Seroprevalence of *Babesia bigemina* and *Anaplasma marginale* in domestic animals in Erbil, Iraq

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Abstract

Seroprevalence of *Babesia bigemina* and *Anaplasma marginale* in cattle, sheep, goats and wild goats was studied in Erbil district, between January to December 2010. A total of 184 blood samples were collected from 44 cattle, 59 sheep, 70 goats and 11 wild goats for the preparation of blood smears and serum samples which tested against *B. bigemina* and *A. marginale* using the SVANOVI® B. bigemina-Abs and A. marginale-Abs ELISA Kit. The overall prevalence of *B. bigemina* infection was 12 (27.27%), 4 (6.77%), 5 (7.14%) and 1 (9.09%) in cattle, sheep, goats and wild goats respectively. The co-infections between them peaked in both spring and summer as revealed by blood smear examination and ELISA.

Keywords: Babesia bigemina, Anaplasma marginale, Domestic animals, ELISA.

Introduction

Tick-Borne Diseases (TBDs) are a constraint to livestock production in many developing countries of the world. They are responsible for high morbidity and mortality resulting in decreased production of meat, milk and other livestock products and the loss of draught power.

They are also a significant impediment to the improvement of indigenous breeds of cattle, sheep and goats, since they prevent the introduction of more productive exotic breeds (1). Ticks transmit a greater variety of pathogenic microorganisms, than any other arthropod vector group, and are among the most important vectors of diseases affecting livestock. In general, tick-borne protozoan diseases (e.g. *Babesia bigemina* and *Babesia microti*, while *Anaplasma marginale* and *Anaplasma phagocytophilum* are the most common rickettsial diseases transmitted by ticks. These parasites cause significant economic loss due to reduced productivity, increased mortality, and decreased meat and milk production. The prevalence of these diseases can vary widely depending on the area, host species, and the tick species involved.
Theileriases and Babesiosis and rickettsial diseases (e.g., Anaplasmosis and Heartwater or Cowdriosis) are pre-eminent health and management problems of cattle, small ruminants and buffaloes, affecting the livelihood of farming communities in Africa, Asia and Latin America (2).

Babesiosis is a worldwide tick-borne hemoprotozoosis affecting many mammalian species and caused by intraerythrocytic multiplication of apicomplexans in the Babesia genus. The evolutionary success of this parasite is attested by the large number of species described more than 100, with numerous species probably remaining to be discovered and/or described (3). Babesiae are the second most common blood-borne parasites of mammals after the trypanosomes. More than 100 species of Babesiae have been identified which are traditionally divided on the basis of their morphology into the small and large groups. To date, only ixodid ticks have been identified as vectors for Babesia spp. The specific tick vector must feed on a vertebrate reservoir that is competent in maintaining the Babesia organisms in an infectious state. (4).

Anaplasmosis is an arthropod-borne, haemolytic disease of ruminants caused by the rickettsial haemoparasite, A. marginale (5). A. marginale is the most prevalent tick-borne pathogen of animals worldwide and is responsible for severe morbidity and mortality in temperate, subtropical, and tropical regions (6). Anaplasmosis reduces the animal’s body weight, reduces milk production, causes abortions, and frequently leads to death (7 and 8). Anaplasma spp transmitted by at least 20 ticks’ species, including Argas persicus, Ornithodoros lahorenensis, Boophilus annulatus, B. decoloratus, B. microplus, Dermacentor albipictus, D. andersoni, D. occidentalis, D. variabilis, Hyalomma excavatum, Ixodes ricinus, Rhipicephalus bursa, R. sanguineus and R. simus (9). The aim of the present study were to investigated B. bigemina and A. marginale in cattle, sheep, goats and wild goats using indirect ELISA test in Erbil, Iraq.

Materials and methods

Blood samples were collected from 44 cattle, 59 sheep, 70 goats and 11 wild goats in Erbil district for a period of a year 2010. Samples were selected randomly from each animal. Information about age, breed and sex was recorded. Sera were separated by centrifugation and stored at -20°C.

The study was conducted in north, east, south and west of Erbil district including 15 location. The distribution of location as shown in Figure 1. The north part extend Shagalwa to the Mergasur. East includes Bnaslawa, Kasnazan and Koyisnjaq. South covers Gushtapa and Mahmur area and west include Khabat, Aenkawa and Bahrka.

Thin and thick blood smears were prepared from the peripheral blood and jugular vein blood of the goat, sheep and cattle. The smears were air dried, fixed in absolute methanol and stained for 30 min in a 5% dilution of Giemsa solution in PBS, pH 7.2 or stained for 3 min by malaria kit stain. The slides were examined with oil immersion x100.

The sera of animal’s samples were detected for presence of antibodies against B. bigemina and A. marginale using ELISA technique. The kits were supplemented by SVANOVIR ® Company for the two micro-organisms.

The ticks collected from the animals were put into tubes containing 70% ethanol and were examined under a stereo microscope. Morphological characterization of ticks was carried out using a stereoscopic microscope according to the keys given by (10-13). For the confirm identification the specimens were sending to the Iraq Natural History Research Center and Museum in Bagdad according to the letter No.787 in 4-10-2011.

Results

Based on the microscopical and serological test for B. bigemina and A. marginale it have been found that 16, 6, 8 and 2 in cattle, sheep, goats and wild goats respectively were sero-positive for B. bigemina and A. marginale antibodies as showed in Table I. In case of B. bigemina infection eight from 22 samples positive in serology were also positive in microscopic examination but in A. marginale were two from 10 samples.

The Figure 1 showed that highest prevalence of B. bigemina was found in cattle (27.27%) and lower value (6.77%) was detected in sheep, also the highest rates of positive prevalence A. marginale (9.09%) were diagnosed in cattle and wild goats while lower value (3.36%) in sheep.

Table 1: Number of infected animals by B. bigemina and A. marginale and co-infection between them.

<table>
<thead>
<tr>
<th>Animal</th>
<th>No. of examined animals</th>
<th>No. of sero-positive with B. bigemina</th>
<th>No. of sero-positive with A. marginale</th>
<th>Total no. of sero-positive</th>
<th>Total no. of co-infection between B. bigemina and A. marginale in same animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>44</td>
<td>12</td>
<td>4</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>Sheep</td>
<td>59</td>
<td>5</td>
<td>4</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Goats</td>
<td>70</td>
<td>5</td>
<td>3</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Wild goats</td>
<td>11</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>184</td>
<td>22</td>
<td>10</td>
<td>32</td>
<td>10</td>
</tr>
</tbody>
</table>
Figure 1: Percentage of infection by *B. bigemina* and *A. marginale* in domestic and wild animals.

Result of Figure 2 show that *B. bigemina* was observed more frequently in April (45.45%) than in both June and July (18.18%) than in both August and September (9.09%). *B. bigemina* could not be identified in any of the blood sera analyzed in the other time periods, also *A. marginale* observed only in April, June, July and September (30%), (40%), (20%) and (10%) respectively.

Result in Figure 3 indicated that cattle infection by *B. bigemina* peaked in April (66.66%) and *A. marginale* were peaked in June (50%).

The infection sheep by *B. bigemina* was equal separated between April, June, August and September but in the *A. marginale* only observed in April and June (Figure 4).

The results in Figure 5 showed that the samples positive for *B. bigemina* and *A. marginale* antibodies in wild and domestic goats was peaked in July.
It was found that out of the 32 positive infections there was only 10 animals infected by *B. bigemina* and *A. marginale* in the same time (co-infection between *B. bigemina* and *A. marginale*) and which represented of (25%) cattle, (33.33%) sheep, (37.5%) goats and (50%) in wild goats as showed in Figure 6, on the other hand the highest prevalence of co-infection was in June (40%) Figure 7.

![Figure 6: Percentage of co-infection between *B. bigemina* and *A. marginale* in domestic and wild animals.](image)

![Figure 7: Co-infection between *B. bigemina* and *A. marginale* in domestic and wild animals.](image)

Table 2: Numbers of tick species and there stags collected from indigenous cattle, sheep and goats during the period from January to December 2010.

<table>
<thead>
<tr>
<th>Tick species</th>
<th>Cattle</th>
<th>Sheep</th>
<th>Goats</th>
<th>General</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>♂</td>
<td>♀</td>
<td>Total</td>
<td>♂</td>
</tr>
<tr>
<td><em>R. sanguineus</em></td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td><em>R. turanicus</em></td>
<td>3</td>
<td>4</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td><em>H. anatolicum excavatum</em></td>
<td>4</td>
<td>2</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td><em>H. anatolicum anatolicum</em></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td><em>H. marginatum marginatum</em></td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td><em>H. turanicum</em></td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><em>H. detritum</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><em>B. annulatus</em></td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>17</td>
<td>35</td>
<td>52</td>
</tr>
</tbody>
</table>

**Discussion**

This study was the first in Erbil province on Seroprevalence of *B. bigemina* and *A. marginale* in domestic Animals. No work has been previously done in regard to Seroprevalence of *B. bigemina* on sheep, goat and wild goats in Iraq. The low incidence of both *B. bigemina* and *A. marginale* indicates the low spread distribution of Babesiosis and Anaplasmosis in the area of study, this might be due to the geographical distribution of tick vectors transmitted these diseases and the low distribution of the principle vector of *B. bigemina* as to reviewing. *B. bigemina* can transmitted by *Boophilus microplus*, *B. decoloratus*, *B. annulatus*, *B. geigyi*, *Rhipicephalus evertsi* and *Rhipicephalus sanguineus* in Africa, Asia, Australia, Central and South America and Southern Europe (14 and 15). (16) reported that small ruminants are not severely affected by *A. marginale* and cattle show an innate resistance to *A. ovis*. The earliest survey on piroplasmosis in Erbil province were done by (17) in an FAO report, he showed that 12.5% of sheep were infected by *B. motasi*. (18) mentioned that 22.5% of cattle infected by *A.
marginalae in Mosul province. (19) evaluation prevalence of sheep blood parasites in 1987 in Mosul, Iraq and was declared that 36.3% of animals were theileria and babesia positive. Also (20) demonstrated that 15.42% of the native goats were infected with Babesia ovis, B. motasi, B. foliata and B. taylori in Mosul. (21) Showed that 5.6% of sheep were infected with B. motasi in Sulaimanyah province. (22) Observed that a total of 704 sheep were examined, 80 (11.36%) were found infected with A. ovis and 19 (2.70%) with B. motasi in the central part of Iraq. Babesiosis in domesticated animals in Mazandaran, North of Iran was 18.13% in cows, 16.03% in sheep and 22.27 in goats (23). The antibody activity against B. ovis antigen was high with an overall prevalence of 41.02% in Awassi sheep in Urfa, Turkey (24). In the seroepidemiology study of B. bigemina in cattle in the Konya province, Turkey, samples from 770 cattle from 74 barns were examined microscopically and serologically for B. bigemina, based on these examinations, 15 (1.95%) cattle were found to be positive for B. bigemina and 331 (42.9%) cattle were diagnosed as positive for B. bigemina antibodies (25). According to original data from serological studies conducted in different regions of the world, seroprevalence of B. bigemina varies between 40% and 93% (26, 27, 28, 29 and 30).

In the presence study there was clear differences between cattle infection by B. bigemina and other examined animals (Table 1), this result was usual because of the B. bigemina considered the most important bovine causative agent worldwide, and due to cattle infection by B. annulatus the principle vector of B. bigemina in the area of the study as they showed in the table 2. (15) showed that the bovine babesiosis in Asia are B. bovis and B. bigemina. This study demonstrated the high co-infection between B. bigemina and A. marginale in all infected animal (table 1). (31) reported high mixed infections between Anaplasma spp. and Babesia spp. in infected sheep. PCR-based techniques allow detection of parasites at low parasitaemia while discriminating various species of co-infecting agents (32). TBDs can co-infect cattle causing considerable losses to the livestock industry. (33). The prevalence of infection between seasons was found to be difference (Figure 3, 4, 5 and 6) the infection by B. bigemina and A. marginale in domestic and wild animals was found between April and October. In order to determine the epidemiology of tick borne diseases, it is crucial to know the seasonal activities of the ticks. So in the present study all infection by tick and TBDs arise in spring and summer. (34) showed that the seasonal distribution of B. ovis antibodies peaked in spring (76% and 74.3%) in sheep and goats respectively followed by summer (75.6% and 73.7%) in sheep and goats, respectively. The highest prevalence of Babesia spp. was observed during spring and summer which are considered the seasons of high activity of tick vector, (35-37). Seasonally, the prevalence of Babesia spp. infection started to increase in April and reached highest values in August, while a decrease was observed in September, reaching the lowest levels In February and March (38).

Many ticks were identified during the investigation which were seropositive animals in farms, and the goats showed high infection by Ixodid ticks 43.9% (table 2), R. sanguineus showed more frequent (22.4%) while B. annulatus showed lower percentage.

Conclusion

It was found that 17.39% of domestic animal in Erbil district using of ELISA were infected by B. bigemina and A. marginale.

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