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Risk factors associated with brucellosis among slaughtered cattle: Epidemiological insight from two metropolitan abattoirs in Southwestern Nigeria

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ABSTRACT

Objective: To investigate risk factors responsible for the epidemiology of brucellosis among cattle slaughtered in Nigeria in a bid to implement control strategies.
Methods: This was a cross-sectional and sero-epidemiological survey of bovine brucellosis in two metropolitan abattoirs in Southwestern Nigeria. Between March and August 2013, cattle were screened for antibodies to *Brucella* spp. by using Rose Bengal test (RBT), and positive samples were subjected to competitive ELISA (cELISA). Parameters of individual animal were also obtained. Data were analyzed by using STATA version 12 and *Chi*-square; and logistic regression statistics were used to test association.
Results: Overall, 2 480 cattle (1 241 in Oyo; 1 239 in Lagos) were screened. Analysis using RBT revealed a total sero-prevalence of 4.9% (121/2 480), with 7.8% and 1.9% from Oyo and Lagos States respectively. The cELISA result supported 77.7% (94/121) (90.7% in Oyo; 25.0% in Lagos) of the total RBT positive samples. Logistic regression analysis showed that only sex ($P \leqslant 0.001$) and location ($P = 0.001$) of animal screened had statistically significant effects on seropositivity to *Brucella abortus* antibodies.
Conclusions: Our findings reveal low sero-prevalence of brucellosis among slaughtered cattle in Southwestern Nigeria. Sex and location of abattoirs where animals are slaughtered are major risk factors to be considered in the epidemiology of the disease. Therefore, to promote public health, trade cattle meant for slaughter in Nigeria and African countries where brucellosis is endemic, should be monitored, and positive animals be excluded from the food chain.

1. Introduction

Brucellosis is one of the most important and widespread zoonoses in the world[1]. It is a major livestock disease, especially in developing countries and largely a reproductive disease, characterized by abortion, retained foetal membranes and impaired fertility[2-5]. Bovine brucellosis is caused primarily by *Brucella abortus*, which comprises nine serotypes and a number of variant strains[6-8]. Infection of animals with brucellosis could result in huge economic losses due to decreased calving percentage, delayed calving, culling for infertility, cost of treatment, decreased milk production, abortion, stillbirth, birth of weak calves and loss of man-hours among infected people[8-10].

Bovine brucellosis is distributed throughout the world, but has been eradicated from livestock populations of most European countries, Japan, Canada and the United States of America[11-13]. However, it remains widely distributed in developing countries. Its occurrence and prevalence have been documented in various countries in Africa, Asia and South America. A high prevalence was reported in Uganda[14], Egypt, Sudan and Senegal[15], with moderate prevalence rates recorded in Tanzania, Zambia, Ghana and Botswana[16], while low prevalence was reported in Kenya[17], Djibouti and Eritrea[18,19].

In Nigeria, the problem of brucellosis in livestock has been documented by various authors[20-29], with evidence to show that it has greatly militated against livestock productivity, with accompanying huge economic losses. In the past, sero-prevalence ranging from 0.20% to 79.70% were reported in different regions of the country[29-32]. Recently, different infection rates have been reported in various abattoirs in Nigeria: 7.08% in Ibadan[33], 8.60% in Lagos (both in Southwestern Nigeria)[34], and 20.00% in Zamfara State, Northwestern Nigeria[35]. Cadmus *et al*. also reported a sero-prevalence of 5.82% among trade cattle in Ibadan, Southwestern Nigeria while a herd sero-prevalence of 84.60% was reported by Mai and co-workers in Northern

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Nigeria[29,36].

Majority of animals slaughtered in Southwestern Nigeria are sourced from northern region of the country and other neighboring African countries where there are no animal health programmes including control of brucellosis[29]. Therefore, abattoir workers and the general public are at risk of exposure to several zoonotic diseases including bovine brucellosis.

In Nigeria, like most other developing countries, risk factors for transmission of *Brucella* infection to humans abound among livestock handlers (especially abattoir workers) who are engaged in unhygienic practices[37]. Though inhalation is a major route of infection among livestock workers[38], majority of them do not wear personal protective equipment. Thus, they get infected through uncovered wounds, while others get infected by handling wasted fetuses with bare hands and more precarious is the habit of eating raw meat[39].

Available information shows that most studies on bovine brucellosis among slaughtered cattle in Southwestern Nigeria are old[27,39,40,41], and others are limited in scope and often involved epidemiological reports from abattoirs with very few population/sample size of animals slaughtered[34,37]. Therefore, to provide better insight into the disease in Southwestern Nigeria where majority of trade animals are slaughtered, we investigated the epidemiological factors responsible for the disease in two major metropolitan abattoirs in the region. The main objectives were to determine sero-prevalence of the disease as well as the disease prevalence in relation to breed, sex, body score and age of animals in the two abattoirs.

2. Materials and methods

2.1. Selection of study sites and duration of study

The states selected (*i.e.* Oyo and Lagos States) for this study are the two most populated ones in Southwestern Nigeria where the two largest abattoirs are operated due to the very high demand for meat.

2.2. Oyo State

Oyo State has a total land area of 28454 km^2 lying in latitude 8°00' N and longitude 4°00' E. The average daily temperature in the state ranges between 25 °C and 35 °C, almost throughout the year. It has a population of 5.2 million people[42]. The main abattoir in Oyo State is the Bodija municipal abattoir, and it is located in Ibadan north local government area of the state. The abattoir was established in 1972 to serve the entire Ibadan metropolitan city and its environs, with a daily slaughter capacity of 200–250 cattle.

2.3. Lagos State

Lagos State lies between latitude 6°20' N and longitude 2°45' E to 4°20' E. It occupies a total geographical area of about 3475.1 km^2. About 787 km^2 or 22% of Lagos territory is made up of water. The maximum temperature ever recorded in Lagos was 37.3 °C and the minimum was 13.9 °C. It has a population of 9.5 million people according to the 2006 population census[42]. The Oko–Oba, Agege abattoir is the main abattoir in Lagos State and it is located in Agege local government area of the state. Though commissioned in 1992, Oko–Oba abattoir is the largest abattoir in Southwestern Nigeria and presumably the largest in the country with a daily slaughter capacity of 800–1000 cattle per day.

The entire study was conducted between March and August 2013.

2.4. Animals screened, sampling and sample collected

Animals slaughtered in Bodija and Oko–Oba abattoirs were mostly sourced from Northern Nigeria and neighboring African countries of Cameroon, Chad, Niger, Bukina Faso, Mali and Libya. The animals were generally from herds that were extensively managed, assumed to be unvaccinated (since vaccination is not routinely practiced against brucellosis in Nigeria and poor vaccination history exists in most of the neighboring African countries) and with limited or no veterinary care.

A systematic random sampling technique was used for animal selection. By using a sampling fraction of 20%, every fifth animal was sampled. Daily, the first animal was selected by balloting from one to five. Relevant information such as breed, age, body score and sex of each sampled cattle was recorded. Animals' body conditions were scored based on four categories, namely, good, moderate, emaciated and highly emaciated according to van Niekerek[43].

2.5. Sample processing and laboratory tests

For each animal, approximately 5 mL of blood was collected in 15 mL sterile tubes during slaughter. Blood samples were allowed to clot and centrifuged at 800 r/min for 5 min. Serum samples were decanted and stored at -20 °C until they were assayed. Serum samples were examined by the Rose Bengal test (RBT) according to Alton *et al.*[44], and positive samples were further screened by using the competitive ELISA (cELISA) as described by Stack *et al.*[45]. The RBT and cELISA reagents were sourced from Veterinary Laboratories Agency, Surrey KT15 3NB, UK.

2.6. Statistical analysis

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

3. Results

3.1. Rose Bengal test

Over the period, a total of 2480 cattle were screened (1241 from Oyo State and 1239 from Lagos State) with the respective serum samples from the two locations. An overall sero-prevalence of 4.9% was recorded by RBT. About 55.2% of the animals were of Bunaji breed, almost two-third (61.9%) were female and 97.3% were adult. The body score results revealed that 46.0% of the animals screened were emaciated and 54.0% had moderate body condition score (Table 1).

Analysis with RBT revealed that 80.2% of the seropositive animals were from Oyo State, while only 19.8% were from Lagos State (Table 2). The breed-specific sero-prevalence revealed that the highest sero-prevalence was reported among Kuri (15.0%), as compared with other breeds of cattle like Bunaji (5.1%), Rahaji (4.3%), Sokoto Gudali (7.4%), Adamawa Gudali (11.1%) and mixed breed (3.4%) (Table 3). In addition, older animals (5.0%) had higher sero-prevalence compared with younger ones (1.5%), while sex-specific result showed higher sero–prevalence among female animals (6.4%) in contrast to the males (2.4%) (Table 3).

The bivariate analysis identified location (OR = 4.3; 95% *CI*: 2.73–6.76), sex (OR = 2.7; 95% *CI*: 1.73–4.35) and body condition (OR = 2.2; 95% *CI*: 1.51–3.24) as factors associated with seropositivity to *Brucella* infection among the slaughtered cattle.

However, factors such as breed and age were not significantly associated ($P > 0.05$) with positivity to bovine brucellosis. Analysis using logistic regression showed that location ($P = 0.001$) and sex ($P = 0.001$) of cattle were the only significant risk factors associated with seropositivity of cattle to *Brucella* species antibodies. Again, our result revealed that cattle slaughtered in Oyo State are 3.7 times more likely to be seropositive to *Brucella* species antibodies when compared to those from Lagos State (OR = 3.7; 95% *CI*: 2.22–6.25) (Table 4).

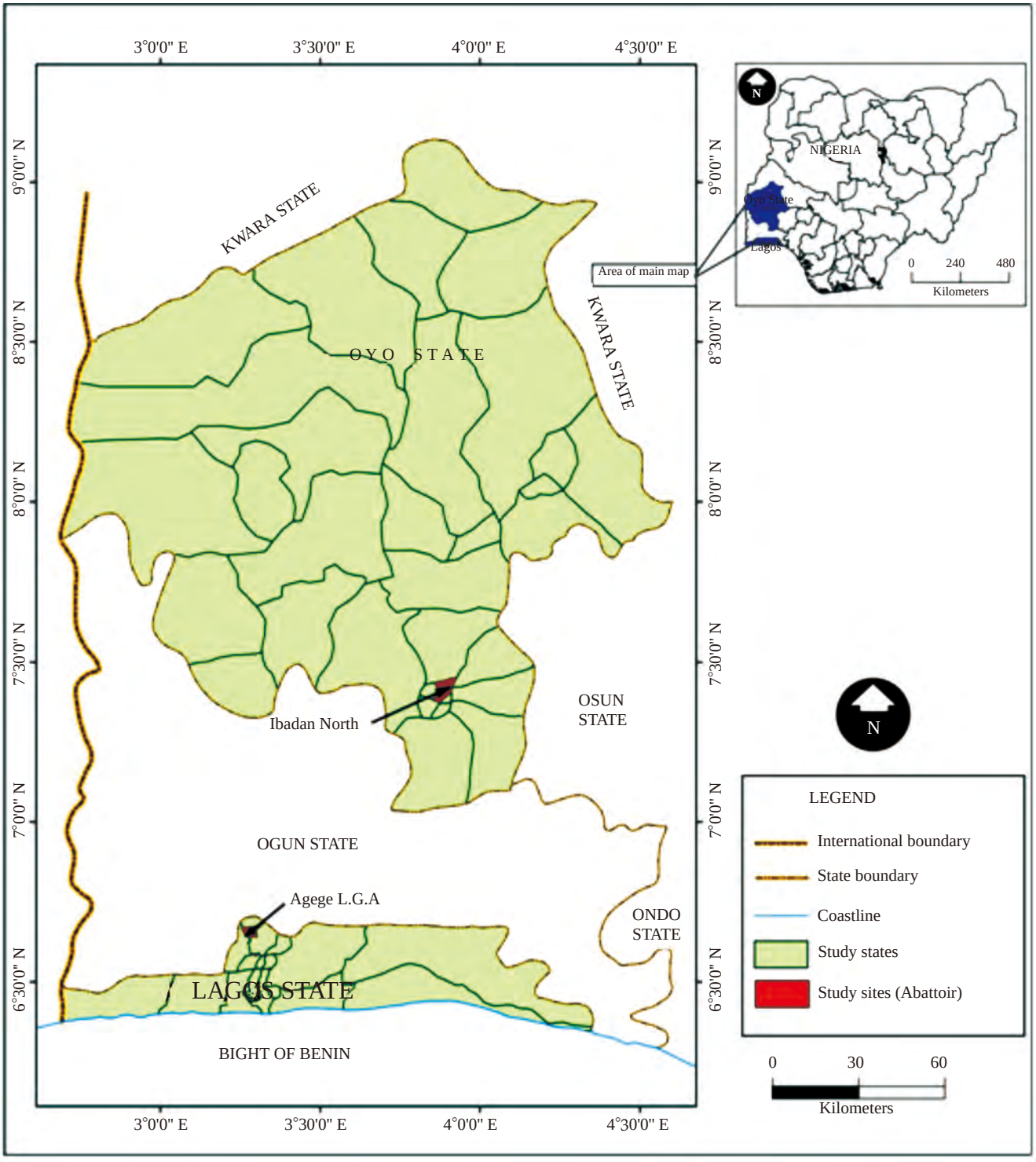

Figure 1. Map of Nigeria showing the states and local government areas in Southwestern Nigeria where the study was carried out.

Table 1
Distribution of cattle screened according to location, breed, sex, body score and age. *n* = 2480.

Variables	Category	Frequency	Percent
Location	Oyo	1241	50.1
	Lagos	1239	49.9
Breed	Bunaji	1368	55.2
	Rahaji	705	28.4
	Sokoto Gudali	54	2.2
	Adamawa Gudali	36	1.4
	Kuri	20	0.8
	Mixed	297	12.0
Sex	Male	946	38.2
	Female	1534	61.8
Body condition score	Moderate	1339	54.0
	Emaciated	1141	46.0
Age	Adult	2413	97.3
	Young adult	67	2.7

Table 2
Factors associated with sero-prevalence of brucellosis among slaughtered cattle tested in two abattoir locations in Nigeria.

Variables		*Brucella* infection [*n*(%)]		OR	95% *CI*	*P*
		Positive	Negative			
Location	Oyo	97 (80.2)	1144 (48.50)	4.3	2.73–6.76	0.00
	Lagos	24 (19.8)	1215 (51.50)			
Breed	Bunaji	70 (57.9)	1298 (55.00)			
	Rahaji	30 (24.7)	675 (28.60)	0.8	0.53–1.27	0.45
	Sokoto Gudali	4 (3.3)	50 (2.10)	1.4	0.52–4.22	0.67
	Adamawa Gudali	4 (3.3)	32 (1.40)	2.3	0.79–6.73	0.22
	Kuri	3 (2.5)	17 (0.72)	3.3	0.94–11.4	0.14
	Mixed	10 (8.3)	287 (12.20)	0.6	0.33–1.27	0.26
Sex	Male	23 (19.0)	923 (39.10)			
	Female	98 (81.0)	1436 (60.90)	2.7	1.73–4.35	0.00
Body condition score	Moderate	43 (35.5)	1296 (54.90)			
	Emaciated	78 (64.5)	1063 (45.10)	2.2	1.51–3.24	0.00
Age	Adult	120 (99.2)	2293 (97.20)			
	Young adult	1 (0.8)	66 (2.80)	3.2	0.38–26.67	0.26

Table 3
Sero-prevalence of brucellosis in cattle screened as measured by RBT and cELISA. *n* (%).

Variables	Category	RBT positive	Positive by cELISA and RBT
Overall		121 (100.0)	94 (77.7)
Locations	Oyo	97 (7.8)	88 (90.7)
	Lagos	24 (1.9)	6 (25.0)
Breed	Bunaji	70 (5.1)	56 (80.0)
	Rahaji	30 (4.3)	19 (63.3)
	Sokoto Gudali	4 (7.4)	4 (100.0)
	Adamawa Gudali	4 (11.1)	4 (100.0)
	Kuri	3 (15.0)	2 (66.7)
	Mixed breed	10 (3.4)	9 (90.0)
Sex	Male	23 (2.4)	11 (47.8)
	Female	98 (6.4)	83 (84.7)
Body condition score	Moderate	43 (1.7)	28 (65.1)
	Emaciated	78 (3.2)	66 (84.6)
Age	Adult	120 (5.0)	94 (78.3)
	Young adult	1 (1.5)	0 (0.0)

3.2. Competitive ELISA

Out of the 121 positive samples by RBT, 94 (77.7%) were also positive by cELISA. In the two locations, 88 (90.7%) and 6 (25.0%) of the RBT positive samples respectively, further tested positive when subjected to cELISA (Table 3). Again, going by the results obtained from the different breeds using RBT, among Sokoto Gudali and Adamawa Gudali, there was 100% agreement between RBT and the cELISA, while 80% and 63% out of the RBT positive samples were also tested positive by cELISA for Bunaji and Rahaji respectively. Furthermore, 84.6% of the female samples and 47.8% of the males that were initially positive by RBT were also tested positive by cELISA (Table 3).

Table 4
Multivariable logistic regression analysis of factors associated with seroprevalence of brucellosis among slaughtered cattle tested in the two abattoirs in Nigeria.

Variables		OR	95% *CI*	*P*
Location	Oyo	3.7	2.22–6.25	0.001
	Lagos	1.0		
Sex	Male	1.0		
	Female	3.3	1.75–6.35	0.001

4. Discussion

Our findings reiterate the fact that brucellosis is prevalent at a moderately low level (4.9%) among slaughtered cattle in Southwestern Nigeria. Therefore, there is an indication that the disease is prevalent in areas where the animals are sourced in Northern Nigeria and neighboring African countries[33,46-49]. Again, given the lack of official policy on the control of brucellosis in Nigeria, its transmission is worsened by unrestricted movement of animals, a factor that has contributed to the circulation of *Brucella* in herds as well as trade cattle in the country[29,50]. Accordingly, livestock sourced from high–risk areas with brucellosis, portends great risk of transmission to areas to which it is imported[51]. Cumulatively therefore, majority of poorly performing and infected animals sourced from various herds in Northern Nigeria and African countries end up in abattoirs in Southwestern Nigeria where the demand for beef is high[52].

The prevalence of brucellosis recorded in this study is similar to previous studies in other parts of Nigeria[53-55], although lower than the 14.0% and 5.8% earlier reported by Cadmus *et al.*[29,37], in the same area in Southwestern Nigeria. However, our current finding is relatively higher in comparison to earlier reports from other developing countries: 3.95% in Pakistan[56], 2.90% in Ethiopia and 2.21% reported in South Africa[57,58]. These variations may be due to differences in management practices, geographical divergence, sources of animals, sampling techniques, individual differences in interpretation of tests and the number of animals sampled[36,55].

Importantly, our findings showed a significant ($P = 0.001$) and higher sero-prevalence of brucellosis in slaughtered cattle in Oyo State (7.8%), compared to Lagos State (1.9%). A plausible reason for this could be the fact that more female animals are slaughtered in Oyo State than in Lagos. Earlier reports have shown that female cattle are more susceptible to *Brucella* infection and represent a greater risk of spreading the disease than males[51,59,60]. Also, livestock farmers in Africa do not often sell off their female animals unless they are not doing well[50]. Therefore, reproductive failure and low milk production, the most important indicators of "not doing well" in female animals, are the primary clinical signs of brucellosis. Thus, it can be inferred, that most female animals slaughtered in Nigerian abattoirs (especially southwestern region) are those of little or no economic values to the farmers, unlike the male

animals that are mostly reared for beef[48]. However, most male animals are in excellent body conditions and costlier in the market. Therefore, it requires good financial standing for butchers to slaughter male animals. Incidentally, such butchers are relatively in higher abundance in Lagos (the commercial capital in Nigeria) than in Oyo State which has less affluent butchers. Furthermore, another possible reason that could be adduced for the detection of more seropositive animals in Oyo State is the fact that more emaciated female animals are slaughtered in Oyo State, compared to Lagos State. In addition to this, body condition of animals can be directly linked to their immunity and/or health status. Since brucellosis is chronic in nature, emaciation in animals could be a result of existence of an underlying chronic disease like brucellosis. This becomes statistically important since the bivariate analysis revealed an association between the body score and seropositivity (P = 0.001); however, the logistic regression revealed no such association.

The variation observed in breed–specific prevalence in this study is consistent with that reported by Cadmus *et al.*[55], which pointed out that breed of cattle was not significantly associated with risk of infection with brucellosis. In addition, our findings recorded the highest breed–specific prevalence among the Kuri breed of cattle. This is, however, in contrast to the findings of Cadmus *et al.* and Junaidu *et al*. where the highest prevalence was reported in Bunaji, Rahaji and Sokoto Gudali breed of cattle respectively[55,61,62]. One of the factors implicated in conferring resistance or tolerance of breeds of cattle to diseases, is the genetic factor. In cattle, variation in resistance and/or susceptibility of animals to brucellosis has been associated with the 3' un–translated polymorphism in the *SLC 1A1* gene[63,64], which still requires elucidation in Nigerian cattle.

From our findings, age–specific sero–prevalence of brucellosis was higher in animals older than three years (5.0%) than younger ones (1.5%), a result similar to earlier reports[59,65,66]. Coincidentally, majority (97.3%) of animals sampled in this study were older than three years. Again, sexually mature and pregnant cattle have been found to be more susceptible to *Brucella* infection than the sexually immature ones[67]. Younger animals tend to be more resistant and frequently clear infections, although re–infection could occur at a later time[68]. The higher prevalence of brucellosis in older cattle could be attributed to persistent exposure to infectious agents. Conversely, a study by Matope *et al.* reported higher sero–prevalence in younger (< 4 years) animals[69], and they posited that older animals may not exhibit a detectable level of antibody to *Brucella* infection, which is a common feature in chronic disease.

Consistent with our findings that revealed significantly (P = 0.001) higher sero–prevalence in female animals (6.4%) than males (2.4%), reports from other investigators also showed similar observations between female and male animals[46,59,67,69,70]. Possible reasons attributed for this include the fact that male animals are generally retained in breeding herds for shorter periods than females[71]; thus reducing the likelihood of exposure for male animals. Again, Berhe and co–workers reported that immunological response of male animals to *Brucella* infection is limited[59].

Despite the findings from our study, some limitations were observed. First, sample collection was restricted to six months of the year, thus this may not have reflected the overall picture of the disease burden in the study area. Second, sample size of some of the cattle breeds encountered in the study was relatively smaller in comparison to the more popular breeds at the two abattoirs. Third, more female animals were encountered in Oyo State, while in Lagos, more males were seen. Overall, these factors may also have created some bias with respect to the sero–prevalence rates in relation to breed, sex and age of animals screened. Furthermore, we reported different infection rates based on the RBT and cELISA results. This disparity may not be unconnected with lack of information regarding the vaccination status of the animals since vaccination is not routinely carried out in Nigeria. Again, many of the trade cattle screened were from neighbouring African countries, whose practice regarding routine vaccination against brucellosis is not known. More importantly, another contributory factor to this disparity could be linked to the fact that the cELISA kits used in this study were based on the manufacturer's instructions, but were never validated under local conditions. Hence, cut-off points established in brucellosis-free and good hygienic conditions cannot be extrapolated to endemic areas[72]. Lastly, culture was not performed. This would have validated the serological assays and confirmed the true status of brucellosis among animals screened.

This study highlights the endemicity (though at a moderate prevalence) of brucellosis among slaughtered cattle in two important metropolitan abattoirs in Southwestern Nigeria. Again, it shows that female animals and location of abattoirs where trade animals are slaughtered are key risk factors responsible for the epidemiology of bovine brucellosis in Nigeria. This is evident from the statistically significant association between seropositivity and sex, and significantly higher seropositivity recorded in Bodija abattoir, Oyo State in comparison to the Oko–Oba abattoir, Lagos State. Therefore, to promote the control and eradication of brucellosis in Nigeria, more attention and efforts should be directed at vaccination of young animals (particularly the females) and separation of clean and infected animals destined for the food chain. This can be achieved through active government intervention in the areas of regulations/monitoring of trans–border animal movement within and across Nigeria. In addition, policies concerning routine screening of cattle and other small ruminant populations, including trade animals destined for slaughter at abattoirs in Nigeria, should be encouraged. Finally, we advocate coordinated awareness programmes involving stakeholders in the livestock industry as well as consumers, in order to safeguard public health and avert economic losses associated with brucellosis in Nigeria and other African countries where brucellosis is endemic.

Conflict of interest statement

We declare that we have no conflict of interest.

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