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ELISA-based serological survey of *Mycoplasma bovis* in cattle in three local government areas in Adamawa State, Nigeria

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ABSTRACT

A serological survey for the detection of antibodies to *Mycoplasma bovis* was conducted in Adamawa State, Nigeria during the year 2012. A total of 400 serum samples were collected from three local government areas (LGA) namely Yola (n=140), Mubi (n=130) and Ganye (n=130), and the samples were examined for the presence of *M. bovis* antibodies using BIO-X *M. bovis* antibody ELISA Kit. The overall seroprevalence of *M. bovis* was recorded as 19.5% (n=78/400). The highest seroprevalence was recorded in Ganye LGA (27.7%; n=36/130), followed by Yola LGA (20.0%; n=28/140) and Mubi LGA (10.8%; n=14/130). The cattle aging <1-year had the highest prevalence (25.8%), followed by 4-year-old cattle (22.4%). Based on sex distribution, 20.4% of the cows and 17.4% of the bulls were seropositive to *M. bovis*. Breed susceptibility to *M. bovis* infection showed that White Fulani had the highest prevalence (21.8%), and Sokoto Gudali had the lowest (11.9%). No statistical significant association was found between *M. bovis* infection and age, sex and breed of the cattle. In conclusion, the above findings are indicative for the presence of *M. bovis* in the study area.

Keywords

Adamawa State, ELISA, Cattle, *Mycoplasma bovis*, Seroprevalence

ARTICLE HISTORY

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INTRODUCTION

Mycoplasma bovis is a common inhabitant of the upper and lower respiratory tracts of healthy and pneumonic cattle (Thomas et al., 2002). *M. bovis* is a significant but sometimes neglected bacterial pathogen of adult dairy cattle, intensively reared beef, and dairy calves (Maunsell et al., 2011; Rérat et al., 2012). In 1961, the bacterium was first isolated in the United States from a mastitis affected cow (Hale et al., 1962). The organism is belonging to the Class *Mollicutes*, Order Mycoplasmatales, Family *Mycoplasmataceae*, and the Genus *Mycoplasma* (Razin et al., 1998). *M. bovis* is considered as a significant cause of several diseases like mastitis (Byrne et al., 2000), arthritis (Stipkovits et al., 1993), genital disorders and abortion (Byrne et al., 1999), bovine pneumonia (Pfützner and Sachse, 1996), and reduction of *in vitro* fertility (Eaglesome and Garcia, 1990).

Mycoplasmas cause several economically costly diseases in cattle. Approximately 157,000 calves die each year due to pneumonia and related diseases, which have a approximate market value of about €99 million (Vanden-Bush and Rosenbusch, 2003). *M. bovis* may be responsible for at least one quarter to a third of these losses, although this may be underestimated (Nicholas et al., 2000).

Diagnosis of *M. bovis* organism can be performed through several methods including immunohistochemical staining (Adegboye et al., 1995), isolation of the agent (Stipkovits et al., 2001), and use of specific PCR probe on lung tissues (Hayman and Hirst, 2003). Besieds, detection of specific antibodies in the serum

can be performed (Le Grand et al., 2001). 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 (Ball and Nicholas, 2010; Maunsell et al., 2011).

Because the status of *M. bovis* infection in cattle in Adamawa State remains unknown, awareness of serological status is important in instituting control measures and preventing economic losses. Cattle are considered as one of the most important farm animals for meat production in Adamawa State. The objective of this study was to assess the current status of *M. bovis* antibodies in serum samples obtained from cattle in Adamawa State. The results would provide baseline data for the implementation of effective strategies for the control of *M. bovis* infection in cattle in Adamawa State.

MATERIALS AND METHODS

This study was conducted during August to November 2012 in three Local Government Areas (LGA; Ganye, Mubi and Yola) representing the three senatorial zones of Adamawa State, Nigeria.

Study Area: Adamawa State is located in the north eastern part of Nigeria, roughly where the River Benue enters Nigeria from the Cameroun Republic. It lies between longitude 11°E of the Greenwich Meridian and latitude 10°N of the Equator. The State shares boundaries with Taraba State in the south and west; Gombe State to its north-west; and Borno State to the north. Adamawa State has an international boundary with the Cameroun Republic up along its eastern side. The State has an estimated cattle population of 2.8 million (Anonymous, 1994), comprising of White Fulani (Bunaji), Red Bororo (Rahaji), Sokoto Gudali (Bokoloji) breeds and their crosses, which together constitutes 88% of cattle breeds in Nigeria (Ngere, 1983).

Sample collection: The cattle were selected using systematic random sampling method. Each animal was properly restrained and its age, sex and breed were recorded before sample collection. About 6 mL of blood sample was aseptically collected from the jugular vein of the animal, using a sterile 10 mL syringe and 18 G hypodermic needle. The blood was dispensed into sample bottles free from anticoagulant and centrifuged at 1500 g for 10 min. The serum portions were decanted into serum vials and stored at -20^{0}C until analyzed.

Serological examination: Serum samples were examined with BIO-X *M. bovis* antibody ELISA Kit (Bio-X Diagnostics, Jemelle-Belgium). The test was carried out according to the manufacturer's protocol. All the reagents were brought to a temperature of 21^{0}C +/-3^{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 (100 µL/well). The plates were incubated at 21^{0}C +/- 3^{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 +/-3^{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 21^{0}C +/- 3^{0}C. Then 50 µL of stop solution was added to each well. The optical density (OD) at 450 nm in the microwell were read using APPAR Plate Reader (UniEquip GmbH®, Martinsried, Munich, Germany).

The OD was calculated from the measured OD values, and negative and positive serum samples using the following formula-

$$Sample's\ Coeff. = \frac{\text{Sample OD} - \text{Negative serum OD}}{\text{Positive serum OD} - \text{Negative serum OD}} \times 100$$

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: Differences in *M. bovis* prevalence in cattle of different age groups, breed and sex were performed using statistical package for social science (SPSS) version 17.0 (SPSS Inc. Chicago, IL, USA). The difference was considered statistically significant when p value was <0.05.

RESULTS AND DISCUSSION

Serum samples (n=400) of cattle from three LGAs in Adamawa State were examined using BIO K260 ELISA kits [(BIO-X Diagnostics, Belgium) for *M. bovis* antibodies.

Out of the 400 serum samples, 19.5% (n=78/400) were found to be seropositive for *M. bovis* (**Table 1**). Out of the positive samples, 36 (27.7%), 28 (20.0%) and 14

(10.8%) were collected from Ganye LGA, Yola LGA and Mubi LGA, respectively. From the results, the highest seroprevalence was recorded in Ganye (27.7%), followed by Yola (20.0%) and Mubi (10.8%) LGAs, these showed a statistical significant difference (p=0.003; p<0.05).

Table 1: Seroprevalence of *M. bovis* in Cattle in three Local Government Areas in Adamawa State.

L. G. A	Examined number	Positive number (Prevalence %)
Yola	140	28 (20.0)
Mubi	130	14 (10.8)
Ganye	130	36 (27.7)
Total	400	78 (19.5)

X^2=11.893, df=2, p=0.003, p<0.05

The ages of the cattle ranged between <1-year and >5-year. Seroprevalence in different age groups varied from 2.9 to 25.8% (**Table 2**). The cattle aging <1-year-old had the highest seroprevalence (25.8%; n=8/31), followed by 4-year-old cattle (22.4%; n=22/98) and 1-year-old cattle (2.9%; n=1/35), although there were no statistically significant differences among the age groups (p=0.690; p>0.05).

Table 2: Seroprevalence of *M. bovis* in Cattle of different ages in Adamawa State.

Age in years	Examined number	Positive number (Prevalence %)
<1	31	8 (25.8)
1≥yr<2	35	1 (2.9)
2≥yr<3	100	21 (21.0)
3≥yr<4	93	19 (20.4)
4≥yr<5	98	22 (22.4)
≥5	43	7 (16.3)

X^2=0.159, df=1, p=0.690, p>0.05

Table 3: Seroprevalence of *M. bovis* in Cattle based on Sex in Adamawa State.

Sex	Examined number	Positive number (Prevalence %)
Male	115	20(17.4)
Female	285	58(20.4)

X^2=0.457, df=1, p=0.499, p>0.05

Sex-specific prevalence showed that the females had the highest seroprevalence (20.4%; n=58/285) as compared to males (17.4%; n=20/115) (**Table 3**), although the difference was not statistically significant (p=0.499; p>0.05).

Seroprevalence of *M. bovis* varied in different breeds of cattle, ranging from 11.9 to 21.8% (**Table 4**). The highest seroprevalence was found in White Fulani (21.8%; n=33/243), followed by Cross breeds (18.5%; n=10/54) and Adamawa Gudali (11.9%; n=5/42). No relationship was observed between the seroprevalence and breed (p=0.427; p>0.05).

Table 4: Seroprevalence of *M. bovis* in Cattle based on breed in Adamawa State.

Breed	Examined number	Positive number (Prevalence %)
White Fulani (Bunaji)	243	53 (21.8)
Sokoto Gudali (Bokoloji)	42	5 (11.9)
Red Bororo (Rahaji)	61	10 (16.4)
Cross breed	54	10 (18.5)

X^2=2.778, df=3, p=0.427, p>0.05

The findings from the seroprevalence indicated that *M. bovis* antibodies could be detected in 19.5% (n=78/400) cattle. The overall seroprevalence was lower than the earlier report of Tambuwal et al. (2011) who reported a prevalence of 66%. The cattle sampled in this study were extensively managed (free range), a practice which predisposes them to nutritional deficiencies and diseases leading to immuno-suppression and vulnerability to infection. The lower seroprevalence reported in this study might be attributed to inadequate storage of samples and distance in transporting the sample. This might explain the causes of low rate of *M. bovis* infection found in this study. This finding was in agreement with the previous report of Byrne et al. (2000) in Ireland and Ayling et al. (2004) in Great Britain who reported a prevalence of 13- 23% and 22%, respectively, eventhough cattle sampled by both authors were intensively managed. The seroprevalence obtained was higher than the report of Fu et al. (2011) and Zhao et al. (2012) in China who reported a prevalence of 7.69% and 5.95%, respectively. This might be attributed to samples from different regions and adequate management and biosecurity practices.

The seroprevalence of *M. bovis* varied in different age groups (2.9 to 25.8%), with cattle of <1-year-old having the highest seroprevalence (25.8%) followed by cattle of 4 years old (22.4%). This agreed with the earlier report of Zhao et al. (2012) who also reported the highest seroprevalence in dairy cattle of <1-year-old with no statistical difference between the age groups. The varied seroprevalence in different age groups suggested the possibility of horizontal transmission. The highest *M. bovis* seroprevalence in calves <1-year-old might be due to ingestion of infected milk which

could be an important means of *M. bovis* transmission. Cows might become intermittent shedders of *M. bovis* as it had been shown that as many as 40% of cows could shed <100 cfu/mL *M. bovis* in the milk, since contaminated milk could be the source of infection to young calves. 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.

In this study, female cattle had higher prevalence (20.4%) as compared to males (17.4%). This agreed with the earlier report of Tambuwal et al. (2011) 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/epidemiology 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.

Breed distribution showed White Fulani (21.8%), Red Bororo (16.4%), Adamawa Gudali (11.9%), and Cross breed (18.5%) even though there was no statistical significant difference. The higher prevalence was observed in White Fulani. However, *M. bovis* infection had not been reported to have breed specificity. Therefore, all cattle irrespective of the breed had equal chance of contracting the infection.

The presence of *M. bovis* infection as immunomodulator and/or co-factor might have an important role in the development of contagious bovine pleuropneumonia (CBPP) due to *M. mycoides* subspecies *mycoides* small colony (*Mmm* SC), being very much endemic in Nigeria. Certain similarity in pathogenesis of *M. bovis* infection (Stipkovits et al., 2001) with that of *Mmm* SC infection might contribute to inefficiency of vaccination against CBPP of cattle in the study area. Thus, the presence of *M. bovis* in these animals might account for the endemicity of CBPP in Nigeria especially that no vaccination campaigns were vigorously pursued in Nigeria.

The result obtained from this study might indicate the importance of *M. bovis* in cattle as a respiratory disease (Le Grand et al., 2001). Other workers had demonstrated the organism to be involved in arthritis, mastitis and reproductive diseases (Adegboye et al., 1996; Abbas, 1996; Le Grand et al., 2001) as frequently observed in cattle herds in Nigeria. Therefore, it was likely that some of the previously diagnosed cases of bovine respiratory, reproductive problems as well as other pathologic conditions of the udder (mastitis), otitis and arthritis might have been associated with *M. bovis* either singly or in combination with other infectious agents incriminated with the disease.

CONCLUSION

This study reports the occurrence of *Mycoplasma bovis* infection with a prevalence rate of 19.5%. Cattle of all ages, sexes and breeds are at risk with the organism. To the best of our knowledge, this is the first time serological survey of *M. bovis* conducted in this trans-boundary State in Nigeria. Therefore, baseline data for *M. bovis* provided in this study may help in taking an effective control strategy of the disease in Nigeria.

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REFERENCES

Abbas SI (1996). First reported isolation of Mycoplasma bovis from an outbreak of bovine mastitis in Sudan. Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux, 49: 108-109.

Adegboye DS, Hallbur PG, Cavanaugh DL, Werdin RR, Chase CC, Miskimins DW, Rosenbusch RF (1995). Immunohistochemical and pathological study of Mycoplasma bovis-associated lung abscesses in calves. Journal of Veterinary Diagnostic Investigation, 7: 333-337.

Adegboye DS, Hallbur PG, Nutsch RG, Kadlec RG, Rosengarten RF (1996). Mycoplasma bovis-

associated pneumonia and arthritis complicated with pyogranulomatous tenosynovitis in calves. Journal of American Veterinary Medical Association, 109: 647-649.

Anonymous (1994). Adamawa State livestock census. Adamawa State Ministry of Agriculture and Natural Resources.

Ayling RD, Bashiruddin SE, Nicholas RA (2004). Mycoplasma species and related organisms isolated from ruminants in Britain between 1990 and 2000. Veterinary Record, 155: 413-416.

Ball HJ, Nicholas RAJ (2010). Mycoplasma bovis-associated disease: here, there and everywhere. Veterinary Journal, 186: 280-281.

Byrne WJ, Ball HJ, Brice N, McCormack R, Baker SE (2000). Application of an indirect ELISA to milk samples to identify cows with Mycoplasma bovis mastitis. Veterinary Record, 146: 368-369.

Byrne WJ, Brennan P, McCormack R, Ball HJ (1999). Isolation of Mycoplasma bovis from the abomasal contents of an aborted bovine fetus. Veterinary Record, 144: 211-212.

Eaglesome MD, Garcia MM (1990). The effects of Mycoplasma bovis on fertilization processes in vitro with bull spermatozoa and zona-free hamster oocysts. Veterinary Microbiology, 21: 329-337.

Fu JH, Liu QY, Xu MJ, Shi DS, He XH, Pan Y, Guo RB, Gao Q, Yi SX, Si HS, Zhu XQ (2011). Seroprevalence of Mycoplasma bovis infection in dairy cows in subtropical southern China. African Journal of Biotechnology, 10: 11313-11316.

Hale HH, Helmbolldt CF, Plastridge WN, Stula EF (1962). Bovine mastitis cause by Mycoplasma species. Cornell Veterinarian, 52: 582-591.

Hayman B, Hirst R (2003). Development of semi-nested PCR for the detection of Mycoplasma bovis from bovine milk and mucosal samples. Veterinary Microbiology, 91: 91-100.

Le Grand D, Phillippe S, Calavalas D, Bezille P, Poumarat F (2001). Prevalence of Mycoplasma bovis infection in France. In: Poveda JB, Fermandez A, Frey J, Johansson, KE (Eds). Mycoplasma of ruminants: Pathogenicity, Diagnostics, Epidemiology and Molecular Genetics, Volume, 5. Brussels: European Commission; pp 106-109.

Maunsell FP, Woolums AR, Francoz D, Rosenbusch RF, Step DL, Wilson DJ, Janzen ED (2011). Mycoplasma bovis infection in cattle. Journal of Veterinary Internal Medicine, 25: 772-783.

Ngere LO (1983). The white Fulani (Bunaji) of Nigeria. Animal Genetics Resources in Africa: High potential and endangered livestock. Proceedings of the 2nd OAU Expert Committee Meeting on Animal Genetic Resources in Africa. November 24- 28, OAU Nairobi, Kenya, pp 67-77.

Nicholas RAJ, Baker SE, Ayling RD, Stipkovits L (2000). Mycoplasma infection in growing cattle. Cattle Practice, 8: 115-118.

Pfützner H and Sachse K (1996). Mycoplasma bovis as an agent of mastitis, pneumonia, arthritis and genital disorders in cattle. Review in Science and Technology, 15: 1477-1494.

Razin S, Yogev D, Naot Y (1998). Molecular biology and pathogenicity of mycoplasmas. Microbiology and Molecular Biology Review, 62: 1094-1156.

Rérat M, Albini S, Jaquier V, Hüssy D (2012). Bovine respiratory disease: Efficacy of different prophylactic treatments in veal calves and antimicrobial resistance of isolated Pasteurellaceae. Preventive Veterinary Medicine, 103: 265-273.

Stipkovits L, Rady R, Glavits R (1993). Mycoplasmal arthritis and meningitis in calves. Acta Veterinaria Hungarica, 41: 73-88.

Stipkovits L, Ripley RH, Varga J, Palfi V (2001). Use of Valnemulin in the control of Mycoplasma bovis infection under field conditions. Veterinary Record, 148: 399-402.

Tambuwal FM, Stipkovits L, Egwu GO, Junaidu AU, Abubakar MB, Turaki UA (2011). Enzyme-Linked Immunosorbent Assay (ELISA) based detection of antibodies to Mycoplasma bovis in Cattle naturally infected with haemoparasites in Institutional farms in Sokoto State, Nigeria. Current Research Journal of Biological Sciences, 3: 12-16.

Thomas A, Ball H, Dizier I (2002). Isolation of mycoplasma species from the lower respiratory tract of healthy cattle and cattle with respiratory disease in Belgium. Veterinary Record, 151: 472-476.

Vanden Bush TJ, Rosenbusch RF (2003). Characterization of the immune respons to Mycoplasma bovis lung infection. Veterinary Immunology and Immunopathology, 94: 23-33.

Zhao FR, Zhu XL, Xu MJ, Huang SY, Zhou DH, Xia HY, Song HQ, Zou FC (2012). First report of Mycoplasma bovis infection in dairy cattle in Guangzhou, subtropical southern China. African Journal of Microbiology Research, 6: 5668-5671.

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