Seroprevalence of contagious bovine pleuropneumonia in three selected south-eastern states of Nigeria

KC Anyika[1]*, SO Okaiyeto[2], AK Sackey[3], CN Kwanashie[4] & LT Ikpa[5]

1. *Livestock Investigation Division, National Veterinary Research Institute, Vom, Nigeria*
2. *Veterinary Teaching Hospital, Ahmadu Bello University, Zaria, Nigeria*
3. *Department of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria*
4. *Department of Veterinary Microbiology, Ahmadu Bello University, Zaria, Nigeria*
5. *Mycoplasma Laboratory, National Veterinary Research Institute, Vom, Nigeria*

*Correspondence: Tel.: + 2347030564505; E-mail: chinetoanyika@gmail.com

Abstract

Contagious bovine pleuropneumonia is a trans-boundary animal disease caused by *Mycoplasma mycoides* subsp. *mycoides*. This study was designed to determine the seroprevalence of contagious bovine pleuropneumonia (CBPP) in three selected south eastern states (Anambra, Enugu and Imo) of Nigeria using competitive enzyme-linked immunosorbent assay (c-ELISA). A total of 438 bovine sera samples were collected randomly for four months (December 2019 to March 2020) and screened for antibodies to *Mycoplasma mycoides* subsp. *mycoides* (Mmm) using IDEXX CBPP antibody ELISA kit (CIRAD, France). Results showed an overall prevalence of 59.4% for the three states screened. Antibodies to Mmm were detected in all the three states. Enugu state had the highest prevalence (64.3%) followed by Imo state (63%) and Anambra state (50.7%). Female animals had higher prevalence of CBPP than male. However, it was not statistically significant (P> 0.05). This study confirms the presence of CBPP in south eastern Nigeria, and could be used as a base line data for future studies in this region. It is recommended that active surveillance and vaccination protocol should be undertaken in the region for the control and prevention of this disease.

Introduction

Contagious Bovine Pleuropneumonia (CBPP) is a highly contagious disease of cattle caused by *Mycoplasma mycoides* subsp. *mycoides* (Mmm) (Egwu *et al.*, 1996; Campbell, 2015). Based on its transmissibility and economic impacts, CBPP is recognized as a priority trans-boundary disease (TAD) and has thus been categorized as the only bacterial disease in the OIE list A diseases (Litamoi *et al.*, 2005). CBPP is characterized by a relatively long incubation period and highly inconsistent clinical course (Litamoi *et al.*, 2005). Clinical signs of the disease include anorexia, fever, dyspnea, cough and nasal discharges (Campbell, 2015). In adult, most infections are limited to the respiratory tract, while

in calves the disease is usually characterized by arthritis (Campbell, 2015). The disease is considered to be the most economically important cattle disease in Africa causing greater losses in cattle than any other disease (OIE, 2003). The annual economic loss due to CBPP in the northern states of Nigeria was then estimated at NGN 498 million (Fayomi & Aliyu, 1997). A five-year retrospective study at Mubi, Adamawa state by Halle *et al.* (1998) reported that 238 infected cattle lungs were condemned due to CBPP out of 43, 810 cattle slaughtered (0.54%) and the worth of organs condemned was estimated at NGN 28,580.

The establishment of the true prevalence rates of CBPP in infected countries is an important prerequisite to mounting a successful control programme of the disease (FAO, 2004). Serodiagnosis plays an important role in the survey and control programs of CBPP (Le Goff & Thiaucourt, 1998). There are two OIE approved serological tests for the diagnosis of CBPP, the complement fixation test (CFT) and the competitive ELISA.

CBPP is an economically important disease condition; this emphasizes the need for an accurate data on the prevalence of the infection, if prompt and effective control measures are to be instituted. Presently, in Nigeria, the extent and pattern of CBPP in south east Nigeria is largely unknown, as many studies have centered within the northern region of the country (Nwanta & Umoh, 1992; Nwankpa, 2008; Okaiyeto *et al.*, 2011; Jasini *et al.*, 2016). The south east region of Nigeria is estimated to have a cattle population of 4.5 million, there is an apparent successful settlement of pastoralists within the southern region of Nigeria in the past two decades

with thousands of zebu cattle (Blench, 1994). Furthermore, data generated from this research will serve as baseline data for future studies within these States.

Materials and Methods

Study area
This study was conducted in three selected south eastern states of Nigeria (Anambra, Enugu and Imo states). South east Nigeria is one of the six geopolitical zones in the country. The region consists of Anambra, Enugu, Imo, Ebonyi and Abia states (Figure 1). Anambra state lies between latitude 5° 32' and 6° 45' N and longitude 6° 43' and 7° 22' E; Enugu state lies between latitude 5° 27'and 6° 33' N and longitude 6° 28' and 7° 32' E and Imo state is located between latitude 4° 45' and 7° and 15' N and longitude 6° 50' and 7°25' E. The south eastern region of Nigeria has an estimated cattle population of 4.5million from the total 16.3 million estimated cattle population in Nigeria (Ikhatua, 2011).

Sample collection
Blood samples were collected randomly from cattle in Fulani sedentary settlements within the three selected south eastern states. Furthermore, blood samples were only collected from cattle that were not vaccinated against CBPP in the past 6 months and apparently sick animals. Sex of animals sampled was also documented.

Blood samples (5ml) were aseptically collected through the jugular vein of each animal using sterile 18G needle and 10ml syringe. The blood samples were allowed to stand in a slanting position for about 5 hours to allow for separation of the serum from the cellular components of the blood. The serum was then harvested into labeled sample bottles and packed on ice for onward transport to the Mycoplasma laboratory of the National Veterinary Research Institute Vom for serological examination.

Serological analysis
The serological analysis was performed using IDEXX CBPP antibody Test Kit (P05410/10, CIRAD, France) according to manufacturer's instruction. Briefly, the serum test samples were pre-mixed with a specific monoclonal
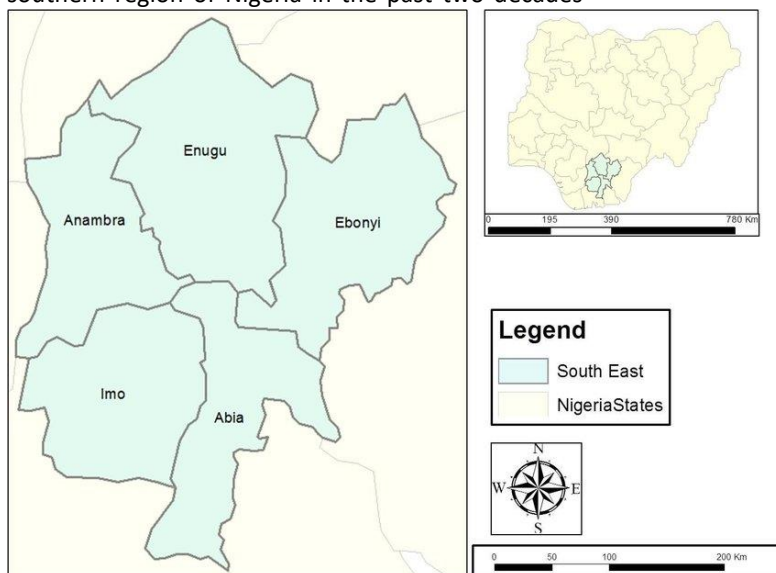

Figure 1: Map of Nigeria showing the south-eastern states

antibody Mab 117/5 in a separate plate called the 'pre-plate' and the content of the pre-plate was then transferred into the pre- coated micro-plate lysed with *Mycoplasma mycoides* subsp. *mycoides* antigen and incubated for 1 hour at 37℃ under gentle agitation. The unbound materials were washed twice with phosphate-buffered saline (300µl) and an anti-mouse conjugate concentrate (100µl) was then added to each well. This was again incubated for 30minutes at 37℃ under gentle agitation, avoiding the desiccation of the plates. After incubation, the unbound materials were again washed three times with phosphate-buffered saline (300µl). Subsequently, 100µl of an enzyme substrate (TMB) was then added to each well and incubated for 20 minutes at 37℃. Finally, the reaction was stopped by adding 100µl of a stop solution (sulphuric acid) into each well. The optical densities of the reactions were then read at 450nm using Multiskan EX (Lab-systems). Samples were considered negative if the percentage inhibition was below 50% and positive above 50%.

Data analysis

The data obtained are presented in tables. Chi-square test using SPSS Version 20 for windows was used to determine any significant difference in the prevalence rate of the disease based on sex of cattle. Values of P ≤0.05 were considered significant at 95% confidence Interval. Prevalence rate was calculated using the formula:

$$Prevalence = \frac{Positive\ samples}{Total\ samples\ analyzed} \times 100$$

Results

The result for the sero-prevalence of CBPP in the three south east states using competitive ELISA is presented in Table 1. Out of the total of 438 sera samples collected, 260 were positive for CBPP, giving an overall prevalence of 59.4% (Table 1). Out of the 146 sera samples collected from each state, 74 (50.7%) were positive for CBPP antibodies in Anambra State, while 92 (63%) were positive in Imo state and 94(64.3%) were positive for CBPP antibodies in Enugu state (Table 1).

Based on sex, 19 (19%) bulls tested for CBPP antibody in the three selected south east states were positive for CBPP, while 241 (71%) cows out of 338 sampled were also positive (Table 2) however, it was not statistically significant (P >0.05).

Discussion

In this study, the overall seroprevalence of CBPP using c-ELISA in the study area was 59.4%. This high prevalence recorded in the study area may be attributed to several factors such as; breakdown in veterinary services within this region. This has adversely affected the quality of veterinary services within this region and as such most of the herdsmen/ cattle owners resort to self-help in the management of the disease. This is similar to study by Ankeli *et al.* (2017). Similarly, indiscriminate use of antibiotics by herdsmen/pastoralists for the treatment of CBPP as reported by Egwu *et al.* (2012) could also account for the high prevalence recorded in the study.

Indiscriminate use of antibiotics could lead to the establishment of chronic carriers of the disease, thereby facilitating the spread of the disease to susceptible animals within the herd. In Nigeria, a live with the disease attitude has prevailed over the past five years; farmers rarely report cases of CBPP but resort to treatment with antibiotics like any other bacteria disease (Chima *et al.*, 2001). Furthermore, lack of access to quality and well preserved CBPP vaccines by herdsmen in the study area could also

Table 1: Sero-prevalence of CBPP in the three selected south east states, Nigeria

States	Number sampled	Number Positive by c-ELISA (%)
Anambra	146	74 (50.7)
Enugu	146	94 (64.3)
Imo	146	92 (63)
Total	438	260 (59.4)

Table 2: Sero-prevalence of CBPP based on sex of cattle in the three selected south east states, Nigeria

State	Sex				P-value
	Male		Female		
	Sampled	Positive (%)	Sampled	Positive (%)	
Anambra	32	6(18.7)	114	68(59.6)	
Enugu	40	6(15)	106	88(83)	
Imo	28	7(25)	118	85(72)	
Total	100	19(19)	338	241(71)	0.063

account for the high prevalence of CBPP recorded in this study, as most of them purchase CBPP vaccines from the open markets rather than NVRI outstations within the region. This is similar to report by Billy (2014), who also reported high patronage of CBPP vaccine from open market by pastoralists. Similarly, improper vaccination practices undertaken by the herdsmen could also attribute to the high seroprevalence of CBPP recorded in this study, as some of the herdsmen engage in the act of mixing different vaccines types within the same bottle before vaccinating their cattle (personal experience). This practice could affect the potency of the vaccine due to under dosage of the vaccine and wrong route of vaccination. The high seroprevalence of CBPP recorded in the study area, could also be because apparently healthy animals not showing clinical signs of the disease were sampled. Consequently, some infected animals within the incubatory stage of the disease and also those recovered from CBPP (lungers) would have been sampled. The high prevalence of CBPP recorded in this region may indicate the endemicity of the disease in south east Nigeria. This is in agreement with the annual report by OIE (2009) which stated that CBPP is gradually spreading across Nigerian states from the northern Region to other regions because of constant migration of cattle herders. It is noteworthy to state that this is the first time such study was undertaken within the south eastern region of Nigeria using this method. Most of the cases recorded in this study are likely due to natural infection as vaccination history was taken before sample collection. Moreso, this technique is capable of detecting field infections even in areas where vaccination is practiced as antibodies produced when T1/44 vaccine is administered to animals do not persist for more than 3 months (Le Goff & Thiaucourt, 1998).

The findings in this study are similar to works done by Olorunshola *et al.* (2020), who recorded a high prevalence rate of 56.2% in the Middle-Belt region of Nigeria. Nwankpa (2008), also reported high prevalence of CBPP in some northern states of Nigeria such as; Bauchi state with 32.4%, Gombe state 50.5% and Taraba State with 40% CBPP seroprevalence rate. Similarly, Danbirni *et al.* (2010) reported a high CBPP seroprevalence of 47% in Kaduna State. This may be attributed to the endemicity of CBPP within the northern region of country where most of the cattle population is located (Egwu *et al.*, 1996; Nwankpa, 2008). However, this finding is in contrast with studies by Olabode *et al.* (2013) who reported a lower CBPP prevalence of 10.65% in Kwara State and Jasini *et al.*

(2015) who also reported CBPP prevalence rate of 0.63% in Borno state. This lower prevalence recorded by these authors may be attributed to the absence of infection at the point of sampling and the smaller number of sera samples collected for screening of Mmm antibodies.

There was higher prevalence of CBPP observed in cows compared to bulls in this study. However, this was not significant. This finding is in accordance with studies by Nwankpa (2008) and Billy (2014) who both reported higher prevalence in cows than bulls. However, this is in contrast to works by Ankeli *et al.* (2017) who reported higher prevalence of CBPP in bulls than cows. This high prevalence could be because fewer bulls are usually kept in the herd than the cows. Most of the cattle owners fatten and sell most of the bulls in the herd so as to prevent competition during breeding. It could also be due to the fact the female animals stay longer within the herd, thereby increasing the chances of repeated exposure to the disease.

This study confirms the presence of contagious bovine pleuropneumonia in south east Nigeria and also indicates its endemicity in the region. Data from this study can be used for future studies and also in designing control measures for the control of the disease in this region.

It is therefore recommended that active disease surveillance and routine vaccination campaigns should be carried out within this region for the control and prevention of CBPP.

Conflicts of Interest
The authors declare no conflict of interest.

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