***Original Research Article***

# *Mycoplasma bovis* Seroprevalence in Khartoum state-Sudan


## ABSTRACT

**Background:** *Mycoplasma bovis* is an important and emerging cause of respiratory disease and arthritis in cattle. It contributes in chronic pneumoniae and mastitis so it causes economical losses.

**Aim:** Serological surveillance for antibodies detection of *Mycoplasma bovis* in Khartoum state, Sudan.

**Methodology:** A total of 180 random serum samples were collected and examined for the presence of *M. bovis* antibodies using BIO-X *M. bovis* antibody ELISA Kit.

**Results**: The overall seroprevalence of *M. bovis* was recorded as 7.2% (n=13/180). The less highest seroprevalence was recorded in the cattle aging 2-5year (9.1%), followed by than 2 year-old (6.6%) and older than 5 year-old (5.6%). Based on sex distribution, 8.8% of the females and 5.5% of the males were seropositive to *M. bovis*. 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.

**Conclusion:** The above findings are indicative for the presence of *M. bovis* in the study area for the first time *M. bovis* seroprevalence in Sudan.



**Keywords:** Seroprevalence, *Mycoplasma bovis,* Cattle, Khartoum state, ELISA.


## INTRODUCTION

*Mycoplasma bovis* is one of the most pathogenic agents in the Mycoplasma species that cause disease in cattle. It was first identified in 1961 from a case of mastitis [1]. *Mycoplasma bovis* is a significant but sometimes neglected bacterial 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],[5 ]. In addition *M. bovis* has been associated with keratocon-junctivitis[6]), abortion and other diseases[7].

Respiratory tract and nasal secretions are important for 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*. On the other hand, *M. bovis* can be transmitted by consumption or contact with infected milk and recently [9] determined the presence of *Mycoplasma bovis* in colostrum with real-time PCR and this plays another source of infection.

*M. bovis* appears to be of no significance as a human pathogen, as there is only one report of its association with human disease: it was cultured from the sputum of a patient with lobar pneumonia and probable myocarditis, nephritis and hemolytic anemia [10].

The economic impact of *M. bovis* infection are likely to include reduced weight gain or feed efficiency, pharmaceutical and labor costs for treatment of ill animals, death losses, and a portion of the cost of preventative measures such as conditioning [ 4].

Diagnosis of *M. bovis* organism can be performed through several methods including immunohistochemical staining [11], isolation of the agent [12], and use of specific PCR probe on lung tissues [13], [2] and [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 for herd diagnosis 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 results would provide baseline data for the implementation of effective strategies for the control of *M. bovis* infection in cattle in Khartoum state.

## MATERIALS AND METHODS

### *2.1.* Study populations

Serum samples (*n*=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 2to 5 years and >5year using a randomized field trial design.

### 2.2. Sample collection

The blood samples were taken from jugular vein. These were immediately placed into an ice bath slanted and transported to the laboratory. The sera were later transferred into

tubes and centrifuged at 3000 rpm for 20 min and then decanted into cryovials, which were identified before storage at −20 °C until analyzed.

*2.3.* **Serological examination:**

Serum samples were examined using BIO K260/2 (Bio-X Diagnostics, Jemelle-Belgium) *M. bovis* antibody ELISA kits. The test was carried out according to the manufacturer's protocol. All the reagents were brought to a temperature of $21^0$C +/-$30^0$C before use. 1 mL aliquot of the dilution buffer was prepared in 5 or 10 mL hemolysis tubes. 10 µL serum samples were added in each tube (dilution 1/100) and were shaken briefly on mechanical agitator. 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 $21^0$C +/- $30^0$C for 1 h. Then the plates were rinsed with the washing solution, emptying the contents by flipping it over sharply above a sink. The washing step was repeated two more times. 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 $21^0$C +/-$30^0$C, 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 OD was calculated from the measured OD values, and negative and positive serum samples using the following formula-

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

The signal read for each sample well was divided by the corresponding positive control serum signal and multiply this result by 100 to express it as a percentage. A sample was considered negative if its coefficient was less than 37%. A sample was taken as positive if its coefficient was greater than or equal to 37%.

**Statistical Analysis:**

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

## RESULTS

From 180 total serum tested; seropositive were 7.2% (n=13/180) for *M. bovis* antibodies.

Seroprevalence in different age groups varied from 9.1% to 5.6 %. The cattle aging 2-5year-old had the highest seroprevalence (9.1%; n= 6/66), followed by <2year-old cattle (6.6%; n= 4/60) and >5-year-old cattle (5.6%; n=3/54).**Table1**.

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

| Age in years | No. tested | No.positive | 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

Sex-specific seroprevalence showed that the females had the highest seroprevalence (8.8%; n=8/90) as compared to males (5.5%; n=5/90).**Table 2.**

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

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

**Std. Deviation**=.50139

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.

## DISCUSSON

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

The presence of *M. bovis* previously was reported by [15], who isolated thirty-seven isolates 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 was found to be more useful method than PCR to detect *M. bovis* infection because of the persistence of *M. bovis* antibodies especially in chronic infections. On the other hand, [4] reported that serology is effective in indicating exposure to *M. bovis* and may be more sensitive than culture in chronic cases or those treated with antibiotics, as

these factors may interfere with the *in vitro* growth of *M. bovis*. *M. bovis* has both lipid and protein antigens which can elicit antibody responses and antibody levels remain high for many months [4].

The seroprevalence finding was in agreement with the previous reports of [17] and [18] in China who reported a prevalence of 7.69% and 5.95%, respectively. The seroprevalence obtained was lesser than the reports of [19] [20] [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* varied in different age groups (9.1% to 5.6 %). The cattle aging 2-5year-old have the highest seroprevalence (9.1%) this age considered the maximum lactating period in which cows exposed to infection through milking procedure. Higher *M. bovis* seroprevalence in cattle of 4-year-old might increase the risk of milk contamination with *M. bovis*. This was because the cattle of 3 to 4 years old cattle were the bedrock of herds that were of breeding age. They were in their first or second stage of gestation (calving), they had potential of the spread of *M. bovis*, harboring the organism sub-clinically or chronically. The milking system of herdsmen also had a potential for contaminating udders as hand milking encouraged mastitis. Therefore, carrier cows were the most likely the source for the infection for naive calves in herds.

The high *M. bovis* seroprevalence in calves <2-year- old (6.6%) might be due to ingestion of infected milk 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 varied seroprevalence in different age groups suggested the possibility of horizontal transmission. Cows might become intermittent shedders of *M. bovis* , since contaminated milk could be the source of infection to young calves.

In this study, female cattle had higher prevalence (8.8%) as 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 a herd in comparison to the males for the purpose of reproduction and milk production. The fact that female cattle predominates pastoral herds meaning that any maintenance of *M. bovis* in such hers would be done by these female cattle. They could play important roles in the spread of *M. bovis* because they remained for longer periods in herds, passed through more stress of reproduction, calving and nursing, had greater chance of coming in contact with contaminated water, pasture, fomites and environment. For this reason, they could transmit the infection from one generation to another.

## CONCLUSION

This study reports the occurrence of *Mycoplasma bovis* infection with a seroprevalence rate of 7.2%. Cattle of all ages, and sexes are at risk with the organism. First serological survey of *M. bovis* conducted in this transboundary state in Sudan. Therefore, baseline data for *M. bovis* provided in this study may help in taking an effective control strategy of the disease in Sudan.

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***Conflict of interest*:** The authors declare that there is no conflict of interest.

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