PREVALENCE OF BRUCELLOSIS IN INDIGENOUS CATTLE BREEDS IN NSUKKA AGRICULTURAL ZONE, ENUGU STATE, NIGERIA

[1,2]OGUGUA, Akwoba Joseph and [1]ONUNKWO, Joseph Ikechukwu

[1]Department of Veterinary Public Health and Preventive Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.

[2]Veterinary Public Health and Preventive Medicine Specialty, College of Veterinary Surgeons of Nigeria.

Corresponding Author: Ogugua, A. J. Department of Veterinary Public Health and Preventive Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria. **Email:** ogugua.akwoba@unn.edu.ng **Phone:** +234 8033844475

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ABSTRACT

Brucellosis is a zoonotic bacterial disease prevalent in Nigeria. However there are few reports of it in local cattle breeds. In order to determine the prevalence and risk factors for the disease in Muturu and Ndama breeds of cattle in Nsukka Agricultural Zone, Enugu State, Nigeria, a cross-sectional study was conducted. Blood samples from the herds were collected via snowball sampling, and the characteristics of each herd observed and noted. Using the conventional and modified Rose Bengal Test (RBT), sera from the samples were tested for brucellosis and the positive samples retested with the cELISA. The standard RBT recorded zero prevalence. In contrast, the modified RBT indicated individual animal and herd prevalence of 34.78 and 42.10%, respectively and 45.3 and 42.10% of these were supported by the cELISA test. It was observed that the herd size (p = 0.019) and presence of calves (0.0049) were all significantly associated with the prevalence of brucellosis in the herds. The study found brucellosis to be prevalent in the herds screened. However, more research is required to determine why the reactive antibody levels in the positive animals were low. To get local herd owners to accept preventive health practices, it is necessary to educate farmers on brucellosis.

Keywords: Prevalence, Modified RBT, Standard RBT, Muturu, Ndama

INTRODUCTION

Brucellosis is a zoonotic disease caused by facultative, intracellular, Gram–negative bacteria of the genus *Brucella*. The disease has had a substantial negative influence on animal productivity and health, resulting in significant financial losses such as abortion, decreased milk output, poor fertility rates and high animal treatment costs (McDermott and Arimi, 2002). In humans, brucellosis is characterized with undulating fever, headache, malaise, anorexia and other problems that if untreated can cause serious man-hours losses and even death (Falade, 2002).

Animals can become infected by drinking contaminated water and or eating contaminated food. Infections can also occur via conjunctiva, contact with aborted materials as well as in-*utero* (Mai *et al.*, 2012). Contact with sick animals, consumption of contaminated unpasteurized milk or dairy products, contact with contaminated surfaces, and inhalation all contribute to human infection (Refai, 2002). Therefore, human infections are synonymous to the presence of the disease in the animal population.

Although ubiquitous, brucellosis has been eradicated in many developed countries but remains endemic in developing ones (Corbel,

2006). In Africa, various epidemiological studies have found prevalence rates at the herd level ranging from 8.4 to 35.3% (Bayemi *et al*., 2009; Folitse *et al.*, 2014). In Nigeria, infection rates reported in cattle herds range from 9.7 to 29.9% (Alhaji *et al*., 2016; Ogugua *et al*., 2018). However, there is no brucellosis control scheme being implemented in the country, therefore cattle populations are often not vaccinated against the disease. The last organized campaign on record to vaccinate cattle against brucellosis in the country took place in 1956 (Ocholi *et al.*, 1993). However, the existing literature does not indicate that that vaccination program took place in Eastern, Nigeria. Consequently, generations of animals in various herds across the nation are susceptible to *Brucella* infection. It is important to note that the RBT is the best option for screening the population of cattle for brucellosis in the absence of vaccination (Ducrotoy *et al*., 2014).

The Muturu and Ndama are short horn, humpless breeds of cattle that traditionally exist in West Africa. These two breeds that share a more recent ancestry (Tijjani *et al.*, 2019) have exhibited unique adaptive traits by surviving tough environmental stress, water scarcities as well as nutritional and disease challenges (Ibeagha-Awemu *et al*., 2019). In history, the Muturu is said to be widely distributed throughout West Africa down to Ethiopia but has become threatened into extinction by urbanization and the influx of Zebu breeds because it is of less economic value (Gwaza *et al*., 2018a, b). The Muturu and Ndama breeds gained back what they lost in size and productivity by being able to survive in the tsetse fly-infested savannah and rain forest. They are also more resistant to trypanosomosis, tick-borne diseases, and streptotrichosis, and these traits lower their production costs in the tsetse-infested area (Adebambo, 2001; Ode *et al*., 2017). They also owe their existence to the fact that they are used in several traditional ceremonies, sacrifices, and funerals as well as for medical purposes. They also have a significant role in traditional culture, and there is a strong spiritual attachment to them (Gwaza *et al*., 2018 a, b). Despite the fact that these breeds have existed for many centuries in West Africa and that their resistance to numerous diseases has been extensively documented, little research has been done on them in terms of infectious diseases. Although, there is high burden and variation in prevalence of brucellosis in many breeds in Nigeria, more studies of the disease in Muturu and Ndama herds are needful for proper management in the phase of environmental changes due to climate change. Factors attributed to the continued transmission of the disease in the different herds in Nigeria range from intrinsic (breed, sex and age) (Nanven *et al*., 2013a) to extrinsic (like poor management practices) (Alhaji *et al*., 2016). In addition, infected nomadic herds play a major role in the transmission of the disease in Nigeria (Ibironke *et al*., 2008) where the annual dry season migration predisposes in - contact herds (like Muturu and Ndama) along their routes to *Brucella* infection (Ogugua *et al*., 2018). On the other hand, having existed for centuries in the West African sub-region (Adebambo, 2001), there is possibility that these breeds have harboured *Brucella* species for centuries. There is therefore the need to determine the occurrence of brucellosis as well as identify the factors that expose Muturu and Ndama herds to *Brucella* infections in the area. This will help to establish well-structured and effective management measures against the disease in Nigeria.

MATERIALS AND METHODS

Study Area: The study was conducted in Nsukka agricultural zone, Enugu State Nigeria. The zone is made up of six local government areas (LGAs) including Nsukka, Igbo-Eze South, Igbo-Eze North, Isi-Uzo, Udenu and Uzo-Uwani LGAs earlier described (Ulo *et al.*, 2021). The zone is among the few areas in Nigeria where Muturu and Ndama cattle can be found in appreciable numbers. These indigenous breeds of cattle are reared mainly by rural farmers and others in different occupations as complement to their earnings. They are kept for their use in traditions and funerals rites (Adebambo, 2001). The Muturu are usually tethered in the school fields or grass lands during the rainy seasons but in dry seasons near the homes, being fed mainly on concentrates and kitchen leftovers.

Most Ndama in the state are found in government farms, university and research institutions where they are kept in fenced enclosures but also fed in fields also used by local and migratory herds. Muturu males are sold at maturity and the few kept as studs roam between communities in search of females on heat. For the fact that feeding the animals during dry seasons is difficult when grasses are scarce, herds sizes are usually small (average of 2.4 animals) (Gwaza *et al.*, 2018a) and this low herd size is maintained by giving out calves as gifts or for share of the proceeds or sold.

Study Population and Design: This study was conducted among Muturu and Ndama cattle herds in three (Uzo-Uwani, Igo-Etiti and Nsukka LGAs) selected LGAs in Nsukka agricultural zone (Ulo *et al.*, 2021), Enugu State using the cross-sectional study design. Other cattle breeds were excluded from the study.

Sample Size, Sampling and Consent: Using the six LGAs in the Nsukka agricultural zone as the sampling frame, three were selected (Uzo-Uwani, Igbo-Etiti and Nsukka LGAs) using simple random sampling by balloting. A total of 138 animals was calculated with 9.6% (Nanven *et al.*, 2013b) expected prevalence using the statistical formula for cross-sectional studies as cited (Agada *et al.*, 2017) but 138 animals (Uzo-Uwani, 36; Igbo-Etiti, 69 and Nsukka 33, LGAs) were sampled from 38 herds (Uzo-Uwani, 3; Igbo-Etiti, 31 and Nsukka 4, LGAs) to improve clarity. Due to scarcity, each herd was selected using snowball (chain referral) sampling technique with all animals in each herd sampled. Oral consent was obtained from each participant herd owner/care taker. A total of 42 owners were approached and 38 consented (90.48% response rate).

Herd Observations, Sample Collection, Handling and Transportation: Observations made during sample collections were herd size, presence of animals with hygroma and presence of calves in the herds. Blood samples (10 ml) were aseptically collected from the jugular veins of cattle into centrifuge tubes with the important parameters recorded. The age of the animals were determined by dentition technique (Lasisi *et al.*, 2002). The blood samples were allowed to clot and transported in flasks with ice packs (4 – 8°C) to the Department of Veterinary Public Health and Preventive Medicine, University of Nigeria, Nsukka Laboratory. In the laboratory, the samples were centrifuged at 3000 rpm for five minutes, the sera decanted and stored at –20 °C until they were assayed.

Rose Bengal Test (RBT): The RBT antigen consisting of standardized *B. abortus* antigen (Animal and Plant Health Agency (APHA), Surrey KT15 3NB, United Kingdom) was used to conduct the test employing standard (Alton *et al.*, 1988) and modified (Díaz *et al.*, 2011) methods. Briefly, the standard method involved mixing 30 µl of *Brucella abortus* S19 antigen and 30 µl test serum, while the modified method entailed mixing 25 µl of *B. abortus* S19 antigen and 75 µl test serum thoroughly on a plate using a stick applicator. The plate was then rocked for 4 minutes with the appearance of agglutination scored positive (+) and the absence negative (-).

Competitive ELISA: For economic reasons, only samples positive by the RBT were subjected to the cELISA. The cELISA sourced from Animal and Plant Health Agency (APHA), Surrey KT15 3NB, United Kingdom, was conducted according to the manufacturers' instructions and read with the ELISA plate reader. Briefly, into the ELISA plate pre-coated with *Brucella melitensis* LPS antigen, 20 µl of each test sera was added per well but wells 11 and 12 were left empty to serve as controls. A 20 µl of negative control was added to wells A11, A12, B11, B12, C11 and C12. Also, 20 µl of positive control was added to well F11, F12, G11, G12, H11 and H12. The remaining 4 wells had no serum added and acted as conjugate controls where there was no competition. The100 µl of the prepared conjugate solution gave the serum a final dilution of 1/6. The plate was initially shaken for 30 seconds followed by 10 seconds hand-shakings every 10 minutes for a total of 1 hour. The content of the plate was poured out and rinsed 5 times with washing solution and thoroughly dried by tapping on an

absorbent paper. Immediately before use the substrate and chromogen solution were prepared by dissolving one tablet of urea H_2O_2 in 12 ml of distilled water. The OPD tablet was then added and mixed thoroughly and 100 µl of this solution was added to each well. The plate was then left at room temperature for 10 minutes. A 100 µl of stopping solution was then added to all wells and the plates were read. 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 (B. Brian Scientific and Instrument Company, England, Model GM2000 SN: RE10DEC2022®). 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 scored negative

Statistical Analysis: Data collected were entered into MS Excel work sheets and analyses were performed using the statistical software packages STATA Version12 and Open Epi. Descriptive statistics was used to show the levels of seropositivity, group differences were tested using chi-square statistics for categorical variables and p-values less than α (0.05) considered significant.

RESULTS

Over all, the result of the tests showed that out of the samples tested with the standard RBT, none was positive to *Brucella* antibody. However, the individual animal prevalence of 34.78% (48/138) with the modified RBT with 45.3 (22/48) supported by cELISA and herd level prevalence of 42.10% (15/38) were obtained with the modified RBT and 39.47 (15/38) supported by cELISA.

Individual Animal Prevalence as Tested with the RBT: The location specific prevalence showed Uzo-Uwani as having the highest prevalence (38.89%) as compared to the Igbo-Etiti (33.33%) and Nsukka (33.33%) LGAs by the RBT but 39.13, 39.13 and 54.54% were supported by the cELISA respectively. In the same vein, the Muturu/Ndama mix had highest prevalence (42.86%) with 66.67% supported by the cELISA among the cattle breeds screened. The study also found the male animals as having the prevalence of 35.71% with the RBT of which only 30.00% were supported by cELISA which is slightly higher than in the females (34.55%) where 50% of the RBT positives were supported by the cELISA. The prevalence among the young animals (calves and other cattle less than the age of puberty) was found to be 37.50% each. The individual animal brucellosis prevalence was not found to be significantly associated ($p>0.05$) with any of the factors considered in the study area (Tables 1 and 2).

Table 1: Prevalence of brucellosis in individual animal of the Muturu and Ndama herds in Nsukka Agricultural Zone, Enugu State by breed, sex, age as measured by RBT

Variable	Category	RBT	
		Positive n(%)	Negative n(%)
	Prevalence n=138	48 (34.78)	90 (65.22)*
Location	Nsukka LGA	11(33.33)	22(66.67)*
	Igbo-Etiti LGA	23(33.33)	46(66.67)*
	Uzo-Uwani LGA	14(38.89)	22(61.11)*
Breed	Muturu	21(34.43)	40(65.57)*
	Ndama	21(33.33)	42(66.67)*
	Muturu/Ndama	6(42.86)	8(57.14)
Sex	Male	10(35.71)	18(64.29)*
	Female	38(34.55)	72(65.45)*
Age	Less than puberty	9(37.50)	15(62.50)*
	Young adults	15(37.50)	25(62.50)*
	≥ 2 years	24 (32.43)	50 (67.57)*

** Significantly different ($p<0.05$)*

Seroprevalence of Brucellosis in Muturu and Ndama Herds: The results of the study showed that out of the 38 herds of cattle screened, 39.47% (15/38) prevalence was recorded with the RBT but 60% of the positives were supported by the cELISA. The chi-square analysis showed the seropositivity to brucellosis to be significantly associated with herd size ($p = 0.019$) and presence of calves in the herds ($p = 0.049$) (Tables 3 and 4).

Table 2: RBT positive samples from individual animal of Muturu and Ndama herds in Nsukka Agricultural Zone, Enugu State by breed, sex, age supported by cELISA

Variable	Category	cELISA	
		Positive	Negative
	Prevalence n = 48	22(45.83)	26(51.67)
Location	Nsukka LGA	6(54.54)	5(45.46)
	Igbo-Etiti LGA	9(39.13)	14(60.87)*
	Uzo-Uwani LGA	7(50.00)	7(50.00)
Breed	Muturu	8(38.10)	13(61.90)
	Ndama	10(47.62)	11(52.38)
	Muturu/Ndama	4(66.67)*	2(33.33)
Sex	Male	3(30.00)	7(70.00)*
	Female	19(50)	19(50)
Age	Less than puberty	3(33.33)	6(66.67)*
	Young adults	7(46.67)	8(53.33)
	≥ 2 years	12(50)	12(50)

** Significantly different (p<0.05)*

Table 3: Prevalence of brucellosis in Muturu and Ndama herds in Nsukka Agricultural Zone, Enugu State as measured by the RBT

Variables	Category	RBT	
		Positive n(%)	Negative n(%)
Herds	n = 38	15 (39.47)	23 (60.53)*
Location	Nsukka LGA	2(50.0)	2(50.00)
	Uzo-Uwani	1 (33.33)	2 (66.67)*
	Igbo-Etiti	12 (39.29)	19 (60.71)
Herd size	≥11	6 (85.71)*	1 (14.28)
	≤10	9 (29.03)	22 (70.97)*
Presence of calves	Yes	12 (75.00)*	4 (25.00)
	No	3 (13.64)	19 (86.36)*
Presence of hygroma	None observed		

** Significantly different (p<0.05)*

Table 4: RBT positive samples from Muturu and Ndama herds in in Nsukka Agricultural Zone, Enugu State supported by the cELISA

Variables	Category	cELISA	
		Positive	Negative
Herds	n = 15	9(60.00)	6(40.00)
Location	Nsukka LGA	2(100.00)*	0(0.00)
	Uzo-Uwani	1(100.00)*	0(0.00)
	Igbo-Etiti	6(50.00)	6(5.00)
Herd size	≥11	2(40.00)	3(60.00)
	≤10	7(70.00)*	3(30.00)
Presence of calves	Yes	8(66.67)*	4(33.33)
	No	1(33.33)	2(66.67)*

** Significantly different (p<0.05)*

DISCUSSION

The standard method of RBT (Alton *et al*., 1988) showed no positive reactor among the cattle screened but with the modified method (Ferreira *et al*., 2003) 34.78% seropositivity to brucellosis was observed.

Such zero prevalence with standard RBT was also recorded in Muturu in Plateau State (Agada *et al*., 2017). The seronegativity of the tests observed using the standard RBT may be due to postzone effect as a result of too much antigen preventing agglutination and giving rise to false negative results (Kojima *et al*., 2018). In agglutination reactions like the RBT, the antibody being too high or too little results in prozone or postzone effects, respectively preventing reaction between the antigen and antibody (Casadevall, 2003). Thus in the present study, agglutination (zone of equivalence) occurred only when the antigen quantity was reduced (modified method RBT) but negative when the antigen and serum quantities were of equal quantities (standard RBT). Therefore, the amount of acquired antibodies in the positive animals in this study was smaller than other *Brucella* infected cattle breeds detected with the standard RBT (Asmare *et al*., 2010; Akinseye *et al*., 2016; Ogugua *et al*., 2018). The impacts of innate or constitutive immunity, in addition to acquired immunity, which has been described in vertebrates, may be responsible for the low antibody levels (Matson, 2006; Martin *et al*., 2008). Natural antibodies (NABs) play a crucial role in innate immunity against infectious pathogens by decreasing acquired immune response through epitopes masking (Baumgarth *et al*., 2000; 2005; Parmentier *et al*., 2008; Ujvari and Madsen, 2011). This is because by binding to antigens, NABs limit the quantity of free

antigens and the magnitude of the ensuing acquired response. This process is referred to as masking of epitope (Janeway *et al.*, 2005). This suggests that the animals sampled contained significant amounts of NABs. The NABs are polyspecific, and it is believed that their abundance indicates a high level of resistance to pathogenic infections (Flajnik and Rumfelt, 2000; Baumgarth *et al.*, 2005). The NABs have been reported to play a major role in the host defense against *Brucella* infections and persistence (Rolán *et al.*, 2009). Additionally, it is believed that NABs variation is heritable and cows with high levels of them were resistant to mastitis (Ploegaert *et al.*, 2010). Thus, from this study, brucellosis tolerance may be a natural trait of the Muturu and Ndama breeds. Similar findings have also been reported in other animals. High titres of NABs in chickens have been shown to result in resistance to other infectious agents (Parmentier *et al.*, 2004). In fish, levels of NABs and levels of acquired antibody generated in response to vaccines appear to be generally inversely correlated (Sinyakov *et al.*, 2002; Sinyakov *et al.*, 2006; Sinyakov and Avtalion, 2009). The masking effect of NABs was also implicated for the failure of water snakes (*Liasis fuscus*) to produce effective acquired antibody response to vaccinations (Madsen *et al.*, 2007). Presently, little is known about the reactivity of NAB of Muturu and Ndama to brucellosis. However, the Muturu and Ndama are known to be tolerant to other diseases like trypanosomosis, parasitic protozoa diseases, tick and tick-borne diseases and streptotrichosis (Adebambo, 2001) but susceptible to rinderpest (Rege *et al.*, 1994). Contrary to the findings of this study, Esuruoso and Van Blake (1972), employing a serum agglutination test, discovered that the Ndama breed of cattle had a higher frequency of brucellosis than the zebu varieties (SAT). However, differences in the tests (SAT and RBT) used in the two studies may be responsible for the observed dissimilarities. On the other hand, genetic polymorphism has been implicated as a key factor in conferring resistance or tolerance in breeds of cattle to antibody response to *Brucella* infection, which differs significantly between resistant and susceptible animals, may also account for the difference in prevalence recorded in Muturu and Ndama as compared to other breeds (Martínez *et al.*, 2010). Further research is needed on these breeds to determine the role of the 3' untranslated polymorphism in the Slc11a1 gene, which has been linked to the high genetic diversity associated with resistance and susceptibility to brucellosis shown in cattle (Barthel *et al.*, 2001).

The findings of the study showed that the Muturu and Ndama cattle herds in Nsukka Agricultural Zone, Enugu State, have a high prevalence of brucellosis. The herd prevalence recorded (48.48%) is higher than the 29.2% obtained in cattle herds in Lagos and Oyo States (Ogugua *et al.*, 2018) and the 10.3% in Plateau State (Agada *et al.*, 2017), but lower than the 77.5% reported in three Northern states (Mai *et al.*, 2012). The lack of a bovine brucellosis control policy in Nigeria could be responsible for the high prevalence found in this study (Rikin, 1988). This lack of coordinated control of the disease in the country may have resulted in generations of animals in various herds being exposed to the *Brucella* infection, particularly because most herds in the country share common pastures. These practices have been recognized as potential risk factors for *Brucella* infections in Africa (Barthel *et al.*, 2001). The seasonal movement of cattle herds by Fulani herders from the north to the south of Nigeria (Teshale *et al.*, 2006; Dinka and Chala, 2009) may also aid in the spread of brucellosis due to potential contamination of grazing grounds in the south. It has been reported that more than 70% of the migratory Fulani cattle herds in Kaduna State had at some point made a seasonal migration to the south, according to Mbuk *et al.* (2011). These infected herds contaminate grazing pastures and pose a serious risk to the indigenous herds for *Brucella* infection (Mai *et al.*, 2012).

In this study, older animals had lower levels of seropositivity to brucellosis than the young though not at a significant level. This was contrary to data from earlier studies, which found that older animals had significantly higher seropositivity than younger animals (Kubuafor *et al.*, 2000; Mai *et al.*, 2012). However, *Brucella* infections lead to chronic conditions

since they are intracellular organisms. Therefore, it is quite possible that the disease had progressed to the chronic stage in the older animals. The immunoglobulin isotopes usually present in chronic and recurrent cases of brucellosis which are the IgG2, IgG3 and IgA are difficult to diagnose with conventional serological tests (Kubuafor *et al.*, 2000; Diaz *et al.*, 2011). However, it is possible that the younger animals may have inherited antibodies from their mothers or they may be at the acute stage of the disease where the IgM and IgG1 are predominant and these immunoglobulins are easier to detect by agglutination reactions (Ismail *et al.*, 2002). The study also found having calf to be a risk factor for *Brucella* antibody presence in the herds screened. The secretions from the uterus represent a primary method of *Brucella* transmission, despite the fact that it was reported that abortions rarely occurred in the herds screened (Olsen and Tatum, 2010). It is also important to note that the herds that were screened lacked special calving pens, and since the organism is released in large quantities in infected cows during normal birth as well, areas where calving occur may be seriously contaminated and serve as sources for further transmission (Muflihanah *et al.*, 2013).

This study found herd size being large to be a risk factor for the occurrence of brucellosis ($p = 0.007$). This supports the findings of other researchers (Unger *et al.*, 2003; Dias *et al.*, 2009) who reported that large herds had a greater prevalence of brucellosis than small herds. The epizootiological rule of large herds, large incidence and small herds, low incidence (Akakpo and Bonarel, 1987) is reiterated by this work. This might be due to the fact that more animals increase the risk of coming into contact with *Brucella*-contaminated materials. This result concurs with reports from four West African countries (Unger *et al.*, 2003), Myanmar (Than, 2007), Brazil (Dias *et al.*, 2009), Uganda (Makita *et al.*, 2011) and Ethiopia (Megersa *et al.*, 2011) but contrary to the no association between herd size and seropositivity to brucellosis recorded in herds in Ogun State (Cadmus *et al.*, 2013).

Hygroma, a pathognomonic sign of brucellosis in the tropics, was not observed during the course of this study. This was contrary to reports in the (Fulani) Zebu cattle herds (Smits *et al.*, 2003; Mai *et al.*, 2012), camel (Salisu *et al.,* 2018), horse (Ocholi *et al.*, 2004), sheep (Onoja *et al.*, 2008) in Nigeria and in other African countries including other traditional cattle breeds in Togo (Verger *et al.*, 1982), Senegal (Akakpo and Bonarel, 1987), Burkina Faso (Corbel, 1997), sub-Sahara Africa (McDermott and Arimi, 2002), Benin Republic (Koutinhouin *et al.*, 2003), Gambia (Unger *et al.*, 2003), Guinea and Guinea Bissau (Unger and Münstermann, 2004), South Africa (Hesterberg *et al.*, 2008), Zimbabwe (Matope *et al.*, 2009), Mozambique (Manhica, 2010), Uganda (Kungu *et al.*, 2010) and Sudan (Madut *et al.*, 2018). In tropical areas, hygroma is a typical sign of brucellosis in livestock. Its absence in Muturu and Ndama lends support to the theory that these breeds have some sort of resistance to the disease.

There are some limitations in this study, notwithstanding all of our findings. First of all, although the calculated sample size was met, few herds were examined; more herds should have provided a more representative picture. Additionally, because Muturu breeds, females, and adults were more in number in the study area, more samples were taken from them. These numerical differences may have given the study a bias. Lastly, the animal handlers were not screened for brucellosis in order to have better insights into the sero-epidemiological situation of the disease between humans and animals at the points of sample collection. Most importantly, the study did not culture samples from these animals to confirm the disease in the herds neither did it characterize the strains responsible.

Conclusion: This study underscores the fact that there is high prevalence of bovine brucellosis among Muturu and Ndama cattle herds reared under semi-extensive management system in Nsukka Agricultural Zone, Enugu State, Nigeria. This study has also highlighted the fact that the two breeds seem to have some level of innate antibody indicating possible

resistance to brucellosis. Buttressing this is the fact that the classical clinical signs of the disease in the tropics such as hygroma were not observed in the course of the study. There is an urgent need for extensive study of the level and nature of resistance and susceptibility of the breeds to the disease both phenotypically and genotypically. Results from such studies will be useful for breeding purposes that could help to genetically control the disease thereby avoiding antibiotic use or other control methods and their consequences. In addition, there is need to isolate and characterize the *Brucella* strains present in the two breeds in the area. This will shed more light on the nature of the disease in the breeds tested. Furthermore, epidemiological studies involving brucellosis among the owners and other occupationally exposed groups are required, given the close contact between these groups and the animals.

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