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

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**Abstract:** This was a cross-sectional study involving cattle from four (4) institutional farms (Prison farm, Livestock Investigation and Breeding Centre (LIBC), Usmanu Danfodiyo University Teaching and Research (UDUTRF) and Kebbe Cattle Ranch (KCR) in Sokoto state, Nigeria. A total of 62 cattle comprising 49 females and 13 males were randomly selected and bled from a total population of 205. The cattle sampled were local breeds comprising Gudali, Rahaji, White-Fulani and their crosses. They were aged 1-10 years and are managed semi-intensively. The enzyme-linked immunosorbent assay was used for the detection *M. bovis* antibody. Of the 62 cattle screened, *M. bovis* antibody was detected in 41(66%). Also, 24 out of the 41 *M. bovis* positive cattle were found infected with haemoparasites. Similarly, 11 out of the 21 serologically negative cattle were infected with one or more haemoparasites. Seven (17%), 3 (7.3%) and 7 (17%) of the *M. bovis* positive cattle were infected with *Babesia bigemina*, *Anaplasma marginale*, and or *B. bigemina* and *A. marginale* respectively. In the overall, 27 of the 62 screened cattle were infected with one of blood parasites or a combination of both. However, there is no significant statistical relationship (p>0.05) between the number of cattle positive for *M. bovis* and the presence of haemoparasites among the examined cattle.

**Key words:** Antibodies, cattle, ELISA, haemoparasitism, institutional farms, *Mycoplasma bovis*, Nigeria

**INTRODUCTION**

The two most important mycoplasmal disease agents in cattle are *Mycoplasma mycoides subspecies mycoides* small colony type (*MmSC*) and *Mycoplasma bovis* (Bashiruddin et al., 2001; Thomson, 2005). Whilst the former is known to cause Contagious Bovine Pleuropneumonia (CBPP), the later is known to be involved in a variety of bovine diseases but neglected in most African countries (Nicholas and Ayling, 2003, Nicholas, 2004). *M. bovis* is widely spread and causes major economic losses in cattle in Europe and North America (Boothby et al., 1983; Rebhun et al., 1995). It is a proven cause of contagious mastitis in dairy cattle, pneumonia, septic arthritis (Pfutzner and Sachse, 1996) and otitis media (Walz et al., 1997). It is also reported to cause keratoconjunctivitis (Kirby and Nicholas, 1996), meningitis (Stipkovits et al., 1993) and abscesses (Kinde et al., 1993). *M. bovis* infection was also incriminated in reproductive disorders of cows (endometritis, salpingitis, reduction of conception rate, abortion) (Hirth et al., 1966 as well as seminal vesiculitis, epididymitis, orchitis and impaired spermatozoa motility in bulls (Lein, 1974; Jurmanova and Sterbova, 1977; Kissi et al., 1985). Furthermore, *M. bovis* is one of the mycoplasmas that colonizes bovine respiratory tract (Muesster et al., 1979).

Diagnosis of *M. bovis* infection can be performed by several methods including isolation of the agent (Sachse et al., 1993; Stipkovits et al., 2001), immunohistochemical staining (Adegoye et al., 1995; Knudtson et al., 1996) and the use of specific PCR probe in the lung samples (Ghadersohi et al., 1997; Hayman and Hirst, 2003) as well as detection of specific antibodies in the serum (Byrne et al., 2000; Le Grand et al., 2001). Serological detection of *M. bovis* antibody is a more reliable diagnostic method for evidence of previous or recent infections, as antibody level detected by ELISA remains high for many months (Nicholas, 2004). This diagnostic technique is also suitable for detection of
M. bovis antibody in milk (Byrne et al., 2000). Awareness of serological status of M. bovis in the herd/farm is important for preventing economic losses and instituting control measures (Pfutzner and Sachse, 1996). On the other hand, haemoparasites of cattle such as Babesia bigemina and A. marginale are known to cause serious anaemia and debilitations in this species (Jongejan et al., 1988). Concurrent infections of M. bovis with some haemoparasites can be more devastating in cattle than single infection. Cattle exposed to M. bovis naturally or experimentally are immunosuppressive (Thomas et al., 1991, Vanden Bush and Rosenbusch, 2004). This could precipitate other co-existing latent infection(s) in debilitated cattle with delitarious consequences. The present study therefore examines the presence of M. bovis infection in cattle concurrently infected with some haemoparasites.

**MATERIALS AND METHODS**

This study was conducted in 2008 (January to September) involving registered farms (only) in Sokoto metropolis and it’s environ of Sokoto state, Nigeria.

**Study area:** Sokoto state is located between the longitude 11° 30’ to 13° 30’ East and latitude 4° to 6° 40’ North. The state is semi-arid area of Nigeria with tropical climate where the temperature reaches 10 - 15°C from November to January, and 38-42°C from March to May with scanty rainfall that starts late in April and ends early in September with a mean range between 500-1300 mm (Abdullahi, 1985). The state is one of the leading producers of livestock in the country (RIM, 1992).

**Sampling:** A total of 62 cattle comprising 49 females and 13 males were randomly selected from four institutional farms with total population of 205. Samples collected were only from registered (Institutional) farms with update records of their stock in the state. Other farms short of these were not included in the studies. The number of samples collected from each farm was 17, 15, 15, and 15 from cattle population of 66, 51, 42 and 46, respectively. The animals sampled were local breeds that comprised Gudali, Ruhaji, White Fulani and their crosses. They were aged 1-10 years and managed semi-intensively, fed dry legume hay/pasture and supplemented with local concentrate (Wheat offals). Water was provided ad libitum (ad-lib) in the farms.

**Collection of sera:** Blood was collected in vacutainers tubes from jugular vein, allowed to clot and sera separated and stored at -20°C until analysed.

Sera were examined with CHECKIT Mycoplasma bovis Sero ELISA kit (Bommeli AG, Liebefeld-Bern, Switzerland (Stipkovits et al., 2001). The test was carried out according to the manufacturer's instruction. Serum samples as well as positive and negative controls were diluted 1:100 with CHECKIT diluting solution. 200 µL of the diluted sera were distributed into the wells of sensitized plates and incubated at room temperature for 90 min. Then the plates were washed three times with CHECKIT washing solution. 200 µL of the included peroxidase-labelled anti-ruminant IgG conjugate was added to the wells in a dilution of 1: 600 and incubated as earlier described. After washing off the excessive conjugate, the reaction was visualized with 200 µL of CHECKIT chromogen solution. The absorbance values were read at 405 nm with a Titrtek Multiscan MS plate reader. The OD (optical density) value was calculated from the measured OD values of the samples and the negative and positive sera as shown:

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\frac{\text{OD sample} - \text{OD negative serum}}{\text{OD positive serum} - \text{OD negative serum}} \times 100 = \text{OD%}
\]

Based on the protocol of the original kit, cattle having OD% values between 60 and 80% are classified as suspicious for M. bovis infection and a value above 80% is a clear sign of infection. However, since our aim was to detect any trace of M. bovis antibodies, OD% values above 60% were regarded as positive.

**Parasitological investigation:** Five ml of blood was collected from the same animal population and transferred into bijou bottles containing 1 mg/mL of Ethylene Diaminetetra Acetate (EDTA) and analysed for haemoparasites as described by FAO (1986).

**Statistics:** Data was analysed by descriptive and inferential statistics using Graph Pad InStat (2000) Version 3.05, 32bit for Windows 95/NT.

**RESULTS**

Details of results of serology and haemoparasites examination are shown in Table 1. Out of the 62 cattle examined, 41 (66%) were positive for M. bovis antibodies, while 21 (34%) cattle were found to be negative. More females (65.3%) showed antibodies to M. bovis than males (69.2%) although the difference was not statistically significant (p>0.05). Similarly, 24 (58.3%) of the 41 M. bovis positive cattle were infected with haemoparasites. Eleven (52.3%) out of 21 M. bovis negative cattle were infected with one or mixed haemoparasites.

Seven (17%), 3 (7.3%) and 7 (17%) of the 41 M. bovis positive cattle were infected with B. bigemina, A. marginale and B. bigemina and A. marginale infection,
respectively. In the same vein, 7 (33.3%), 1(4.7%) and 2(9.5%) of M. bovis negative cattle were infected with B. bigemina, A. marginale and B. bigemina and A. marginale respectively. Overall, 27 out of the 62 screened cattle were infected by one of haemoparasite or a combination of both. However, there is no significant relationship between the number of cattle positive for M. bovis and the presence of haemoparasites amongst the examined cattle (p>0.05).

**DISCUSSION**

M. bovis in spite of its importance is under estimated in Nigeria (Tambuwal et al., 2009). Consequently, there is paucity of information on the sero-prevalence of M. bovis in Nigerian cattle except for the restricted studies in the North eastern Nigeria (Egwu et al., 1999). Prior to this investigation, no serological examination has been performed on the prevalence of M. bovis in Sokoto state. Therefore, it is likely that some of the previously diagnosed cases of bovine respiratory problems may have been associated with M. bovis either singly or in combination with other infectious agents incriminated with the disease. Moreover, Mannheimia haemolytica has been reported to act synergistically with M. bovis, producing very severe exudative pneumonia in calves and aggravates the disease caused by the latter (Doherty et al., 1994). This sometimes creates confusion in the accurate aetiological diagnoses in the herds with persistent disease problem as common with the mycoplasma infections (Adegboye, 1986).

M. bovis antibody was detected in 66% of the cattle examined. Cattle used in these farms were managed semi-intensively; a practice which predisposes them to nutritional deficiencies and diseases (RIM, 1992). This probably explains the high rate of M. bovis infection recorded in this study. It also probably explains its causal role in endemic respiratory diseases and mastitis as indicated by the respective farms record. The figure 66% is a true position of serological prevalence rate since the disease (M. bovis) is not vaccinated against in the state and Nigeria as a whole. Thus, the recorded prevalence rate in the study provides a reliable sero-prevalence baseline data for M. bovis in the state.

Haemoparasitic diseases are endemic in Nigeria (Dipeolu, 1975; Ajayi, 1978; Osiyemi and Agbonlahor, 1980; Dogo, 2001; Ameh et al., 2004) with tremendous impact on livestock production. Immunosuppression which is a common characteristic among some of these diseases will exacerbate other intercurrent infections (Ajuwape et al., 2004). The concurrent detection of M. bovis antibody and haemoparasites in more than 50% serologically positive cattle may have been due to stress induced immunosuppression. It may as well be linked by system of husbandry practice of the farms which exposes them to health threat through contact with other potentially infected animals. However, there is no statistical correlation (p>0.05) between the occurrences of the two diseases.

**CONCLUSION**

Paradoxically, the presence of M. bovis infection singly or with other concurrent infections in these cattle may have some synergistic roles in precipitating Contagious Bovine Pleuropneumonia (CBPP) disease which is a constant threat to cattle industry in Nigeria (Egwu et al., 1996; Egwu, 2001; PACE, 2004). Importantly, the results obtained from this study may indicate the importance of M. bovis in cattle especially as a respiratory disease (Rodriguez et al., 1996; Le Grand et al., 2001), arthritis (Romvary et al., 1977; Adegboye et al., 1996), mastitis (Taoudi et al., 1988; Abbas, 1996) and reproductive disorders frequently observed in herds in Nigeria (Tambuwal, 2009). It as well suggests a neglect of simple management practice such as vaccination, deworming, dipping/spraying and proper housing in these farms.

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**REFERENCES**


