Pathogenicity of Boophilus Species Hard Tick among Engorged Female in Naturally Infested Cattle with Ixodidae Piroplasmosis in Sulaimani Province-Kurdistan Region/ Iraq.

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Keywords:
Cattle, Boophilus spp., (Hemolymph and Histological analysis), blood smear.

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ABSTRACT
Isolated seventy-five cattle (female local breed) infested naturally with Ixodidae, identified into three genera: Boophilus, Hyalomma and Rhipicephalus. According to chi-square test for independence, shows highly significant value 50.75** which compared to tabular chi-square, it means that there were an association between the genus of hard tick in different locations with cattle groups. The prevalence rate of Boophilus spp. was the highest 286 (52.8%) followed by Hyalomma spp. 173 (32%) and Rhipicephalus spp. as the lowest 82 (15.2%). When using chi-square test, the overall prevalence rate of Piroplasmosis in all three types of Boophilus spp. examination were 4.9%, whereas; in adult female hymolymph 8 (10.7), followed by histological examined 2 (2.7%) than larva hymolymph 1 (1.3) with still the Anaplasma spp. the highest prevalence 54.5% followed by Babesia spp., 4 (36.4%), than in Theileria spp., 1 (9.1%) in all examination occurred in Boophilus spp., and shows not significant differentiation in their investigation among the hemolymph examined in adult and larva stage and histological examination. The prevalence rate of piroplasmosis in cattle (blood smear examined) was equally distributed and a not significant difference shows among different locations. According the chi-square test for independence, nonetheless that the result shown, the highest prevalence of piroplasmosis observed with blood smear 18.7%, whereas; Anaplasma spp. 9 (12 %), followed the Theileria spp., 3 (4 %) than the Babesia spp., 2 (2.7%) as a lowest. The prevalence rate of infection with piroplasmsps in cattle blood smear infested with hard ticks was higher than hemolymph and histological cutters examined.

في الماشية في piroplasmosis ixodidae ضمن الإناث المحققة المصابة طبيعيا بـ Boophilus

محافظة السليمانية، أقيم كردستان/ العراق

بهزاد حمه صالح مصطفى
جامعة السليمانية/ كلية العلوم الزراعية/ قسم الإنتاج الحيواني

الخلاصة
: تم عزل خمسة وسبعين بقرة (جماعة محلية) مصابة طبيعيا بـ Ixodidae وتمت توصيفها إلى ثلاثة أنواع Ixodidae , و Pinixodidae و Rhipicephalus و Hyalomma و Boophilus.

50.75** عند مقارنتها مع مراعات كاي تعبير عن وجود اختلاف عالي القيمة مع جميع المواقع المختلفة مع Boophilus spp. و Hyalomma spp. و Babesia spp.، 173 (32%) و 82 (15.2%) % في جميع المواقع

Inference: Anaplasma spp. was the highest prevalence 4.9% (286) followed by Babesia spp. 3 (4%), Babesia spp. 2 (2.7%) as lowest. The prevalence rate of infection with piroplasmosis in cattle blood smear infested with hard ticks was higher than hemolymph and histological cutters examined.
Introduction:

The one-host tick *Rhipicephalus* (*Boophilus*) *microplus* is an economically important ectoparasite of cattle involved in the transmission of the apicomplexan protozoan *Babesia bovis*, the etiological agent of bovine Babesiosis and Anaplasmosis is the most globally prevalent tick-borne pathogen of cattle, with regions of endemicity on the six populated continents (Polar et al., 2005; Mekonnen et al., 2002). Although *A. marginale* has a global distribution, the prevalence and incidence are highest in regions where the tropical cattle fever tick *Boophilus microplus* is endemic (Lincoln et al., 1987 and Palmer et al., 2001) and transmitted transstadially in cattle (Samish et al., 1993). Adult females of *R. microplus* acquire *B. bovis* merozoites by ingesting blood from an infected bovine and pass the protozoan transovarially to their larval offspring that can transmit *B. bovis* sporozoites to cattle during subsequent feeding (Friedhoff, 1988; Mahoney and Mirre, 1979; Bock et al., 2004). Observed that *R. microplus* females can be acquire *B. bovis* from both acute and persistently infested cattle, and efficiently transmit the protozoan transovarial transmission to their larval progeny (Howell et al., 2007a and b). Bovine tropical theileriosis transmitted by *Hyalomma anatolicum anatolicum* is an economically important disease of cross-bred cattle in India which causes heavy economic losses in terms of high morbidity, mortality and reduced production in recovered animals (Haque et al., 2010). Theileriosis caused by *Theileria annulata* is the most economically important cattle disease in Iran, causing major losses in livestock production, which shows the rate of *Theileria annulata* alone was 45.1% while in mixed infections with *Babesia* was 11.7%. The total rate of *Theileria* infection was 56.9% carried out with direct blood smear examination (Hashemi-Fesharki, 1990). In Iraq, the main vectors causes Theileriosis in cattle were: *Hyalomma a. anaticum*, followed by *Boophilus annulatus* and *Rhipicephalus sanguineus* (Tarish, 1982). The purpose of this study are to investigate and to detection the relationship between pathogenicity of piroplasmosis in infested cattle blood smears with whole *Boophilus* species examined (engorged female, larva (lab incubation) hemolymph and histological cutters examined) which collected in same cattle.

Materials and Methods:

1-Cattle sampling and Microscopic examination:

Isolated seventy-five cattle (female) local breed (karadi) infested with ixodidae from 5 different regions and flock in the Sulaimani governorate, each group content 15 cattle, thin blood smears were prepared from each group. These smears were air-dried, fixed in methanol for 3 min and stained in Giemsa 10% for 45 min and examined at 10× oil immersion objective lens of a Nikon SE light microscope for detection of blood parasites.

2-Ticks Collection.

In each group, removed the different stage of hard tick from different site of cattle and identified according to Walker et al. (2003), using only forty five of adult female of *Boophilus* spp. in each groups carried out the study as the following: 15 for hemolymph and 15 of histological examination and additional 15 of engorged female incubated to hatching and growing in laboratory to getting the larva stage to detection of piroplasmosis in hymolymph.
A-Hemolymph (Hemocyte slides) Smear.

Hemolymph was collected by severing the forelegs at the coxal-trochanteral joint and drawing hemolymph into a glass micropipette (Fujisaki et al., 1975). For the purpose of light microscopy of hemocytes, hemolymph was smeared on a glass slide, immediately dried, and fixed in methanol for 10 min. The specimens were stained with Giemsa solution10% for 10 min. Examined under a light microscope with high power100X.

B-Tick inoculations.

Ten of *Boophilus microplus* engorged females were collected from naturally infested cattle from a different region (five regions) farm in the Sulaimani governorate. The ticks in each region were individually placed into sterile glasses container and incubated at 28°C and relative humidity at 85%. Under these conditions, larvae starting to hatching after 21 days, the larvae become ossification after 25 days. Killed the larva and prepare to notice of *piroplasma* spp. in hymolymph.

C- Histological analysis.

15 engorged female of *Boophilus* spp. were collected and amputated legs for better penetration fixation, fixed with 10% formaldehyde for 72hr, transferred to sodium phosphate buffer solution (pH 7.2), after 24 hr, embedded in paraffin, 3–4 μm sections were cut in a microtome as a longitudinal sagittal plane and staining with Hematoxylin and Erosin (H&E) technique for the observation and evaluation of the *Piroplasma* spp. through histological section of adult female by microscopically examination (Hlatshwayoa et al., 2004).

Statistical analysis:

The association test between the parameters under the study was done using chi-square test for independence (p˂ 0.05 or p ˂ 0.01)

RESULTS:

In this study, a survey of piroplasmosis from 75 cattle (female) naturally infested locally breed, totally 541 hard tick, above average 7tick / cattle (Min 6 to mix 11) isolated in different location in Sulaimani governorate. According to chi-square test for independence, the prevalence rate of *Boophilus* spp. was the highest 286 (52.8%) followed by *Hyalomma* spp. 173 (32%) and *Rhipicephalus* spp. as the lowest 82 (15.2%). The highest prevalence of genus *Boophilus*. 49 (68%) and 73 (78.5%) in Zharawa and Sangasar respectively. While the highest prevalence of *Hyalomma* spp. shows in Medan 59 (43.1%) followed in Kalar 52 (39.1%), with a highest prevalence 25 (18.8%) of *Rhipicephalus* spp. in Kalar. However, the results confirmed that this survey shows that the genus *Boophilus* spp. dominance in cattle compared to the other two genera (Hyalomma and Rhipicephalus) as shown as on Table (1).

Data in table (2) shows the distribution of *piroplasma* spp., among infested cattle (blood smears examined) and whole *Boophilus* spp. (hymolymph (larvae and adult stage)) and histological section examination in adult *Boophilus* spp. female. Testing the hypothesis theory the equally probability of the observation, the prevalence rate of *piroplasma* spp., using chi-square test, shows significant (P< 0.05) value, which mean there were not equally observed, the most significant observation occurred in hemolymph examined 8 (10.7) ticks followed by histological examined 2 (2.7%), and the lowest type was showed by the larva hemolymph examined 1 (1.3%) with overall prevalence of piroplasmosis 11(4.9%).

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Table 1. Number and % of ticks collected from experimental cattle in different region (Batwen- Pashder).

<table>
<thead>
<tr>
<th>Region and Groups</th>
<th>No. of Boophilus spp.%</th>
<th>No. of Hyalomma spp.%</th>
<th>No. of Rhipicephalus spp.%</th>
<th>Total of tick collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kalar/ G1</td>
<td>15</td>
<td>56 (42.1)</td>
<td>52 (39.1)</td>
<td>25 (18.8)</td>
</tr>
<tr>
<td>Medan / G2</td>
<td>15</td>
<td>57 (41.6)</td>
<td>59 (43.1)</td>
<td>21 (15.3)</td>
</tr>
<tr>
<td>Halabja/G3</td>
<td>15</td>
<td>51 (48.1)</td>
<td>37 (34.9)</td>
<td>18 (17)</td>
</tr>
<tr>
<td>Zharawa/ G4</td>
<td>15</td>
<td>49 (68)</td>
<td>16 (22.3)</td>
<td>07 (9.7)</td>
</tr>
<tr>
<td>Sangasar/ G5</td>
<td>15</td>
<td>73 (78.5)</td>
<td>09 (9.7)</td>
<td>11 (11.8)</td>
</tr>
</tbody>
</table>

Overall 75 286 (52.8) 173 (32) 82 (15.2) 541

\[^2\text{cal} = 50.75^{**}] x^2 (8) = 20.09

Table 2. Prevalence rate of Piroplasma species in Boophilus whole collected examined in different groups of cattle.

<table>
<thead>
<tr>
<th>Types of examination of Boophilus spp.</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>Overall examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hemolymph examined</td>
<td>15</td>
<td>13</td>
<td>15</td>
<td>14</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>histological examined</td>
<td>15</td>
<td>0</td>
<td>14</td>
<td>15</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Larvae hemolymph examined</td>
<td>15</td>
<td>n.s.</td>
<td>14</td>
<td>15</td>
<td>n.s.</td>
<td>15</td>
</tr>
<tr>
<td>Overall</td>
<td>45</td>
<td>43</td>
<td>45</td>
<td>43</td>
<td>45</td>
<td>11 (4.9)</td>
</tr>
</tbody>
</table>

Overall the prevalence rate in all examined

\[x^2 \text{cal} = 8.21\]

\[x^2 (2) = 5.99\]

0.05

n.s. not significant

Chi-square test for independence for distribution of piroplasma spp. namely, Anaplasma spp., Theileria spp., and Babesia spp. shows no difference among the Hemolymph and Histological examined in adult and larva stage. But still the Anaplasma spp. was dominant in hemolymph examined with (5), followed by Babesia spp. (2), than the Theileria spp. (1). Histological technique in adult female Boophilus spp., shows equally infested with both genus; Anaplasma and Babesia spp., but the genus of Theileria spp. was not observed. In the larva hemolymph examined, only the Babesia spp. was seen, Anaplasma spp. and Theileria spp. were not appeared.
Table 3. *Piroplasma* specis investigated in genera *Boophilus* (only fifty whole) collected in different groups of cattle.

<table>
<thead>
<tr>
<th>Boophilus Examination</th>
<th><em>Piroplasmosis species</em></th>
<th><em>Anaplasma spp</em></th>
<th>%</th>
<th><em>Theileria spp</em></th>
<th>%</th>
<th><em>Babesia spp</em></th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total No. of piroplasmosis</td>
<td>Anaplasma spp</td>
<td>%</td>
<td>Theileria spp</td>
<td>%</td>
<td>Babesia spp</td>
<td>%</td>
</tr>
<tr>
<td>hemolymph examined/ 15 tick</td>
<td>2</td>
<td>1</td>
<td>50</td>
<td>1</td>
<td>50</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Histological Examined/ 15 tick</td>
<td>0</td>
<td>1</td>
<td>100</td>
<td>0</td>
<td>1</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Larvae hemolymph examined/ 15 tick</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Overall no. of *Anaplasma spp* | 6 (54.5%) |

Overall no. of *Theileria spp* | 1 (9.1%) |

Overall no. of *Babesia spp* | 4 (36.4%) |

Total no. % of piroplasmosis in hemolymph = 8 (10.7), A=5(6.6%), The=1(1.3%), Ba=2 (2.7%)

Total no. % of piroplasmosis in Histological examination = 2 (2.7); A=1 (1.3%), The=0%, Ba=1 (1.3%)

Total no. % of piroplasmosis in Larvae hemolymph = 1 (1.3); A=0%, The=0%, Ba=1(1.3%)

x² cal=2.53**n.s**

x² (4) = 9.48

0.05

In this study only fifty isolated cattle were infested naturally with *Boophilus* spp., for the investigation of piroplasmosis by blood smear testing. Table (4) shows, equally distribution of the prevalence rate of piroplasmosis in different locations of this study, through the testing of blood smear when using chi-square test. It was observed that the prevalence of piroplasmosis (blood smear) in isolated cattle were equally distributed in different location and the differences of this observations were not significant.

Table 4. Prevalence rate of piroplasmosis in cattle with blood smears (Gimsa stain).

<table>
<thead>
<tr>
<th>Cattle microscopic examination (thin blood smears)</th>
<th>Fifty cattle examined (Blood smear) in each group.</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>Total No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P%</td>
<td>N</td>
<td>P%</td>
<td>N</td>
<td>P%</td>
<td>N</td>
<td>P%</td>
</tr>
<tr>
<td></td>
<td>3 (20)</td>
<td>12</td>
<td>4 (26.7)</td>
<td>11</td>
<td>3 (20)</td>
<td>12</td>
<td>1 (6.7)</td>
</tr>
</tbody>
</table>

x² ca=2.00**n.s**

x² (4) = 9.48

0.05

n.s= Not significant. P= Positive. N= Negative

(Table 5) shows the results of the microscopic examination by Giemsa stain (thin and thick blood smears) for prevalence of piroplasmosis spp. in cattle in different locations. 18.7% as a overall prevalence of piroplasmosis, whereas; the highest number 12% *Anaplasma* spp., followed by 4% *Theileria* spp., and the lowest rate was 2.7% of *Babesia* spp., In the same table it was revealed that the fluctuations between the observed genus of Anaplama, *Theileria* and *Babesia* spp.
and the locations where the blood cattle were sampled is common by chance and not significant according the chi-square test for independence.

Table 5. Prevalence of *Piroplasma* species in different groups' cattle with blood smears (Gimsa stain).

<table>
<thead>
<tr>
<th>Cattle microscopic examination by Giemsa stain (thin blood smears)</th>
<th>Twelve cattle examined (Blood smear) in each group (12 cattle)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Piroplasmosis species-G1</td>
</tr>
<tr>
<td>Total No. of Anaplasma spp.</td>
<td>3</td>
</tr>
<tr>
<td>Total No. of Babesia spp.</td>
<td>1</td>
</tr>
<tr>
<td>Overall no. % of Anaplasma spp.</td>
<td>9 (12%)</td>
</tr>
<tr>
<td>Overall no. % of piroplasmosis</td>
<td>14 (18.7%)</td>
</tr>
</tbody>
</table>

\[ \chi^2 \text{cal} = 3.69^{ns} \]
\[ \chi^2 (8) = 15.51 .05 \]

Discussion

Isolated the 75 female cattle may be the hard tick remain and sucking large quantities of blood from the female than the male cattle because productive age; that similar with Tarish, (1982) who recorded the piroplasmosis (*Theileria* and *Babesia* spp.) more in female than male hard tick. In this study, observed that the *Boophilus* spp. are the highest 286(52.8%) and predominant ticks followed by *Hyalomma*, 173 (32%) and *Rhipicephalus* 82 (15.2%) in all groups infested cattle isolated in different distract and subdistract in Sulaimani governorate, this results in agreement with Mustafa, et al. (2013), which they observed a highly significant differences between different tick species when calculated by general test (LSD) in Kurdistan region/Iraq. The predominant of *Boophilus* spp. may be due to their ability to tolerate the dry and harsh environment with little hiding places (Kettle, 1995). *Boophilus* among the genera of ticks using large mammals as host and feed primarily on cattle, less frequently on other large herbivores (Cumming, 1998) and constitute major problem for the cattle industry in tropical and subtropical areas of the world (Fragoso et al., 1998), this results contrast with other surveys in some Iraq’s governorates (Robson et al., 1969; Shamsuddin, and Mohammad, 1988; and Hadi, and Fatohi, 2002).

Also, the overall prevalence of piroplasmosis infection investigated among three rough examination of adult and larva stage 11(4.9%), whereas the *Anaplasms* spp. 6 (54.5%) followed by *Babesia* spp. 4 (36.4%), Than the *Theileria* spp. 1(9.1%) was the lowest percentage, shows the most significant observation occurred in engorged female hemolymph examined 8 (10.7) ticks followed by histological examined 2 (2.7%), and the lowest type was showed in larva hemolymph 1 (1.3%). This agreement with Heekin et al., (2013), which revealed that the merozoite stage apicomplexa, migrate from the digestive cells to hemolymph and salivary gland (Bock et al., 2004) discovered the adult females of *R. microplus* acquire *B. bovis* merozoites by ingesting blood from
an infected bovine and pass the protozoan transovarially to their larval offspring that can transmit *B. bovis* sporozoites to cattle during subsequent feeding. (Christensen and Schnittger, 2009) conferred, after fertilization (Male and Female gamonts), the zygote is phagocytozed by the cells of the midgut and change by endomogenesis to the first kinete, which enters the haemolymph of the tick. It becomes a multiplying body and divides by binary divisions to become sporozoites, which are infective for warm-blooded animals. Chauvin et al. (2009) revealed that the sexual development and multiply of piroplasma (sporozoite) occurrence in midgut cells and spread to different tissues including the salivary glands and ovary. In this study, not significant differential investigated among the hemolymph examined in adult engorged female and larva, and Histological examination. But still the *Anaplasma* spp. was dominant in hemolymph examined, may be the cattle previously infected with piroplasmosis, specially infested by *Anaplasma* spp, through larva hemolymph examined only the genus of *Babesia* was seen, and the genus of *Theileria* and *Anaplasma* was not observed. Most of *Babesia*, unlike *Theileria*, are capable of transovarial transmission and newly hatched larvae are infectious to the hosts, as well as (Merck Veterinary Manual) revealed that the *Boophilus* spp. are the main vectors transmitted *Babesia* spp., especially *Babesia bigemina* and *B. bovis* are one host, which transmission occurs transovarially and by blood inoculation experimentally. The transmission of *Theileria* spp. and *Anaplasma* spp., occurred by trans-stadial transmission and the trans-ovarian does not occurs as in *Babesia* transmission, only the genus of *Babesia* spp. was seen during testing of larvae histological. Transmission through these one-host ticks is transovarial. The engorging adult female ticks pick up sporozoites and pass it on to their progeny (larval or seed ticks) through eggs. Following the attachment to another host, the infection is transmitted by the larval, nymphal and adult stages in case of *B. bovis* or by nymphal and adult stages in case of *B. bigemina*. The percentage of larvae infected can vary depending mainly on the level of parasitemia of the host at the time the female ticks engorge (El-Sawalhy, 1999). *Theileria* parasitites are transmitted only trans-stadially. The kinete migrates to the salivary gland as the tick is completing its moult and development begins when the tick commences feeding or is stimulated in some other way (Dolan, 1989)

Through the this study, the blood smear testing of infested cattle by piroplasmosis, showed the prevalence of piroplasmosis (blood smear) in naturally infested cattle 14(18.7%) were equally for distributed of genus of Anaplama 9(12%), *Theileria* 3(4%) and *Babesia* 2(2.7%) species, and the locations, and not significant according the chi-square test for independence, that may be the blood smears were sampled from infested cattle is common by chance in different location, The prevalence of Piroplasmosis by blood smears (Giemsa stain) in naturally infested cattle was a highest than whole ticks type examination, that may be the cattle infected with piroplasmosis before the hard tick infested "and the amount of merozoite sucking by a tick is too little, which leads to difficulty to diagnosis. Using the PCR technique lead to significantly higher efficacy of detection of bovine piroplasmsids compared to microscopically examination of blood smears, may be PCR showed a significantly higher efficacy of detection of *Babesia* spp. and *Theileria* spp. (Ibrahim et al., 2009). In addition, using of ELISA revealed higher babesiosis and theileriosis infection percentage than that of PCR, (39% or 28%) respectively, might be attributed to cross reaction between some protozoan parasites which leading to false positive reaction.

**Conclusion**

During this study Investigated Piroplasmosis in both technique; in cattle blood smear and in *Boophilus* annulata (in adult female, larva stage (hemolymph) and also in histological cutters examination), while the most significant observation of *Babesia* spp. occurred in hemolymph. *Anaplasma* spp. was dominant in adult hemolymph but the *Theileria* spp. and *Anaplasma* spp. we’re not appeared in larva hymolymph *in vitro* and shows susceptibility *Babesia* spp. for transmission trans-ovarian.
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