Seroprevalence of *Theileria equi* and *Babesia caballi* infection in Turkoman breed horses in Iran

Vali Abedi¹, Gholamreza Razmi²*, Hesam Seifi³ and Abolghasem Naghibi²

¹Student of Veterinary Parasitology (Ph.D), Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
²Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
³Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Received: April 6, 2013 Accepted: July 9, 2013

Abstract

Equine babesiosis is a hemoprotozoan tickborne disease with worldwide distribution and caused by *Theileria equi* and *Babesia caballi*. This study was conducted to determine the seroprevalence of *T. equi* and *B. caballi* infection in Turkoman breed horses in North Khorasan Province of Iran. Blood samples were collected from 100 apparently healthy horses and examined by microscopy and indirect immunofluorescent antibody test. *T. equi* was microscopically detected in five blood smears. Antibodies against *T. equi*, *B. caballi* and dual infection were found in 48 (48%), 2 (2%) and 3 (3%) serum samples, respectively. No significant difference was observed between the seroprevalence of piroplasm infection with risk factors such as age, gender and activity in horses. This is the first report of detection of *T. equi* and *B. caballi* infection using IFAT in Iran. It was concluded that the seroprevalence of *T. equi* infection is higher than *B. caballi* infection in Turkoman breed horse in Iran.

Keywords: *Theileria equi*, *Babesia caballi*, Indirect immunofluorescent antibody test, Turkoman breed horse

*Corresponding author: Gholamreza Razmi
Email: razmi@ferdowsi.um.ac.ir
Tel: +98 51 38788944
Fax: +98 51 38763853
P.O.: 91775-1793*
Introduction

Equine piroplasmosis is an important tickborne hemoprotozoan disease in horses, mules, donkeys and zebras. It is caused by Theileria equi and Babesia caballi (Mehlhorn and Schein, 1998, Sellon and Long, 2007).

The disease is characterized by fever, anemia, icterus, hepatosplenomegaly, bilirubinuria and hemoglobinuria (de Waal, 1992). Most of the clinical cases are caused by T. equi while infection with B. caballi tends to be inapparent (Friedhoff et al., 1990). Carrier horses may show clinical disease under stress condition (Hailat et al., 1997).

Detection of Theileria and Babesia parasites can be done by different methods. Microscopical examination of stained blood smear is a traditional diagnostic method of piroplasm detection in acute phase of disease, but it is almost impossible to detect parasites in carrier horses with low parasitemia (Bose et al., 1995).

Several serological techniques have been developed to detect antibodies against blood parasites in subclinical or chronic forms of disease. The indirect immunofluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA) are considered to more sensitive than other serological methods and it is recommended for detection of T. equi and B. caballi infection in international trade of horses (OIE, 2008). B. caballi and T. equi were reported in horses from Iran (Aslani, 2000, Seifi et al., 2000) but there is not any information about epidemiology of piroplasm infection in horses of Iran. The aim of study was to determine the seroprevalence of the T. equi and B. caballi infection in Turkoman breed horses in Iran and evaluating the relationship between risk factors with seropositivity.

Materials and methods

Field study area

This study was carried out in North Khorasan Province located in northeastern of Iran between 36°37′-38°17′ N latitudes and 55°53′-58°20′ E longitudes with an area of more than 28400 km². It is situated next to the northeastern border of Iran, level with the southern Caspian sea and south of Turkmenistan. This province is a mountainous area and receives about 250 mm of rainfall annually. Turkoman horses breed are reared by turkmen tribes in the northern areas of the province.

Sampling

One hundred horses were randomly selected from 14 villages from June to August 2011. Data of each horse including age, gender, activity and any grazing in pasture were recorded. Blood samples were taken from jugular vein and placed into two sterile tubes with and without EDTA. The blood tubes were kept in cooled condition and immediately transmitted to the laboratory. The sera were separated by centrifugation at 4000 rpm for 10 min and stored at -20°C until the serological examination.

Examination of blood smears

The blood smears were prepared from blood samples in EDTA tubes. The smears were fixed in methanol and stained in 10% Giemsa solution in phosphate buffered saline (PBS) pH 7.2. The slides were examined with an oil immersion lens at total magnification of ×1000. In order to identify Theileria equi and Babesia caballi species, the full length of intraerythrocytic mature piroplasm organism was measured by a graded ocular microscopy at magnification of ×1000 (Soulsby, 1982). Parasitemia was assessed by counting the number of infected red blood cells on examination of 50 microscopic fields (approximately 50000 cells). The infected number was then expressed as percentage.

Serology

IFAT was done according to manufacturer’s instructions for T. equi antibodies (Fuller Laboratories, Fullerton, Callifornia, USA) and for B. cabali antibodies (MegaScreen, Horbranz, Austria). Sera will
considered as positive if the parasites showed fluorescence reaction at dilution of 1:80.

Statistical analysis
Any relationship between infection rate and risk Factors such as age, gender and activity were analyzed by Chi-square test. Significant associations were statistically identified when a p-value of less than 0.05 was observed.

Results
*T. equi* infection were microscopically detected in 5 (5%) of blood smears with low parasitemia (approximately 0.001-0.003%). *T. equi* is smaller than *B. caballi*, about 2 μ and frequently form maltese cross (Fig.1). Serological examination revealed that 51 (51%), 2 (2%) and 3 (3%) had antibodies against *T. equi*, *B. caballi* and mixed infection, respectively (*p*<0.05) (Table 1).

The frequency of antibodies against *T. equi* in different groups of ages, activity and gender in horses were not statistically different (*p*>0.05) (Table 2).

Table 1. Results of microscopical and serological examination of *B. caballi* and *T. equi* infection in one hundred horses in North Khorasan Province

<table>
<thead>
<tr>
<th>species</th>
<th>Microscopic examination No(%)</th>
<th>Serology No(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. caballi</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>T. equi</td>
<td>5</td>
<td>48</td>
</tr>
<tr>
<td>Mixed infection</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>53</td>
</tr>
</tbody>
</table>

Table 2. Frequency of antibodies against *T. equi* and *B. caballi* infections by different risk factors in 100 turkoman breed horses (No*: No per group).

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>T. equi</th>
<th>B. caballi</th>
<th>Mixed infection</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>1-2</td>
<td>24</td>
<td>8</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>3-5</td>
<td>31</td>
<td>14</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>5&lt;</td>
<td>38</td>
<td>24</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stud</td>
<td>33</td>
<td>20</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>Racehorse</td>
<td>67</td>
<td>28</td>
<td>2</td>
<td>32</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>21</td>
<td>8</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Female</td>
<td>79</td>
<td>40</td>
<td>1</td>
<td>44</td>
</tr>
</tbody>
</table>
Discussion

In this study, *T. equi* infection was microscopically detected in 5 (5%) blood samples with low parasitemia, while 53% of horses were found seropositive for piroplasms infection using IFAT. The results confirmed low sensitivity of the microscopical examination in comparison with serological methods. Some studies were shown the high sensitivity and specificity of IFAT to differentiate *T. equi* and *B. caballi* in horses (Ogunremi et al., 2007; Ogunremi et al., 2008). In the present study, Antibodies against *T. equi*, *B. caballi* and mixed infection were detected in 48 (48%), 2 (2%) and 3 (3%) of horses, respectively. Seropositivity rates of *T. equi* and *B. caballi* infections were reported 10.4 and 7.5% in Saudi Arabia (Alanazi et al., 2012), 77.1 and 11.4% in Kuwait (Donelly et al., 1980), 97.7 and 40.9% in Oman (Donelly et al., 1980), 12.8 and 9.6% in Turkey (Karatepe et al., 2009) by IFAT and 16.21 and 0.83% in Turkey (Sevinc et al., 2008), 81.11 and 18.88% in Iraq (Al-saad, 2009), 32.45 and 15.2% in United Arab Emirates (Jaffer et al., 2010), 14.6 and 0% in Jordan (Abutarbash et al., 2012), by ELISA, respectively. The serological studies indicated that *T. equi* infection is more prevalent than *B. caballi* in Middle East.

The seropositivity rate of *T. equi* infection in different age groups of horses were non significant. The result was similar to the studies that done in Turkey (Acici et al., 2008; Karatepe et al., 2009), Italy (Moretti et al., 2010), Israel (Shkap et al., 1998, Steinman et al., 2012), Switzerland (Sigg et al., 2010) and Venezuela (Mujica et al., 2011) and on the contrary other studies that done in Mongolia (Ruegg et al., 2007) and Greece (Kouam et al., 2010).

A mare with her foal (one month old) were also seropositive against *T. equi* infection. The foal was healthy without any clinical signs. It seems the antibodies against *T. equi* were transfered to foal via colostrum. The antibodies in colostrum usually decrease after 63-77 days post foaling (Kumar et al., 2008).

The difference of seroprevalence in male and female horses was not statistically significant. This finding agrees with the results of the studies in Turkey (Karatepe et al.,

**Figure 1.** Maltese-cross form of *T.equi* (at magnification of ×1000.)
2009), Israel (Steinman et al., 2012), Switzerland (Sigg et al., 2010), Greece (Kouam et al., 2010), Venezuela (Mujica et al., 2011) and Trinidad (Asgarali et al., 2007) but disagreed with other studies that done in Israel (Shkap et al., 1998) and Mongolia (Ruegg et al., 2007)

Non-significant difference was obtained between the seroprevalence of piroplasm infections with different activity in horses. The turkoman horses with different activity graze in the pasture during spring and summer and chances of tick biting and piroplasm infection is equal. Some studies were shown that the grazing and different kinds of horse management could be pronounced risk factors for equine piroplasmosis (Moretti et al., 2010, Kouam et al. 2010, Sevinc et al., 2008). To the knowledge of the authors, this is the first study of seroprevalence of piroplasmosis in Turkoman horses in Iran. The results showed that T. equi infection is more prevalent than B. caballi infection.

Acknowledgements

We thank Dr. Abdoljalil ghiadi the manager of Turkoman horse breeding company (THBC) for his hospitality and aid for sampling. Mohammad Abedi and Dr. Ali Sarani for their aid for sampling and data collection.

References


بررسی شیوع سرمی ابتلا به تیلریا اکویی و بابزیا کابالی در اسب های نژاد ترکمن در ایران

ولی عابدی، غلامرضا رزمی، حسام الدین سیفی، ابوالقاسم نقیبی

چکیده
پیروپلاسموز اسبی بیماری تک یاخته ای خونی منتقله از کنه با انتشار جهانی است و عامل آن تیلریا اکویی و بابزیا کابالی می باشد. این مطالعه به منظور تعیین شیوع سرمی ابتلا به آلودگی تیلریا اکویی و بابزیا کابالی در اسب های نژاد ترکمن در استان خراسان شمالی صورت گرفت. ارتباط بین ابتلا به عفونت پیروپلاسمی و فاکتورهای خطر از قبیل سن، جنس و نوع قلالیت نیز مورد بررسی قرار گرفت. نمونه های خون از تعداد 100 راس اسب ابتلا به ظاهر سالم اخذ گردید. نتایج نشان داد که ابتلا به عفونت تیلریا اکویی در 5 کسترش خونی مشاهده شد. تنها باید به توجه گرفته شود که شیوع آلودگی تیلریا اکویی، بابزیا کابالی و آلودگی مخلوط دو گونه به ترتیب در 0.1% (0.1%), 0.2% (0.2%) و 0.3% (0.3%) نمونه تعیین گردید. بین فاکتورهای خطر شامل سن، جنس و نوع فعالیت و میزان سرم مثبت بر علیه عفونت، نتایج معنی‌داری مشاهده نگردید. این اولین گزارش در مورد ابتلا به تیلریا اکویی و بابزیا کابالی در ایران با استفاده از روش ایمنوفلوئورسانس غیر مستقیم می باشد. این مطالعه نشان می دهد که میزان شیوع آلودگی تیلریا اکویی بیشتر از شیوع آلودگی بابزیا کابالی در اسب های نژاد ترکمن در ایران می باشد.

واژگان کلیدی: تیلریا اکویی، بابزیا کابالی، ایمنوفلوئورسانس غیر مستقیم، اسب نژاد ترکمن