Molecular detection of *Babesia bovis* and *Babesia bigemina* in Cattle in Al-Qadisiyah Province

Khawla H. Sabbar *Noaman N. A'aiz

Dept.of Microbiology College of Veterinary Medicine, AL-Qadisiyah University
*Email/noaman.aaiz@qu.edu.iq

Summary

This study aims to determine *B. bigemina* and *B. bovis* based on genetic methods. A total of 293 blood and spleen tissue samples were collected from live and slaughtered cows from different regions of Al-Qadisiyah province from December 2013 to August 2014. RT-PCR technique was used to detect the presence of *B. bigemina* and *B. bovis* with study the effect of animals age and sex in the distribution of infection.

Overall 43.77% (83/192) of examined cows showed positive infection with Babesiosis. among them 47.91% (42/96) and 38.54% (37/96) were given positive result to *B. bovis* and *B. bigemina* respectively. The highest infections were shown among the adult cow (≥1 year) While there were a variation regarding to two species of parasite in infection according to the sex but with non-significant difference (P≥0.05).

Key words: Babesiosis, *Babesia bigemina*, *Babesia bovis*, cattle

التحري الجزيئي عن *Babesia bigemina* و*Babesia bovis* في الأبقار في محافظة القادسية عن خوله حسين صبار نعمان ناجي عايز كلية الطب البيطري / جامعة القادسية

الخلاصة

أن الهدف من هذه الدراسة هو تحديد *B. bigemina* و*B. bovis* في الأبقار من مناطق مختلفة في محافظة القادسية خلال الفترات من شهر كانون الأول 2013 ولغاية شهر اب 2014.
**Introduction**

Piroplasms are a tick – transmitted parasitic protozoa parasites divided into two genera *Theileria* and *Babesia*. They are the causative agents of Theileriosis and Babesiosis, respectively (1,2). Many *Babesia* spp. have been described since Victor Babes who first recognized *Babesia* in the red blood cells of cattle in 1888 (3).

Researchers (4) pointed that the species of *B. bovis* and *B. bigemina* affect cattle, and widely important spread in many parts of Asia, Africa, Australia and America because of the presence of the main vector of *Babesia* spp. that represent by *Boophilus microplus*, and is wide spread in the tropics and subtropics areas.

Cattle between 7 and 8 months of age have higher innate resistance to most tick–borne diseases and consequently disease incidence and corresponding mortality are typically lower for this stock class. If a sufficiently high proportion of a herd are consistently exposed to *Babesia* spp. as calves a state of endemic stability may develop in which clinical tick fever is rarely seen (5).
Babesiosis was recorded in various domestic and wild animals in Iraq, with variation in proportions of infection depended upon factors like age, breed, season and activity of ticks (7, 8).

Most of the previous studies depended in detection of parasite upon the microscopical examination, so the aim of this study was to detect the blood parasite (*B. bovis*, *b. bigemina*) genetically in addition to show the effect of age and sex of animals on the disease distribution.

**Materials and Methods**

This Study was conducted during the period from December 2014 to August 2015 in different areas of AL-Qadisiyah province. A total of 157 blood and spleen tissue samples were collected from cows clinically suspected to be infected with babesiosis.

The examined cows included 137 males and 56 females where distributed according to the age into three groups involved calves less than or equal to six months (≤ 7 m), young cows ranged between six months to one year and adult cows with age of equal or more than one year (≥ 1 y).

Two – five ml of blood sample were collected directly from the Jugular vein or during the slaughtering of suspected animal and kept in anticoagulant EDTA tubes, in addition to the collection of 5 – 10 grams of spleen tissue in sterile plastic containers. All samples were transferred in cooling conditions to the laboratory of parasitology in Veterinary Medicine Collage in AL-Qadisiyah University to conduct the necessary tests to determine the infection with babesiosis according to the experimental design. DNA extraction from blood
and tissue samples was done by using the Genomic DNA extraction Kit (Bioneer/Korea) according to the manufacturers instruction.

The extracted DNA were tested by RT-PCR method through used the RT-PCR kit (Genkam/Germany) for *B. bovis* and *b. bigemina*, the thermocycler conditions was done according to primer annealing temperature and probe that included one cycle of Pre-Denaturation in 95 °C for 6 min, and 40 cycles of Denaturation in 95 °C for 15 sec, Annealing/Extension in 71 °C for 41 min and Detection (Scan) was 71 °C for 41 min.

**Results:**

1- Prevalence of Babesiosis in cattle according to real–time PCR test:

The results showed that among (197) babesiosis suspected cases were examined by real – time PCR, 87(44.24%) gave a positive result to babesiosis infection. Among these infected cases and regarding to the species, the results revealed that 47,91% (42/92) from which were infected with *B. bovis* and 38.54% (37/97) with *B. bigemina* (Table 1, Figure 1, 2).

Table (1) : Real – time PCR positive cases of Babesiosis

<table>
<thead>
<tr>
<th>Babesia Species</th>
<th>Examined No.</th>
<th>Positive No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Babesia bigemina</td>
<td>96</td>
<td>37</td>
<td>38.54a</td>
</tr>
<tr>
<td>Babesia bovis</td>
<td>96</td>
<td>46</td>
<td>47.91a</td>
</tr>
<tr>
<td>Mixed infection</td>
<td>192</td>
<td>10</td>
<td>5.20  b</td>
</tr>
</tbody>
</table>

- Similar letters refer to the non-significant differences among species while different letters refer to significant differences at \( P \leq 0.05 \).
Present Prevalence of Babesiosis cases according to the sample:

Related to the used samples in detected of the parasite species, the results showed that there is a relative highest were recorded in blood samples (35 %, 29%) when compare with spleen samples (29%, 22%) in both B. bovis and B. bigemina respectively (Table 3).

Table (3): Real – time PCR positive cases according to the sample.

<table>
<thead>
<tr>
<th>Babesia Species</th>
<th>Examined No.</th>
<th>Positive No.in</th>
<th>%</th>
<th>positive No.in</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Babesia bovis</td>
<td>96</td>
<td>34</td>
<td>25a</td>
<td>22</td>
<td>22.91a</td>
</tr>
<tr>
<td>Babesia bigemina</td>
<td>96</td>
<td>19</td>
<td>19.97a</td>
<td>18</td>
<td>18.76a</td>
</tr>
<tr>
<td>Total</td>
<td>192</td>
<td>53</td>
<td>27.39</td>
<td>40</td>
<td>20.83</td>
</tr>
</tbody>
</table>

- Similar letters refer to the non-significant differences among samples at (P ≥0.05).

Figure (1): Real-Time PCR amplification plot for B. bovis in positive and negative samples.
Figure (3): Real-Time PCR amplification plot for *B. bigemina* in positive and negative samples

 résultats of Babesiosis cases according to the Age.

In both species the highest infection (73.26% and 78.17%) were seen among cattle with age of equal or more than 1 year (≥1 y) (Table 4).

Table (4): Real – time PCR positive cases of different species of Babesiosis according to age.

<table>
<thead>
<tr>
<th>Babesia species</th>
<th>Age</th>
<th>Examined No.</th>
<th>Positive No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Babesia bovis</td>
<td>≤ 7 mns</td>
<td>32</td>
<td>6</td>
<td>18.76a</td>
</tr>
<tr>
<td></td>
<td>7 mns – 1 y</td>
<td>32</td>
<td>10</td>
<td>46.87b</td>
</tr>
<tr>
<td></td>
<td>≥ 1 y</td>
<td>32</td>
<td>20</td>
<td>78.17c</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>96</td>
<td>46</td>
<td>47.91</td>
</tr>
<tr>
<td>Babesia bigemina</td>
<td>≤ 7 mns</td>
<td>32</td>
<td>6</td>
<td>18.76a</td>
</tr>
<tr>
<td></td>
<td>7 mns – 1 y</td>
<td>32</td>
<td>11</td>
<td>34.37b</td>
</tr>
<tr>
<td></td>
<td>≥ 1 y</td>
<td>32</td>
<td>20</td>
<td>72.5c</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>96</td>
<td>37</td>
<td>38.54</td>
</tr>
</tbody>
</table>

- Similar letters refer to the non-significant differences among ages while different letters refer to significant differences at (P ≤ 0.05).

- Prevalence of Babesiosis cases according to the sex:
Regarding to the sex the results appeared that the females cows recorded the higher rate (\( \overline{61\%} \)) in \( B. \text{bovis} \) infection while the male recorded the higher rate (\( \overline{49\%} \)) in \( B. \text{bigemia} \) infection (Table 5).

Table (5): Positive cases in Real – time PCR according to animal sex

<table>
<thead>
<tr>
<th>( \text{Babesia species} )</th>
<th>Sex</th>
<th>Examined No.</th>
<th>Positive No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{Babesia bovis} )</td>
<td>Male</td>
<td>66</td>
<td>31</td>
<td>46.96( \text{a} )</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>30</td>
<td>16</td>
<td>53.4( \text{a} )</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>96</td>
<td>46</td>
<td>47.91</td>
</tr>
<tr>
<td>( \text{Babesia bigemina} )</td>
<td>Male</td>
<td>66</td>
<td>26</td>
<td>39.35( \text{a} )</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>30</td>
<td>11</td>
<td>37.66( \text{a} )</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>96</td>
<td>37</td>
<td>38.55</td>
</tr>
</tbody>
</table>

* Similar letters refer to the non-significant differences among sexes at (\( P \geq 0.05 \)).

Discussion

In the current study and according to the RT-PCR test the result showed that \( \overline{54.2\%} \) of suspected cases appeared positively to infection with babesiosis. Regarding to the species of parasite the results showed that the rates of infection with \( B. \text{bigemina} \) and \( B. \text{bovis} \) were \( \overline{38.54\%} \) and \( \overline{37.91\%} \) respectively, while the mixed infection was \( \overline{5.2\%} . \)

Through access to the results of other studies it found that most of them had the lowest results than which came in the current study, as indicated by Abdo-Sakaya (\(^{8} \)) from Egypt who said that the rate of infection with \( \text{Babesia} \)
spp. reached \%3.4\% also scores of\%9,10 and\%11) ratios close of approximately 1/12. The infection rate in study conducted by Devos and potgieter\%12) in France was 1/10, as well as the rates were amounted in other studies like \%13,14 and\%15, the difference among the current study and other different studies may be attributing to difference in the samples number and type, climate conditions, epidemic of parasite and vector. The prevalence of infection and the occurrence of disease are determined by complex interactions between the bovine host, vector and parasite \%16). PCR does not provide information on herd immunity. Tick-borne pathogen densities in carrier animals appear to fluctuate over time and periodically fall below the levels detectable by PCR\%17,18).

This study appeared a mixed infection of two studied species\%19B. bigemina and\%20B. bovis) which reached to \%8.2\%. In Turkey Dumanli and Ozer \%21) found a mixed infection between\%22B. bigemina and\%23T. anulata reached \%1.8\% in cattle, also Co-infection with Babesia, Theileria and Anaplasma was seen in a Bangladesh by Abdul karim\%\%24, and in Egypt Nayel\%25) found mixed infection of Babesia and Theileria\%26\%27.

The present study pointed to that infection with babesiosis according to age revealed high percentage\%28\%8.1\% and\%8.5\%) in animals with equal or more than one year, where as the lowest result were recorded in animal with age under six month\%≤/7mons). The highest rate of infection in adults animal may be due to the chronicity of infection which can easily detectable by real time-PCR method. Results above don’t correspond with \%11) who recorded rate only 8.8\% in calves and \%16\% in adult cows.
According to sex the results appeared that non significant differences between males and females in *B. bovis* and *B. bigemina* infections, and this may be due that both sexes subjected to the same condition of rearing like nutrition and climate.
References


Oliveira-Sequeira, T.C; Oliveira, M.C; Araujo, J.P. and Amarante, A.F. (2000). PCR-based detection of *Babesia bovis* and


Geysen, D. (2009). The application of Molecular Biology detection techniques to analyse diversity of Theileria parva populations in Zambia. PhD thesis, Brunel University, UK.


