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Nigeria has the highest cattle population of about 16.3 million in West Africa (Ikhatua, 2011), but is constantly threatened with CBPP (Ajuwape et al., 2004), and "a live with the disease" attitude has always prevailed in the last few years among pastoralist and farmers who hardly report cases of CBPP but rather resort to treatment with antibiotics like any other bacterial disease (Chima et al., 1999). Animal Diseases Information System (NADIS) classified Nigeria as an endangered zone based on her CBPP status. CBPP is considered to be a disease of economic significance due to its ability to compromise food security through loss of protein, increased production costs due to costs of disease control and inhibition of sustained investment in livestock production (Ikede and Taiwo 1988).

Latex agglutination test is a rapid agglutination test that is easier to interpret and can be performed in the field when compared to Slide Agglutination Test (SAT), which lack sensitivity, and detects only animals in the early (acute phase) stage of the disease and can only be used on herd basis (Ayling et al., 1999b; Turner and Etheridge, 1963). Although Latex agglutination is not capable of differentiating between antibodies from vaccinated animals and those of natural infection, it however provides, a fast and easy to perform diagnostic technique in the field, and therefore, it is good for early detection of cattle with CBPP. This test uses a "specific" polysaccharide antigen extracted from the *Mmm*SC capsule, which is then bound to latex beads. "specific" means found to be specific by empirical means, testing it against different antigens until the one was found that did not cross-react or gave false-positive results (John et al., 2003). This test has been evaluated using CBPP negative sera from England and CBPP positive sera from Africa, Portugal and Italy (Ayling et al., 1999a). Sensitivity was comparable to the internationally recognized complement fixation test, but is far simpler and more rapid to perform. This test may have great potential in parts of Africa where there are great distances between the outbreaks, usually in nomadic herds, and diagnostic laboratories enabling control measures to be implemented rapidly (March et al., 2003). This study was aimed at detecting the presence of CBPP antibodies in the field using BoviLAT Latex Agglutination Test as an alternative to other tests in the three senatorial districts of Kaduna State, Nigeria.

MATERIALS AND METHODS

Study Area

The research was carried out in 3 senatorial districts of Kaduna State, which is located in the North-West geo-political zone of Nigeria. Kaduna state lies between Longitude 30'' and 0900 East of the Greenwich Meridian and has a Latitude of 0910 and 11°30'' North of the Equator (KADP) (Figure1). Kaduna State has 23 Local Government Areas (LGAs) and a human population of 6.07 million (NPC, 2006). The state is an agrarian state and also has potentials for livestock industry, with about 70-75% of the population engaging in farming activities (NLR, 1992).

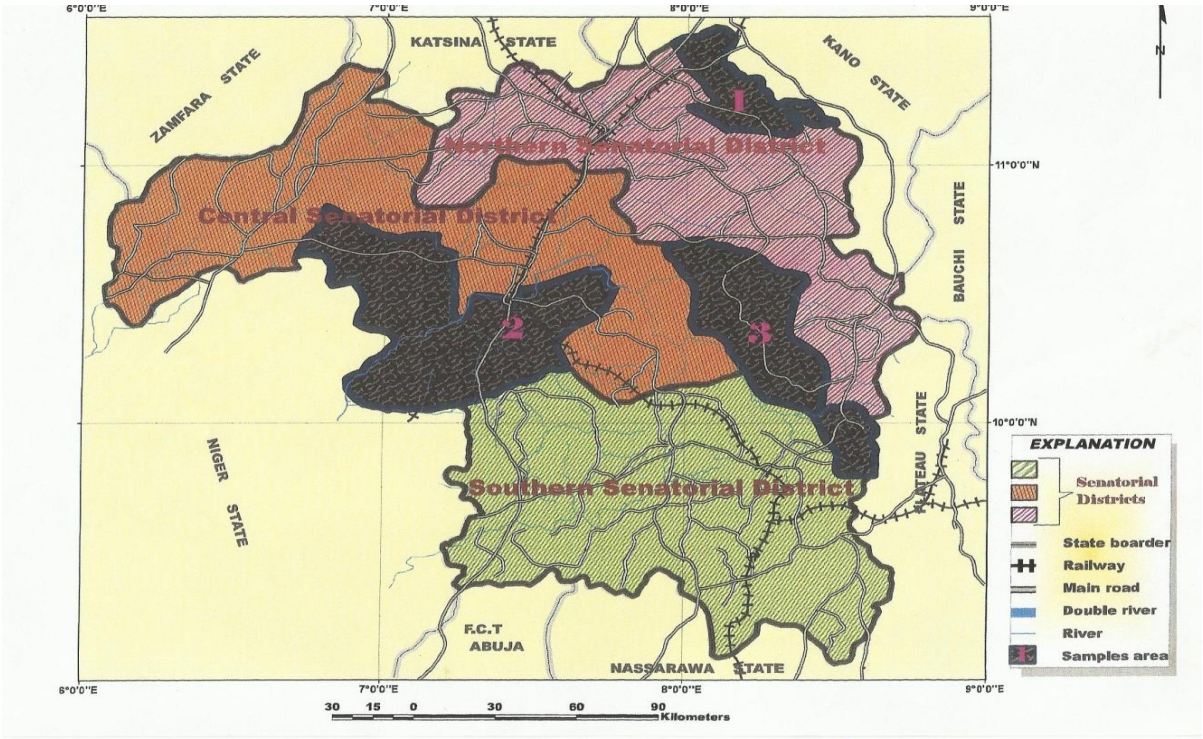

Figure 1. Map of Kaduna State showing the three Senatorial Districts with Sampled areas (**1:** Ikara Local Government Area -representing the Northern Senatorial District, **2**: Chikun Local Government Area -representing the Central Senatorial District and **3**: Kauru Local Government Area -representing the Southern Senatorial District).
Source: Department of Catography; National Geoscience, Kaduna Zonal Office, Barnawa, Kaduna, Kaduna State, Nigeria (Production map from KADP, 2012).

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Sample Size

Sample size was determined as described by the method of Mugo (2011).

$$n = \frac{Z^2 Pq}{d^2}$$

Where:
n= sample size
q = 1-p
P = expected prevalence of 47% (Danbirni, 2010)
d = desired absolute precision = 5% or 0.05
Z = appropriate value for the standard normal deviation for the desired confidence = 1.96
Therefore,

$$n = \frac{1.96^2 \times 0.47(1-0.47)}{0.05^2} = 382.7$$

This sample size shows a minimum of 382.7 cattle heads that can be investigated. However, to increase precision, 900 heads of cattle were bled and sera obtained.

Collection and processing of blood samples

One local government area (LGA) in each of the senatorial district (Northern, Central and Southern) was randomly selected to represent the district. The LGAs are; Ikara for (Northern), Chikun (Central) and Kauru for Southern senatorial districts. Each of the 3 selected LGAs has 11 political wards and 3 political wards were randomly selected from each of the LGAs. A cattle population of a minimum of 20 heads was considered as a herd in this study (Tambuwal et al., 2011), and 10 herds were randomly identified in each of the selected ward. Ten heads of cattle from 10 herds were randomly sampled and properly restrained in each of the 3 selected wards in a LGA (making a total of 300 heads of cattle in each LGA). Ten millilitres of blood was aseptically collected from the jugular vein of each cattle using an 18G needle mounted on a 20ml syringe and their respective ages, sexes and breeds labelled and recorded on each sample bottle. The blood samples collected were kept in a slanting position at ambient temperature for about 6 hours and the sera separated from the cellular component of the blood were collected and transferred into labelled plain sterile sample bottles and stored at 4^{O}C until use.

Sera Analysis (BoviLAT Test Procedures)

The test was carried out according to the manufacturer's procedure for BoviLAT test Kit (BoviLAT PA6223). To perform the test, the sera was brought out from the cold box and allowed to attained ambient temperature. Twenty micro litre of serum was dropped onto a black reaction card using plastic dropper. This was carefully dispensed to avoid air bubbles. The BoviLAT Latex reagent was well shaken and a drop of the reagent was added close to the spot where the serum was dropped. The BoviLAT Latex reagent and the serum were mixed together using a wooden stick (sterile tooth pick) and the mixture spread out inside the reaction cell. The reaction card was rocked from left to right for three minutes and any agglutination or otherwise was recorded. A maximum of six reactions were done at a time.

Reaction Process

The latex cards are coated with Capsular Polysaccharide (CPS) purified from *Mmm*SC cells. Antibodies recognising the CPS that banded and cross-linked the latex particles causing agglutination at different degrees in the positive cases are as follow; (i) Positive (+++), Strong clumping of latex beads, and agglutination beginning within one minute, (ii) Positive (++), Clear agglutination of latex beads, agglutination beginning between one and two minutes and (iii) Positive (+), Fine agglutination of latex beads, agglutination formed between two and three minutes. The sera from animals that might not be suffering from CBPP will not show any reaction, and therefore, it is recorded as negative (-), No agglutination formed within three minutes (Figure 2 A, B, C, D, E).

Data Analysis

The data obtained were presented in tables using Microsoft Excel 2007 and analyzed with Graphpad Prism version 4.0 for Windows and Chi-square (X^2). Values of $P \leq 0.05$ were considered significant. The prevalence of CBPP was determined using the formula:

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

Ethical approval

This work was jointly approved by the Ahmadu Bello University Zaria ethics committee on the use of animals for research purposes and the department of livestock resources, Kaduna State ministry of Agriculture, Nigeria.

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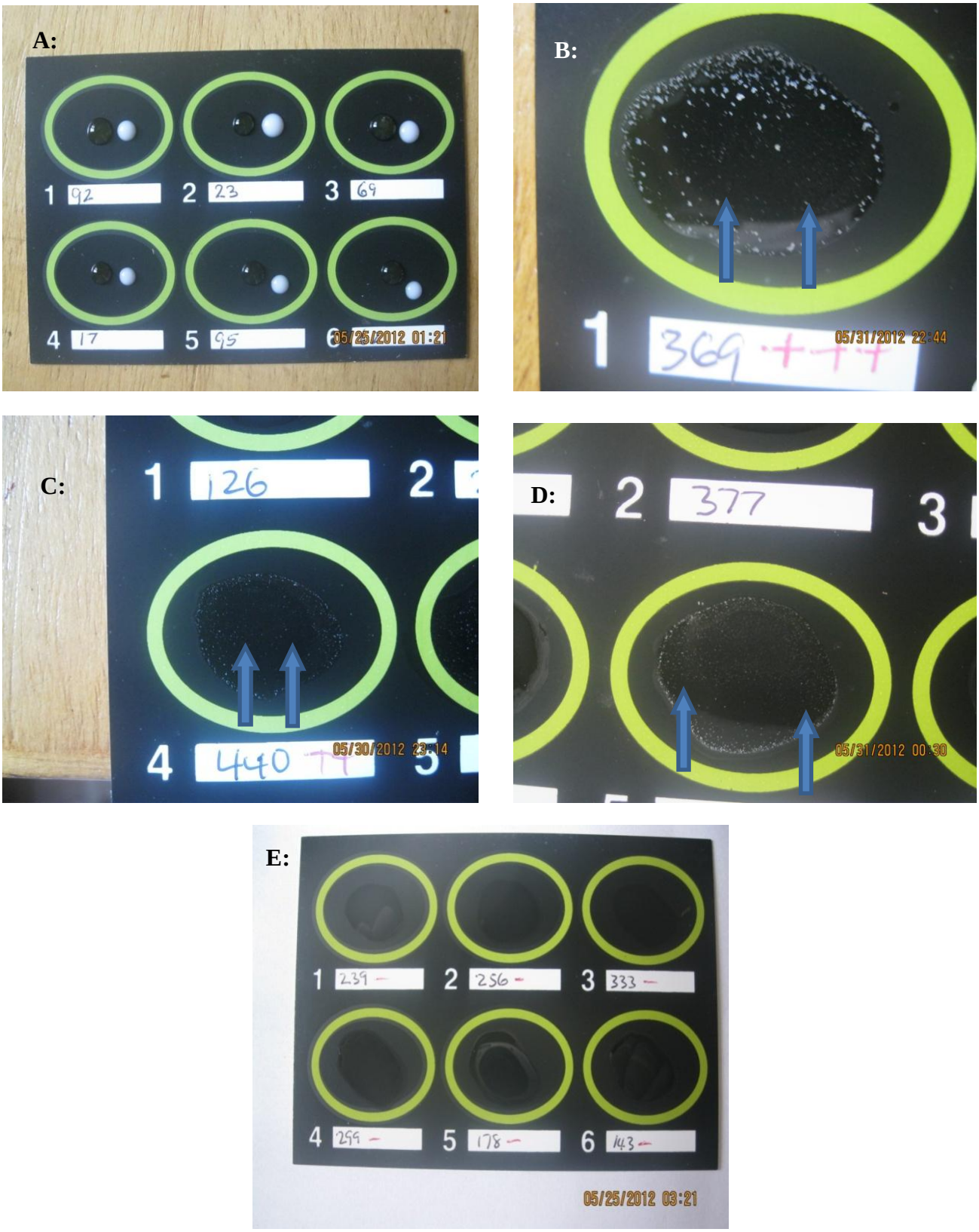

Figure 2.

A: Reaction beads card coated with capsular polysaccharides purified *Mmm*SC made up of 6 reacting cells containing spot of serum and reagent next to each other in the cells (arrows), for the latex agglutination test to diagnosise CBPP in cattle.

B: Strong clumping of latex beads (arrows). Agglutination of CBPP antibodies began within one minute, 3 positives (+++) indicates a positive case.

C: Clear agglutination of latex beads (arrows).

D: Fine aggluttination of the latex beads (arrows). agglutination of CBPP antibodies formed between two and three minutes, 1 positive (+) indicates a positive case. Agglutination of CBPP antibodies began between one and two minutes, 2 positives indicates a positive case.

E: Negative (-). No agglutination formed in all the reaction cells of *Mmm*Sc after three minutes (homogeneuos solution), indicating negative cases.

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RESULTS

An overall sero-prevalence of 26.0% (234/900) was recorded. Forty six percent (138/300) of the 300 cattle sampled in Kauru LGA were sero-positive, while, 17.0% (51/300) and 15.0% (45/300) of the 300 cattle sampled from Ikara and Chikun LGAs respectively, were similarly sero-positive for CBPP. Sero-prevalence of CBPP was significantly associated with LGAs sampled (P=0.0001) (Table 1).

From the 275 heads of cattle in the 3 senatorial districts with an age range of <1-3 years, 17.1% (47/275) were sero-positive for CBPP. This consist of 31.0% (27/87) sero-positivity for cattle in Kauru, 11.9% (12/101) for Ikara and 9.2% (8/87) for Chikun LGA. Similarly out of a total of 375 heads of cattle within the age range of 4-6 years old from the 3 districts, 24% (90/375) were sero-positive for CBPP out of which, Kauru, Ikara, Chikun LGAs 47.8% (56/117), 11.1% (19/171) and 10.2% (15/147) sero-positivity respectively.

Two hundred and fifty heads of cattle with ages >6 years old from the 3 districts were sampled and 38.8% (97/250) of the cattle were sero-positive for CBPP. Among the cattle with ages >6 years old in the 3 selected LGAs from the 3 district, Kauru LGA had a higher 57.3% (55/96) sero-prevalence rate of CBPP than Chikun 33.3% (22/66) and Ikara 22.7% (20/88) LGAs. There is a significant association (P=0.0027) in the sero-prevalence for CBPP between the age groups with the sero-prevalence being higher 38.8% (97/250) in ages >6 years old, and lowest 17.1% (47/275) in the younger cattle aged<1-3 years old (Table 2).

Table 3 shows that 196 male cattle were sampled for sero-prevalence for CBPP from the 3 senatorial district, and 21.9% (43/196) were found to be sero-positive for CBPP. While out of the 704 females sampled, 27.6% (191/704) were sero-positive for CBPP. Male cattle from Kauru LGA had higher 31.4% (17/54) sero-prevalence for CBPP than Chikun 18.2% (10/55) and Ikara 18.4% (16/87) LGAs. Similarly, female cattle sampled from Kauru had a higher 49.2% (121/246) sero-prevalence rate for CBPP than Ikara 16.4% (35/213) and Chikun 14.3% (35/245). There is no significant association (P=0.1424) between sexes in the CBPP sero-prevalence in the cattle sampled and equally no association between sexes in the CBPP sero-prevalence in the cattle sampled in the 3 LGAs.

Among the cattle sampled for this study in the 3 senatorial district, 97% (879/900) were of the White Fulani (Bunaji) breed, while, 1.6% (14/900) and 0.8% (7/900) were Red Bororo (Rahaji) and Sokoto Gudali (Bokoloji) breeds respectively. The White Fulani breed of cattle in the 3 district had a sero-prevalence rate of 25.4% (223/879), out of which the white Fulani breed of cattle from Chikun LGA had the lowest sero-prevalence rate 13.9% (41/295) while those from Kauru LGA had the highest 45.9% (134/292) sero-prevalence rate. Of the Red Bororo breed of cattle sampled in the 3 senatorial district, 50% (7/14) were sero-positive. None of the Red Bororo breed of cattle from Ikara LGA but 40% (2/5) were positive for CBPP. There was no significant association in the sero-prevalence between the breeds of cattle sampled (P=0.0572) (Table 4).

Table 1. Sero-prevalence of CBPP in Cattle by Local Government Area in the three Senatorial Districts of Kaduna State, Nigeria

LGA*	No. Sampled	No. Positive	% Positive
Ikara	300	51	17.0
Chikun	300	45	15.0
Kauru	300	138	46.0
Total	900	23.4	26.0

P-value=0.0001, X^2= 93.87 df=2; *LGA= Local government area

Table 2. CBPP Sero–prevalence by age in the cattle of three senatorial districts of Kaduna state, Nigeria, during 2016

Age (years)	Local government areas						Total sampled	Total positive (%)
	Ikara		Chikun		Kauru			
	Sampled	Positive (%)	Sampled	Positive (%)	Sampled	Positive (%)		
<1-3	101	12(11.9)	87	8(9.2)	87	27(31.0)	275	47(17.1)
4–6	171	19(11.1)	147	15(10.2)	117	56(47.9)	375	90(24.0)
>6	88	20(22.7)	66	22(33.3)	96	55(57.3)	250	97(38.8)
Total	300	41(13.7)	300	45(15.0)	300	138(46.0)	900	234(26.0)

P value = 0.0027, X^2 = 9.010, df = 1, OR = 0.5299

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Table 3. Sero-prevalence of CBPP by sex in the cattle of three senatorial districts of Kaduna State, Nigeria during 2016

Sex / LGA*	Male		Female	
	Sampled	Positive (%)	Sampled	Positive (%)
Ikara	87	16(18.4)	213	35(16.4)
Chikun	55	10(18.2)	245	35(14.3)
Kauru	54	17(31.5)	246	121(49.2)
Total	196	43(21.9)	704	191(27.1)

P value=0.1428, X^2=2.148, df=1, OR=0.7549; *LGA= Local government area

Table 4. Sero–prevalence of contagious bovine pleura pneumonia in cattle by breed in three senatorial districts of Kaduna state, Nigeria during 2016

Breed / LGA*	White Fulani (Bunaji)		Red Bororo (Rahaji)		Sokoto Gudali (Bokoloji)	
	Sampled	Positive	Sampled	Positive	Sampled	Positive
Ikara	292	48(16.4%)	3	-	5	2(40%)
Chikun	295	41(13.9%)	4	3(75.0%)	1	1(100%)
Kauru	292	134(45.9%)	7	4(57.1%)	1	1(100%)
Total	879	223(25.3%)	14	7(50.0%)	7	4(57.0%)

P value = 0.00573, X^2 = 5.718, df = 2; *LGA= Local government area

DISCUSSION

Contagious Bovine Pleuropneumonia (CBPP) has been an endemic disease in Nigeria since it was first reported in 1924 (Foluso, 2003). This may be due to the transhumance and nomadic nature of cattle rearing and inadequate control measures for CBPP in Nigeria (Egwu et al., 1996). Latex Agglutination Test (BoviLAT PA6223) for CBPP is a fast and easy diagnostic technique to perform in the field (Ayling et al., 1999b).

The 26.0% sero-prevalence of CBPP found in this study is, lower than the 47% sero-prevalence reported by Danbirni et al. (2010), in a herd of cattle with concurrent infection of CBPP and bovine Tuberculosis in Igabi LGA of Kaduna State and that of Suleiman et al. (2015) who reported 30.2% of seropositivity to CBPP within agro-pastoral areas of Nigeria. However, the result of this study was higher than that of Okaiyeto et al. (2011) who reported a sero-prevalence of 16.7% and 17.5% for adults and young cattle respectively, in a herd of cattle with CBPP outbreak in Kafur LGA Katsina State, using LAT and Musa et al. (2016) who also reported 3.33% prevalence of CBPP from lung samples sampled in Maiduguri and Yola abattoir both in north eastern Nigeria. The result of this study also differ from the work of Nawathe (1992) and Adamu and Aliyu (2006) who in their separate studies recorded a lower sero-prevalence of 0.52% and 0.33% respectively in Borno State and Aliyu et al. (2000) who recorded a sero-prevalence of 0.29% in 5 other States in Northern part of Nigeria. The higher sero-prevalence rate recorded in this study could be as a result of the inadequate prevention and control measures that resulted in absence or irregular vaccination programmes for cattle over the years, as well as the introduction of infected cattle into the areas (particularly through transhumance and nomadism) that were initially thought to be free of the disease (Aliyu et al., 2000). It could also be as a result of epidemiological trend of the disease with the presence of carriers in some herds which might not have been detected clinically and hence the maintenance and gradual spread of the disease (Egwu et al., 1996).

Southern senatorial district (Kauru) has a higher sero-prevalence of 46.0% when compared to northern (Ikara) and central (Chikun) senatorial district with 17.0% and 15.0% sero-prevalence respectively. The higher sero-prevalence of CBPP in cattle in the southern district may not be unconnected to the strategic location of Kauru LGA, where a major cattle route, from the North- western part of Nigeria passes through to the southern part of the country. Kauru LGA shares boundary with Kajuru and Zangon kataf LGAs where two grazing reserves, (Libere and Laduga) are located, and harbours large population of cattle. The movement of these cattle in and out of Kauru might be responsible for the introduction and easy spread of CBPP. Furthermore, a previously reported outbreak of CBPP by Danbirni et al. (2010) in a herd of cattle in Igabi LGA, which shares boundary with Kauru LGA, might also have contributed to the higher sero-prevalence of the disease in southern district due to uncontrolled movement of cattle between the 2 LGAs. The presence of the CBPP in older than younger animals agrees with the findings of Boelert et al. (2005), who reported that age is a measure of exposure and chances of being infected. Thus, younger animals have a lower risk of being infected. The result of the study shows that sex and breed is not a factor in the epidemiology of CBPP, agrees with Santini et al.

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(1992), who reported that ruminants of *Bos* genus are generally susceptible to CBPP indicating uniform susceptibility among breed and sex.

CONCLUSION

Sero-prevalence of CBPP in cattle was found to be high in the study area. Since there was no history of recent vaccination against CBPP in the area prior to this study, and the sero-prevalence of CBPP in cattle was found to be high; there is therefore, a need for the authorities concerned to intensify vaccination of cattle against the disease. BoviLAT should be encouraged due to its simple, fast and easy to perform technique for the diagnosis of CBPP in the field.

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Conflict of interest

There is no conflict of interest

Author's contribution

Billy Ishaya La'ah and Arhyel Gana Balami conducted the research and wrote the manuscript, A.K.B Sackey, S.N.A Sa'idu and L.B Tekdek supervised the research and corrected the manuscript while S.O Okaiyeto provided the test kits.

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