Epidemiology and Seasonal Variation of Ixodid Ticks and Piroplasmida Detection in Cattle of Basrah Province, Iraq

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Abstract

Four hundred and twenty cattle belonging to different breed and age groups were investigated for infestation by ticks during the period from October 2018 to September 2019 in Basrah governorate, Iraq. Investigated cattle were found to be infested by four species of hard ticks namely (Hyalomma anatolicum anatolicum, Hyalomma marginatum turanicum Rhipicephalus (Boophilus) annulatus, Rhipicephalu sturanicus) . No significant difference in infestation rate was observed according to the method of cattle raising (Χ²=0.455, p=0.500), however, seasonal variation in infestation with significant difference was found, higher infestation rate reported in June (63.3%) and the lowest was in January (20%) (Χ² =76.740 ,p = 0.05). In the meantime blood smears samples from the same cattle were also examined by microscopy for hemoprotozoan pathogens. The examination revealed that those cattle are infected by Babesia spp. (27.14%) and Theileria spp. (19.52%). No, significant difference in infection rate was found between male and females, but a significant variation was seen among age groups, however, age group 1-3 years revealed a high rate of infection. Seasonal variation in the infection rates were observed in infected cattle. Higher infection rates of Babesiosis and Theileriosis reported in June (50%) and (36.7%) respectively.

Keywords: Epidemiology, Babesia bovis, Theileria annulata , Hyalomma, Rhipicephalus, Basrah, Iraq.

Introduction

Cattle are most important source of national income for countries. The directorate of animal wealth estimated the number of cattle in Iraq about 2.5 million in 2007. We have no specific information on the races and strains of cattle that are raised in Iraq but are believed to be most of the indigenous cattle breeds and fall within the following races: AL-Janobi cows; AL-Restaki cows, AL-Sharabi cows and AL-Karadi cows; the last two races are confined to the northern region of Iraq, in addition, there are few numbers of the strain Holstein – Friesian introduced to improve local of dairy production. There are a number of obstacles facing the progress and development of livestock industry in Iraq, mainly diseases including ticks and tick-born disease (TBDs) which are most prevalent and exert their huge impact in tropical and sub-tropical regions. TBDs cause enormous losses through mortality, morbidity, productive losses and the cost control and their effect on the immune status of infected animal. The passive impact of ticks does not only acts as vectors for pathogens, but also causes significant effects on animals, such as lack of milk production, weight loss, skin grafting, and predispose animal to other bacterial and fungal diseases. The climatic condition of Iraq is favorable for growth of tick species which is contribute to potential occurrence of Babesiosis and Theileriosis which are caused by Babesia spp. and Theileria spp., respectively. Routine diagnosis of babesiosis and thileriosis is performed by microscopic examination of Giemsa stained blood smears and parasite viewing as well as clinical signs in severe cases, but in subclinical infections parasite microscopically undetectable and lead to relatively high rate of false negative diagnosis. Moreover, Bilgic etal. (2013) pointed out that it is difficult to differentiate between species of Babesia based on morphological characteristics, especially in mixed infection. If animals

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recover from infection, along with lasting carrier status. This occurs in animals in which low numbers of erythrocytes remain infected with parasites and act as a carrier or reservoir for the parasites. These carriers play an important role in the transmission of the infection by ticks.

Materials and Methods

A - Study Area and Field Sampling

The study was carried out in Basrah province, which is located in the southern part of Iraq, at a latitude of 30°30'N and longitude 47°48'W. A total number of 420 cattle were randomly sampled during the period from October 2018 to September 2019.

Samples were collected from farms with two different methods of raising, those that are grazed in unimproved natural pasture and those that were kept in pens and hand fed and watered. All cattle without regular acaricide treatment. The sample-level variables included sample size and location (northern, central, east, west, and southern areas). Cattle were categorized into age classes (<1 year old to ≥10 years) and divided into two categories: cattle with tick burden and no tick burden.

B - Collection of tick and Blood samples

Ticks were collected with rubbing alcohol pads surrounding the skin of cattle and removed by forceps and kept in labeling screw plastic tubes containing ethanol. All collected ticks were examined under the stereomicroscope. Species, sex, and state of feeding were recorded.

Ticks identification was done according to Hoogstootal et al. (1981) and Shubber (2014). Some of the female ticks were frozen at -20°C for DNA extraction.

Blood samples were collected from the vena jugularis from a total of 420 cattle with a 10ml disposable syringe under aseptic precautions. About 5ml of blood was collected in tubes containing Ethylene Diamine Tetraacetic Acid (EDTA) for DNA extraction and then stored in iceboxes at 4°C. The samples were transported to the parasitology laboratory at Basrah University, Education College for Pure Sciences, where blood smears were prepared, and fixed by using methanol and stained by Giemsa, then examined under an oil immersion 1000x objective.

C - DNA Extraction

The DNA was extracted from Blood and tick samples using DNA extraction kit (GeneiabioTech, Taiwan) according to the manufacturer instructions. The extracted DNA were tested by Nano drop spectrophotometer (Type Implen) at wave length 260/280 nm.

D - Polymerase Chain Reaction (PCR)

For the molecular diagnosis of T.annulata, B.microti, B.bovis, B.ovis and B.motasi in ticks and cattle, PCR reactions were performed using the specific primers for detection T.annulata (Cytob1: F ACTTTGGCGTAATGTAAAC, R CTCTGGACCAACCTTTTG) 312bp (Bilgic et al., 2010), B.microti ISSrRNA (F CTTAGTATAAGCTTTTATACAGC, R ATAGGTCAGAAACTTGAATGATACA) 238bp (Inoue et al., 2015), B.bovis SSrRNA (F CTGTCGTACCGTTGGTGC, R CGCACGGACGGAGACCCA) 541bp (Chaudhry et al., 2010), B.ovis SSrRNA (F TGGGCAGGACCTGGTTTGC, R CCGCAAGACGGAGACCCA), 549bp Aktas (2005) and B.motasi Rap1b (F TGGCGCTTCAGGTGTTGAC, R GACGGGTTGCTAGGGCTGAC, 565bp Niu, 2016).

The amplification protocol was as follows: initial denaturation at 95°C for 1 minute followed by 35 cycles of 95°C for 50 sec for T.annulata, 40 cycles of 94°C for 1 min for B.microti, 35 cycles of 94°C for 30 sec for B.bovis and B.motasi and 35 cycles of 94°C for 1 min for B.ovis, annealing 35 cycles at 55°C for 50 sec for T.annulata, 40 cycles of 54°C for 1 min for B.microti, 35 cycles of 50°C for 30 sec for B.bovis and 35 cycles of 62°C for 1 min for B.ovis and B.motasi, 35 cycles of 58°C for 30 sec for B.motasi. Extension at 72°C for 1 min. for T.annulata and 72°C for 90 sec. for B.microti, 45 sec for B.bovis and B.motasi. Extension at 72°C for 1 min. for T.annulata and 72°C for 90 sec. for B.microti, 45 sec. for B.bovis, 72°C for 1 min. for B.ovis and B.motasi with final extension at 72°C for 10 min. for all pathogens (except for B.bovis at 72°C for 7 min.) in the MiniAmp plus thermocycler. The amplification products were separated on 1.5 agarose gel stained with ethidium bromide.
E- Statistical analysis:

Chi-square test was used according to the SPSS statistical program (software, Version 23). 

Results

Out of 420 cattle examined in Basrah governorate, an overall 42.5% cattle were infested by *Hyalomma anatolicum anatolicum*, 32.5% with *H.turanicum*, 23.1% with *Rhipicephalus turanicua*, 21.8% with *R.(Boophilus) annulatus* and 6.2% with mixed infestation. 485 specimens of ticks were collected from 160 animals that mean the intensity of infestation for each animal was 3.0 ticks. Cattle raised in pens recorded the highest prevalence of ticks reaching 39.5%, while cattle grazing in pastures recorded a lower prevalence (34.9%), but the differences were insignificant ($X^2 = 0.455, P=0.500$). No marked differences were observed between sex or between age groups regarding the prevalence of ticks infestation. However, the monthly prevalence of infestation in cattle, *H. a. anatolicum*, *H. turanicum*, *R. turanicus* and *R.B.annulatus* was highest in June (63.3%) and the lowest in January (20%). There were as significant difference ($X^2 = 76.7, P=0.05$) in monthly prevalence (Table 1).

![Table (1) Monthly prevalence of ticks](image)

<table>
<thead>
<tr>
<th>Months</th>
<th>No.of examined cattle</th>
<th>No. of cattle infested with ticks</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>October</td>
<td>25</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>November</td>
<td>25</td>
<td>10</td>
<td>36</td>
</tr>
<tr>
<td>December</td>
<td>30</td>
<td>7</td>
<td>23.3</td>
</tr>
<tr>
<td>January</td>
<td>25</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>February</td>
<td>30</td>
<td>8</td>
<td>26.7</td>
</tr>
<tr>
<td>March</td>
<td>25</td>
<td>12</td>
<td>48</td>
</tr>
<tr>
<td>April</td>
<td>40</td>
<td>18</td>
<td>45</td>
</tr>
<tr>
<td>May</td>
<td>50</td>
<td>22</td>
<td>44</td>
</tr>
<tr>
<td>June</td>
<td>30</td>
<td>19</td>
<td>63.3</td>
</tr>
<tr>
<td>July</td>
<td>50</td>
<td>27</td>
<td>54</td>
</tr>
<tr>
<td>August</td>
<td>50</td>
<td>22</td>
<td>44</td>
</tr>
<tr>
<td>September</td>
<td>40</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>Total</td>
<td>420</td>
<td>160</td>
<td>38.1</td>
</tr>
</tbody>
</table>

$X^2 = 76.740, P=0.05$

Microscopic examination of 420 blood smears showed that 114(27.1%) and 82(19.5%) of cattle were positive for *Babesia spp*. and *Theileria spp*. respectively, according to the sex of cattle, however, there were no significant differences in the prevalence of infection.

Higher prevalence of infection with bovine babesiosis and theileriosis were recorded in cattle of 1-3 years age group 32.3%(50/155) and 22%(34/155) respectively, while lower prevalence recorded in ≥ 10 years for babesiosis 0.1(1/11) and 0%(0/11) for theileriosis. There were significant differences between age groups ($X^2=19.88, P=0.001$) and ($X^2=24.81, P=0.00$) respectively.

The seasonal variation of infection showed that the highest rate of infection were found in June, represented by 50% for Babesiosis and 36.7% for Theileriosis, while lower rates recorded in October 8% and 4% respectively (Table 2). There were significant differences ($X^2=$
88.6, P=0.00; X²=116.69, P=0.00 and X²= 71.5, P=0.00) respectively.

Table (2) Seasonal variation of infected Cattle with Babesia spp. and Theileria spp. in Basrah province.

<table>
<thead>
<tr>
<th>Months</th>
<th>No. of cattle examined</th>
<th>No. of cattle infected with Babesia</th>
<th>Prevalence (%)</th>
<th>No. of cattle infected with Theileria</th>
<th>Prevalence (%)</th>
<th>Mixed infection</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>October</td>
<td>25</td>
<td>4</td>
<td>16</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>November</td>
<td>25</td>
<td>2</td>
<td>8</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>December</td>
<td>30</td>
<td>3</td>
<td>10</td>
<td>2</td>
<td>6.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>January</td>
<td>25</td>
<td>3</td>
<td>12</td>
<td>5</td>
<td>20</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>February</td>
<td>30</td>
<td>4</td>
<td>13.3</td>
<td>4</td>
<td>13.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>March</td>
<td>25</td>
<td>7</td>
<td>28</td>
<td>5</td>
<td>20</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>April</td>
<td>40</td>
<td>15</td>
<td>37.5</td>
<td>10</td>
<td>25</td>
<td>7</td>
<td>17.5</td>
</tr>
<tr>
<td>May</td>
<td>50</td>
<td>15</td>
<td>30</td>
<td>12</td>
<td>24</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>June</td>
<td>30</td>
<td>15</td>
<td>50</td>
<td>11</td>
<td>36.7</td>
<td>5</td>
<td>16.7</td>
</tr>
<tr>
<td>July</td>
<td>50</td>
<td>21</td>
<td>42</td>
<td>12</td>
<td>24</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>August</td>
<td>50</td>
<td>18</td>
<td>36</td>
<td>9</td>
<td>18</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>September</td>
<td>40</td>
<td>7</td>
<td>17.5</td>
<td>10</td>
<td>25</td>
<td>11</td>
<td>27.5</td>
</tr>
<tr>
<td>Total</td>
<td>420</td>
<td>114</td>
<td>27.14</td>
<td>82</td>
<td>19.52</td>
<td>49</td>
<td>11.70</td>
</tr>
</tbody>
</table>

X² = 116.669 , P= 0.000), (X² = 71.542 , P= 0.000), (X² =88.659 , P= 0.000)

Frequency of babesiosis and theileriosis infections were significantly higher in cattle with tick burden than no tick burden.

In the present study using specific primers ,it was found that H.a.anatolicum ticks were infected by T. annulata, B. bovis and B. ovis Fig.(1) and Rh.(Boophilus)annulatus were infected by T. annulata, B. microti and B. bovis Fig. (2) and the cattle had been infected by B. microti, B. ovis, T. annulata, B. bovis and B. motasi Fig. (3),(4).

![Agar gel electrophoresis PCR products of H.a.anatolicum :T.annulata(4) and B.bovis (2) and B.ovis(4) positive](image)

Fig.1: Agar gel electrophoresis PCR products of H.a.anatolicum :T.annulata(4) and B.bovis (2) and B.ovis(4) positive . L. (100-2000bp) represents ladder
Fig. 2: Agar gel electrophoresis PCR products of *Rh.(Boophilus) annulatus*, *T.annulata*(1,2,3,4,5) and *B.microti* (3) and *B.bovis* (3,4) positive. L. (100-2000bp) represents ladder.

Fig. 3: Agar gel electrophoresis PCR products of cattle: *B.microti* (3) and *B.ovis*(1,4) positive. L. (100-2000bp) represents ladder.
Discussion

Iraq is located in the southern part of the northern temperate zone, and this location has significant impact on its climate, which is similar to the climate of the tropical region in terms of temperatures, as it is subtropical.

The current study provides preliminary epizootiological data on ticks and ticks borne diseases (TBDs) in south region of Iraq. However, this study provides useful information about the species of parasitoid ticks in cattle of this area. Cattle were infested with *H. a. anatolicum*, *H. m. turanicum*, *R. turanicus* and *Rh. B. annulatus*. Thus, it does not differ in terms of diversity with the study of Abdul Hussein(2006) and Muhammad(2013) in middle and southern Iraq. Infestation rate in the present study (38.1%) differs from 48.2% recorded by Tuama et al. (2007), 54.3% of AL-Ramahi (2011) and 62% of Mohammad (2015) from other parts of Iraq. These differences in infestation prevalence may be due to the animal raising practices and using or not using of acaricides, difference in vegetation, rainfall rate from year to year and availability of other appropriate host.

There was no significant difference in the rate of infestation of cattle raised in pens and that grazing in the pastures, although the cattle raised in the pens recorded a higher rate of infestation (39.5%).

Although variation was observed in *H.a.anatolicum* population in different seasons, the result indicates that is the predominant tick in all season in Basrah cattle. This population dynamic pattern may be attribute to the fact that hot and dry weather is conducive for the development of *H.a.anatolicum* ticks.

Aktas et al. (2004) have referred the abundance and diversity of ticks as well as the intensity of infestation in animals when studying the epidemiology of the diseases it transmits and this is what the current study has.
The life cycles of *Babesia* and *Theileria* parasites are very similar and closely related to them in that they are transmitted by the same vector which are ticks, but the latter differs by having a development stage in its life cycle, which is the infection of lymphocytes before the erythrocytes are infected, so it is possible to explain the epidemiological picture of both diseases as it is correct on the first can be correct in the second.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Education for Pure Sciences and all experiments were carried out in accordance with approved guidelines.

**References**

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