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ORIGINAL RESEARCH

Serological evidence for brucellosis in *Bos indicus* in Nigeria

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Abstract
Purpose Nigeria is the largest cattle-rearing nation in Africa with most animals kept under traditional husbandry practices. While bovine brucellosis does not receive much attention, a relatively high seroprevalence is found in samples submitted for laboratory testing. The aim of the study was to provide serological evidence of brucellosis in cattle from some of the main cattle-rearing states of the country and to validate a simple and rapid field test for the serodiagnosis of bovine brucellosis.
Method Serum samples collected in various states of Nigeria from cattle because of suspicion of brucellosis were investigated in the Rose Bengal plate test, and results were compared with a newly developed rapid field test for the detection of *Brucella*-specific antibodies.
Results Serological evidence for the presence of brucellosis in cattle was obtained for all states included in the study and a high herd prevalence was observed. The seroprevalence was also high among trade and slaughter animals. Results of a rapid field test for the serodiagnosis of bovine brucellosis correlated well with the Rose Bengal plate test (agreement, 95.7%; kappa value, 0.80).
Conclusions The results indicate that bovine brucellosis is an important veterinarian problem in Nigeria. The easy-to-use and robust field test is most promising for field-based surveillance as it provides an immediate result allowing the prompt instigation of control measures.

Keywords Zoonoses · Disease surveillance · Disease control · Livestock · Transmission · Brucellosis

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Introduction

The prevalence of brucellosis is highest in the Mediterranean area, the Middle East, and Central Asia (Pappas et al. 2006). Bovine brucellosis has recently been reported from different countries in Africa including Ethiopia, Zambia and Zimbabwe, indicating that the disease is present throughout the continent (Chimana et al. 2010; Matope et al. 2011; Megersa et al. 2011; Tesfaye et al. 2011). Bovine brucellosis was also reported from two of Nigeria's neighbour countries, Chad and Cameroon (Schelling et al. 2003; Scolamacchia et al. 2010; Bronsvoort et al. 2009; Bayemi et al. 2009). However, detailed information on the prevalence of the brucellosis is still lacking for most African countries. Such information is essential for the development of control measures aimed to improve productivity (Zinsstag et al. 2005).

Nigeria is the largest cattle-rearing nation on the African continent, and with the vast majority of the cattle

population of over 14 million reared under traditional husbandry practices, improvement of productivity has become a major challenge to meet the increasing demand for meat and dairy. The country does not have a brucellosis control programme in place, and epidemiological information is not available. Here, we summarize seroprevalence data on bovine brucellosis in samples submitted for testing because of suspicion of brucellosis from several of the main cattle-rearing states. Moreover, we examined the use of an easy-to-use rapid field test for bovine brucellosis described by Abdoel et al. (2008).

Methods

Study groups

All samples originated from states in the northern and central parts of the country and were submitted by local veterinarians to the National Veterinary Research Institute (NVRI). The NVRI is the national reference centre for brucellosis in Nigeria. As no control policy for brucellosis exists, samples are submitted for laboratory testing on an individual basis and on the initiative of either the farmer or the veterinarian when brucellosis is suspected. Because of the large distances involved and the poorly organized transportation systems, the number of samples submitted is generally low and in most cases from nearby states only.

The samples investigated consisted of two groups. Group 1 samples (N=1.234) were received between 2004 and 2009 from different states without further epidemiological information. Group 2 (N=1.367) had been received in 2009, and for these samples, epidemiological data could be retrieved after contacting the local veterinarian. Group 1 samples had been received from Nassarawa state in 2004 (N=16 samples); Adamawa state in 2004 (N=8), 2008 (N=26) and 2009 (N=40); Plateau state in 2005 (N=15), 2007 (N=22) and 2009 (N=52); Kano state in 2008 (N=36 from Kumbotso province; N=15 from Kano province); Federal Capital Territory of Abuja in 2008 (N=80); Bauchi state in 2008 (N=230) and 2009 (N=217); Niger state in 2005 (N=176), 2006 (N=65) and 2008 (N=176); and Taraba state in 2009 (N=60). Group 2 samples had been collected during an investigation for brucellosis from production cattle in eight neighbouring villages (Butalawa, Dalili, Guraza, Gidan Dankauye, Rakauna, Kosawa, Dokau A and Dokau B) in Kano state (N=977 animals; N=93 herds) and in one village (Ibi) in Taraba state (N=65 animals; N=9 herds), at slaughterhouses in three villages (Ibi, Wakuri and Jalingo) in Taraba state (N=119) and from three herds kept for trade and slaughter purposes in three villages (Gboko, Katsina Ala and Makurdi) in Benue state (N=206). Information on the sex and breed for the group 2 samples is presented in Table 1.

Laboratory testing

The Rose Bengal plate test (RBPT) was performed by mixing on a ceramic tile 30-μl serum with one drop Rose Bengal antigen (Veterinary Laboratory Agency, UK, Alton et al. 1988). Agglutination was read after 4 min.

The rapid field test (Royal Tropical Institute, the Netherlands) was performed by spotting 5 μl serum and 130 μl test fluid onto the sample pad of the plastic assay device (Abdoel et al. 2008). Results were read after 10–15 min by visual inspection for staining of the test and control line.

The RBPT had been performed on all groups 1 and 2 samples, and the rapid test was performed for all group 2

Table 1 Sex and breed of production and trade and slaughter animals tested for brucellosis from different states

		No. of animals from the following states and kept for the following purpose (percent of all animals)			
		Kano state	Taraba state		Benue state
		Production	Production	Slaughterhouse	Trade and slaughter
Sex	Female	792 (81.1)	53 (81.5)	108 (90.8)	138 (67.0)
	Male	185 (28.9)	12 (18.5)	11 (9.2)	68 (33.0)
Breed	White Fulani	694 (71.0)	50 (76.9)	80 (76.2)	135 (65.5)
	Adamawa Gudali	–	–	–	30 (14.6)
	Brown Sokoto Gudali	281 (28.8)	5 (7.7)	19 (16.0)	23 (11.2)
	Red Bororo	–	10 (15.4)	–	18 (8.7)
	N'dama	–	–	20 (17.8)	–
	Not recorded	3 (0.2)	–	–	–
Total		977 (100)	65 (100)	119 (100)	206 (100)

samples from Taraba state and Benue state and for all RBPT positive and a selection of the RBPT-negative group 2 samples received from Kano state.

Statistical analysis

Descriptive statistics were calculated using Epi Info. The intermethod variation between the RBPT and the rapid field test was determined by calculating agreement and kappa values with standard errors (SE). Generally, a kappa value of 0.80 and above represents almost perfect agreement beyond chance, values between 0.40 and 0.80 represent fair to good agreement, and values below 0.40 represent slight to no agreement.

Results

The RBPT seroprevalence in production cattle with suspicion of brucellosis varied widely and ranged from 0% for samples collected from several herds from Kano state and Bauchi state to 80.1% for a herd tested in Adamawa state (Table 2). The mean RBPT seroprevalence was 20.1% (SD, 24.7; 95% CI, 8.1–93.1) with a median seroprevalence of 9.1%. The mean RBPT seroprevalence in cattle tested at three slaughterhouses in Taraba state and in three herds kept for trade and slaughter purposes in Benue state was 11.5% and 8.4%, respectively, with seropositive animals detected at all three slaughterhouses and in all three herds kept for trade and slaughter purposes (Table 2). The results obtained for the different type of cattle and states are displayed in Fig. 1, indicating that bovine brucellosis is present throughout several of the main cattle-rearing states in the northern and central part of the country.

The herd prevalence in production cattle in eight villages investigated for brucellosis in Kano state was 14.0% with RBPT-seropositive herds detected in four villages and was 22.2% in a village investigated in Taraba state (Table 2). Herds in these villages were small (5–50 animals) with one to three positive animals in positive herds. RBPT-seropositive animals were found among all cattle breeds with a similar seroprevalence among females and males (Table 3). In cattle tested at slaughterhouses or kept for trade and slaughter purpose, the seroprevalence was highest in females.

Seroprevalence results obtained in the rapid field test were fairly similar to those calculated for the RBPT. The overall observed agreement between the two tests for all (N=575) samples tested was 95.7% (kappa value, 0.80; SE, 0.05) with 51 samples testing positive in both test, 507 samples testing negative in both test, 7 samples testing RBPT positive and rapid test negative, and 11 samples testing RBPT negative and rapid test positive. The agreement between the two test was 100% (kappa, 1.0; SE, 0.17) for the samples from Kano state, 96.7% (kappa, 0.75; SE, 0.07) for the samples from Taraba state and 95.8% (kappa, 0.80; SE, 0.07) for the samples from Benue state.

Discussion

Previously, *Brucella abortus* biovar 1 was isolated from livestock in Nigeria (Ocholi et al. 2004). Samples tested in

Table 2 Seroprevalence of brucellosis in cattle in different states of Nigeria

State and year(s)	Type of cattle (no. of animals; no. of herds)	Seroprevalence in RBPT		
		Mean (SD)	Median (range)	Herd prevalence (95% CI)
Abuja (2008)	Production (80)[a]	3.8	–	–[b]
Adamawa (2004, 2008, 2009)	Production (74)[a]	45.3 (32.2)	37.5 (17.5–80.8)	–
Bauchi (2008, 2009)	Production (447)[a]	3.0 (5.2)	0.0 (0.0–9.1)	–
Kano (2008)	Production (51)[a]	0.0	–	–
Kano (2009)	Production (977; 93)[c]	1.8 (2.2)	1.0 (0.0–5.5)	14.0 (8–23)
Nassarawa (2004)	Production (16)[a]	50.0	–	–
Niger (2005, 2006, 2008)	Production (417)[a]	46.4 (14.8)	43.9 (2.3–56.9)	–
Plateau (2005, 2008, 2009)	Production (89)[a]	12.9 (6.1)	9.6 (9.1–20.0)	–
Taraba (2009)	Production (60)[a]	8.3	–	–
Taraba (2009)	Production (65; 9)[c]	6.2	–	22.2 (4–60)
Benue (2010)	Trade and slaughter (206)[c]	8.4 (2.6)	9.0 (5.7–10.7)	–
Taraba (2009)	Slaughter (119)[c]	11.5 (3.1)	11.3 (8.3–14.8)	–

[a] Samples submitted for laboratory testing for brucellosis with no epidemiological information available

[b] No information available on the number of herds tested or all herds tested positive

[c] Samples submitted for laboratory testing for brucellosis with epidemiological information

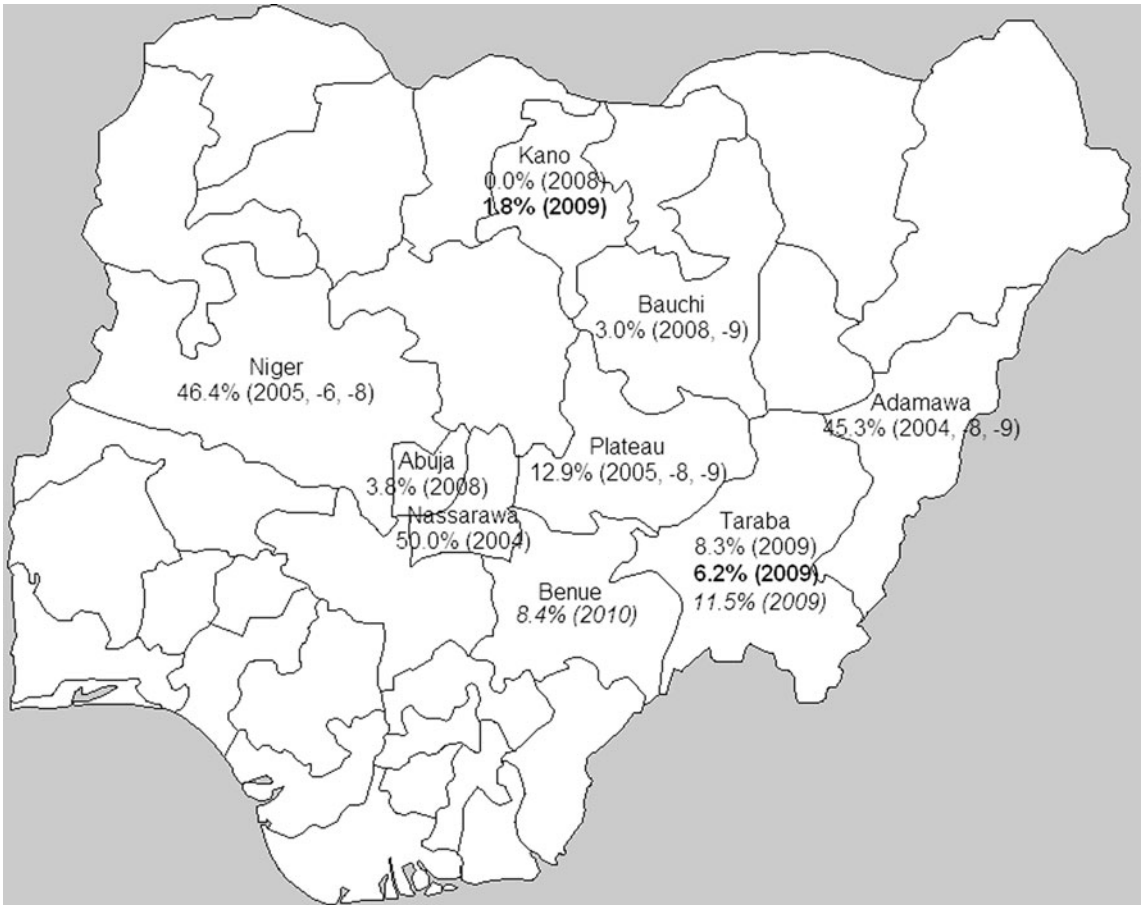

Fig. 1 Seroprevalence of bovine brucellosis in different types of cattle and in different states of Nigeria. Mean values of the Rose Bengal plate test results are presented for samples collected from production cattle herds in different states of Nigeria and submitted for laboratory confirmation of brucellosis to the Brucella Research Laboratory of the National Veterinary Research Institute in Vom between January 2004 and December 2009. *Bold values* are for production cattle herds for which specific epidemiological information was available and values for trade and slaughterhouse cattle are in *italics*

this study were received from several of the main cattle-rearing states in Nigeria, and our results indicate that bovine brucellosis could be a common and widespread infection in this country. Since samples were submitted for laboratory testing because of suspicion of brucellosis, further studies are required to determine the actual prevalence of the infection. The relatively high herd prevalence of 14.0% found in villages in Kano state and of 22.2% in a village in Taraba state indicates that infection is not limited to few individual herds.

Table 3 Seroprevalence of brucellosis in production, abattoir, and trade and slaughter cattle stratified according to sex and breed

		Percentage RBPT positive cattle from different states and kept for the following purposes			
		Kano state	Taraba state		Benue state
		Production (%)	Production (%)	Slaughterhouse (%)	Trade and slaughter (%)
Sex	Female	3.3	5.7	12.1	17.2
	Male	2.7	8.3	9.1	5.9
Breed	White Fulani	2.8	9.3	11.5	10.4
	Adamawa Gudali	–	–	–	26.7
	Brown Sokoto Gudali	3.2	3.6	4.5	13.0
	Red Bororo	–	2.7	–	11.1
	N'dama	–	–	3.1	–

Cattle in Nigeria is predominantly reared in traditional husbandry practices, and a variety of risk factors inherent to these practices including migration with the use of common pastures and water sources with frequent mixing of herds likely contribute to the spread of infectious diseases such as brucellosis (Godfroid et al. 2011). All infected herds in the eight villages in Kano state came from four villages, and discussions with village heads and herd owners lead to the conclusion that livestock from the four villages with infected herds more often were taken to common pastures during the dry season. Thus contact with other infected herds is more likely to occur at these pastures.

The high seroprevalence among cattle kept for trade and slaughter purposes may indicate that trade and transport of infected animals could be another source of infection and cause of the spread of brucellosis. Traders may transport their cattle over long distances before they are finally sold to farmers or presented at slaughterhouses. A large proportion of the animals reared in the northern states of Nigeria are sold in more southern states that are more densely populated and where the number of herds is lower and prices for cattle tend to be higher. Trade and slaughter animals may also include animals originating from Chad and other neighbouring countries as the demand for cattle and cattle meat at local markets in these countries is generally lower than in Nigeria. Earlier, Cadmus et al. (2010) reported a seroprevalence ranging from 5.3% to 6.2% for trade cattle slaughtered between 2004 and 2006 at the Bodija Municipal Abattoir in Ibadan in southwestern Nigeria.

Serosurveillance for brucellosis is most easily done using the rapid field test (Abdoel et al. 2008). Other tests for brucellosis should be performed in a laboratory. This requires careful labelling of samples, the identification of farms and herds with tagging of individual animals and transportation of samples over often long distances. The rapid field test does not require refrigeration and may be performed directly on a drop of whole blood. The use of the rapid field test allows the immediate instigation of control and preventive measures as results are available without delay. The rapid field test has favourable performance characteristics, and a study performed on samples collected in Cameroon showed that the assay is accurate also at relatively low disease prevalence (Bronsvoort et al. 2009).

In conclusion, bovine brucellosis is prevalent and widespread in Nigeria. Long-term chronic infections could be common, providing a steady supply of infectious organisms. While animals with chronic disease may be difficult to detect by serology, the presence of seropositive animals provide a good indication of ongoing transmission. Further studies confirming the presence of brucellosis in livestock by culture and correlating seropositivity in herds with disease manifestations such as abortions are required to determine the impact of the disease. On-site testing using the rapid field test simplifies logistics and allows the immediate instigation of control measures.

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