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Preliminary Prevalence and Risk Factors of *Mycobacterium bovis* in Local and Imported Breeds of Cattle and Buffaloes in Mosul city, Iraq

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THE objectives of the present study were to (i) investigate the prevalence of bovine tuberculosis in cattle and buffalo in Mosul, (ii) examine potential risk factors (source of animals, sex, and age), and (iii) indicate clinical signs associated to BTB in affected animals. A total of 196 animals (106 cattle and 90 buffalo) from different areas in Mosul. Serum antibodies againstbovine TB infection were examined by indirect ELISA. Prevalence was calculated, and potential risk factors and clinical signs associated to seropositive TB were examined using conditional logistic regression. Results indicated that the overall prevalence of bovine tuberculosis was 12.2% (18.9% in cattle, which was significantly higher than that (4.4%) in buffalo). The odds of seropositive for TB in imported cattle and female were 3 times higher, compared to local and male. There was no significant difference between age groups. Seropositive TB animals showed higher odds of having weakness and enlargement of lymph nodes, compared to those tested negative. All animals tested positive for TB exhibited intermittent hacking cough. In conclusion, tuberculosis is evident in bovine in Mosul, particularly in cattle, and the infection in imported cattle is a critical concern. Bovine TB should be considered in the differential diagnosis when the animals weak, exhibiting intermittent hacking cough, and having enlarged peripheral lymph nodes.

Keywords: Bovine tuberculosis, ELISA, *Mycobacterium bovis*.

Introduction

Bovine tuberculosis (BTB) are an important infectious disease affecting a wide range of domestic animals, wildlife species as well as humans. The infection is mainly caused by *Mycobacterium bovis*, and characterized by gradual development of tuberculous lesions in different tissues insidethe infected host [1,2]. Human infection usually occurs through consumption of unpasteurized contaminated milk, ingestion of raw or undercooked meat, inhalation, and transcutaneously through handling of infected carcasses [3,4,5]. In addition to the fact that BTB threats the public health, it constitutes a major economic burden to cattle industry due to decrease cattle productivity (milk yields, meat production, and fertility), and trade restrictions [6, 7, 8].

The disease is widespread in Central and South America, parts of Asia and Middle East countries. While BTB has been controlled successfully in most developed countries through the application of the test and slaughter schemes, meat inspection at abattoirs and pasteurization of milk, it remains a problem in some developing countries because practice of control programs are either at an early stage or not exist [9,10,11].

In Iraq, BTB has been reported in cows and buffaloes in Wasit and Basra provinces based

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on comparative intradermal tuberculin test and antigen rapid bovine TB Ab test [12,13]. Those studies indicated an increased trend in the prevalence of the disease in the area. In Baghdad, *Mycobacterium bovis* was detected in cow's milk [14], and the prevalence of the infection in cattle sera was 19% [15]. In Mosul, however, the epidemiologic situation of the disease is not known in cattle and buffalos. The present study was, therefore, aimed to: (i) investigate the prevalence of bovine tuberculosis in cattle and buffalo in Mosul, (ii) Examine potential risk factors (source of animals, sex, and age), and (iii) Indicate clinical signs associated to BTB in affected animals.

Materials and Methods

Study Design and Animals

This study was designed as cross-sectional study, conducted between January and July 2019. Study animals included 106 cattle and 90 buffalo (a total of 196 animals) from different areas in Mosul city, Iraq.

Diagnosis of TB

In this study, animals were considered positive for TB if their sera tested positive for the presence of antibodies against bovine TB infection by indirect ELISA using a commercial kit (Elabscience Biotechnology Inc., USA).

Data Collection

For each animal, the following data were collected: age (≤ 4, > 4year-old), sex (male, female), a source of animal (local, imported). Data on clinical signs observed in the animals were also collected, including: weakness (yes, no), loss of appetite (yes, no), fever (yes, no), cough (yes, no), and enlargement of peripheral lymph nodes (yes, no). In this study, the animal was considered weak if shown emaciation and failure of support [16] , and have fever if the rectal temperature was greater than 39.5°C.

Statistical Analysis

The overall prevalence was calculated as the number of animals (cattle and buffalo) that were seropositive for TB divided by the total number of study animals. The prevalence at the level of animal type (cattle or buffalo) was calculated as the number of animals (cattle or buffalo) that were seropositive for TB divided by the total number of same type of animal. Finally, prevalence was compared among type of animal (cattle vs. buffalo) by use of chi-square test. Potential risk factors (i.e, source of animal, sex, age) and clinical signs associated to seropositive TB were examined using conditional logistic regression, as variables were matched by type of animal [17]. Odds ratio (OR) was used as an epidemiologic measure of association between the outcome (TB) and a factor. An Odds ratio of 1 indicated no association. In contrast, the greater the departure of the OR from 1, the stronger the association exists. Variables with a value of $P \leq 0.05$ (two-tailed) were considered significant, and the OR and 95% CI were reported. Statistical analysis was performed using STATA 13.0 (Stata Crop., College Station, TX, USA).

Results

The overall prevalence of bovine tuberculosis was 12.2%. The prevalence of TB in cattle was 18.9%, in buffalo was 4.4% (Fig. 1). As determined by use of chi-square test, the prevalence of TB was significantly higher ($P < 0.01$) in cattle than that it was in buffalo.

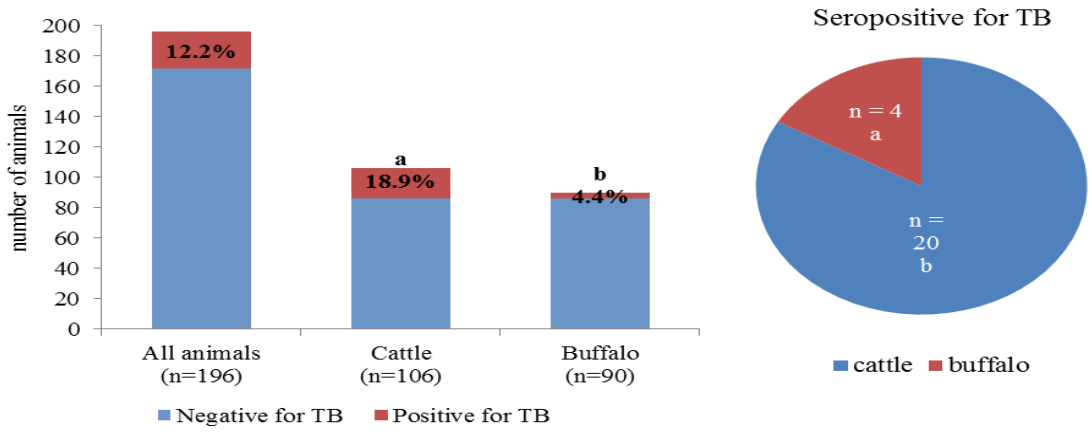

Fig. 1. Prevalence of bovine tuberculosis in cattle and buffalo in Mosul, Iraq, diagnosed on the basis of IgG antibody detection in the sera using ELISA.

Different letters (a, b) mean groups were statistically different at value of $P < 0.01$.

The conditional logistic regression indicated that odds of seropositive for TB in imported animals (cattle) were 3 times higher (P = 0.03), compared to local, and in female were 3 times greater (P = 0.05), compared to male (Table 1). There was no significant difference between age groups (Table 1).

Seropositive TB animals showed higher odds of having a weakness (P = 0.01) and enlargement of lymph nodes (P < 0.01), compared to those tested negative (Table 2). In addition, all animals tested positive for TB exhibited intermittent hacking cough (Table 2). On the other hand, there was no difference between seropositive and negative TB for loss of appetite or fever (Table 2). Finally, the clinical signs were not observed in buffaloes as clearly as observed in cattle.

TABLE 1. Final conditional logistic regression models for potential risk factors associated to seropositive for TB in cattle and buffalo in Mosul, Iraq.

Factor	TB		Odds Ratio	95% CI	*P*
	Positive	Negative			
Model I					
Source of animal					
Local	10	136	1.00	Reference	NA
Imported	14	36	3.20	1.13, 9.10	0.03
Model II					
Sex					
Male	4	66	1.00	Reference	NA
Female	20	106	3.23	1.02, 10.22	0.05
Age (year)					
≤ 4	8	66	1.00	Reference	NA
> 4	16	106	1.18	0.46, 3.05	0.73

TABLE 2. Clinical signs associated to seropositive for TB in cattle and buffalo in Mosul, Iraq.

Factor	TB		Odds Ratio*	95% CI	*P*
	Positive	Negative			
Weakness					
No	6	93	1.00	Reference	NA
Yes	18	79	3.54	1.32, 9.50	0.01
Loss of Appetite					
No	10	98	1.00	Reference	NA
Yes	14	74	1.77	0.73, 4.29	0.20
Fever					
No	16	101	1.00	Reference	NA
Yes	8	71	0.62	0.25, 1.55	0.31
Cough					
No	0	65	1.00	Reference	NA
Yes	24	107	-	-	-
Enlarged Peripheral Lymph Nodes					
No	10	143	1.00	Reference	NA
Yes	14	29	7.54	2.93, 19.41	< 0.01

The* Odds ratio was indicated by conditional logistic regression.

Discussion

In this study, we were able to estimate the overall prevalence of bovine TB in cattle and buffalo, and compare the prevalence between cattle and buffalo. The conditional logistic regression enabled us to examine potential risk factors for TB while controlling for the difference in animal type (i.e., cattle or buffalo) through matching. In addition, we were able to quantify the association between clinical signs observed in animals and TB through calculating the odds ratio with 95% CI and p-value. Finally, our detection of TB is considered sensitive, as an ELISA technique that was used for TB detection is considered a sensitive method for the measurement of antibodies in tuberculosis animal sera.

In the current study, the overall prevalence was 12.2% (18.9% in cattle). In Baghdad, the prevalence in cattle was 19% [15] , which is close to what is reported here. However, in Was it, the prevalence was reported at 75% [12]. The prevalence reported here differ from those reported by [12] Kalaf et al. (2014), which might be due to type of tests used in the diagnosis of the disease, differences in farming practices, cattle breeds, and production systems·; as the prevalence of bovine TB can be influenced by different factors such as geographical region,cattle movement, hygienic status of animals, husbandry system, and type of diagnostic test used [18,19,20] . On the other hand, our study indicated that the prevalence of TB was significantly higher in cattle, compared to buffalo. An explanation is that *Mycobacterium bovis* in buffalois not shed in a high quantity through nasal and oral discharges, which reduces the transmission of bovine tuberculosis among buffaloes [21].

In this study, TB was 3 times higher in imported cattle, compared to local cattle. One possible reason is that imported cattle were already infected. However, the origin of imported cattle is missing in our data, which is a study limitation. Another possible reason is that imported cattle are less resistant to TB infection than local breed [22], which can make imported cattle at high risk of infection. On the other hand, the result showed that the odds of TB were greater in female, compared to male, which is in line with what was previously reported by Worku et al. (2016) [23] and Ahmad et al. (2018) [24] . The stress of calving and lactation might decreasefemale immunity and makes them at high risk of infection. Finally, although animals older than 4 years-old were about 20% at high risk of infection, the result was not statistically significant (P = 0.73), which might be a sample size issue. Other studies indicated that the risk of infection increases as the animals become older [25,26]. Phillips et al. (2002) [27] and Cleaveland et al. (2007) [28] suggested that older animals are more susceptible to tuberculosis. That is, as the animals live longer period, they might have a greater possibility of contact with the infectious agent.

In this study, animals tested positive for TB was 3 times at high risk to suffer from weakness, compared to animals tested negative, indicating the debilitating and chronic nature of the disease. In addition, all animals tested positive for TB exhibited intermittent hacking cough, which reflect the impact of the disease on the respiratory system and confirm that the inhalation is a critical route of infection [29] . Finally, the odds of enlargement of peripheral lymph nodes were significantly higher in animals tested positive for TB, which might reflect the generalization of the infection and development of non-progressive lymph node abscess [16].

Conclusion

Tuberculosis is evident in bovine in Mosul, particularly in cattle. Infection in imported cattle is a critical concern, and highlights the value of animal quarantine and testing at the borders. Bovine TB should be considered in the differential diagnosis when the animals weak, exhibiting intermittent hacking cough, and having enlarged peripheral lymph nodes. Further studies are important to assess the role of other animals, e.g., sheep and goats raised together with cattle.

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Conflicts of interest

The authors report no conflicts of interest.

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الانتشار الأولي وعوامل الخطورة لميكروب السل البقرى فى السلالات المحلية والمستورة من الابقار والجاموس في مدينة الموصل ، العراق

اسامة موفق العراقي ، مدركة محمود الجمالي ، عمر خزعل الحنكاوي، مآب ابراهيم الفروهجي ومحمد أسامة دحل

قسم الامراض الباطنة والطب الوقائى - كلية الطب البيطري - جامعة الموصل - الموصل - العراق.

شملت أهداف هذه الدراسة هي (١) تحديد نسبتي انتشار مرض السل في الأبقار والجاموس في الموصل \ العراق ، (٢) دراسة عوامل الخطورة المحتملة (مصدر الحيوانات والجنس والعمر) في الحيوانات المصابة ، و (٣) وتسجيل العلامات السريرية المرتبطة بالمرض. اجريت الدراسة على ١٩٦ الحيوانات (١٠٦ الماشية و ٩٠ الجاموس) من مناطق مختلفة في الموصل. تم باستخدام فحص الاليزا غير المباشر ضد عدوى السل البقري. أشارت النتائج إلى أن معدل انتشار مرض السل في الأبقار كان ١٢,٢ ٪ (١٨,٩ ٪ في الماشية ، والتي كانت أعلى بكثير من ذلك (٤,٤ ٪) في الجاموس). كانت نسب الإصابة بمرض السل في الابقار والإناث المستوردة أعلى بثلاثة أضعاف ، مقارنة بالحيوانات المحلية و الذكور. لم يكن هناك فرق كبير بين الفئات العمرية. أظهرت الحيوانات الموجبة مصليا للاصابة بالسل الهزال وتضخم العقد الليمفاوية، مقارنةً بتلك السالبة للمرض. جميع الحيوانات التي ثبتت إصابتها بالسل أظهرت السعال المتقطع. نستنتج من الدراسة، إن مرض السل منتشر في الأبقار في الموصل ، وخاصة في الابقار المستوردة، يجب مراعاة السل البقري في التشخيص التفريقي عندما تكون الحيوانات تعاني من الهزال، والسعال المتقطع، وتضخم العقد الليمفاوية المحيطية.