NIGERIAN VETERINARY JOURNAL

ISSN 0331-3026

Nig. Vet. J., June 2017 **Vol 38 (2): 104-116.**

ORIGINAL ARTICLE

Prevalence of Bovine Brucellosis and Analysis of Risk Factors in Resident Cattle Herds of Kanke Local Government Area, Plateau State, Nigeria

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SUMMARY

Brucellosis an important zoonotic disease is endemic in Nigeria resulting to huge economic losses in livestock and loss of man hour in infected people. Information about the prevalence and risk factors for the disease in resident cattle herds in the North Central Zone of Nigeria is however lacking. A cross-sectional study was conducted to determine the prevalence of bovine brucellosis and the risk factors associated with the disease in Kanke Local Government Area (LGA) of Plateau State. A total of 479 resident cattle sera from 39 herds in the four districts of the LGA were examined for antibodies against *Brucella* species using Rose Bengal plate test (RBPT) and competitive Enzyme-Linked Immunosorbent Assay (cELISA). Risk factors responsible for the occurrence of the disease in the herds were investigated using pre-tested structured questionnaire. The strength of association between risk factors and seropositivity to brucellosis was measured using logistic regression analysis. Out of the 479 sera examined, 1.0% (5/479) and 3.6% (18/479) were positive for *B. abortus* antibodies using RBPT and cELISA respectively. The herd prevalences were 10.3% (4/39) and 38.5% (15/39) with the RBPT and cELISA, respectively. There was a significant association between seroprevalence of brucellosis and herd size (OR: 4.3, 95% CI: 1.0.-18.3; P=0.05) as well as a number of milking cows (OR: 4.7, 95%CI: 1.2.-18.9; P=0.03). The study found brucellosis to be prevalent in resident cattle herds in the study area and milk from cows in these herds are likely to transmit the disease to humans.

Key words: Brucellosis, Resident herds, Cattle, Kanke LGA.

INTRODUCTION

Brucellosis is a chronic disease of animals caused by Gram negative, facultative non-motile, intracellular bacteria of the genus *Brucella* (OIE, 2009). It is a contagious systemic disease primarily of ruminants, characterized by inflammation of the genital organs and foetal membranes, abortion, sterility, and formation of localized lesions

in the lymphatic system and joints (CDC, 2011). The disease has a worldwide distribution but has been eradicated from the livestock populations of most European countries, Japan, Canada and the United States of America (USA) (Radostits *et al* 2000; (WHO, 2001). Brucellosis is, however, prevalent in parts of Asia (Chahota *et al*., 2003); South America (Dias *et al*., 2009); and Africa (Ogugua *et al*., 2015).

In cattle, the disease is transmitted by contact with infected uterine discharges and maternal transfer either by suckling or in-vivo (Corbel, 2006). Humans are infected by inhalation, contact of abraded skin with infected materials as well as consumption of unpasteurised milk originating from infected animals (CFSPH, 2009; WHO, 2004). It is, therefore an occupational disease to veterinarians, abattoir workers, herdsmen, hides and skin factory workers as well as laboratory personnel (Falade, 2002; Traxler *et al*., 2013). Infection in cattle may be lifelong and in naïve cattle population, abortion storm (abortion rates varying from 30 to 70%) may occur (CFSPH, 2009; Godfroid *et al.*, 2004; Pappas *et al.*, 2005). After the first abortion, subsequent pregnancies are usually delivered normal but *Brucella* is still shed in the milk and uterine discharges of such animals (CFSPH, 2007). Since the reproductive performance of these carrier animals seems unaffected, they are retained in herds especially in developing countries like Nigeria despite the presence of pathognomonic clinical signs in some cases, making effective control programmes extremely difficult (Mai *et al.*, 2012). In humans, it causes undulating fever, and when left untreated could result in complications such as meningitis, epididymo-orchitis, arthritis (Safirullah *et al*., 2014) and death due to cardiac involvement in about 5% of the cases (Chadda *et al*., 2004; Esuruoso *et al*., 2005).

In cattle herds, brucellosis results in huge economic losses due to decreased calving percentage, culling for infertility, decreased milk production, abortion, stillbirth or birth to weak calves; as well as loss of man hours in infected people (McDermolt *et al.* 2002; Ocholi *et al.* 2004; Adamu, 2009). The presence of brucellosis in cattle herds portends a major public health problem especially to individuals with regular contact with cattle as well as the members of the general public who consume unpasteurised milk and milk products of cattle origin in Nigeria.

Brucellosis remains a problem in Nigeria due to lack of official policy for the control of the disease (Ibironke *et al*., 2008), uncontrolled movement of slaughter cattle within and from neighbouring countries (Ogundipe, 2001; Cadmus *et al*., 2008), nomadism (Mai *et al*., 2012) and poor knowledge and practices concerning the diseases among farmers and other risk groups (Adesokan *et al*., 2013). In Nigeria, varying prevalence rates have been recorded in different parts of the country: the prevalence of 7.8% and 1.9% was recorded from Oyo and Lagos (Ogugua *et al*., 2015); 20.0% prevalence was recorded in slaughter cattle in Zamfara State (Lawal *et al*., 2012), a within herd prevalence of 32.2% was recorded in a prison cattle farm in Sokoto State (Junaidu *et al*., 2008); in three states of Adamawa, Kano, Kaduna prevalence of 29.2%, 26.7% and 23.3%, respectively was recorded (Mai *et al*, 2012); 14.1% prevalence was recorded in Obudu, Cross River State (Nanven *et al*., 2013). In Plateau State, the prevalence of 37.3%, 2.5% and 3.7% was recorded in Bassa, Riyom and Jos South Local Governments Areas (LGAs), respectively (Nanven *et al*., 2013).

In Nigeria, the most popularly consumed animal products are those of cattle origin (Alimi, 2013; Rauf, 2012). The livestock production system in Nigeria includes the nomadic, semi nomadic and intensive system. Although with time, population increase has resulted in corresponding increase in demand for livestock products, cattle production in Nigeria is concentrated

in the hands of the nomadic Fulani herdsmen (Ibironke *et al*., 2008). However, in Plateau State, many farmers are involved in agro-pastoralist farming system whereby cattle are raised in small herds in the backyard of the farmers where grasses are cut and given to the animals or the animals are taken to the nearby communal grazing lands. The animals are therefore resident in the communities and not involved in long distance movement in search of feed and water. In Nigeria, where no control policy is employed to control brucellosis, grazing of cattle herds in communal lands result to exchange of diseases like brucellosis between different herds (Bertu *et al*., 2010; Hesterberg *et al*., 2008). In Plateau State, past studies on brucellosis were focused on pastoral herds (Nanven *et al*., 2013), cattle settlements (Bertu, 2014) and small ruminants (Bertu *et al*., 2010). Therefore, information regarding the prevalence and risk factors for brucellosis in resident herds in the state is scarce. This study, therefore used the RBPT, cELISA and questionnaire to determine the prevalence of bovine brucellosis and the associated risk factors in the resident herds of Kanke LGA of Plateau State, Nigeria.

MATERIALS AND METHODS

Study area

Kanke LGA is in the central zone of Plateau State and located between latitude 80^0 24´ North and Longitude 80^0 32´ and $100^0$38´ East. The LGA shares boundary with Bauchi State in the North, Pankshin LGA in the West, Kanam LGA in the East and Langtang North LGA in the South. The LGA has an area of 7,808. 85 km^2 and population of 268,000 people (NPC, 2006). The majority of the inhabitants are farmers while among others are civil servants, businessmen, artisans etc. Most of the farmers are crop farmers some of whom keep a few herds of cattle that make up the resident herds. These resident herds have grass cut in the fields and brought home for them as well as graze within the vicinity of the homesteads in private or communal grazing lands. There are pastoral herds in the area which are not indigenous but are reared by nomadic Fulani herdsmen that settle briefly and eventually move on in search of feed and water. The pastoral herds were not included in this work. The LGA has four districts namely; Amper, Kabwir, Ampang and Garam in order of decreased livestock population. Out of the four, Amper is the only district that harbours cattle market due to a suitable grazing topography.

Study population and design and animal sampling

A cross-sectional survey was conducted between January and June 2015 among resident cattle aged over six months. With the statistical formula $n = \frac{1.96^2.P.(1-P)}{d^2}$, the sample size of 33 herds was calculated using the prevalence of 9.6% earlier recorded from cattle herds screened in northern Plateau State (Nanven *et al*., 2013). A non-response rate of 10% was added giving a total sample size of 37 although, a total of 39 herds were screened in the study. An interviewer administered questionnaire was issued to each herd owner. Also information about sex, age, breed of individual animal were collected along with the sample. About 5ml of blood collected from the jugular vein of each cattle after proper restraint using sterile needle and syringes was dispensed into centrifuge tubes and labelled accordingly. These tubes were placed in a slanting position to enhance serum separation, kept in a flask containing ice pack and transported to the laboratory at the Department of Public Health and Preventive Medicine College of Veterinary Medicine University of Agriculture, Makurdi. The blood samples were centrifuged at 3000rpm for 5 minutes, the sera decanted into serum vials and stored at -20^0c until assay.

Serological tests
The serum samples were tested for *Brucella* antibodies by RBPT and cELISA.

Rose Bengal plate test (RBPT)
The serum samples were tested for *Brucella* antibodies by RBT as described by OIE (2009). The RBPT antigen consisting of standardized *B. abortus* antigen from the Animal and Plant Health Agency (APHA), Surrey KT15 3NB, U.K. was used to carry out the test. Briefly, equal volumes (30 µl) of antigen and test serum were mixed thoroughly on a plate using a stick applicator and the plate was rocked for 4 minutes. The appearance or absence of agglutination (rough or smooth clumps with rim edges) was scored positive (+) and negative (-), respectively.

Competitive enzyme-linked immunosorbent assay (cELISA)
The cELISA kit was sourced from the APHA. The kit contained cELISA plate and reagents. The plate was coated with the lipopolysaccharide (LPS) of *B. melitensis* M16. The reagents included control sera, diluting buffer, conjugate, washing solution, chromogen and stopping solution. The reagents were reconstituted as directed by the manufacturers. The test was performed according to the manufacturer's instructions. Positive samples had a clear appearance whereas negative samples appeared orange in colour. The optical density (OD) was measured at 450nm using a microplate ELISA reader. A positive/negative cut-off was calculated as 60% (as instructed by manufacturers) of the mean of the OD of the conjugate control wells. Samples in wells with OD equal to or less than the cut-off point were scored positive, while those above were negative.

Data analysis
Data analysis was performed using Stata Version 12. Group differences were tested for by using chi-square statistics for categorical variables. A multivariable adjusted logistic regression was carried out using all the variables that were statistically significant at the 10% level with the main outcome measure (RBPT) in bivariate analysis. All tests were two-tailed and statistical significance was set at $p<0.05$.

RESULTS
The results of the study show the individual prevalence of brucellosis to be 1.0% (5/497) and 3.8% (18/497) as well as the herd prevalence of 10.26% (4/39) and 38.46% (15/39) with the RBPT and cELISA, respectively (Tables I and III). While the brucellosis prevalence was not found to be associated with district, breed, sex and age on individual basis, it was found to be

TABLE I: Factors associated with the individual level prevalence of brucellosis among resident cattle screened in Kanke LGA of Plateau State as measured by RBT

Variable	Characteristic	Seropositive animals based on RBT				Odds ratio	95%CI	p-value
		Positive N=5	% 1.0	Negative N=492	% 99.0			
District	*Others	2	1.1	177	98.9	1		
	Amper	3	0.9	315	99.1	0.84	0.14-5.09	0.84
Breed	**Others	1	5.6	17	94.4	1		
	Bunaji	4	0.8	475	99.2	0.14	0.02-1.35	0.09
Sex	Male	2	0.7	274	99.3	1		
	Female	3	1.4	218	98.6	1.89	0.31-11.38	0.26
Age	1-2 years	1	0.6	180	99.4	1		
	3-8 years	4	1.3	312	98.7	2.31	0.26-20.8	0.25

*Others include Ampang, Garam and Kabwir; **Others include Muturu and Sokoto Gudali

TABLE II; Results of logistic regression analysis of a variable significant at 10% level with main outcome measure RBT in bivariate analysis

Variables	Category	*Brucella* infection		OR	95%CI	P -value
		Positive n=5(1.0%)	Negative N=492(99.0%)			
Breed	**Others	1(5.6)	17 (94.4)	1		
	Bunaji	4 (0.8)	475 (99.2)	7.0		
					0.74-65.88	0.09

*Others include Ampang, Garam and Kabwir; **Others include Muturu and Sokoto Gudali

TABLE III: Factors associated with the individual level prevalence of brucellosis among resident cattle screened in Kanke LGA of Plateau State as measured by cELISA

Variable	Characteristic	Seropositive animals based on cELISA				Odds ratio	95%CI	p-value
		positive N=18	% 3.6	Negative N=479	% 96.4			
District	*Others	5	2.8	174	97.2	1		
	Amper	13	4.1	305	95.9	1.5	0.52-4.23	0.32
Breed	**Others	1	5.6	17	94.4	1		
	Bunaji	17	3.5	462	96.5	0.6	0.1-4.98	0.49
Sex	Male	11	4.0	265	96.0	1		
	Female	7	3.2	214	96.8	0.79	0.30-2.07	0.32
Age	1-2 years	5	2.8	176	97.2	1		
	3-8 years	13	4.1	303	96.5	1.51	0.53-4.31	0.23

*Others include Ampang, Garam and Kabwir; **Others include Muturu and Sokoto Gudali

significantly associated with the number of milking cows (OR: 4.7, 95%CI: 1.2-18.9; P=0.03) and herd size (OR: 4.26, 95%CI: 1.0-18.3; P=0.05) in the herds. (Tables II and V).

DISCUSSION

The prevalence of brucellosis recorded in this study (1.0%) showed that brucellosis is prevalent in cattle in the study area. The prevalence could be attributed to the lack of official policy for the control of the disease in Nigeria (Cadmus *et al*., 2006), uncontrolled movement of livestock within and from neighbouring countries (Ogundipe, 2001), ignorance of the mode of transmission the disease among farmers (Adesokan *et al*, 2013), retaining of animals showing pathognomonic signs of the disease (Mai *et al*., 2012) and many other factors. However, the prevalence recorded is lower than the 26.3% in three northern states of Nigeria (Mai *et al*., 2012), 7.1% in Kaduna State (Mbuk *et al*., 2011), 9.6% in Plateau State (Nanven *et al*., 2013), 42.1% in Obudu Cross River State (Nanven *et al*., 2013) and 8.4% in Cameroon (Bayemi *et al*., 2009). This low prevalence could be due to the fact that the cattle herds in the study area are relatively small in size and are therefore at low risk of exposure to the disease (Megersa *et al*., 2011). It could also be as a result of the fact that the herds in the area are resident cattle and are not involved in seasonal migration which is common with cattle herds in Nigeria (Mbuk *et al*., 2011). Brucellosis prevalence has been reported to be higher in pastoral than resident herds (Unger *et al*., 2003) due to increased exposure potential as a result of movement from one location to another; interacting and sharing grazing lands and watering points with other potentially infected cattle herds and other animals (Mai *et al*., 2012; Matope *et al*., 2011).

TABLE IV: Prevalence and risk factors associated with the occurrence of brucellosis in resident cattle herds as measured by RBT in Kanke LGA

Variable	Characteristic	Seropositive animals based on RBT				Odds ratio	95%CI	p-value
		Positive n=4	% 10.3	Negative n=35	% 89.7			
Herd size	1and2	2	6.5	29	93.5	1		
	3	2	25.0	6	75.0	4.83	0.56-41.41	0.10
Number of milking cows	0, 1 and 2	2	6.3	30	93.7	1		
	3	2	28.6	5	71.4	6.00	0.68-52.89	0.07
Period of existence of herd	1 and 2	1	5.6	17	94.4	1		
	3	3	14.3	18	85.7	2.83	0.27-29.95	0.36
Originating herd	purchases	1	5.3	18	94.7	1		
	Inheritance	3	15.0	17	85.0	3.17	0.30-33.58	0.32
Abortion	No history of abortion	1	5.6	17	94.4	1		
	History of abortion	3	14.3	18	85.7	2.00	0.19-21.57	0.502
Retained placenta	No history of retained placenta	1	7.7	12	92.3	1		
	History of retained placenta	3	11.5	23	88.5	1.57	0.15-16.71	0.59
Knowledge of brucellosis in animals	Good	3	9.4	29	90.6	1		
	Poor	1	14.3	6	85.7	1.61	0.14-18.26	0.56
Attitudes of farmers	Good	0	0.0	6	100			
	Poor	4	12.1	29	87.9			
Practices of farmers	Good	1	25.0	3	75.0	1		
	Poor	3	8.6	32	91.4	0.28	0.02-3.62	0.36

TABLE V: Results of logistic regression analysis of variables significant at 10% level with main outcome measure RBT in bivariate analysis

Variables	Category	*Brucella* infection		OR	95%CI	P value
		Positive n=4(10.3%)	Negative n=35(89.7%)			
Herd size	Small	2 (6.5)	29 (93.5)	1		
	Large	2(25.0)	6(75.0)	4.3	1.0-18.3	0.05
No of milking cows	Few number	2 (6.3)	30 (93.7)	1		
	Large number	2 (28.6)	5 (71.4)	4.7	1.2-18.9	0.03

The study found the prevalence of brucellosis to be significantly associated with herd size (OR: 4.26, 95%CI: 1.0.-18.3; P=0.05). This agrees with other investigators (Jergefa *et al*., 2009; Makita *et al*., 2011; Megersa *et al*., 2011; Unger *et al*., 2003) that recorded the prevalence of brucellosis to be higher in large herds than small herds. This is in consonance with the epizootiological rule of "large herds, large incidence and small herds, low

incidence" as stated by Akakpo and Bornarel, (1987). Large herd sizes have been shown to increase the exposure to brucellosis especially after abortion or calving by *Brucella* infected animals because of the higher stocking density as compared to small sized herds (Megersa *et al*., 2011).

Number of milking cows was found to be significantly associated with the prevalence of brucellosis in the study (OR: 4.7, 95%CI: 1.2.-18.9; P=0.03). The pregnancy period which precedes milking is noted to be associated with brucellosis (Swai and Schoonman, 2010). This is because *Brucella* species have tropism for the pregnant uterus because erythritol sugar which is preferentially metabolised by the organism is produced in the placenta (Neta *et al*., 2008). The multiplication of the *Brucella* organism results in inflammation that leads to abortion which may not occur in subsequent pregnancies (Corbel, 2006). However, such animals may become latent carriers that could only be detected by serological tests during and after pregnancies (CFSPH, 2009). Retaining of such animals in the herds is common in Nigeria (Mai *et al*., 2012) and tests during the milking periods may detect infection in such animals. Also, some cows infected *in-utero* may not be serologically positive until during and after pregnancy (Forbes and Steele, 1989). This indicates a risk of infection to the cattle owners who have regular contact and custom of consuming unpasteurised milk and milk products.

The prevalence recorded with the RBPT (1.0%) is lower than that recorded with the cELISA (3.6%). This is in contrast with the findings of other studies that recorded higher prevalence with the RBPT than the cELISA (Mohammed *et al*., 2011; Mai *et al*., 2012; Cadmus *et al*., 2013). This can be explained by the fact that serological tests differ in their ability to detect a particular immunoglobulin (Beh, 1974). The immunoglobulin isotypes found in the blood in early or acute infections are the IgM and IgG1 (Ismail *et al*., 2002), which may not be seen in cases with insidious onset, in chronic, recurrent and relapse cases (Serra and Viñas, 2004) where IgG2, IgG3 and IgA are predominant (Henk *et al*., 2003; Diaz *et al*., 2011). While the RBPT is better suited for detecting acute cases (Chenais *et al*., 2012) (i.e. the IgM and IgG1), the cELISA is the test of choice in chronic cases (Smits *et al*., 2003). This may, therefore suggest that most of the cases in this study were of the chronic form of brucellosis which is supported by a previous report that many cases of brucellosis in endemic areas could be in chronic or relapse stage of the disease (Serra and Viñas, 2004). The IgG ELISA has been used to monitor chronic and relapse infections because of its better ability to detect IgG and IgA in sera (Smits *et al*., 2003). However, the discrepancy between the two tests could also be due to the presence of non-specific antibodies due to infection with antigenically related bacteria like *Yersinia enterocolitica 0:9*, *Salmonella* Urbana, *Escherichia coli 0:157* and *Francisela tularensis* (Bowden *et al*., 1997; Chenais *et al*., 2012). Although, *Yersinia enterocolitica 0:9*, the most antigenically related bacteria to *Brucella*, has been noted by Shey-Njila *et al*. (2005) to belong to temperate regions, but it has been isolated in Nigeria (Okwori *et al*., 2009).

The use of cELISA to complement the RBPT has been noted as one of the best combinations of specificity and sensitivity especially in areas where vaccination is practiced (Marín *et al*., 1999). In Nigeria however, vaccination is not generally practiced since there is no official policy on control of brucellosis (Aworh *et al*., 2013; Ibironke *et al*., 2008). The RBPT however, has been described as being superior to the cELISA and therefore cannot be confirmed with an inferior test like the cELISA (Ducrotoy *et al*., 2014; Megersa *et al*., 2011). The RBPT is therefore recommended as the test of choice in endemic and resource-poor countries where vaccination is not generally practiced like Nigeria because of its simplicity, relatively low cost and high standard in testing for brucellosis (Mcgiven,

2013). Meanwhile, the ELISA kit used in this study was manufactured in the United Kingdom which is an area of low prevalence and the cut-off point (60%) was set as the *Brucella* infection rate applies to the UK. Since cut-off points are set as the points of highest accuracy (minimal false positive and negative) results, it cannot be extrapolated from areas devoid of brucellosis and with good hygienic conditions to areas where brucellosis is endemic (Greiner and Gardner, 2000). This is because in endemic areas cattle population sometimes acquire antibodies due to possible contact with the organism, but without having the disease (Corbel, 2006). In Adamawa region of Cameroon for instance, 50% was suggested to be a better cut-off point using the cELISA in the cattle (Bronsvoort *et al*., 2009). The OIE, therefore, recommends that cut-off points for ELISA be validated under local settings (OIE, 2009).

Although the study found no association between prevalence of brucellosis and sex, higher seropositivity was recorded among the females than males. This result is in agreement with other studies that found prevalence of brucellosis to be higher in cows than bulls (Dinka and Chala, 2009; Junaidu *et al*., 2011) but contrasts some other studies that reported higher prevalence in males (Chimana *et al*., 2010; Cadmus *et al*., 2013). This finding may be due to the fact that unlike the bulls which are sooner sold for beef once they attain market weight, most cows are retained for a longer period for reproduction and milk production in Africa (Mangen *et al*., 2002). Such longer period of existence in endemic areas has been associated with greater chances of exposure to brucellosis (Kebede *et al*., 2008; Megersa *et al*., 2011). In addition, bulls have been reported to show limited immunological response to *Brucella* infection (Berhe *et al*., 2007).

Despite the findings of the study, some limitations were observed. Most of the cattle screened were of the Bunaji because it is the most common breed in the study area. Also, cattle screened in Amper District were more than that in the other three districts and this was due to the fact that more animals are reared in this district. These discrepancies in numbers might have introduced bias in the study. Also, the *Brucella* organisms responsible for the disease were not isolated to confirm the *Brucella* species responsible for brucellosis in the resident herds screened.

CONCLUSION

This study shows that brucellosis is prevalent in resident herds in the study area although at a low level. There is need to test individuals in regular contact with these animals for brucellosis. Food products derived from these animals should be properly cooked to protect the consumers from *Brucella* infection. In addition, individuals in regular contact with the infected animals should be mindful of personal protection especially when assisting in calving. Also, the herd owners and members of the public who consume unpasteurised milk in the area should be educated on the economic and public health importance of the disease. Finally, further studies should confirm brucellosis in the study area by isolation of the *Brucella* species responsible for the disease as well as validation of cut-off points for serological methods like cELISA in such local setting.

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