Detection of *Theileria*-like organisms in *Hyalomma* ticks (Acarina: Ixodidae) in Khuzestan, Iran

Zahra Asadollahi¹, Mohammad Hossein Razi Jalali²✉, Alireza Alborzi², Hossein Hamidinejat²

¹ - Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran; Current address: Institute of Parasitology, Department of Pathobiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
² - Department of Pathobiology, School of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Correspondence: Tel. +986133330073, E-mail mh.jalali@scu.ac.ir

Abstract. Tropical theileriosis caused by *Theileria anulata* results in serious economic losses in cattle industry in Iran. *Hyalomma* ticks are known as vectors of parasites such as *Theileria* spp. The aim of this study was to detect *Theileria*-like organisms in *Hyalomma* spp. salivary glands gathered from cattle in Khuzestan province, Iran. Totally, 655 *Hyalomma* ticks were collected from infested cattle in five geographical districts of the area under study. The salivary glands of ticks were stained by Methyl green/Pyronin method. The infection intensity and the prevalence rate in ticks were statistically analyzed using Chi-square test based on the difference in species and the gender of the ticks. The most prevalent species was *Hyalomma anatolicum anatolicum* (59.54%), followed by *Hyalomma anatolicum excavatum* (21.83%), *Hyalomma asiaticum asiaticum* (10.38%), *Hyalomma detritum* (5.2%), and *Hyalomma dromedarii* (3.05%). The microscopic study of acini in *Hyalomma* (H) ticks showed 67 (10.22%) infections with *Theileria* spp. The average number of infected acini per infected ticks was 14.3 in *H. anatolicum anatolicum*, 6.5 in *H. anatolicum excavatum*, 4 in *H. detritum*, 7.2 in *H. asiaticum asiaticum*, and 3 in *H. dromedarii*. The results revealed that the highest prevalence of infection with *Theileria*-like organisms belonged to *H. anatolicum anatolicum*.

Keywords: *Theileria*; Salivary glands; *Hyalomma*; Methyl green/Pyronin; Iran.

Received 05.07.2018. Accepted 18.12.2018.
Introduction

*Theileria* spp. are obligate intracellular tick-borne protozoan parasites belonging to the order *Piroplasmida* that cause a worldwide disease called theileriosis in many domestic and wild animals (O.I.E., 2009). The classification and nomenclature of *Theileria* spp. are still controversial, being gradually elucidated by molecular characterization (Radostits et al., 2007). Sporozoites are transmitted to susceptible animals through saliva of feeding ticks. Tropical theileriosis is one of the most prevalent and economically important fatal diseases of cattle in Iran (Hashemi-Fesharki, 1988). The major feature of tropical theileriosis is its progressive lympho-proliferation (Omer et al., 2003).

The common vectors of *Theileria (Th) annulata* are *Hyalomma (H) anatolicum anatolicum, H. anatolicum excavatum, H. detritum, H. marginatum marginatum*, and *H. dromedarii* (Aktas et al., 2004). To date, nine *Hyalomma* species have been reported in Iran and many of these species have been found capable of transmitting *Th. annulata* to cattle (Razmi et al., 2003a).

*Hyalomma* spp., the most prevalent tick in cattle in south-western Iran, is widely distributed all over the country (Asadollahi et al., 2014). Although several researchers have worked on the biology and distribution of hard and soft ticks in Iran (Rahbari, 1995; Razmi et al., 2003b; Razmi et al., 2007; Nabian et al., 2007; Salim Abadi et al., 2010), little information exists regarding the frequency of *Theileria* infection in ticks (Razmi et al., 2003a; Tavassoli et al., 2003). Investigating the potential of pathogen transferring in vectors is among the most important factors in epidemiological studies. So far, several researchers have measured *Theileria* in tick salivary glands using staining techniques (Haque et al., 2010; Irvin et al., 1981; Buscher and Tangus, 1986). However, only a few studies have been conducted in some parts of Iran (Habibpour et al., 2013; Salim Abadi et al., 2010). In our previous study (Asadollahi et al., 2014), we collected 1000 ticks from cattle in five geographic zones of Khuzestan province (north, south, east, west and center). Our findings revealed that the ticks of the study area were of eight species in four different genera. The aim of the current study is to detect the infection of *Hyalomma* species with *Theileria* spp. in south-west of Iran.

Materials and methods

Sampling area and animals

The province of Khuzestan is located in the southwest of Iran. With the co-ordinates 31.3273°N 48.6940°E, it covers an area of 63,238 km². The climate of this region is basically very hot and humid. A total of 1000 ticks were collected from the cows of five geographical regions of Khuzestan (North: Dezfol and Shoosh; South: Shadegan; East: Izeh; West: Soosangerd and Hoveyzeh; Center: Ahvaz). The sampling areas are shown in figure 1. The sampling was carried out in spring and summer because the infestation was very low in autumn and winter due to cold weather. A total of 100 cows (20 cows from each region) were randomly selected and sampled. From each cow, 10 to 12 ticks were harvested. All the cows were female, and almost all of them were infected with ticks on the body surface (abdomen, chest, under the tail, and thighs).

Some cows had severe infestation (more than a thousand elements in the body) in which fever and enlargement of the lymph nodes were seen.

Tick examination

From one thousand of the ticks collected, 655 ticks were from *Hyalomma* spp. and 345 ticks were from other genera. In the current study, the specifications of 655 *Hyalomma* ticks were identified using the general identification keys (Hoogstraal and Kaiser, 1959; Walker et al., 2003) and the mentioned ticks were dissected. Afterward, their salivary glands were removed and transferred to glass slides with a drop of Normal Saline Buffer solution and teased with a sharp needle under the dissection microscope. The smears were fixed with Methanol 10% for 2-5 minutes at room temperature. After air drying, the slides were stained with Methyl green/Pyronin as described by Walker et al. (1979).
The infected acini were investigated using light microscopy at ×40 magnification. The prevalence and the intensity of infected ticks were calculated based on tick species and sex. In order to determine the percentage of intensity of infection in the salivary glands of the ticks, the total number of infected and non-infected acini was counted. The percentage of the intensity of infected acini was calculated via dividing the number of the infected acini by the total number of counted acini. The average rate of infection of acini in the salivary glands of the ticks was calculated for each species. The prevalence and intensity of infection for both genera (male and female) of Hyalomma ticks were identified.

Chi-square test using SPSS ver16 was performed to evaluate the relationships between the variables.

Results

Hyalomma species collected from cattle

A total of 655 ticks (both males and females) from five Hyalomma species were collected from the infested cattle. Among them, 390 ticks were H. anatolicum anatolicum, forming the dominant species in the region (59.54%). Other diagnosed species were H. anatolicum excavatum (21.83%; 143/655), H. asiaticum asiaticum (10.38%; 68/655), H. detritum (5.2%; 34/655), and H. dromedarii (3.05%; 20/655). From the ticks, 452 were female (69%) and 203 were male (31%). Statistical analysis revealed no significant difference in various species and sexes in the area of the study (P > 0.05).

Prevalence and intensity of Theileria-like infection in ticks

The rate of Theileria-like infection in ticks is shown in table 1. Screened salivary glands identified parasite infection in 67 Hyalomma (10.22%), 24 of which were male (35.82%) and 43 were female (64.18%) although the infection rate was not significant among the different species or sexes (P > 0.05).

The percentages of infected acini per infected ticks collected from the cattle were 14.3% in H. anatolicum anatolicum, 6.5% in H. anatolicum excavatum, 4% in H. detritum, 7.2% in H. asiaticum asiaticum, and 3% in H. dromedarii. No significant difference was found in tick species (P > 0.05).

Discussion

In the present study, H. anatolicum anatolicum, H. anatolicum excavatum, H. detritum, H. asiaticum asiaticum, and H. dromedarii were
recognized in the cattle and *Theileria*-like infection was determined in all of these tick species. The mentioned species have been reported in the cattle of different areas in Iran (Razmi et al., 2003a; Rahbari, 1995; Razmi et al., 2007; Nabian et al., 2007; Salim Abadi et al., 2010). The results of this study showed that *H. anatolicum anatolicum* was the major tick species in the cattle raised in Southwest of Iran. The warm climate in this district in most of the year is suitable for the ticks’ life cycle.

The staining of tick salivary glands with Methyl green/Pyronin to study the epidemiological aspect of *Theileria* vectors is a well-known useful tool (Walker et al., 1979). The prevalence of *Theileria* spp. infection in salivary glands of *Hyalomma* ticks in Khuzestan was 10.22%. The prevalence of *Theileria*-like infection in *H. anatolicum anatolicum* (61.2%) was higher than in *H. anatolicum excavetum* (19.4%) although no significant difference was observed in infection rates among the species (p > 0.05). In the same line, Aktas et al. (2004) and Tavassoli et al. (2011) found that *H. anatolicum anatolicum* ticks had a higher infection rate than other species. The differences in the distribution of ticks and their infection rate can perhaps be attributed to the difference in the areas of those studies, the climatic conditions, and the system in which the animals are raised. While cows with moderate to severe contamination usually showed clearer clinical symptoms, low-infestation cows (fewer than fifteen ticks in the body) had no apparent symptoms.

The prevalence rate of *Theileria*-like infection in female ticks was higher than in males but this difference was not statistically significant. The higher prevalence and intensity of *Theileria*-like infection in female ticks, as compared with male ticks, has been reported by Aktas et al. (2004).

### Table 1. *Theileria* organisms of infection rate in *Hyalomma* spp.

<table>
<thead>
<tr>
<th><em>Hyalomma</em> spp.</th>
<th>Dissected ticks</th>
<th>Infected ticks</th>
<th>Prevalence (%) of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. an. anatolicum</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>104</td>
<td>17</td>
<td>4.35</td>
</tr>
<tr>
<td>F</td>
<td>286</td>
<td>24</td>
<td>6.15</td>
</tr>
<tr>
<td>T</td>
<td>390</td>
<td>41</td>
<td>10.5</td>
</tr>
<tr>
<td><em>H. an. excavatum</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>57</td>
<td>5</td>
<td>3.5</td>
</tr>
<tr>
<td>F</td>
<td>86</td>
<td>8</td>
<td>5.6</td>
</tr>
<tr>
<td>T</td>
<td>143</td>
<td>13</td>
<td>9.1</td>
</tr>
<tr>
<td><em>H. asiaticum</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>27</td>
<td>2</td>
<td>2.9</td>
</tr>
<tr>
<td>F</td>
<td>41</td>
<td>8</td>
<td>11.8</td>
</tr>
<tr>
<td>T</td>
<td>68</td>
<td>10</td>
<td>14.7</td>
</tr>
<tr>
<td><em>H. detritum</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>25</td>
<td>2</td>
<td>5.9</td>
</tr>
<tr>
<td>T</td>
<td>34</td>
<td>2</td>
<td>5.9</td>
</tr>
<tr>
<td><em>H. dromedarii</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>14</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>T</td>
<td>20</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

However, in a study by Sangwan et al. (1989), no significantly different prevalence rates between sexes were reported. The greater prevalence in female ticks may be due to more type III acini in this sex (Young et al., 1983). The result of our study is in agreement with previous studies like that of Sangwan et al. (1989). It is clearly known that *Hyalomma* ticks transmit other parasites as well as *Theileria annulata* (Schein et al., 1981; Hashemi-Fesharki, 1997). Since the subtraction of the piroplasmic forms (small *Babesia* and *Theileria*) is difficult via the staining methods, it seems that molecular methods are necessary for differentiating *Theileria* parasites in salivary glands in later stages of the study.
From another point of view, mixed infection of acini with *Th. annulata*, *Th. lestoquardi* and *Th. equi* should be determined in infected ticks. Our study was a preliminary study of *Hyalomma* tick in this district in order to estimate the dynamics of infection transmission. It is important that the focus be shifted on molecular detection of species in the next step.

In conclusion, five *Hyalomma* species (*H. anatolicum anatolicum*, *H. anatolicum excavatum*, *H. detritum*, *H. asiaticum asiaticum*, and *H. dromedarii*) were identified in cattle in south-west of Iran and natural *Theileria*-like infection was observed in all of them. Among these tick species, *H. anatolicum anatolicum* was found to have the highest prevalence and intensity of infection. Therefore, these ticks may play a major role in the transmission of tropical theileriosis in this region.

**Acknowledgements**

Financial support from the vice chancellor of research in Shahid Chamran University of Ahvaz is gratefully acknowledged.

**References**


