

# Serological investigation of bovine brucellosis in three cattle production systems in Yewa Division, south-western Nigeria

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Limited data are available on the risk factors responsible for the occurrence of brucellosis amongst different cattle production systems in Nigeria despite its significant impact on livestock production. Consequently, a cross-sectional study was conducted to determine the prevalence of bovine brucellosis in three cattle production systems in Yewa Division of Ogun State, south-western Nigeria. A total of 279 blood samples (sedentary = 88; transhumance = 64; trade = 127) were examined for antibodies to *Brucella* sp. using the Rose Bengal test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA). Overall, 24 (8.6%) and 16 (5.7%) of the animals tested seropositive for *Brucella* using RBT and cELISA, respectively. The herd seroprevalences based on RBT and cELISA were 31.6% and 15.8%, respectively. The results using cELISA reveal higher seroprevalence in the trade cattle (7.9%; confidence intervals [CI] = 3.2% – 12.6%) and those in a sedentary system (5.7%; CI = 0.9% – 10.5%) than in cattle kept under a transhumant management system (1.6%; CI = 1.5% – 4.7%). Age (> 3 years;  $p = 0.043$ ) and breed (Djali;  $p = 0.038$ ) were statistically significant for seropositivity to brucellosis based on cELISA, but sex (female,  $p = 0.234$ ), production system (trade and sedentary;  $p = 0.208$ ) or herd size (> 120;  $p = 0.359$ ) was not. Since breeding stock is mostly sourced from trade and sedentary cattle, it is important that routine serological screening should be conducted before introducing any animal into an existing herd.

## Introduction

Brucellosis is a disease of major public health importance, causing significant economic losses (Abdou 2000) to the animal industry because of abortion and infertility, and extensive chronic morbidity in humans. Brucellosis as a zoonosis poses a serious hazard for human health worldwide (Hamidy & Amin 2002; Zinsstag *et al.* 2007). The available evidence, although incomplete, shows that bovine brucellosis is widespread (McDermott & Arimi 2002). It is a serious, debilitating disease in humans, causing fever, headaches and further complications if left untreated (Abdou 2000; Zinsstag *et al.* 2007). Whilst some countries have eliminated or substantially reduced the disease by means of extensive eradication programmes, it remains endemic in Africa in particular (Omer *et al.* 2000).

Some of the risk factors responsible for the spread of bovine brucellosis have been studied in some developing countries (Berhe, Belihu & Asfaw 2007; Dinka & Chala 2009; OIE 2011). Recent investigations based on serological studies have shown that bovine brucellosis is endemic in Nigeria (Abdou 2000; Ate *et al.* 2007; Cadmus *et al.* 2010) and that there is an apparent increase in its occurrence in the country (Junaidu, Oboegbulem & Salihu 2011; Ocholi *et al.* 2004). Previous reports have indicated that the prevalence of bovine brucellosis may be influenced by herding different species together (Bale, Nuru & Addo 1982; Junaidu, Oboegbulem & Salihu 2008; Ocholi *et al.* 2004), use of common pastures and water sources (Bertu *et al.* 2012), age (Cadmus, Adesokan & Stack 2008; Junaidu *et al.* 2011; Ocholi *et al.* 1996), breed (Cadmus *et al.* 2008; Esuruoso 1974), sex (Atsanda & Agbede 2001; Junaidu *et al.* 2011), lactation status (Junaidu *et al.* 2011) and season (Bertu *et al.* 2012; Nuru & Dennis 1976). However, the role of different cattle production systems needs to be assessed given the uncontrolled free movement of animals, especially as practised by the Fulani herdsmen, and unsupervised introduction of new animals into herds.

This study was therefore conducted to determine the seroprevalence of bovine brucellosis and identify associated risk factors in different cattle production systems in Yewa Division, south-western Nigeria.

## Materials and methods

### Study area

The Yewa Division of Ogun State (7°15'N, 3°3'E) was used for this study (Figure 1). Yewa is a town on the Nigerian border with the Republic of Benin. It is home to 60% of the cattle population

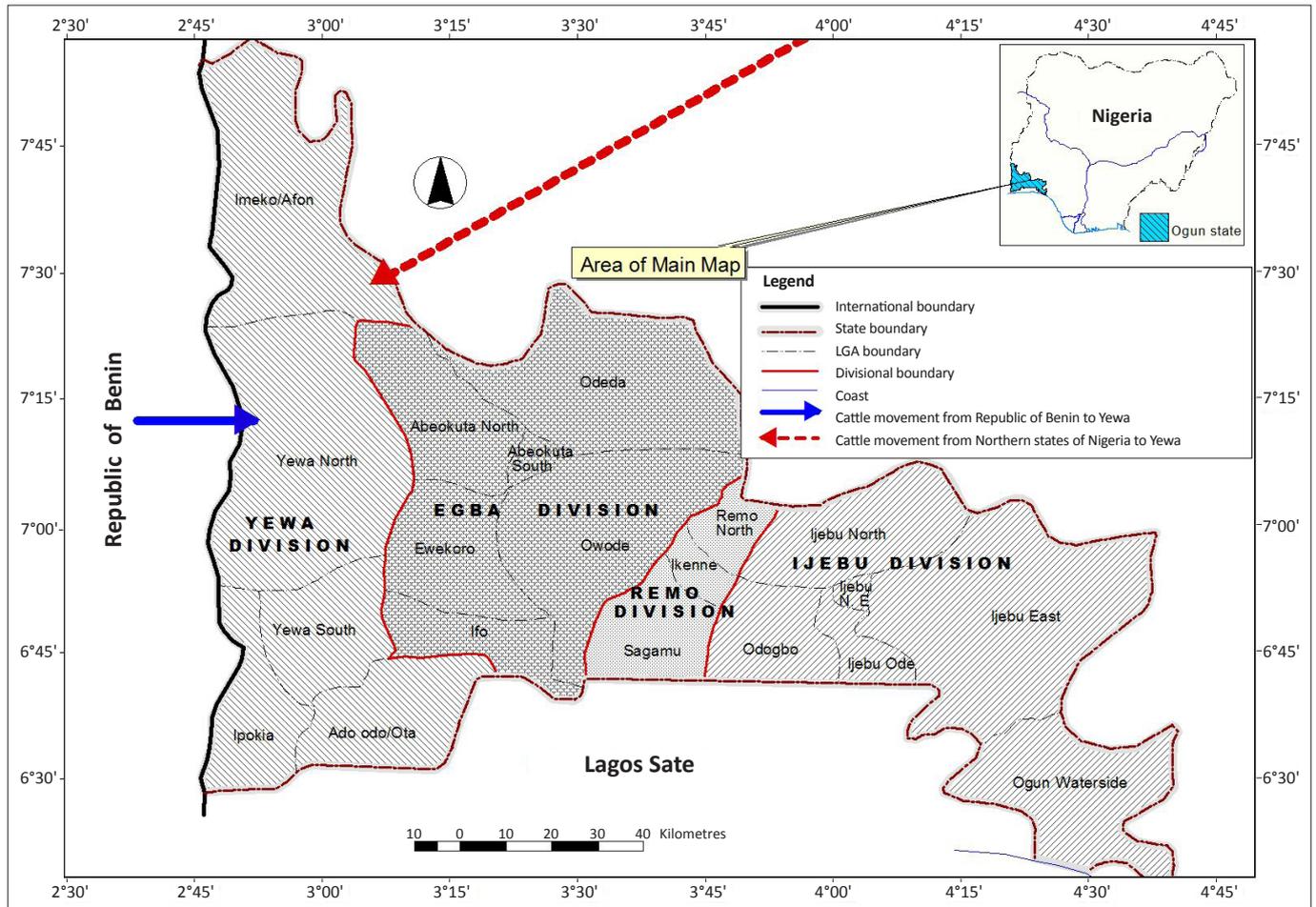


FIGURE 1: Map of Ogun State, showing different geopolitical regions.

in the state and houses a major cattle market that attracts cattle merchants from other African countries (e.g. Burkina Faso, Mali, Togo and Chad) travelling into Nigeria through the Republic of Benin. Cattle production in the region is extensive and includes sedentary, transhumant and trade cattle husbandry systems, which intermingle during grazing or at the market.

Sedentary farming is characterised by herds of cattle resident in the region for a minimum of six years. The transhumant system comprises Fulani<sup>1</sup> herds that migrate southwards from northern Nigeria in search of water and pastures during the dry season (between November and April) and hence can intermingle with resident herds at watering points. They then migrate back to the north at the beginning of the rainy season. Trade cattle comprise mainly cattle brought to the market from neighbouring West African countries that share boundaries and socioeconomic as well as cultural ties with the study site (Figure 1). These cattle are brought directly to the market for sale and hence have no contact with cattle from other production systems until they reach the market. Therefore, the simultaneous presence of cattle from the different production systems in Yewa at the time of this study provided an opportunity to determine the prevalence and other risk factors associated with brucellosis in these animals.

<sup>1</sup>The Fulani is a major tribe that deals in livestock production in Nigeria.

## Animal sampling, sample collection and handling

Herds in the sedentary and transhumant production systems were sampled, as well as trade cattle brought to the cattle market. Livestock owners in the study area were informed about the purpose of the study and its associated benefits to their operations. However, there were limitations to the number of herds/animals that could be screened, as some owners were unsure of the consequences of having their animals tested.

Every third herd from both the sedentary and the transhumant cattle production systems was randomly selected. Of the selected herds, 5% of each population was sampled (sedentary = 88; transhumance = 64). A group representing 5% of the trade cattle ( $n = 127$ ) was randomly selected at the cattle market by picking one in every 20 animals (Table 1). A blood sample of 10 mL was collected from the jugular vein from each selected animal, using a 15-mL sterile vacutainer

TABLE 1: Population size and number of animals sampled from each cattle production system in Yewa Division, Nigeria.

Production system	Cattle population	Sample size
Sedentary	1760	88
Transhumance	1280	64
Trade	2540	127
<b>Total</b>	<b>5580</b>	<b>279</b>

tube. The breed, age and sex of the animals, as well as the herd size, were recorded. History of abortion amongst the cattle in the herds was also documented.

The blood samples were allowed to clot and centrifuged at 3000 g for 5 min. Serum samples were decanted and stored at  $-20^{\circ}\text{C}$  until assay. The sera were examined for antibodies to *Brucella* spp. using the Rose Bengal test (RBT) (Alton *et al.* 1988) and the competitive enzyme-linked immunosorbent assay (cELISA) (MacMillan *et al.* 1990). Both tests were sourced from the Veterinary Laboratories Agency (Surrey, United Kingdom) and standardised according to the stipulations set by the World Organisation for Animal Health (OIE 2008). For the cELISA, some wells were used as controls for the monoclonal antibody as a basis for determining the positive/negative optical density cut-off criteria for each plate. The plates were read at 450 nm using an automated Multiscan reader (Anthos Labtech, Austria) to determine whether a sample was positive or negative. Cut-off points for positive samples were set at 60% or lower when compared with the wells containing the monoclonal antibody, as determined in a previous study (Stack *et al.* 1999).

### Statistical analysis

Data entry and analysis were performed using the statistical software package SPSS for Windows. Data were analysed to determine the association between seropositivity to *Brucella* antibodies and the different cattle production systems. Frequencies and proportions were stratified according to seropositivity and reported for individual, herd and cattle production systems. Group differences were tested using chi-square statistics for categorical variables. All variables significant at the 10% significance level were included in the multivariate logistic regression model. The odds ratios were reported with their 95% confidence intervals (CI).

### Ethical considerations

The protocols for this study were approved by the Ethics Committee, Faculty of Veterinary Medicine, University of Ibadan, Nigeria (Ethics/12/10/03).

### Potential benefits and hazards

There was no appreciable risk for the animals as only 10 mL of blood was collected from each animal. By participating in the study, owners would be aware of their animals' brucellosis status and act accordingly to minimise the spread of the disease.

### Recruitment procedures

Cattle owners' participation was voluntary. The purpose of the study was explained to the cattle owners and they were allowed to withdraw from the study without any attached penalty.

### Informed consent

The cattle owners who allowed their cattle to be sampled consented verbally as most of them could not read or write.

### Data protection

The data from the respective herds were kept separately. Herd-specific results were conveyed to the relevant owners.

## Results

### Rose Bengal test

Of the 279 serum samples examined using RBT, 24 (8.6%) were seropositive to smooth *Brucella* species. The individual and herd seroprevalences obtained were 8.6% and 31.6%, respectively. Seropositivity to *Brucella* was lowest in cattle kept under a transhumant management system (4.7%), followed by those in sedentary systems (7.9%). The highest seropositivity was detected in the trade cattle (11.0%) ( $p = 0.208$ ).

The breed-specific prevalence showed that the highest seroprevalence occurred amongst Djali cattle (20.0%;  $p = 0.038$ ) followed by that amongst Red Bororo (11.3%) and White Fulani (5.8%). The lowest seroprevalence was recorded in Sokoto Gudali (3.3%).

Higher age-specific prevalence was recorded in animals older than three years (12.5%), with this group being more than twice as likely to be seropositive for brucellosis than those younger than three years ( $p = 0.033$ ). Female animals also displayed a higher seroprevalence (11.2%) than male animals (5.5%), although the result was not significant ( $p = 0.226$ ). Female animals therefore were almost twice as likely to be seropositive to *Brucella* (Table 2).

Animals sampled from large herds showed a higher seropositivity rate than those from small herds (33.3% vs 25.0%;  $p = 0.670$ ). Cattle originating from herds larger than 120 animals were approximately one and a half times more likely to be seropositive for *Brucella* than those from herds with fewer than 120 animals. A chi-square test of significance and multivariate logistic regression showed that age ( $p = 0.033$ ) and breed ( $p = 0.038$ ) were statistically significant with regard to seropositivity to *Brucella*, whilst sex ( $p = 0.226$ ), production system ( $p = 0.208$ ) and herd size ( $p = 0.670$ ) were not significant.

**TABLE 2:** Risk factors associated with seroprevalence of bovine brucellosis as measured by the Rose Bengal test and competitive enzyme-linked immunosorbent assay in three cattle production systems in Yewa Division, Nigeria.

Variable	Category	RBT		cELISA		* <i>p</i> -value
		<i>n</i>	%	<i>n</i>	%	
Prevalence	Individual prevalence	24	8.6	16	5.7	0.25
	Herd prevalence	6	31.6	3	15.8	0.45
Age	≤ 3 years	7	4.9	4	2.8	1.00
	> 3 years	17	12.5	12	8.8	
Sex	Male	7	5.5	5	3.9	1.00
	Female	17	11.2	11	7.3	
Breed	White Fulani	9	5.8	6	3.8	0.50
	Red Bororo	6	11.3	5	9.4	
	Sokoto Gudali	1	3.3	0	0.0	
	Djali	8	20.0	5	12.5	
Systemic factors	Transhumance	3	4.7	1	1.6	0.81
	Sedentary	7	7.9	5	5.7	
	Trade	14	11.0	10	7.9	

RBT, Rose Bengal test; *n*, sample size; cELISA, competitive enzyme-linked immunosorbent assay. \*, There was no significant difference in the use of the RBT and cELISA in the diagnosis of brucellosis.

**TABLE 3:** Risk factors associated with seroprevalence of bovine brucellosis as measured by competitive enzyme-linked immunosorbent assay in three cattle production systems in Yewa Division, Nigeria.

Variable	Category	Positive		Negative		Total	OR		p-value
		n	%	n	%		n	95% CI	
Age	≤ 3 years	4	2.8	138	97.2	142	-	-	-
	> 3 years	12	8.8	125	91.2	137	3.31	1.04–10.54	0.043*
Sex	Male	5	3.9	123	96.1	128	-	-	-
	Female	11	7.3	140	92.7	151	1.93	0.65–5.72	0.234
Breed	White Fulani	6	3.8	150	96.2	156	-	-	-
	Red Bororo	5	9.4	48	90.6	53	3.57	1.03–12.35	-
	Sokoto Gudali	0	0.0	30	100.0	30	1.37	1.68–5.10	0.038*
	Djali	5	12.5	35	87.5	40	-	-	-
Individual Animal tested/Herd Size†	≤ 120	1	1.9	52	98.1	53	-	-	-
	≥ 120	5	5.1	94	94.9	99	2.76	0.32–24.39	0.359
Systemic factor	Transhumance	1	1.6	63	98.4	64	-	-	-
	Sedentary	5	5.7	83	94.3	88	1.42	0.47–4.31	0.537
	Trade	10	7.9	117	92.1	127	5.38	0.67–43.48	0.112

n, number; OR, odds ratio; CI, confidence interval.

Individual animal prevalence = 5.7% (3.0%–8.4%).

Herd prevalence = 15.8% (11.5%–20.1%).

†, This did not include the trade cattle as they are not organized into herds.

\*, Risk factors statistically significant for brucellosis.

## Competitive enzyme-linked immunosorbent assay

Results from the cELISA showed the seroprevalence of individual animals and herds to be 5.7% and 15.8%, respectively. The seropositivity results for the different production systems follow a similar trend to those for RBT.

Breed-specific results show that the highest seroprevalence occurred amongst Djali cattle (12.5%;  $p = 0.038$ ). The lowest seroprevalence (zero) was recorded amongst Sokoto Gudali cattle (Table 3).

Seroprevalences according to age and sex were higher in older (8.8%;  $p = 0.043$ ) and female (7.3%;  $p = 0.234$ ) animals, following a similar trend to that of results from RBT. A similar pattern was also obtained with respect to herd size ( $p = 0.359$ ), with cattle originating from herds larger than 120 animals being approximately three times more likely to be seropositive for *Brucella* than those from herds with fewer than 120 animals. A chi-square test of significance and multivariate logistic regression show that age ( $p = 0.043$ ) and breed ( $p = 0.038$ ) were statistically significant for seropositivity, whilst sex ( $p = 0.234$ ), production system ( $p = 0.537$ ) and herd size ( $p = 0.359$ ) were not significant (Table 3). Abortion or sterility was not significantly associated with seropositivity to *Brucella* in individual animals ( $\chi^2 = 1.5$ ,  $p > 0.05$ ) (Table 4).

## Discussion

In this study, 5.7% and 8.6% seroprevalence to brucellosis were obtained as measured by cELISA and RBT, respectively. This implies that the disease is endemic amongst the cattle population in Yewa Division of Ogun State and corroborates the results of previous studies in other parts of Nigeria (Abdou 2000; Ate *et al.* 2007; Ishola & Ogundipe 2000). Although there were differences in the results obtained with the two tests, they were not statistically significant ( $p > 0.05$ ) (Table 2); RBT is, however, known to be a less specific test than cELISA (Nuru & Dennis 1976) owing to its poor ability

**TABLE 4:** Relationship between animals with history of abortion/sterility and brucellosis as measured by competitive enzyme-linked immunosorbent assay amongst the transhumant and sedentary cattle populations screened in Yewa Division, Nigeria.

Serological results	$\chi^2$ (p-value)	Abortion/sterility positive	Abortion/sterility negative	Total
Seropositive	1.49 (> 0.05)	5	11	16
Seronegative	-	25	111	136
<b>Total</b>	-	<b>30</b>	<b>122</b>	<b>152</b>

These results did not include the trade cattle.

to discriminate between antibodies from a cross-reacting organism (Biancafiori *et al.* 2000; Samartino *et al.* 1999). The observation of fewer cELISA positives than RBT positives has also been noted in another study that demonstrated higher specificity of cELISA than RBT in cattle and humans (Mainar-Jaime *et al.* 2005). It has been recommended that when using two serological tests, animals should test positive to both in any serial testing strategy (Mainar-Jaime *et al.* 2005). Therefore, the 5.7% seroprevalence obtained with the cELISA is considered valid as all the samples testing positive with cELISA also tested positive with the RBT.

Overall, our findings reveal higher seroprevalence rates in trade and sedentary cattle than in the transhumance production system, a possible epidemiological factor to be considered in the spread of the disease. The higher seroprevalence recorded amongst cattle in the trade and sedentary systems is contrary to reports that showed highest incidences in pastoral production systems in sub-Saharan Africa (Bekele *et al.* 2000; Dinka & Chala 2009; McDermott & Arimi 2002; Schelling *et al.* 2003). This may be attributed to keeping large herds of cattle with a high turnover of animals infected with *Brucella* (Musa 1995), as replacement stock was often from similar husbandry systems. Keeping flocks of other species (such as goats) in close contact with cattle herds, especially under sedentary conditions, could also have contributed to this finding.

Furthermore, the higher seroprevalence of *Brucella* infection in the sedentary system may be due to the introduction of newly purchased animals from similar husbandry systems

into existing herds without prior screening. This is often the case, as farmers' decisions in such transactions are based on the cost of the animal, with lower prices being favoured. Again, the sedentary herds used in this study had been established in the same region for more than the past six years. Given the years of existence and close contact with other potentially infected animals, it was therefore possible that *Brucella* circulated within the herds.

The difference in breed-specific prevalence is contrary to the findings of Cadmus *et al.* (2010), who showed that the breed of cattle was not significantly associated with the disease. Meanwhile, age-specific prevalence was higher in animals older than three years (8.8%) than in younger animals (2.8%), which is consistent with several reports (Bekele *et al.* 2000; Berhe *et al.* 2007; Kubuafor, Awumbila & Akanmori 2000). Sexually mature and pregnant cattle have been found to be more susceptible to infection by *Brucella* than sexually immature animals (Walker 1999). Younger animals tend to be more resistant to infection and frequently clear infections, although re-infection could occur at a later time (Radostits, Blood & Gay 1995). The higher prevalence of brucellosis in older cattle could be attributed to consistent exposure of the cattle to the infectious agent.

Similar to the findings in this study, other studies also recorded a higher seroprevalence in female animals than in male animals (Bekele *et al.* 2000; Berhe *et al.* 2007; Dinka & Chala 2009; Kebede, Ejeta & Ameni 2008; Kubuafor *et al.* 2000; Tolosa, Regassa & Belihu 2008). According to Kebede *et al.* (2008), male animals are generally kept in the breeding herd for a shorter time than female animals, thus making the chances of exposure lower for male animals. Berhe *et al.* (2007) also stated that the serological response of male animals to *Brucella* infection is limited and that the testes of serologically positive male animals were usually observed to be culture negative.

The difference in the herd sizes did not significantly affect the number of seropositive animals in this study. However, this is contrary to reports by some authors, who asserted that large herd size is one of the major risk factors for bovine brucellosis (Berhe *et al.* 2007; McDermott & Arimi 2002). It has also been reported that a large herd size increases the exposure potential when a large number of animals are in contact with each other at common feeding and watering points, with higher risk following cases of abortion (Dinka & Chala 2009). Nonetheless, the overriding factor for infection in this study may be common exposure of these animals, irrespective of the herd size at watering and feeding points, particularly during the calving period.

The findings of this study had some limitations. Firstly, the number of animals sampled was small; more samples would probably have provided better epidemiological information about each production system. Consequently, limited data were obtained regarding the roles of age, sex and breed as risk factors in each group. Secondly, there were no records of the pregnancy status of the cows screened.

This could have introduced some bias in the observed ratio of infections amongst female and male animals, as it has been noted that pregnant cattle at more than five months of gestation are more susceptible to *Brucella* infection. This is due to the preferential localisation of *Brucella* in the uterus, where allantoic fluid factors such as erythritol stimulate the growth of *Brucella* (Godfroid *et al.* 2004). Thirdly, we obtained different infection rates from the RBT and cELISA, which might be due to the unknown vaccination status of some of the animals, especially those within the trade cattle production system. Vaccination against *Brucella* spp. using *Brucella* S19, which is the vaccine available in Nigeria, is not routinely carried out. However, many of the trade cattle were from neighbouring African countries, whose practice regarding routine vaccination is not known. It is therefore possible that some of the animals were already vaccinated, resulting in the different rates found with the RBT and cELISA. Furthermore, cultures were not performed; this would have helped to confirm the true status of the animals with *Brucella* infection.

## Conclusion

This study highlighted the endemicity of brucellosis in the different cattle production systems screened in Yewa, south-western Nigeria. It also highlighted the fact that trade and sedentary cattle production systems are risk factors associated with exposure of animals to *Brucella*. As our findings show lower seroprevalence amongst transhumant cattle, it is suggested that additional vaccination efforts should be considered for this group of cattle to protect them from other infected groups. In addition, further work should be carried out using a larger sample size in order to achieve the best control measures in countries with endemic bovine brucellosis.

Finally, given the close contact of cattle herders with their animals further epidemiological studies to investigate the link between bovine and human brucellosis in the present study area are recommended. This will go a long way towards formulating strategies that will help in the control of brucellosis, given its public health implications in Nigeria and its neighbours.

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## Competing interest

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

## Authors' contribution

S.I.B.C. (University of Ibadan) was responsible for the project concept and design. P.I.A. (University of Ibadan) and H.K.A. (University of Ibadan) collected the samples and performed some of the sample processing. E.J.D. (Veterinary Laboratories Agency) and J.A.S. (Veterinary Laboratories Agency) performed some of the sample processing. All authors contributed to the writing and final approval of the manuscript.

## References

- Abdou, A.E., 2000, 'Fifty years of veterinary public health activities in the Eastern Mediterranean region', *Eastern Medical Health Journal* 6, 796–807. PMID:11794086
- Alton, G.G., Jones, L.M., Angus, R.D. & Verger, J.M., 1988, *Techniques for the brucellosis laboratory*, Institut National de la Recherche Agronomique, Paris.
- Ate, I.U., Andrew, P.I.R., Nok, J. & Tekdek, L.B., 2007, 'Seroprevalence of brucellosis in puerperal cows and its public health implications in Zaria, Northern Nigeria', *Journal of Animal Veterinary Advances* 6, 863–866.
- Atsanda, N.N. & Agbede, S.A., 2001, 'Seroprevalence of brucellosis antibodies in cattle herds and human beings in some parts of Adamawa state of Nigeria', *Sokoto Journal of Veterinary Science* 3, 34–38.
- Bale, J.O., Nuru, S. & Addo, P.B., 1982, 'Serological study of sheep and goat brucellosis in Northern Nigeria', *Bulletin of Animal Health and Production in Africa* 30, 73–79. PMID:6985296
- Bekele, A., Molla, B., Asfaw, Y. & Yigezu, L., 2000, 'Bovine brucellosis in ranches and farms in southeastern Ethiopia', *Bulletin of Animal Health and Production in Africa* 48, 13–17.
- Berhe, G., Belihu, K. & Asfaw, Y., 2007, 'Seroprevalence investigation of bovine brucellosis in the extensive cattle production system of Tigray region of Ethiopia', *International Journal of Applied Research in Veterinary Medicine* 5, 65–71.
- Bertu, W.J., Gusi, A.M., Hassan, M., Mwankon, E., Ocholi, R.A., Ior, D.D. *et al.* 2012, 'Serological evidence for brucellosis in *Bos indicus* in Nigeria', *Tropical Animal Health and Production* 44, 253–258. <http://dx.doi.org/10.1007/s11250-011-0011-2>, PMID:22086409
- Biancafiore, F., Garrido, F., Nielsen, K., Moscatti, L., Duran, M. & Gall, D., 2000, 'Assessment of a monoclonal antibody-based competitive enzyme-linked immunosorbent assay (C-ELISA) for diagnosis of brucellosis in infected and Rev. 1 vaccinated sheep and goats', *Microbiologica* 23, 399–406.
- Cadmus, S.I.B., Adesokan, H.K., Adedokun, B.O. & Stack, J.A., 2010, 'Seroprevalence of bovine brucellosis in trade cattle slaughtered in Ibadan, Nigeria, from 2004–2006', *Journal of the South African Veterinary Association* 81, 50–53. PMID:20649155
- Cadmus, S.I.B., Adesokan, H.K. & Stack, J., 2008, 'The use of the milk ring test and Rose Bengal test in brucellosis control and eradication in Nigeria', *Journal of the South African Veterinary Association* 79, 113–115. <http://dx.doi.org/10.4102/jsava.v79i3.256>, PMID:19244818
- Dinka, H. & Chala, R., 2009, 'Seroprevalence study of bovine brucellosis in pastoral and agro-pastoral areas of East Showa Zone, Oromia Regional State, Ethiopia', *American-Eurasian Journal of Agricultural and Environmental Science* 6, 508–512.
- Esuruoso, G.O., 1974, 'Brucellosis in Nigeria', *Veterinary Record* 95, 54–58. <http://dx.doi.org/10.1136/vr.95.3.54>, PMID:4217017
- Godfroid, J., Bosman, P.P., Herr, S. & Bishop, G.C., 2004, 'Bovine brucellosis', in J.A.W. Coetzee, G.R. Thomson & R.C. Tustin (eds.), *Infectious diseases of livestock*, 2nd edn., pp. 1510–1527, Oxford University Press, Cape Town.
- Hamidy, M.E.R. & Amin, A.S., 2002, 'Detection of *Brucella* species in the milk of infected cattle, sheep, goats and camels by PCR', *Veterinary Journal* 163, 299–305. <http://dx.doi.org/10.1053/tvjl.2001.0681>
- Ishola, O.O. & Ogundipe, G.A.T., 2000, 'Seroprevalence of brucellosis in trade cattle slaughtered in Ibadan, Nigeria', *Bulletin of Animal Health and Production in Africa* 48, 53–55.
- Junaidu, A.U., Oboegbulem, S.I. & Salihu, M.D., 2008, 'Seroprevalence of brucellosis in prison farm in Sokoto, Nigeria', *Asian Journal of Epidemiology* 1, 24–28. <http://dx.doi.org/10.3923/aje.2008.24.28>
- Junaidu, A.U., Oboegbulem, S.I. & Salihu, M.D., 2011, 'Serological survey of *Brucella* antibodies in breeding herds', *Journal of Microbiological and Biotechnological Research* 1, 60–65.
- Kebede, T., Ejeta, G. & Ameni, G., 2008, 'Seroprevalence of bovine brucellosis in smallholder farms in central Ethiopia (Wuchale-Jida District)', *Revue de Elevage et Médecine Vétérinaire des Pays Tropicaux* 159, 3–9.
- Kubuafor, D.K., Awumbila, B. & Akanmori, B.D., 2000, 'Seroprevalence of brucellosis in cattle and humans in the Akwapim-South district of Ghana: Public health implication', *Acta Tropica* 76, 45–48. [http://dx.doi.org/10.1016/S0001-706X\(00\)00088-7](http://dx.doi.org/10.1016/S0001-706X(00)00088-7)
- MacMillan, A., Greiser-Wilke, I., Moennig, V. & Mathias, L., 1990, 'A competition enzyme-linked immunoassay for brucellosis diagnosis', *Deutsche Tierärztliche Wochenschrift* 97, 83–85. PMID:2178906
- Mainar-Jaime, R.C., Muñoz, P.M., De Miguel, M.J., Grillo, M.J., Marin, C.M., Moriyon, I. *et al.*, 2005, 'Specificity dependence between serological tests for diagnosing bovine brucellosis in *Brucella*-free farms showing false positive serological reactions due to *Yersinia enterocolitica* O:9', *Canadian Veterinary Journal* 46, 913–916. PMID:16454384
- McDermott, J.J. & Arimi, S.M., 2002, 'Brucellosis in sub-Saharan Africa: Epidemiology, control and impact', *Veterinary Microbiology* 20, 111–134. [http://dx.doi.org/10.1016/S0378-1135\(02\)00249-3](http://dx.doi.org/10.1016/S0378-1135(02)00249-3)
- Musa, M.T., 1995, 'Brucellosis in Darfur states: The magnitude of the problem and methods of diagnosis and control', PhD thesis, Faculty of Veterinary Medicine, University of Khartoum.
- Nuru, S. & Dennis, S.M., 1976, 'Abortion and reproductive performance of cattle in Northern Nigeria: A questionnaire survey', *Tropical Animal Health and Production* 8, 213–219. PMID:996934
- Ocholi, R.A., Ezeokoli, C.D., Akerejola, O.O. & Saror, D.I., 1996, 'Use of enzyme-linked immunosorbent assay for screening cattle for *Brucella* antibodies in Nigeria', *Veterinary Quarterly* 18, 22–24. <http://dx.doi.org/10.1080/01652176.1996.9694606>, PMID:8833608
- Ocholi, R.A., Kwaga, J.K.P., Ajogi, I. & Bale, J.O., 2004, 'Phenotypic characterization of *Brucella* strains isolated from livestock in Nigeria', *Veterinary Microbiology* 103, 47–53. <http://dx.doi.org/10.1016/j.vetmic.2004.06.012>, PMID:15381265
- OIE, 2008, *Manual of diagnostic tests and vaccines for terrestrial animals*, World Organisation for Animal Health, Paris.
- OIE, 2011, *Manual of diagnostic tests and vaccines for terrestrial animals*, World Organisation for Animal Health, Paris, viewed 10 July 2012, from [http://www.oie.int/fileadmin/Home/eng/Health\\_standards/tahm/2.04.03\\_BOVINE\\_BRUCCELL.pdf](http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.04.03_BOVINE_BRUCCELL.pdf)
- Omer, K., Holstad, G., Skjerve, E., Woldehiwet, Z. & MacMillan, A.P., 2000, 'Prevalence of antibodies to *Brucella* spp. in cattle, sheep, goats, horses and camels in the State of Eritrea; influence of husbandry system', *Epidemiology and Infection* 125, 447–453. <http://dx.doi.org/10.1017/S0950268899004501>, PMID:11117970
- Radostits, O.M., Blood, D.C. & Gay, C.C., 1995, *Veterinary Medicine: A textbook of the diseases of cattle, sheep, pigs, goats and horses*, Baillière Tindall, London.
- Samartino, L., Gall, D., Gregoret, R. & Nielsen, K., 1999, 'Validation of enzyme-linked immunosorbent assays for the diagnosis of bovine brucellosis', *Veterinary Microbiology* 70, 193–200. [http://dx.doi.org/10.1016/S0378-1135\(99\)00122-4](http://dx.doi.org/10.1016/S0378-1135(99)00122-4)
- Schelling, E., Diguimbaye, C., Nicolet, J., Boerlin, P., Tanner, M. & Zinsstag, J., 2003, 'Brucellosis and Q-fever seroprevalences of nomadic pastoralists and their livestock in Chad', *Preventive Veterinary Medicine* 61, 279–293. <http://dx.doi.org/10.1016/j.prevetmed.2003.08.004>, PMID:14623412
- Stack, J., Perrett, L., Brew, S. & MacMillan, A., 1999, 'Competitive ELISA for brucellosis suitable for testing poor quality samples', *Veterinary Record* 145, 735–736. PMID:10972112
- Tolosa, T., Regassa, F. & Belihu, K., 2008, 'Seroprevalence study of bovine brucellosis in extensive management system in selected sites of Jimma Zone, Western Ethiopia', *Bulletin of Animal Health and Production in Africa* 56, 25–37. <http://dx.doi.org/10.4314/bahpa.v56i1.32823>
- Walker, R.L., 1999, '*Brucella*', in C.H. Dwight & Z.Y. Chunge (eds.), *Veterinary Microbiology*, pp. 196–203, Blackwell Science, Massachusetts.
- Zinsstag, J., Schelling, E., Roth, F., Bonfoh, B., Savigny, D. & Tanner, M., 2007, 'Human benefits of animal interventions for zoonosis control', *Emerging Infectious Disease* 13, 527–532. <http://dx.doi.org/10.3201/eid1304.060381>, PMID:17553265