Mycoplasma bovis Seroprevalence in Khartoum State-Sudan

Original Research Article

ABSTRACT

Background: *Mycoplasma bovis* causes several diseases in cattle. It contributes in chronic pneumoniae and mastitis so it causes economical losses.
Aim: Serological surveillance of *Mycoplasma bovis* in Khartoum state, Sudan.
Methodology: Serum samples (No.180) were collected randomly and tested using BIO-X *M. bovis* antibody ELISA Kit.
Results: The seroprevalence of *M. bovis* was 7.2% (13 out of 180). The cattle aging 2 to 5year recorded the highest seroprevalence (9.1%), followed by less than 2 year-old (6.6%) and older than 5 year-old (5.6%). Based on gender distribution, females record 8.8% seropositive of *M. bovis* antibodies in comparison of 5.5% of the males. There is correlation between serum tests and gender (.391) with confidence intervals 95%, P value .05. The correlation between serum tests and age of tested group was (.839) with confidence intervals 95% with P value .05.
Conclusion: These findings showed that *M. bovis* is existed and special consideration must be taken to control the disease. No previous serological investigation for the disease has been attempts in Sudan.

Keywords: Seroprevalence; Mycoplasma bovis; cattle; Khartoum state; ELISA.

1. INTRODUCTION

Mycoplasma bovis one of the most important pathogenic agents causes disease in cattle. First isolation was attempts in 1961 from of mastitis case [1]. "The disease is sometimes considered as neglected pathogen of adult dairy cattle, intensively reared beef, and dairy calves" [2,3]. The agent contributes in considerable economical losses to the beef and dairy industries [4], [2] and [5]. On the other hand, *M. bovis* has been associated with keratocon-junctivitis [6], abortion and other diseases [7].

Respiratory tract and nasal secretions the main source of infection and increase the epidemiology of infection [8], [2] [5]. Recently [2] reported that "*M. bovis* has been isolated from air in shed containing diseased calves and calves may be experimentally infected by inhalation of *M. bovis*. The disease can be transmitted direct consumption or contact with infected milk" .Recently [9] determined "the presence of *Mycoplasma bovis* in colostrum with real-time PCR".

"Although *M. bovis* is not a zoonotic disease, there is only one report of its association with human disease: it was cultured from the sputum of a patient with lobar pneumonia, nephritis and hemolytic anemia" [10].

The economic impact of *M. bovis* infection is associated with weight gain reduction and drug and labor costs for treatment .Death losses and aportion also has major economic impact.

"Diagnosis of *M. bovis* organism can be performed using several methods including immunohistochemical staining" [11], isolation of the agent [12], and "use of specific PCR probe on lung tissues" [13,2,5]. "Many serological tests have been developed, including indirect hemagglutination, and indirect ELISA. Indirect

ELISAs using whole cell or treated antigen are the principal methods used for serological testing. Serology-based detection of antibody against *M. bovis* by ELISA is considered as a reliable method as herd test for evidence of previous or recent infections" [14,2].

"First isolation of *M. bovis* from an outbreak of bovine mastitis in Sudan was reported by" [15]. Because the serological status of *M. bovis* infection in Khartoum state is not determined yet, the objective of this study was to assess the current serological status of *M. bovis* in serum samples obtained from cattle in Khartoum state. The outcomes offer baseline data for the application of successful tactics for the management of M. bovis infection in cattle in Khartoum state.

2. MATERIALS AND METHODS

2.1 Sample Collection

Serum samples (No. =180) of cattle were randomly selected from Khartoum state dairy herds in December 2019.From both sexes with range of age as follows: <2year and between 2 to 5 years and >5year using a randomized field trial design.

2.2 Serological Examination

Serum samples were examined using BIO K260/2 (Bio-X Diagnostics, Jemelle-Belgium) *M. bovis* antibody ELISA kits .According to the manufacturer's protocol; the reagents were brought to a temperature of 21^0C +/-30^0C before use. 1 mL aliquot of the dilution buffer was prepared in 5 or 10 mL tubes. 10 µL serum samples were added in each tube (dilution 1/100) and shaken gently. The positive and negative control sera were diluted as 1/100 in a dilution buffer. Sera samples and the positive and negative sera were distributed to the wells (100µL/well). The plates were incubated at 21^0C +/- 30^0C for 1 hour. Following that, the washing solution was used to rinse the plates, emptying the contents. Two additional times were spent washing. The conjugate was diluted as 1:50 in the dilution buffer and 100 µL of the conjugate solution was added to each well and incubated for 1 h at 21^0C +/-30^0C, and the plates were washed as mentioned earlier. After washing, 100 µL of the chromagen solution was added to each well on the plate. The plates were incubated for 10 min at the same temperature. Then 50 µL of stop solution was added to each well. The optical density (OD) at 450 nm in the micro-well were read using (Star Fax –USA) ELISA Reader.

The following formula was used to determine the OD from the measured OD values of the samples, as well as the negative and positive serum samples:

$$\text{Value} = \frac{\text{OD} * 100}{\text{Delta OD positive}}$$

Values less than 37% are considered negative sample and greater than or equal to 37% is positive ones.

2.3 Statistical Analysis

The data was entered in Microsoft excel spread sheet and then transferred to SPSS version 17 for analyzing the results. The correlations between tested serum to gender and age were tested. The confidence level and absolute precision were 95% and 5%, respectively, and $p < 0.05$ was set for significance.

3. RESULTS

From 180 total serum tested; seropositive results were 7.2% (No.=13/180) for *M. bovis* antibodies. Seroprevalence in different age groups ranged from 9.1% to 5.6 %. The cattle aging 2-5 year-old scored the highest seroprevalence (9.1%; No. = 6/66), followed by <2year-old cattle (6.6%; No. = 4/60) and >5-year-old cattle (5.6%; No. =3/54) Table 1.

Seroprevalence showed that the females scored the highest seroprevalence (8.8%; No.=8/90) when compared to the males (5.5%; No.=5/90) Table 2.

Statistically, there is correlation between serum tests and gender (.391) with confidence intervals 95% with P value .05. The correlation between serum tests and age of tested group was (.839) with confidence intervals 95% with P value .05.

4. DISCUSSON

This research results showed high individual animal and herd seroprevalence, this indicates that *M. bovis* causes infection like mastitis or other respiratory disorders in cattle in Khartoum state-Sudan.

The presence of *M. bovis* previously was reported by [15], in which 37 isolates were recovered from 42 milk samples from imported

Friesian cows in Khartoum state. Importing live animals or semen without investigation can lead to introducing new *M. bovis* Strains. The seroprevalence of *M. bovis* was estimated using ELISA test [16]. Concluded that the ELISA test is better than using PCR to detect the presence of *M. bovis* infection as a result of the persistence of *M. bovis* antibodies especially in chronic infections. On the other hand, [4] reported that "serology is effective in indicating infection with *M. bovis* and may be more sensitive than culture either in chronic cases or in case of using antibiotics.The fact that *M. bovis* has both lipid and protein antigens lead to high antibody responses and antibody levels for many months" [4]. There is similarity in seroprevalence findings with the previous reports of [17] and [18] "in China who reported a seroprevalence of 7.69% and 5.95%, respectively. The seroprevalence obtained was lesser than the reports of" [19-21] in Great Britain and [16] in Turkey who reported "a prevalence of 19.5%, 13- 23%, 22% and 35.4% respectively, even though cattle sampled by the mentioned authors were intensively managed".

The seroprevalence of *M. bovis* differed in targeted age groups (9.1% - 5.6 %). The first group (2-5year-old) has the highest seroprevalence (9.1%) this age considered the maximum lactating period in which cows exposed to infection through milking procedure. The cattle of 3 to 4 years old -the breeding age- scored highest seroprevalence when using milking system or hand milking of herdsmen which had a potential for contaminating udders as encouraged mastitis.

The high *M. bovis* seroprevalence in calves <2-year- old (6.6%) might be due to vertical transmission which could be an important means of *M. bovis* transmission. This agreed with the earlier report of [18] who also reported "the highest seroprevalence in dairy cattle of <1-year-old. The variation in seroprevalence in different age groups suggested the possibility of horizontal transmission. Cows might become source of infection to young calves".

Table (1): *M. bovis* Seroprevalence in different ages of Cattle in Khartoum state

Age	No. tested	No.positive result	Seroprevalence%	p value
<2year-old	60	4	6.6%	.839
2-5 year-old	66	6	9.1%	
>5-year-old	54	3	5.6%	

Std. Deviation=.79734

Table 2. Seroprevalence of *M. bovis* in Cattle based on sex in Khartoum state

Sex	No. tested	No.positive result	Seroprevalence%	p value
Male	90	5	5.5	.391
Female	90	8	8.8	
Total	180	13	7.2	

Std. Deviation=.50139

In this study, female cattle had higher seroprevalence (8.8%) when compared to males (5.5%). This agreed with the earlier report of [22] who reported that "females showed antibodies to *M. bovis* more than the males. This was because more females were kept in for the purpose of reproduction and milk production in comparison to the males. They could play important roles in the spread of *M. bovis* because they remain for longe periods in herds and exposed to stress of reproduction, calving and nursing. For this reason, they could transmit the infection from one generation to another".

5. CONCLUSION

This investigation indicated that *Mycoplasma bovis* is present in Sudan with a seroprevalence rate of 7.2%. Cattle of all ages, and genders are exposed to the organism. First serological survey of *M. bovis* conducted in this transboundary state in Sudan. Therefore, special consideration must be taken for effective control strategy of the disease in Sudan.

ACKNOWLEDGEMENT

The Author acknowledges Dr.Annita Genter – Bio-X Diagnostics-Belgium for kindly providing the ELISA kit.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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