

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

S.A. Esmaeel¹ and B.A. Albadrani²

Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Email: ¹salamaljubori96@gmail.com, ²basima1971@yahoo.com

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Abstract

The objective of current study was to determine the prevalence of bovine heamotropic 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 heamotropic 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 heamotropic mycoplasma was widely distributed in Nineveh province associated with several risk factors.

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

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

سلام عبد اسماعيل و باسمة عبد الفتاح البدراني

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

الخلاصة

إن الهدف من الدراسة الحالية تحديد نسبة انتشار المايكوبلازما البقرية المحبة للدم في الماشية باستخدام طريقة تفاعل البلمرة المتسلسل التقليدي PCR في محافظة نينوى، وتحديد بعض عوامل الخطورة الوبائية المرتبطة بحدوث المرض. من شهر أيلول ٢٠١٧ ولغاية شهر أيلول ٢٠١٨، تم اخذ ٤٠٠ عينة دم من الأبقار والعجول من كلا الجنسين وبمختلف الأعمار والمنشئ ونظم التربية والمناطق في محافظة نينوى. تم جمع ١٠ مليلتر من دم الوريد الوداجي لحيوانات الدراسة لغرض إجراء فحص PCR، واستحصلت جميع البيانات الوبائية من مالكي الحيوانات. أظهرت نتائج الدراسة أن نسبة الإصابة بالمايكوبلازما البقرية المحبة للدم في عينات الدم بلغت ٧٥%. شملت عوامل الخطورة المرتبطة بالنسبة المعنوية العالية لانتشار المرض للأعمار الأكبر من ٣ سنوات والإناث والحيوانات المستوردة والمربأة داخل الحظائر ٨٦.٨ و ٨٠ و ٩١.٧ و ٧٧.٥% على التوالي، وكانت نسبة انتشار المرض مرتفعة معنويًا في المناطق الغربية حيث بلغت ٩٣.٧%، سجلت أعلى نسبتين معنويتين للإصابة بالمرض في فصلي الربيع والصيف حيث بلغتا ٧٩.٦ و ٧٨.٩% على التوالي. استنتج من هذه الدراسة ان المايكوبلازما البقرية المحبة للدم واسعة الانتشار في محافظة نينوى ومرتبطة بعوامل خطورة متعددة.

Introduction

Haemotropic mycoplasma (heamoplasma) include small, cell wall-free, epierythrocytic 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 *Haemotropic 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 haemotropic 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 haemotropic 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 electrophoresis 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-Info™ 7 software (version 7).

Results

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

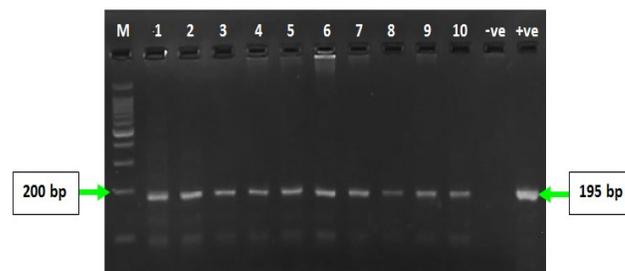


Figure 1: PCR 16S rRNA Gene for bovine haemotropic mycoplasma (195bp) on agarose gel the, lane 1-10 positive blood sample, -ve lane negative sample, +ve lane positive control.

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

Factors	No. tested cattle	PCR technique			
		No of positive (%)	RR	95% CI	P
Age					
1-30 days	16	7 (43.7%) ^a	1		
<1 years	107	73 (68.2%) ^b	1.55	0.88-2.75	0.05
1-3 years	125	88 (70.4%) ^b	1.60	0.9-2.83	0.032
> 3 years	152	132 (86.8%) ^c	1.98	1.13-3.47	0.0001
Gender					
Male	150	100 (66.6%) ^a	1		
Female	250	200 (80%) ^b	1.20	1.05-1.36	0.002
Origin					
Native	291	200 (8.7%) ^a	1		
Imported	109	100 (91.7%) ^b	1.33	1.212-1.469	0.0001
Husbandry system					
Outdoor feeding	93	62 (66.6%) ^a	1		
Indoor feeding	307	238 (77.55%) ^b	1.11	0.99-1.35	0.034

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

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

Regional factors	No. tested cattle	PCR technique			
		No. positive (%)	RR	95% CI	P
North regions	67	36 (53.7%) ^a	1		
City center	93	62 (66.6%) ^a	0.71	0.95-1.6	0.09
East regions	212	176 (83%) ^b	0.44	1.22-1.94	0.0001
South regions	12	11 (91.6%) ^b	0.21	1.28-2.25	0.01
West regions	16	15 (93.7%) ^b	7.47	1.35-2.25	0.003

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

Factors	No. cattle tested	PCR test			
		No. positive (%)	RR	95% CI	P
Autumn 2017	56	34 (60.7%) ^a	1		
Winter 2017	52	34 (65.3%) ^a	1.07	0.80-1.43	0.61
Summer 2017	76	60 (78.9%) ^b	1.30	1.02-1.65	0.02
Spring 2017	216	172 (79.6%) ^b	1.31	1.05-1.63	0.003

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 be 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 >3years, 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.

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