The reports of the disease have submitted from some counties since 1999 (9). Although human infection and CCHF are recorded from 23 provinces of Iran, the most prevalence of the diseases is reported from Isfahan, Fars, Khorassan and Yazd provinces (10). Studies on the fauna of livestock ticks in relation to CCHF during 2000-2011 show 17 hard tick species are active in the studied areas as follows: *Boophilus annulatus, Dermacentor marginatus, Haemaphysalis pucntata, Hae. sulcata, Hyalomma aegyptium, Hy. anatolicum anatolicum, Hy. anatolicum excavatum, Hy. asiaticum asiaticum, Hy. detritum, Hy. dromedarii, Hy. marginatum marginatum, Hy. schulzei, Hy. detritum, Ixodes ricinus, Rhipicephalus bursa, R. sanguineus* and *R. Turanicus* (11-24).

Because the Darreh Shahr county includes nomad people that, their main job is animal husbandry. Eery year many animals are migrating to different parts of this area. Therefore, studing infection rate of livestock to ticks, tick fauna as well as the population at risk of CCHF seems to be necessary. Also survey on the infection rate of the collected ticks either introduce the vectors of CCHF in the area or is an important step toward improving planning for prevention of CCHF outbreak.

Materials and Methods

Ilam province is located in south west of Iran. This province is surrounded by Kermanshah province in the north, Lorestan province in the east, Khuzestan province in south and Iraq country in west. Darreh Shahr County with an area of 1480 km^2 and coordinates of 33^{o}7'N and 47 ^{o} 21' E, and elevation of 650 m above the sea level, is l42 km away from the Ilam city, the capital of the province (Fig. 1). This county has a tropical climate with long and hot summers and short and temperate winters. The highest and lowest temperatures in 2012 were recorded as 42 and -6 °C, respectively. Darreh Shahr County has a population of 56346. Nomads can be found in different areas of this county and animal husbandry is the main job of inhabitants.

This cross-sectional study was conducted during 2011-2012 in four seasons. Sampling was random in four villages of the county: Gholamabad in north, Markazi in center, Farhadabad in south and Kolejoob in the east. These sampling sites were selected after consultation with the veterinary staff of the county.

Tick collection and virus detection

Sampling was carried out from all parts of the body of visited livestocks (sheep, goat and cow). Collected ticks were put in the caped tubes, and all details were recorded, including weather information. The samples were then transferred to the laboratory for species identification (25). Regarding the type of the animal host, season and study village, a number of hard ticks were selected at random and RT-PCR were used to detect the viral infection (26). Sample size for this part of the study was calculated based on the infection rate of hard ticks in different parts of the country using the following equation.

$$n_0 = \frac{Z^2_{1-\alpha/2} p(1-p)}{d^2},$$

where Z= 95%, P= 0.2 and d= 0.59.

SPSS 16, Spearman and $\chi 2$ tests were used for statistical analysis of the data.

Results

During the period of tick sampling, 640 livestock were visited; 28.59% were found to be infected to hard ticks. A total of 592 hard ticks were collected from different parts of the infected animal bodies. Two genera of the hard ticks were identified: *Hyalomma* (90.21%) and *Rhipicephalus* (9.79%). The first genus included five species: *Hy. marginatum, Hy. anatolicum, Hy. asiaticum, Hy. dromedarii* and *Hy. detritum;* while two species were found to be related to the second genus: *Rh. sanguineus* and *Rh. bursa.* All species were collected from the four studied villages, although their frequency was variable in those villages in different seasons. Sex ratio of the collected specimens showed 82.1% females versus 17.9% males.

Seasonal activity of the hard tick was considered, and it was found that the highest activity is happening during spring and summer, whereas the lowest density of this arthropod observed in winter (Table 1). Statistical analysis showed a significant difference in the study seasons (P<0.05). None of the *Rhipicephalus* species was captured during autumn and spring visits.

Sheep was found to be the most infected animal host (59.6%), followed by goat (30.24%) and cow (10.13%). All species were found on all hosts, except for *Rh.*

bursa. There was no record of a collection of this species on cow (Table 2).

Out of 592 collected hard tick, 137 (23.1%) were selected at random for the study of CCHF virus infection. Among this sample 109 and 28 ticks were due to *Hyalomma* and *Rhipicephalus* genera, respectively. Result of this survey confirmed viral infection in nine (6.6%) specimens. All infected species were due to the *Hyalomma* genus: *Hy. marginatum, Hy. dromedarii, Hy. asiaticum, Hy. anatolicum* and *Hy. detritum.*

Table 1.Frequency and prevalence of hard ticks of the study area in different seasons, Darreh Shahr County, West of Iran, 2011-2012

Tick species	Season								Total
	Spring		Summer		Autumn		Winter		
	No.	%	No.	%	No.	%	No.	%	
Hy. anatolicum	11	4.85	68	39.77	26	21.31	20	27.78	125
Hy. asiaticum	75	33.04	49	28.65	34	27.87	6	8.33	164
Hy. detritum	23	10.13	0	0	3	2.46	0	0	26
Hy. dromedarii	10	4.41	5	2.92	51	41.80	0	0	66
Hy. marginatum	57	25.11	42	24.56	8	6.56	46	63.89	153
Rh. bursa	14	6.17	1	0.58	0	0	0	0	15
Rh. sanguineus	37	16.30	6	3.51	0	0	0	0	43
Total	227	100	171	100	122	100	72	100	592

Table 2. Frequency and prevalence of hard ticks of the study area in different seasons, Darreh Shahr County, West of Iran, 2011-2012

Tick species	Animal host						Total
	Goat		Sheep		Cow		
	No.	%	No.	%	No.	%	
Hy. anatolicum	23	12.84	90	25.50	12	20	125
Hy. asiaticum	61	34.09	82	23.23	21	35	164
Hy. detritum	9	5.02	16	4.53	1	1.66	26
Hy. dromedarii	8	4.46	39	11.05	19	31.67	66
Hy. marginatum	43	24.05	105	29.75	5	8.34	153
Rh. bursa	11	6.14	4	1.13	0	0	15
Rh. sanguineus	24	13.40	17	4.82	2	3.33	43
Total	179	100	353	100	60	100	592

Discussion

Findings of this study show an infection rate of 28.59% in the livestock. This rate was higher than previous reports from several Iranian provinces, Ilam (19), Hamedan (12), Ghaemshahr (27), North West of Iran (28), and Azarbaijan-e-Sharghi (29); and lower from reports of Golestan (30), and some selected areas of Iran (31). The infection rates based on the animal host is consistent with the results of Sarani (2011) and Nasiri *et al.* (2009) that show the highest infection rate in sheep, goats and cattle, respectively (19,30).

Most of the collected ticks were species of the *Hyalomma* genus. This is the same as previous studies in Ilam province (19,32), Yazd province (18) and Meshkinshahr (33). The dominant species varies in different studies. It indicates that the natural fauna of ticks is related to the ecological factors. The frequency of ticks is dependent on the season and climate, although in almost all previous studies, spring and summer had the highest infection rates, while winter is reported to have the lowest numbers (15,19,22,30,34-37). This may be the result of the change in the grazing pattern. So that in the spring and summer, when pastures have adequate forage and provide the food for livestock, host-seeking ticks have more chance to infect the livestock there.

Unlike, in the cold season, there is no favorable food in pastures and shepherds keep the flock in the fold, where they use acaricides for tick control.

Although minor differences exist between the results of this study and the surveys mentioned above, they can be justified due to difference in climate, weather, management and rearing of livestock farms and their plans for spraying and other tick control methods.

The highest infection rate of the tested ticks to CCHF virus was found to be in the spring and summer seasons. All infected ticks to CCHF virus were found to be species of *Hyalomma*. This genus had the highest infection rate in different parts of Iran, although some other species have also been infected (16,30,38-40). In Turkey the northwestern neighbor of Iran, it is found that *Rhipicephalus* ticks had more infection than *Hyalomma* (41). More than 90% of the collected ticks in this study were identified as *Hyalomma,* and so it is expected to find more infection rate in this genus. Of particular interest was finding the CCHF virus in all five collected species of *Hyalomma* in the study area.

Given that ticks are the most important vectors of CCHF and according to the results of this study, particular attention should be paid to combat these pests. In planning to control the ticks in the study area, it should be considered the effect of ticks on public health and economy of the community, sanitation, construction of anti-tick bath in the infected areas, organized distribution and using acaricides and training programs should be considered.

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