Prevalence and some risk factors of bovine haemotropic mycoplasma in Nineveh province - Iraq

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Abstract

The objective of current study was to determine the prevalence of bovine haemotropic mycoplasma in cattle using conventional polymerase chain reaction (PCR) in Nineveh province/ Iraq, to investigate some of the epidemiological risk factors associated with occurrence of the disease. From September 2017 - September 2018, four hundred blood samples were obtained from cattle both sexes, different ages, origin, different management systems and from different regions in Nineveh province, ten milliliter blood were collected from the jugular vein for PCR test, whatever epidemiological data were collected through interview with the farms owners. The results of this study appear that the prevalence of bovine haemotropic mycoplasma was 75%. The risk factors associated with increased prevalence of disease include age, increased prevalence was at >3 years, females, imported animals, indoor animals which were 86.8, 80, 91.7, 77.5% respectively. The significantly increased prevalence of the disease 93.7% in the western regions of Nineveh province. In spring and summer months a significantly increased prevalence of disease which were 79.6 and 78.95 respectively. In conclusions, this study detected that bovine haemotropic mycoplasma was widely distributed in Nineveh province associated with several risk factors.

Keywords: Haemotropic mycoplasma, Prevalence, Bovine, Nineveh, Iraq

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نسبة الحدوث وبعض عوامل الخطورة لمرض المايكوبلازم البقرية المحبة للدم في محافظة نينوى

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فرع الطب الباطني والوقائي، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

إن الهدف من الدراسة الحالية تحديد نسبة انتشار المايكوبلازم البقرية المحبة للدم في الماشية باستخدام طريقة تفاعل البلمرة المتسلسل PCR التقليدي في محافظة نينوى، وتحديد بعض عوامل الخطورة الوبائية المرتبطة بحدوث المرض. من شهر أيلول 2017 ولغاية شهر أيلول 2018، تم أخذ عينة دم من الأبقار بالعجل من كلا الجنسين ومختلف الأعمار والمنشئات ونظم التربية والمناطق في محافظة نينوى. تم جمع 10 ملليتر من دم الوريد الوداعي لحيوانات الدراسة لإجراء فحص PCR، واستخلصت جميع البيانات الوبائية من مالتا الحيوانات. أظهرت نتائج الدراسة أن نسبة الإصابة بالمايكوبلازم البقرية المحبة للدم في عينات الدم بلغت 75%، شملت عوامل الخطورة المرتبطة بالربية المتنوعة. وارتفاع المعدلات في المناطق الغربية حيث بلغت 93.7%، سجلت أعلى نسبتين معنويتين للإصابة بالمرض في فصلي الربيع والصيف حيث بلغتا 79.6 و 78.9% على التوالي. استنتج من هذه الدراسة أن المايكوبلازم البقرية المحبة للدم واسعة الانتشار في محافظة نينوى ومرتبطة بعوامل خطورة متعددة.
Introduction

Haemotropic mycoplasma (heamoplasma) include small, cell wall-free, epiehrocytic bacteria infect the blood of various mammalian (1,2) and consider an emerging bacterial pathogen to a wide variety of animals including livestock, wildlife and companion animals (3-5). Formerly the causative agent classified as Haemobartonella and Eperythrozoon species within the order Rickettsiales, and recently reclassified in the genus Mycoplasma (1,6-8). Bovine Heamotropic mycoplasmas clinically relevant as causative agents of acute, life-threatening hemolytic anemia in animals. Whatever, few animals may develop only mild clinical signs or be asymptomatic (1). Factors such as gender, age, immune status, or concurrent infection with other pathogenic agents, have been suggested implicated in the development of disease (9-12). Infections caused by Mycoplasma wenyonii and Candidatus Mycoplasma haemobos have been documented in cattle in countries in the Asia-Pacific region, including Japan (13-15), China (16,17) and Europe (18,19). The transmission of hemoplasma is believed to occur through various blood-feeding arthropods, including ticks (20). Due to the lack information about the epidemiology of bovine heamotropic mycoplasma in Mosul/Iraq, this study aimed to determine the prevalence of the disease in cattle and some of the risk factors associated with the disease.

Material and methods

Animal and Specimens collection

The epidemiological survey was performed a total of 400 blood samples from cattle (imported, native origin) were collected from jugular vein between September 2017 - September 2018 from both sex, different ages animals (1-30 days, <1 years, 1-3 years, >3years) and different region in Nineveh province (Iraq). The recorded relative risk factors analysis, the blood samples were Immediately placed in EDTA tubes and stores at -20°C prior to DNA extraction.

Polymerase chain reaction technique

A total genomic DNA was extracted from 300 micro litter blood samples with a commercially available kit (blood samples Bioingentech veterinary extraction, purification kit, Chile) and stores -20 °C until PCR testing. PCR was carried out using primers Mycoplasma genus - specific primers: specific primers for the 16S rRNA gene of bovine heamotropic mycoplasma, forward primer: 5’- ATATTCCTACGGGAAGCAGC-3’, equivalent to nucleotide numbers 328 to 347 of M. wenyonii, reverse primer :5’-ACCGCAGCTGCTGGCACATA-3’, equivalent to nucleotide numbers 503 to 522 of M. wenyonii amplified a 195 base pair and 173 base pair product for M. wenyonii and ‘Candidatus M. haemobos’, respectively (21) with few modification. With final volume 20 microliter PCR reaction consist of 2 microliter of DNA samples, 1 microliter forward primer (10 Pico mole) and 1 microliter reverse primer (10 Pico mole), 4 microliters of 5× Taq master mix, 12 microliter PCR Grade water. The PCR program included initial denaturation at 94°C for 30 sec. Denaturation 94 °C for 30 sec. 31 cycles, Anneling 57 °C 30 second, extension 72 °C for 1 minute and final extension 72 °C for 2 min., cooling 4 °C. The electrophorese run on 1.5% gel agarose and visualized using UV imager.

Statistical analysis

The Statistical analysis was done by using computed 2 by 2 tables in Epi-InfoTM 7 software (version 7).

Results

In the present study overall prevalence of bovine heamotropic mycoplasma in Nineveh Province was 75% (300 out of 400) by conventional polymerase chain reaction (Figure1).

Figure 1: PCR 16S rRNA Gene for bovine heamotropic mycoplasma (195bp) on agarose gel the, lane 1-10 positive blood sample, -ve lane negative sample, +ve lane positive control.

This study revealed that the Prevalence of the disease was significantly increased in cattle aged more than >3 years RR: 1.98 times, CI: 1.13 - 3.44 Compare to other ages (Table 1). The current study appears that the prevalence was significantly increased in female compared to male cattle (P<0.0001) RR: 1.20 times, CI: 1.05 - 1.36. The prevalence was also significantly increased among imported cattle (RR: 1.33 times, CI: 1.212 - 1.46) compared to native cattle (P<0.0001) (Table 1). Also, the study indicated that the prevalence of the disease was significantly higher in indoor feeding 77.5% in compared to outdoor feeding 66.6% (P<0.034) RR: 1.11 times, CI: 0.99 - 1.35 (Table1). Based on regional factors the west, south and east regions of the city showed significantly
increased prevalence of the disease (P<0.003) (RR: 7.47 times, CI: 1.35-2.25) compared to the city center and north regions (Table 2). This study also demonstrated that the prevalence was significantly increased in spring and summer seasons, 79.6 and 78.7% respectively (RR: 1.31 and 1.30 times respectively) compared to the winter and autumn seasons (Table 3).

Table 1: Relative risk factors of cattle associated with the prevalence rate of the Haemotropic mycoplasma

<table>
<thead>
<tr>
<th>Factors</th>
<th>No. tested cattle</th>
<th>PCR technique</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No of positive (%)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-30 days</td>
<td>16</td>
<td>7 (43.7%)</td>
</tr>
<tr>
<td>&lt;1 years</td>
<td>107</td>
<td>73 (68.2%)</td>
</tr>
<tr>
<td>1-3 years</td>
<td>125</td>
<td>88 (70.4%)</td>
</tr>
<tr>
<td>&gt; 3 years</td>
<td>152</td>
<td>132 (86.8%)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>150</td>
<td>100 (66.6%)</td>
</tr>
<tr>
<td>Female</td>
<td>250</td>
<td>200 (80%)</td>
</tr>
<tr>
<td>Origin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native</td>
<td>291</td>
<td>200 (8.7%)</td>
</tr>
<tr>
<td>Imported</td>
<td>109</td>
<td>100 (91.7%)</td>
</tr>
<tr>
<td>Husbandry system</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outdoor feeding</td>
<td>93</td>
<td>62 (66.6%)</td>
</tr>
<tr>
<td>Indoor feeding</td>
<td>307</td>
<td>238 (77.55%)</td>
</tr>
</tbody>
</table>

Values significantly different (P < 0.05) are labeled with different letters (a, b or c).

Table 2: Relative risk of regional factors associated with the prevalence rate of the Haemotropic mycoplasma

<table>
<thead>
<tr>
<th>Regional factors</th>
<th>No. tested cattle</th>
<th>PCR technique</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No of positive (%)</td>
</tr>
<tr>
<td>North regions</td>
<td>67</td>
<td>36 (53.7%)</td>
</tr>
<tr>
<td>City center</td>
<td>93</td>
<td>62 (66.6%)</td>
</tr>
<tr>
<td>East regions</td>
<td>212</td>
<td>176 (83%)</td>
</tr>
<tr>
<td>South regions</td>
<td>12</td>
<td>11 (91.6%)</td>
</tr>
<tr>
<td>West regions</td>
<td>16</td>
<td>15 (93.7%)</td>
</tr>
</tbody>
</table>

Values significantly different (P < 0.05) are labeled with different letters (a, b or c).

Table 3: Relative risk of seasonal factors associated with the prevalence rate of the Haemotropic mycoplasma

<table>
<thead>
<tr>
<th>Factors</th>
<th>No. cattle tested</th>
<th>PCR test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No of positive (%)</td>
</tr>
<tr>
<td>Autumn 2017</td>
<td>56</td>
<td>34 (60.7%)</td>
</tr>
<tr>
<td>Winter 2017</td>
<td>52</td>
<td>34 (65.3%)</td>
</tr>
<tr>
<td>Summer 2017</td>
<td>76</td>
<td>60 (78.9%)</td>
</tr>
<tr>
<td>Spring 2017</td>
<td>216</td>
<td>172 (79.6%)</td>
</tr>
</tbody>
</table>

Values significantly different (P < 0.05) are labeled with different letters (a, b, c).

Discussion

Haemotropic mycoplasma is of great economic importance in relation to the infectious agents affecting cattle (20,22). This is the first epidemiological study of disease in Nineveh province. The study shows overall of the prevalence rate of disease in Mosul was 75%. Lower and/or near similar prevalence has been reported in earlier studies of the disease in Iraq and other countries, the prevalence was 83.3% in cattle by using PCR in Basrah (23). In China 21.5% (17), In Japan 38.5% (24), in Malaysia 50% (25), In Ghana 32% (26), In Brazil 60.9% (27) and In Switzerland 85% (28). The prevalence of the disease may differ from country to other and even within
regions of the same country and this might due to the different in management practices, number of samples, sensitivity of the diagnostic methods, incidence of competent vectors, efficacy of control programs, climatic variations, extensive cattle trade, uncontrolled animal movement, population size and biosecurity (3,14).

The current study revealed a considerable difference in the prevalence of disease among the age range of cattle, higher prevalence rate was revealed in cattle more than >3 years, which may be due to stress factors or concurrent infection and Immune States. This finding was consistent with (25,29,30).

The current study appears increased prevalence of disease in female which agreed with (25) and may be due to stress factors such as lactation, pregnancy, immunosuppression. The result revealed that the prevalence was significantly higher among imported cattle than native cattle, due possibly to the fact that most of the cattle population in Nineveh province are imported from Iran, Turkey and Syria in which the disease is prevalent (31,32,33). It should also be noted that some of the imported cattle were often brought into Nineveh province without border control and quarantine.

The result also revealed that the prevalence significantly higher among indoor feeding cattle in compared to outdoor feeding cattle, due possibly to overcrowding cattle in stockyard and present of ticks, this result agreed with (24). According to the geographical regions this study demonstrated that the prevalence of disease was significantly higher in west, south and east regions compared to with city center and north regions. This difference could partly be explained by factors such as high cattle population density, close distances between animals, poor management, animal movements or livestock trade, large animal markets, meeting between the owners and attenders, climatic factors.

This study demonstrated that the prevalence was significantly higher in spring and summer seasons in compared to autumn and winter season. This finding was similar to the results mentioned by (34). The causations may be relevancy with climate which related with tick's reproduction in these seasons.

Acknowledgement

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References


