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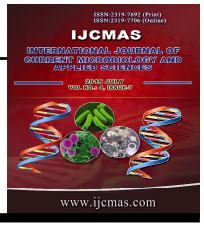

Original Research Article

Detection of Antibodies to *Mycoplasma mycoides* subspecies *mycoides* in Cattle using Competitive Enzyme-Linked Immunosorbent Assay

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A B S T R A C T

Contagious bovine pleuropneumonia caused by *Mycoplasma mycoides* subsp. *mycoides* has remained a threat to livestock producers and to the well-being of cattle population in Africa, where the disease is still prevalent and endemic. Competitive enzyme linked immunosorbent assay (cELISA) is now been recommended as an alternative to complement fixation test (CFT) and for the OIE official method that is applicable for the diagnosis of CBPP. Competitive ELISA is principled on the basis of monoclonal antibody named Mab 117/5. Detection of antibodies to *Mmm* was carried out according to the standard method using CBPP serum competition ELISA - Version P05410/02 from CIRAD / Institut POURQUIER. Out of the total of 160 sera tested, the Percentage Inhibition (PI) of sera at OD=450nm revealed 1(0.63%). CBPP positive sera for sex was 1.03% in a female animal, 1.32% in one aged >4-6 years and 2.27% for Borno red (Wadara) breed. The overall finding revealed that 0.63% of the detectable antibodies to *Mmm* was found in a single animal as compared to 159 (99.38%) which tested negative with cELISA. Consequently, our finding has detected the presence of an antibody to *Mmm* in a serum of cattle at slaughter in Maiduguri abattoir, Northeastern Nigeria.

Introduction

Mycoplasma mycoides subsp. *mycoides* (*Mmm*) is the causative agent of contagious bovine pleuropneumonia (CBPP), an insidious, infectious and highly contagious disease of cattle and water buffaloes (Thiaucourt *et al.,* 2013; Amanfu, 2009; Schubert *et al.,* 2011). It is a trans-boundary animal disease that is notifiable to the World Organization of Animal Health- *Office International des Epizooties* (OIE).

Contagious bovine pleuropneumonia has remained a threat to livestock producers and to the well-being of cattle population in Africa due to its prevalence, endemic nature, huge economic losses and social consequences (Egwu *et al.,* 1996; Aliyu *et al.,* 2000; Tambi *et al.,* 2006; Swai *et al.,* 2013). Clinically, CBPP is characterized by anorexia, abduction of the fore-limbs, arched back, distention of the head,

dyspnoea, polypnoea, moderate to high temperature, nasal discharge and coughing precipitated by forced exercise (Thiacourt *et al.*, 2003; OIE, 2014).

In Africa, CBPP has continued to spread given its epidemiology and other factors such as breakdown of veterinary services, lack of strict observance of formulated policies on disease control, political and economic limitations, as well as inaccessibility to diagnostic methods (Thiacourt *et al.,* 2003; Nwankpa *et al.,* 2004).

Serological techniques for the detection of antibodies to *Mmm* have been examined using enzyme linked immunosorbent assay. It was applied for the detection of antibodies to *Mmm* in sera of cattle at least 19 months after recovery from an infection and 23 months from the period of vaccination. Antibody was rarely detected when the same sera were examined by other established serological tests, emphasizing the sensitivity of ELISA (Onoviranand Taylor-Robinson, 1979). Competitive enzyme linked immunosorbent assay (cELISA), now been recommended as an alternative to complement fixation test (CFT) and for the OIE Official method that is applicable for the diagnosis of CBPP (Legoff and Thiacourt, 1998; OIE, 2014).

Competative ELISA is principled on the basis of monoclonal antibody named Mab 117/5 (Legoff and Thiacourt, 1998; Regalla and Levefre, 2000). Although the serological methods are saddled with limitations, they are still useful in terms of herd diagnosis (Schubert *et al.,* 2011). Presumptively, the set-back with respect to specificity can be conquered by using cELISA which is useful in detecting chronic cases of CBPP (Amanfu *et al.*, 1998; Legoff and Thiacourt, 1998).

In spite of the fact that cELISA is used for the detection of antibodies to *Mmm*, it does not, assess vaccination efficiency. This is because post-vaccinal antibodies fail to appear in circulation after 3 months. In natural infection, cELISA can be used for antibody detection even in areas where vaccination against CBPP has been carried out (Provost *et al.,* 1987; Legoff and Thiacourt, 1998; Regalla and Levefre, 2000). Additionally, detection of *Mmm* antibodies can be performed in sera samples collected from animals as opined by Brocchi *et al.* 1993).

It is well known that infection due to *Mmm* is confined to the respiratory system with sole involvement of the lungs and pleural membrane. Presence of low titre or not at all may be presented as a result of asymptomless carriers (lungers), which do not manifest visible detectable signs of disease (Niang *et al.*, 2006; Gon□alves *et al.*, 2008).

The study is aimed at detecting specific antibodies to *Mmm* in sera samples collected from cattle at slaughter in Maiduguri abattoir and to assess the suitability of using cELISA for targeted abattoir surveillance.

Materials and Methods

Study area

The study area is Maiduguri, the Borno State capital. It lies between Latitude 11^{o} 50′ 42″ N and Longitude 13^{o} 9′ 36″ E. It shares border with Konduga Local Government to the Northeast and Jere Local Government to the south and northwest. It occupies an area of 50,778 square kilometers. The climate of Maiduguri has a mean annual rainfall and temperature of about 650 mm and 32^{o}C respectively. Maiduguri metropolitan area has a population of 521, 492 people (NPCC,

2006). The vegetation is characterized by Sahel savannah.

Sampling and sample collection

A total of 160 blood samples were collected from Maiduguri township abattoir during daily slaughter using random sampling technique (Portney and Watkins, 2007). Five to ten milliliter of blood were collected immediately after slaughter into a Vacutainers® tubes free of anticoagulant and thereafter were labeled properly and kept in a Coleman box containing ice parks at 4^{o}C. The blood samples were then transported to theVeterinary Microbiology Laboratory, Faculty of Veterinary Medicine, University of Maiduguri were they were processed.

Sera collection from sampled blood

The blood samples in the Vacutainers® tubes were placed in a slanting position for 20-30 minutes at 4^{o}C, followed by centrifugation at 1500g for 10 minutes and quick removal of 1000 µl to 1,500µl of serum from individual tubes and then transferred into labeled cryo vials. The samples were kept under -20^{o}C prior to transportation to the National Veterinary Research Institute (NVRI), Vom in Jos, Plateau State, for processing.

Detection of Antibodies to *Mycoplasma mycoides* subsp. *mycoides* with cELISA Technique

Detection of antibodies to *Mmm* was carried out according to the method outlined by Legoff and Thiaucourt (1998) and OIE (2014) using CBPP serum competition ELISA - Version P05410/02 from CIRAD / Institut POURQUIER.

The microplates wells coated with *Mmm*SC lysate were set. It was followed by the dilution of the serum samples to be tested, and mixed with the specific monoclonal antibody (Mab 117/5) in a dilution plate or "pre-plate". This mixture was transferred into the *Mmm*SC- coated microplate. After washing, an anti-mouse IgG serum conjugated to horseradish peroxidase (HRP), which will bind to any Mab fixed to the wells, was added. Following another series of washes, the HRP substrate (TMB) was added, then the reaction was stopped.

The cut-off point was calculated using a monoclonal control (Cm), percentage (%) inhibition (PI) or Cm, 0% inhibition and conjugate control (Cc), 100% inhibition.

$$\frac{\text{OD Sample} - \text{OD negative}}{\text{OD Positive serum} - \text{OD negative serum}} \times 100 = \text{OD\%}$$

$$\frac{\text{OD Cm} - \text{OD Test}}{\text{OD Cm} - \text{OD Cc}} \times 100 = \text{PI (Percentage inhibition)}$$

The Optical density (OD) was read in an ELISA reader at 450 nm and the cut off points was calculated to validate the results. All sera with percentage Inhibition (PI) > 50% were considered as positive. Sera with PI between 40-50% were considered doubtful and those sera with PI less than 40% were negative (cELISA-Version P05410/02 from CIRAD / Institut POURQUIER).

Statistical analysis

Data was summarized and expressed as percentages. Descriptive statistics was applied using Microsoft Excel Version, 2010.

Results and Discussion

The percentage inhibition (OD= 450 nm) of sera of slaughtered cattle in Maiduguri, Nigeria is documented on Table 1. It ranges from less than 40 to ≥50. Only one serum was found to be within ≥50, regarded as positive. Four (2.50%) of the sera tested were doubtful, between 40-49 percentage of inhibition, while 155 (94.38%) were found to be devoid of *Mmm* antibodies.

Sex distribution of CBPP positive sera of slaughtered cattle in Maiduguri, Nigeria is presented on Table 2. Out of the total of 97 sera samples tested, 1(1.03%) was found to be positive in a single female animal, while 96(98.97%) appeared negative with cELISA. Table 3 gave the age distribution of CBPP positive sera of slaughtered cattle in Maiduguri. Only one animal was found to be positive appearing within the age group of >4-6 years. Thus the animals within the earlier stated age group had the percentage of detectable antibody at 1(1.32%) as opposed to other age groups that were 0(0.0%).

Breed distribution of CBPP positive sera of slaughtered cattle in Maiduguri, Nigeria is presented on Table 4. Both local and non-indigenous breeds were stated. Of the total number of 8 breeds from which sera were sampled, others inclusive, one breed (Borno Red or Wadara) was found to be indicative of positive serum, signifying the presence of *Mmm* antibody. It was additional examined and found in only 1(2.27%) Borno Red (Wadara) from among the 44 members of the same breed. On opposite side, the other breeds: Bunaji, Rahaji, Adamawa and Sokoto Gudalis, Kuri, Ambala and others constitute the remaining larger number (159) cattle, but had 0(0.0%) when tested with cELISA.

Figure 1 present the comparison of positive percentages of parameters: percentage inhibition (OD=450nm), sex (Female), age (>4-6 years) and breed (Borno Red or Wadara). All the parameters had 1 as a common value, but varies on percentage level, ranging from the highest, 2.27% for breed (Borno Red or Wadara), followed by 1.32 % for age (>4-6 years), then 1.03% for sex (Female) and the lowest percentage level is 0.63% for percentage inhibition (OD=450nm). However, the overall percentage of antibody detection remained as 0.63%.

Contagious bovine pleuropneumonia has been widely studied in different continents of the world (Thiacourt *et al.*, 2003; Tambi *et al.*, 2006). The presence of antibodies to Mmm SC in sera collected from cattle at slaughter in Maiduguri abattoir reveals that CBPP is present in this geographical location. This work agrees with the findings of Musa *et al.* (2011) in detection of antibodies to Mmm SC from the sera of cattle at slaughter using serological technique. Although the technique used by Musa *et al.* (2011) was complement fixation test, the idea in both conveyed the fact that CBPP is present in the area of study. Furthermore, cELISA is a recommended test by OIE (2014) and as an alternative to CFT an official method for the diagnosis of CBPP. The percentage of detection was 0.63% which was lower than the 16.8% reported by Olabode *et al.* (2013) from cattle market in four local government areas of Niger State, Nigeria. The same type of cELISA was used in both cases, although from different areas and different conditions during sampling. The findings of Adamu *et al.* (2006) reported 1.1%, using Agar gel Precipitation Test (AGPT) in Maiduguri, Borno State, which share international border with Cameroon, Chad and Niger.

Table.1 Percentage inhibition (OD=450 nm) of sera of slaughtered cattle in Maiduguri, Nigeria

Percentage inhibition (OD = 450nm)	Number (n)	Percentage (%)	Outcome
≥50	1	0.63	Positive
40-49	4	2.50	Doubtful
<40	155	96.88	Negative
Total	160	100.00	

Table.2 Sex distribution of CBPP positive sera of slaughtered cattle in Maiduguri, Nigeria

Number of assay N =160	Sex		Location
	Male	Female	Maiduguri
Number of positive (%)	0(0.00)	1(1.03)	1(0.63)
Number of negative (%)	63(100.00)	96(98.97)	159(99.38)
Total (%)	63(100.00)	97(60.63)	160 (100.00)

Table.3 Age distribution of CBPP positive sera of slaughtered cattle in Maiduguri, Nigeria

Age	Number sampled	Number positive	Percentage (%)
6 months-2years	0	-	
>2-4 years	26	-	0.00
>4-6 years	76	1	1.32
>6 years	58	-	0.00
Total	160	1	0.63

Table.4 Breed distribution of CBPP positive sera of slaughtered cattle in Maiduguri, Nigeria

Type of Breed	Number of samples	Positive value	Percentage (%) value
Bunaji (White Fulani)	36	-	0.00
Rahaji (Red Bororo)	34	-	0.00
Adamawa Gudali	24	-	0.00
Sokoto Gudali (Bokoloji)	1	-	0.00
Borno Red (Wadara)	44	1	2.27
Kuri	16	-	0.00
Ambala	2	-	0.00
Others	3	-	0.00
Total	160	1	0.63

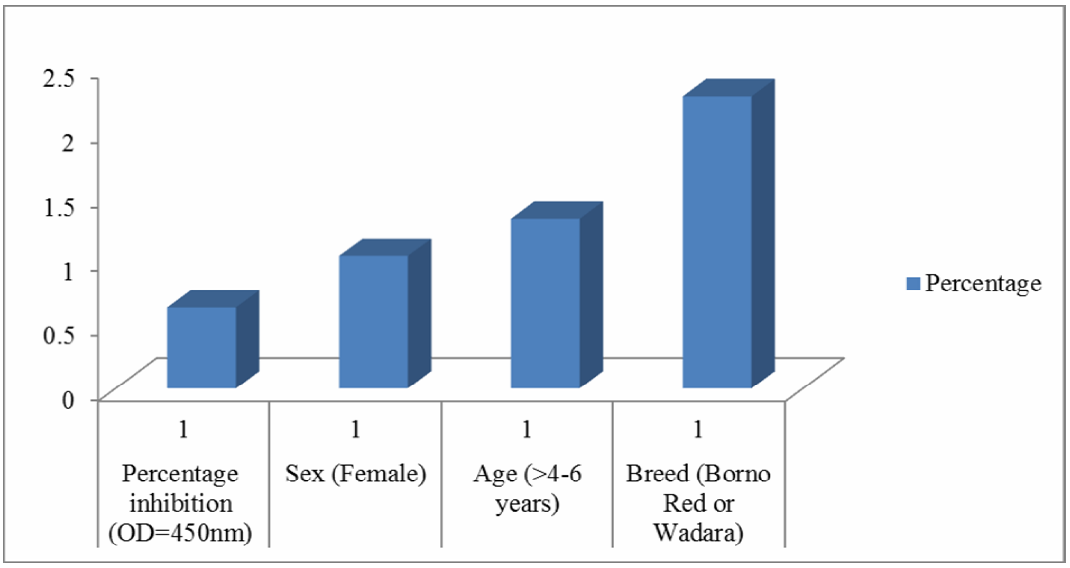

Figure.1 Comparison of positive percentages of parameters: percentage inhibition (OD=450nm), sex (Female), age (>4-6 years) and breed (Borno

Even though cELISA does not allow cross reaction with vaccinal antibodies, concomitant with other studies, it has been shown that cELISA was incapable of detecting all CBPP infected cattle. This may possibly be a reason in addition to the absence of infection, as to why only one positive serum was detected in the present study. Absence of *Mmm* antibodies were reported to be due to; low specific antibody titres at early and chronic cases. Also, an *invivo* variability in antigen expression by *Mmm*, may lead to lack of expression of relevant proteins at a given point in time as documented by Schubert *et al.* (2011).

Variability within the sex population was found to exist, where the female animal had a percentage detection of 0.63% as compared to the male animals with no percentage of detection. However, sex variation does not play an important role in the epidemiology of CBPP. The diversity observed may be due to inter-animal differences in cellular immune response as reported in previous studies (Jores *et al.*, 2008; Sacchini *et al.*, 2011). Additionally, efficiency of the individual animal or host defense plays an important role in determining the progress of *Mmm* infection and variation subsequently from one animal to another (Schubert *et al.*, 2011).

The present study has unveiled the presence of antibody to *Mmm* in a serum collected from cattle at slaughter in Maiduguri abattoir, which further confirm the presence of CBPP. Negative results, however, are inconclusive, since the technique applied is unable to detect all CBPP infection at different levels.

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