

Serological Evidence of Contagious Bovine Pleuro-Pneumonia antibodies in trade cattle (*Bos Indicus*) sold in Kwara state-Nigeria

*Olabode HOK¹, Mailafia S¹, Adah BMJ¹, Nafarnda WD², Ikpa LT³, Jambalang AR³, and Bello RH⁴

¹Department of Veterinary Microbiology, University of Abuja, Nigeria.

²Department of Veterinary Public Health, University of Abuja, Nigeria.

³Mycoplasma laboratory, National Veterinary Research Institute, Vom, Plateau State, Nigeria.

⁴Department of Pharmaceutics and Pharmaceutical Microbiology, University of Ilorin, Kwara State, Nigeria.

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A serological survey for the detection of antibodies to *Mycoplasma mycoides* subspecies *mycoides* small colonies (MmmSC) in trade cattle was conducted in four (4) cattle markets (Ilesha baruba, Ilorin, Bode Sadu, Ajashe Ipo) in four local government areas in Kwara State. This was to ascertain the prevalence of Contagious Bovine Pleuro-Pneumonia in trade cattle. Out of 169 cattle sera screened for mycoplasma antibodies 18 (10.65%) were positive to serum competitive ELISA (CIRAD) version: P05410/02 antibody detection kit. Ilesha baruba (Baruten LGA) recorded the highest prevalence of 9 (50%). Others indicated 6 (33.3%) in Ilorin (Ilorin East), 2 (11.1%) Bode Sadu (Moro LGA), and 1 (5.6%) in Ajashe (Irepodun LGA) respectively. Based on breed susceptibility, white Fulani breed of cattle showed 13 (72.2%), Sokoto Gudali 3 (16.7%), and 2 (11.1%) red Bororo. Based on sex susceptibility, 12 (66.7%) cows and 6 (33.3%) bulls were reactors. Age distribution, indicated young cattle 8 (44.4%) and Adult 10 (55.6%) were also affected. Although percentage proportional distribution of Contagious Bovine Pleuro-Pneumonia carriers showed white Fulani, adult cows had more association with the disease. The occurrence thus confirms susceptibility of all sex, ages and some breed of cattle. In conclusion, this finding provides preliminary information on the prevalence and endemicity of the disease amongst trade cattle in the study area. Hence, suggesting Contagious Bovine Pleuro-Pneumonia could be an emerging trans-animal border disease. It is recommended that continuous surveillance and quarantine of cattle within herds and inter-state as well as national border control should be practiced. Reinforcement of eradication strategy through compulsory annual vaccinations is also advocated.

Keywords: Prevalence, Contagious Bovine Pleuro-Pneumonia antibodies, Trade cattle, Serology, Kwara State.

INTRODUCTION

Contagious Bovine Pleuro-Pneumonia (CBPP) is an infectious and highly contagious [1] economically important disease of cattle which is widely distributed in the world [2]. It is caused by *Mycoplasma mycoides*

subspecies *mycoides* small colonies (MmmSC) [3]. The *Mycoplasma mycoides* cluster is made of six species, subspecies or group of strains that are pathogenic for ruminants that are closely related both genetically and phenotypically [4]. The two of particular importance include *M. mycoides* subsp. *mycoides* SC causing contagious bovine pleuropneumonia and *M. capricolum* subsp. *capripneumoniae* causing contagious caprine pleuropneumonia [5,6]. CBPP is transmitted by direct

*Corresponding Author's Email: olabodeok@yahoo.com;
Telephone: +2348023806741.

contact and inhalation of droplets from lungs especially within susceptible animals. Other factors that enhance the spread of the disease include movement of trade cattle, seasonal migration, and trans-human nomadism as the disease is characterized by severe exudative pleuropneumonia and pleurisy [7].

In Europe, the disease has low morbidity and mortality and appears to be less severe than in Africa with a high percentage of infected animals with chronic lesions [8], and often characterized by high morbidity and mortality losses to cattle. The financial implications of these losses are of great significance to cattle owners especially in Sub-Saharan Africa [9] with heavy estimated economic impacts of CBPP in Burkina Faso, Chad, Côte d'Ivoire, Ethiopia, Ghana, Guinea, Kenya, Mali, Mauritania, Niger, Tanzania and Uganda. In Nigeria, CBPP has been reported to be endemic in various parts of the country [10,3] with serious economic losses as well. Outbreaks of CBPP had occurred in large numbers in cattle confined to stalls or herds ranging freely [11]. These animals are usually the source of a variety of clinical samples including nasal swabs, lung and lymph node tissues, and pleural fluid that may be tested for the presence of MmmSC [12]. Confirmatory diagnosis of CBPP is achieved by the demonstration of typical pathology and/or the presence of MmmSC after postmortem examination and isolation of the causative agent from pneumonic lesions characteristic of CBPP when combined with other serological tests [13].

Thus the need for specific and sensitive detection tests, which are capable of screening large numbers of samples simultaneously, efficiently, and at a relatively low cost such as Immunoblotting test (IBT) [14], Complement Fixation Test (CFT) and especially cELISA which it does not allow cross-reaction with any of the other *Mycoplasma* species as well as identifying false-negative cattle, favors its diagnostic significance in areas where CBPP is enzootic [15] especially in Africa. Although, colorimetric polymerase chain reaction (PCR) test for the detection of *Mycoplasma mycoides* subsp. *mycoides* SC (MmmSC), has proved diagnostic at a cost effective and alternative to gel electrophoresis for the screening of moderately large sample numbers in surveillance programmes [16]. This is not easily accessed and affordable for use in Nigeria. In spite of various control efforts, outbreaks of this disease are often reported in cattle herds [11].

Therefore, serological assessment of exposure to the disease agent using cELISA kit is necessary to facilitate quick design and implementation of control policy amongst affected member states and neighboring countries is highly desirable. This premised the investigation in Kwara State which hosts the cattle international market in Ilesha baruba (Baruten LGA) that shares an international border with Benin Republic.

Hence, the aim of this study is to establish the prevalence of contagious bovine pleuro-pneumonia

amongst trade cattle using cELISA kit.

MATERIALS AND METHODS

Study Area

Kwara State is also referred to as the 'gateway' State because of its location between the Northern and Southern parts of Nigeria. This State is a first order administrative division located on the longitude N 8° 30' 0" and latitude E 5° 0' 0" 8.5 / 5 (Geo Name Id : 2332785) [17] with 16 Local Government Areas namely: Asa, Baruten, Edu, Ekiti, Ifelodun, Ilorin East, Ilorin West, Irepodun, Isin, Kaiama, Moro, Offa, Oke-Ero, Oyun, Ilorin south and Pategi. This state shares common boundaries with Niger and Kebbi States to the North, Oyo, Ondo and Edo States to the South, Benue, Plateau and Federal Capital Territory to the East [18].

Study Design

A cross-sectional study was designed using convenient sampling based on availability of cattle and the willingness of the cattle owners at various location covered by this study. The sampling was conducted forth nightly over a period of three months (August, September, and October, 2011). Four cattle markets in different local government areas was considered for this study based on their location and proximity to other States and international border (Benin republic, Niger, Oyo, Osun and Ekiti States). The markets include: Ilesha baruba, Bode Sadu, Ilorin and Ajasse in Baruten, Moro, Ilorin East, and Irepodun LGAs respectively for the collection of blood from trade cattle for separation into sera. Sex and breed was being noted as blood samples were collected for risk factor analysis. Ageing of the cattle was conducted as traditionally classified in Hausa/Fulani languages during participatory epidemiology with the cattle marketers as earlier conducted [19]. The ages were designated in Hausa as Marki (male)/ Marka (female)/Wege (Fulani) as age from 6 months-2years. Bijimi (male)/Karsana (female) in Hausa while Nghari (Fulani) as from 3-5years above. Age was grouped into two categories: 6 months - 2 years and 3 years and above.

Sample Collection

Five milliliters of blood was collected from each well restraint animal through the jugular vein by using 10 ml syringe and 18G needle into 5 ml sample bottles and kept at room temperature at an angle of 45°C for 6-8 hrs to allow for clotting of blood and sera separation. The sera was aspirated into new set of 5 ml plastic tubes, labeled, stored on ice pack and transported to the laboratory, National Veterinary Research Institute, Vom where it was

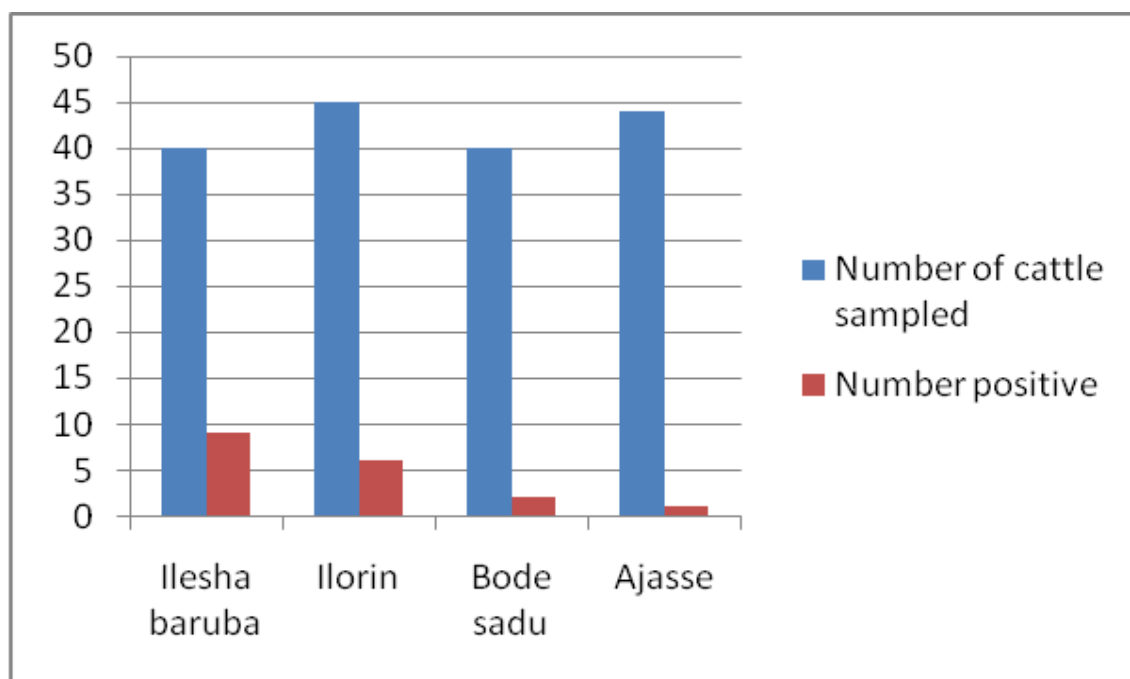


Figure 1. Distribution of CBPP carriers in four local government areas in Kwara state.

Table 1 Percentage Inhibition (PI) of sera samples from CBPP carrier trade cattle at Optical density (OD) of 450nm.

Percentage Inhibition	Frequency	CBPP carrier status
Less than 40%	117	Negative
40-49	34	Doubtful
50 and above	18	Positive
Total	169	

kept at -20°C until used.

Serological Procedures

Competition ELISA (cELISA) was conducted as recommended by CIRAD-UMR15 (FAO and OIE World reference centre for CBPP) based on a monoclonal anti-MmmSC antibody named Mab 177/5 as described [15]. Sera samples were mixed with specific monoclonal antibody (Mab 117-5) in a dilution plate and incubated with gentle agitation for 37°C for 1hr, then transferred into the MmmSc –coated microplate. After washing, anti mouse IgG serum conjugated-horse radish peroxidase (HRP) was added.

After series of washings the HRP substrate (TMB) was added forming a blue compound that turned yellow when the reaction was stopped. 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.

Statistical Analysis

The positive reactors as well as the associated risk factors were expressed as simple descriptive statistics such as percentage/frequency and chats.

RESULTS

The percentage inhibition (PI) of sera greater than 50% considered as positive was 18 (10.65%) and 34 sera was in the doubtful range of 40-49% while 117 sera were negative (Table 1). Ilesha baruba cattle market had the highest positive sera 9 (50.0%) and the least in Ajasse market (5.6%) in (Figure 1). Breed distribution of CBPP carriers showed white Fulani 13 (72.2%) was mostly

Table 2. Distribution of CBPP carriers according to breeds in Kwara state.

Breed	Number sampled	Number positive	Percentage (%)
White Fulani (Bunaji)	101	13	72.2
Red Bororo (Rahaji)	11	2	11.1
Sokoto Gudali (Bokoloji)	57	3	16.7
Total	169	18 (10.65)*	100

* Prevalence of CBPP carriers in cattle

Table 3. Distribution of CBPP carriers according to Age and Sex in Kwara state.

Age	Positive carriers	Percentage (%)	Sex	Positive carriers	Percentage (%)
Young (6months-2 years)	8	44.4	Male	6	33.3
Adult (3 - 5 years)	10	55.6	Female	12	66.7
Total	18 (10.65)*	100		18 (10.65)*	100

* Prevalence of CBPP carriers in cattle

affected and least occurrence in Red Bororo 2 (11.1%) as represented in Table 2, while the age distribution indicated highest occurrence in adults on comparison with the young cattle (Table 3). Sex distribution amongst reactors showed more female than males (Table 3). The percentage proportional distribution of CBPP carriers showed white Fulani, adult cows had more association with the disease.

DISCUSSION

The CBPP positive cattle seen in these markets during this survey could be said to be in chronic stages as the number of positives is known to decrease after recovery from natural disease course [2]. Thus they are MmmSc carriers showing no clinical signs but have evidence of detectable antibodies giving an overall prevalence of 10.65% using ELISA kit (CIRAD). This finding is higher than 1.1% reported [11] using Agar gel Precipitation Test (AGPT) in Borno State a Nigerian border State neighboring the French speaking countries of Chad, Cameroun and Niger. This observation could be attributed to the fact that ELISA is more sensitive than AGPT used in this previous studies, also this cELISA technique does not allow cross reaction with vaccination induced antibodies thus detecting exposures to only natural infections even in areas where vaccination is used. The study also observed 34 sera (20.11%) with percentage inhibition between 40-50% which was classified as doubtful and this could be associated with slight color development due to the quality of washing and of the water used, pipetting precision, and or temperature of the reaction as suggested by the manufacturers (Institut Pourquier- CIRAD).

The distribution of reactors according to the markets (Figure 1) indicates the highest number of carriers 9 (50.0%) in Ilesha baruba which is a border town and usually the first portal of entry for these cattle that have crossed 2-3 ECOWAS countries contaminated trade cattle routes along the West African coast into Nigeria through Benin Republic (Paraku) before getting to its destination in Ilesha baruba cattle international market. This is evidenced as indicated by previous reported prevalence rates of CBPP in Burkina Faso 2.9% and 5.4% for Mauritania [20], as well as 10.5% for Mali [21] and Benin. The Ilorin market also indicated 6 (33.3%) of CBPP carriers which ranked second highest, probably due to the high demand for trade cattle for the robust human consumption and marketing activities as Ilorin represents the capital and administrative centre of the State. The least occurrence in Bode Sadu and Ajassee markets could be attributed to their distant location to Ilesha (entry point of cattle). It may also be associated with low prevalence rate of CBPP amongst trade cattle brought from various parts of the State for marketing.

Breed distribution showed that white Fulani had 13 (72.2%), Red Bororo (11.1%), and Sokoto Gudali (16.7%) suggesting more of the white Fulani were sampled thus the reason for the observed breed predisposition, as CBPP may not have breed specificity as earlier documented [22]. Thus a Case-control study is suggested to further elucidate the breed association.

Adult CBPP cattle carriers' showed higher numerical value over the young (6 months-2 yrs) this contrast to previous studies [23] which states that animals less than three years are less resistant to experimentally challenged cattle but connotes other report [22]. This could be associated to the fact that chronic stages of the disease are usually seen in adult cattle as the age

progresses.

Based on sex distribution, cows had the highest reactors in comparison with the bulls, which contrast previous findings [22]. This could be attributed to lowered immunity following continuous reproductive stress as buttressed by the age frequency of the disease in adult cows.

CONCLUSION

In conclusion, this study provides preliminary information on the prevalence and endemicity as well as risk factors associated with CBPP as CBPP carriers was found in all sex, ages and some breeds of trade cattle in Kwara State of Nigeria as well as those imported by cattle trading partners along the West African coast. This may suggest CBPP is could be an emerging transboundary cattle disease.

It is therefore, recommended that continuous surveillance and quarantine of cattle within herds and inter-state and national borders should be encouraged. Re-enforcement of eradication strategy through compulsory annual vaccinations using more cost-effective vaccines and more effective control as earlier advocated is thus suggested [24].

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Corresponding Author:



Dr. Olabode, HOK

Qualification: DVM (ABU Zaria), MSc Medical Microbiology (UNIBEN), PhD In View (ABU Zaria)

Lecturer: Department of Veterinary Microbiology, University of Abuja, Nigeria.