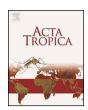
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Serological survey of bovine brucellosis in Fulani nomadic cattle breeds (*Bos indicus*) of North-central Nigeria: Potential risk factors and zoonotic implications



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ABSTRACT

A cross sectional study was conducted to investigate seroprevalence and associated risk factors of bovine brucellosis in Fulani nomadic herds in the 3 agro-ecological zones of Niger State. North-central Nigeria between January and August 2013. A total of 672 cattle in 113 herds were screened for Brucella antibodies using Rose Bengal Plate Test (RBPT) and confirmed by Lateral flow Assay (LFA). Data on herd characteristics and zoonotic factors were collected using structured questionnaire administered on Fulani herd owners. Factors associated with Brucella infection were tested using Chi-square test and multivariable logistic model. The overall cattle-level seroprevalence was 1.9% (95% CI: 1.1-3.2) with highest in agro-zone C (3.2%). Herd-level seroprevalence was 9.7% (95% CI: 5.23-16.29) and highest in agro-zone C (13.5%). Sex and agro-ecological zones were significantly (P < 0.006 and P < 0.01, respectively) associated with Brucella abortus seropositivity. Herd composition, abortion in herd, exchange of bulls for mating, introduction of new cattle, and socio-cultural practices were significantly associated with brucellosis occurrence. Inhalation of droplets from milk of infected cows, and drinking raw milk were less likely [OR 0.27; 95% CI: 0.09-0.82 and OR 0.27; 95% CI: 0.08-0.99, respectively] not to predisposed to brucellosis in humans. Eating infected raw meat, and contact with infected placenta were more likely [OR 7.49; 95% CI: 2.06–28.32 and OR 5.74; 95% CI: 1.78–18.47, respectively) to be risks for the disease in humans. These results highlighted the important risk factors for bovine brucellosis in Fulani herds. Thus, brucellosis control programs which take these factors into consideration will be beneficial.

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1. Introduction

Brucellosis is an infectious and contagious disease caused by gram-negative bacteria of the genus *Brucella*, which comprises many species ranked according to their host preferences and pathogenicity: *Brucella abortus*, *Brucella melitensis*, *Brucella suis*, *Brucella canis*, *Brucella ovis*, and *Brucella neotomae* (FAO, 2009a,b; OIE, 2009). It is one of the neglected zoonotic diseases with a serious worldwide public health importance (WHO, 2006, 2009; OIE, 2009), and often persists in the poorest and most vulnerable populations (FAO, 2009a,b). However, the disease is not sustainable in humans and human infection is often associated with brucellosis in domestic or wild animals (Godfroid et al., 2005). It has worldwide

distribution but more endemic in African countries (Matope et al., 2010).

Bovine brucellosis is a contagious disease of cattle, primarily caused by B. abortus and occasionally by B. melitensis where there is mixed keeping of cattle together with infected sheep or goats (McDermott and Arimi, 2002; OIE, 2009). Clinically, the disease is characterized by abortion, metritis, orchitis and epididymitis (Radostits et al., 2007; Seleem et al., 2010; Anka et al., 2013). It has been associated with high economic losses due to decreased calving percentage, delayed calving, culling for infertility, cost of treatment, decreased milk production, abortions, stillbirth, and birth of weak calves (Gwida et al., 2010; Mekonnen et al., 2010; Megersa et al., 2011a,b). Although bovine brucellosis has been controlled and eradicated in most of the developed nations (Makita et al., 2008), it remains a significant major neglected disease for both cattle and human health in developing countries, especially those in sub-Saharan Africa (Apan et al., 2007; McDermott et al., 2013), such as Nigeria (Cadmus et al., 2010).

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The epidemiology of bovine brucellosis is complex and influenced by several factors that include those predisposing humans to *Brucella* infection, those associated with the transmission of the disease between herds, and factors influencing the maintenance and spread of infection within herds such as management factors of herd biosecurity, herd size and composition, population density, type of animal breed and biological features such as herd immunity, and environmental factors like climate (McDermott and Arimi, 2002; Makita et al., 2011; Megersa et al., 2011a,b).

Many published works have been reported on burdens of bovine brucellosis in developing countries (Dean et al., 2012; Cadmus et al., 2013; McDermott et al., 2013). In West Africa, the prevalence of the infection in cattle varies greatly from one production system to another (Cadmus et al., 2008), with much higher prevalence in the pastoral grazing systems (Chimana et al., 2010; Matope et al., 2010). However, most reports made so far in many African countries are on either agro-pastoral and transhumant production systems or are relatively confined to a single agro-ecology (Holt et al., 2011; Mohammed et al., 2011; Sanogo et al., 2012; Alhaji and Wungak, 2013), without reported associated risks for the infection concurrently in both humans and animals. This warrants the need for a comprehensive survey on the disease burden and potential associated risk factors, specifically in the Fulani nomadic cattle herds and the herders in Nigeria.

This study was, therefore, aimed at investigating prevalence of bovine brucellosis and associated potential risk factors that could predisposed to the disease in Fulani nomadic cattle herds and humans in North-central Nigeria. Knowledge about important determinants for *Brucella* infection in animals and humans is vital, as these factors can be further explored in strategizing evidence based disease surveillance and intervention programs in the country. We hypothesized that potential risk factors cannot predispose to bovine brucellosis in Fulani nomadic cattle herds and herders.

2. Materials and methods

2.1. Study area

The study was conducted in Niger State, located in the Northcentral geopolitical zone of Nigeria, between latitude 8°20′N and 11°30′N; longitude 3°30′E and 7°20′E. It has an estimated cattle population of 2.4 million cattle, mostly in the custodies of nomadic pastoralists, and also provides transit routes for the Fulani nomadic pastoralists on seasonal migrations from the northern parts to the south-western and south-southern parts of Nigeria (MLFD, 2013). The state has 3 agro-ecological zones, with variable climatic conditions. These are: agro-ecological zone A (Southern) with 8 local government areas (LGAs), agro-ecological zone B (Eastern) with nine LGAs, and agro-ecological zone C (Northern) with eight LGAs. Also, it has an international border with the Republic of Benin, which is porous.

2.2. Study design, population and definitions

The study was a cross-sectional survey conducted in the 3 agroecological zones of the state between January and August 2013. It involved blood samples collection from Fulani nomadic cattle as well as biodata (age, sex and breeds) of the sampled cattle. Also, questionnaire based interview was administered on Fulani herd owners to obtain information on predisposing risks for bovine brucellosis in herds and as well as in humans.

The target populations were Fulani nomadic herd owners and their cattle domiciled in the state during the period of the survey. Inclusion criteria for the participants were, that the pastoralist must be a cattle herd owner, and aged 30 years and above. Pastoralists at

this age and above were traditionally considered to be in possession of existing veterinary knowledge and traditional oral history about cattle diseases and management because of their long time relationships.

Fulani nomadic herd was defined as cattle herd in Fulani ethnocultural group that keeps mainly cattle, usually large herd, and takes part in year-round long movements on large range for grazing and in search for water, without permanent homestead in the study area.

2.3. Sample size and sampling procedure

The sample size was determined using random sampling method (Thrusfield, 2009) and expected prevalence of 37% (Mai et al., 2012) at 95% confidence level. Sample sizes for the herds and the questionnaires were each determined at 10% desire precision, giving sample size of 90 for each. However, a contingency of 20% was added (Boukary et al., 2013) and sample sizes were adjusted to 113 questionnaires and 113 herds to increase allocations to agro-ecological strata. Sample size for cattle was determined at 4% margin of error and 560 cattle were obtained. Also, a contingency of 20% was added and the number was adjusted to a total of 672 cattle

Sampling was performed using a two-stage procedure; first the herds and herd owners were selected by purposive sampling approach, and then cattle in each herd were selected proportional to the herd weight by simple random sampling. The herds were spatially selected across each agro-ecological zone.

2.4. Sample collection and laboratory analysis

Ten (10) milli liters of whole blood was collected from jugular vein of each selected cattle, using a sterile 10 ml syringe and $18\frac{1}{2}$ " gage needle for each animal. The sera were transferred into sterile plain tubes and centrifuged at $3000\,\mathrm{rpm}$ for $10\,\mathrm{min}$ and then decanted into cryovials, identified before storage at $-20\,^{\circ}\mathrm{C}$ until analyzed. The sera were transported to the Brucella Research Laboratory Unit, Bacterial Research Division of the National Veterinary Research Institute Vom, Nigeria, also stored at $-20\,^{\circ}\mathrm{C}$. They were screened for antibodies against natural *Brucella* infection using Rose Bengal Plate-agglutination test (RBPT) and confirmed by use of $10\,\mathrm{Im}/\mathrm{IgG}$ Lateral Flow Assay (LFA) to complied with the standard protocol (OIE, 2012).

The RBPT was performed on all samples using the standard protocol available in the 2009 Terrestrial Manual (OIE, 2009). Thirty micro liters of antigen (Institute Pourquier, Montpellier, France) was placed on a glass slide and equal volume of test serum was dropped on the slide. The antigen and test serum were mixed thoroughly by sterile plastic applicator, and shaken gently for 4 min, and occurrence of agglutination was observed. The degree of agglutination was visually recorded immediately by formation of distinct pink granules (agglutination) which was recorded as positive, while the absence of agglutination was recorded as negative.

Positive screened sera were further subjected to Lateral Flow Assay as validation test. The LFA is a simplified form of ELISA (Christopher et al., 2010) and used in detecting specific IgM and IgG antibodies (Nielsen and Yu, 2010). The design and composition of the *Brucella* IgM and IgG flow assays have been described previously (Smits et al., 2003). Five micro liter of serum was added onto the sample application pad in the sample well of the plastic assay device (Organon Teknika Ltd, Dublin, Ireland), followed by the addition of 130 µL of running fluid. The test result was read by visual inspection of staining antigen and control lines in the test zone of the device. The result was scored negative when no staining of the antigen line was observed and positive when a distinct staining of the antigen line was observed. The antigen line stained at different

intensities and was subjectively rated 1+ when staining was weak, 2+ when staining was moderate, 3+ when staining was strong, and 4+ when staining was very strong. Undetermined staining represented by very weak (+/-) staining was considered negative. The sensitivity of LFA to confirmed brucellosis was more than 95% and the specificity was 98.2% (Abdoel et al., 2008; Baddour, 2012).

2.5. Questionnaire design, pretesting and data collection

The questionnaire was designed containing mostly close-ended questions to ease data processing, minimize variation, and improve precision of responses (Thrusfield, 2009). It contained questions that focused on various sub-themes like the pastoralists' demographic characteristics of gender, age, tribe, occupation and formal education; knowledge/attitude on bovine brucellosis; risk factors predisposing to Brucella infection in herds with specific questions on herd size, herd composition (presence of small ruminants), and occurrence of abortions in the herds. Others are exchange of bulls for mating, husbandry management system, introduction of new cattle bought at livestock market into herd, and socio-cultural practices of giving out cattle as gifts or payments for dowries. In addition, were some questions on risk factors predisposing to Brucella infection in humans, which included inhalation of droplets from infected cow during traditional milking, drinking of raw or unpasteurized milk, and eating infected raw meat. Others were consumption of contaminated cheese, butter and yoghurt, and contacts with infected placenta tissues and vaginal discharges from aborted fetuses.

The questionnaire was pre-tested prior to the study on few Fulani nomadic herders on whom the actual study was conducted. The questionnaire was designed in English but verbally translated into Hausa language, during the process of administering since many of the respondents do not possessed formal education. Hausa was the local language generally used in the study area.

Data were collected using interviewer-administered, paper-based questionnaires on herd owners. Before commencement of each questionnaire administering, informed consent was verbally obtained from the respondents who were assured of voluntary participation, confidentiality of their responses and the opportunity to withdraw at any time without prejudice in line with the Helsinki Declaration (WMADH, 2001). Data collections were completed at the selected herd sites on a single visit.

2.6. Defined variables

In this study, covariates (hypothesized explanatory variables) were assessed at cattle, herd and human levels. At the cattle-level, age, sex, breeds and agro-ecological zones were the independent (explanatory) variables while positive and negative serological outcomes constituted the dependent (outcome) variables. At the herd-level, herd characteristics constituted the explanatory variables, while 'poor' and 'satisfactory' existing knowledge responses of the nomadic herders on the herd characteristics were the outcome variables. Also, factors predisposing to *Brucella* infection in humans constituted the explanatory variables; while 'poor' and 'satisfactory' existing knowledge responses of the herders were the outcome variables.

To measure existing knowledge responses, the scoring system range between 1 and 20 points, which were converted to 100%. The score range was further categorized into 'poor' (\leq 10 points, \leq 50%) and 'satisfactory' (\geq 11 points, \geq 51%) to keep them as binary variables.

2.7. Data management and statistical analyses

Collected data were summarized and entered into Microsoft Excel 7 spreadsheet (Microsoft Corporation, Redmond, WA, USA) and stored. Open Source Epidemiologic Statistics for Public Health (OpenEpi) software version 2.3 (Dean et al., 2009) was used for the statistical analysis. Descriptive and analytical statistics were used to describe the obtained data. Only cattle positive to both RBPT and LFA were classified as been true seropositive to *Brucella* infection. However, a herd was classified as *Brucella* seropositive if at least one animal tested seropositive on both tests. As there has never been any history of vaccination, seroprevalence was considered to be due to natural infection. Separate estimates were established for each agro-ecological zone.

The associations between *Brucella* seropositivity and individual cattle as well as herd-level and human risk factors for the disease were investigated using univariable analyses by Chi-square test. All factors found to be biologically plausible and significant were subjected to multivariate analyses using Likelihood stepwise backward logistic regression models to control for confounding and test for effect modification. *P* < 0.05 was considered statistically significant at both analyses.

3. Results

3.1. Demographic information

Mean age of the Fulani pastoral respondents was 52.8 ± 10.6 SD years. The majorities (80.5%) of the respondents were males and most (81.4%) were married. The majorities (33.6%) of the respondents were in the age group 51-60 years, about 61.1% of them were illiterates (without formal education) and all were of Fulani tribe domicile in North-central Nigeria.

3.2. Cattle-level sero-prevalence and associated factors

Of the 672 cattle sampled and sera examined for antibodies to *Brucella* antigen, 3.6% (24/672, 95% CI: 2.36–5.19) were seropositive with Ross Bengal Plate test, and 1.9% (13/672, 95% CI: 1.1–3.2%) tested positive for *B. abortus* antibodies with Lateral Flow Assay and this constituted the true cattle-level sero-prevalence (Table 1). The geographical pattern of the disease burden in the state is shown in Table 1. In the agro-ecological zone A, the individual animal level true prevalence was 1.9% (95% CI: 0.6, 4.4). The highest seroprevalence of 3.2% (95% CI: 1.4, 6.3) was observed in the agro-ecological zone C, while the lowest of 0.8% (95% CI: 0.1, 2.7) was observed in the agro-ecological zone B (Table 1).

At the individual cattle level, brucellosis seropositivity was determined according to the age group, breed, sex, and agroecological zone (Tables 3 and 4). However, the final multivariable logistic model identified only sex and agro-ecological zone to be significantly associated with *B. abortus* seropositivity. Cows were significantly more likely [OR 4.14; 95% CI: 1.37–12.55] to be seropositive than bulls. Also, agro-ecological zones C was significantly less likely [OR 0.22; 95% CI: 0.05–1.01] not to have risk factors associated with sero-positivity to *B. abortus* (Table 4).

3.3. Herd-level sero-prevalence and associated risk factors

The overall herds with at least one sero-positive cattle having antibodies to *Brucella* antigen or true herd-level sero-preorvalence was 9.7% (95% CI: 5.23–16.29). The highest herd-level seroprevalence of 13.5% (95% CI: 5.1, 27.4) was observed in the agro-ecological zone C, while the lowest of 5.1% (95% CI: 0.9, 15.9) was observed in the agro-ecological zone B (Table 2).

 Table 1

 Cattle-level sero-prevalence of bovine brucellosis based on RBPT and LFA in Fulani nomadic herds of Niger State, Nigeria.

Agro-ecological zone	Number of cattle tested	RBPT No. positive (%; 95% CI)	LFA No. positive (%; 95% CI)	False positive (%; 95% CI)
A	216	6 (2.8; 1.1, 5.7))	4 (1.9; 0.6, 4.4)	2 (0.9; 0.2, 3.0)
В	240	4 (1.7; 0.5, 4.0)	2 (0.8; 0.1, 2.7)	2 (0.8; 0.1, 2.7)
С	216	14 (6.5; 3.7, 10.4)	7 (3.2; 1.4, 6.3)	7 (3.2; 1.4, 6.3)
Overall	672	24 (3.6; 2.4, 5.2)	13 (1.9, 1.1, 3.2)	11 (1.6; 0.9, 2.8)

Note: No. number of animals positive; %—proportion; False positive—proportion of animals that were RBPT positive but LFA negative.

Table 2Herd-level sero-prevalence of bovine brucellosis by RBPT and LFA in Fulani nomadic herds of Niger State, Nigeria.

Agro-ecological zone	Number of herd tested	RBPT No. positive (%; 95% CI)	LFA No. positive (%; 95% CI)	False positive (%; 95% CI)
A	37	5 (13.5; 5.1, 27.4)	4 (10.8; 3.5, 24.1)	1 (2.7; 0.1, 12.6)
В	39	4 (10.3; 3.3, 22.9)	2 (5.1; 0.9, 15.9)	2 (5.1; 0.9, 15.9)
С	37	10 (27.0; 14.6, 42.9)	5 (13.5; 5.1, 27.4)	5 (13.5; 5.1, 27.4)
Overall	113	19 (16.8; 10.7, 24.6)	11 (9.7; 5.2, 16.3)	8 (7.1; 3.3, 13.0)

Note: No. number of herds positive; %-proportion; False positive-proportion of animals that were RBPT positive but LFA negative.

 Table 3

 Univariate analysis of potential factors associated with cattle-level antibody sero-positivity to Brucella abortus in Fulani nomadic herds of Niger State, Nigeria.

Factors	Numberpositive	Numbernegative	Chi-square test	<i>P</i> -value
Age (years)				
≤1	2	115	0.31	0.86
2-4	6	254		
≥5	5	290		
Breed				
Bokoloji	4	155	0.37	0.54
Bunaji	9	504		
Sex				
Bull	6	113	7.36	0.006
Cow	7	546		
Agro-ecological zone				
A	2	214	8.37	< 0.01
В	2	238		
С	9	207		

 Table 4

 Multivariate logistic model of potential factors associated with cattle-level antibody sero-positivity to Brucella abortus in Fulani nomadic herds of Niger State, Nigeria.

Factors	Number positive(Col.%)	Number negative(Col.%)	Odds ratio (95% CI)	<i>P</i> -value
Sex				
Bull	6 (46.2)	113 (17.1)	1.00 (ref.)	
Cow	7	546	4.14 (1.37–12.55)	<0.01
Agro-ecological z	zone			
A	2 (15.4)	214 (32.5)	1.00 (ref.)	
В	2	238	1.11 (0.16-7.96)	0.92
C	9	207	0.22 (0.05-1.01)	0.03

 Table 5

 Univariable analysis of risk factors associated with occurrence of bovine brucellosis in Fulani nomadic cattle herds of Niger State, Nigeria.

Factors	Categories	Satisfactory knowledgeN (Col.%)	Poor knowledge N (%)	Chi-square andP-value
Herd size	Yes	53 (63.9)	21 (70.0)	0.37
	No	30	9	0.55
Herd composition (mix with small ruminants)	Yes	60 (77.9)	9 (30.0)	28.91
	No	17	27	<0.0000001
Occurrence of abortion in the herd	Yes	94 (94.0)	7 (53.8)	19.54
	No	6	6	0.000009
Exchange of bull for mating	Yes	87 (87.9)	6 (42.9)	17.07
	No	12	8	0.00003
Husbandry management system	Yes	58 (79.5)	12 (30.0)	26.81
	No	15	28	0.0000002
Introduction of new cattle bought at livestock market	Yes	84 (88.4)	6 (33.3)	29.33
	No	11	12	<0.000001
Socio-cultural factor (gift or dowry)	Yes	32 (59.3)	19 (32.2)	8.34
	No	22	40	0.003

N-number; Col-column; %-percentage.

Table 6Multivariable logistic regression model for risk factors associated with the occurrence of bovine brucellosis in Fulani nomadic cattle herds of Niger State, Nigeria.

Factors	Satisfactory knowledgeN (Col.%)	Poor knowledge N (%)	Odds ratio(95% CI)	P-value
Herd composition	on			
Yes	60 (77.9)	9 (25.0)	10.59	< 0.0000001
No	17	27	(4.19, 26.75)	
Occurrence of a	bortion in the herd			
Yes	94 (94.0)	7 (53.8)	13.43	0.0005
No	6	6	(3.42, 52.71)	
Exchange of bul	ll for mating			
Yes	87 (84.5)	6 (42.9)	8.08	0.0008
No	12	8	(2.48, 26.39)	
Husbandry man	nagement system			
Yes	58 (79.5)	12 (30.0)	9.02	0.0000003
No	15	28	(3.73, 21.82)	
Introduction of	new cattle bought at livestock market			
Yes	84 (88.4)	6 (33.3)	15.27	0.000003
No	11	12	(4.77, 48.92)	
Socio-cultural fa	actor (gift or dowry)			
Yes	32 (59.3)	19 (32.2)	3.06	0.004
No	22`	40	(1.42, 6.61)	

N-number; Col-column; %-percentage.

The herd size, herd composition, abortion in the herd, exchange of bulls for mating, husbandry management system, introduction of new cattle bought at markets into herd, and socio-cultural practices were all found to be significantly associated with the occurrence of bovine brucellosis in the herds at the univariable analysis, (Table 5). In the final multivariable logistic, herd composition, abortion in the herd, exchange of bulls for mating, husbandry management system, introduction of new cattle bought at markets into herd, and socio-cultural practices were significantly identified to be more likely to be risk factors predisposing brucellosis in the nomadic cattle herds of Niger State in Nigeria (Table 6).

3.4. Predisposing factors to Brucella infection in humans

Of the 113 pastoralist respondents that participated in the survey, only 69.0% (n = 78) accepted, after informed consent was obtained, to respond to questionnaires on bovine brucellosis as a zoonotic disease while the remaining 31.0% (n=35) declined for lack of existing knowledge on the subject matter. Pastoralists' responses indicated that inhalation of droplets from milk of infected cows during traditional milking, drinking raw or unpasteurized milk, eating infected raw meat and contact with infected placenta and vaginal discharges from aborted fetuses were significantly associated with the zoonotic brucellosis at univariate analysis, while consumption of contaminated cheese, butter or yoghurt was not statistically significant (Table 7). On subsequent logistic regression, inhalation of droplets from milk of infected cows during traditional milking and drinking raw or unpasteurized milk were less likely [OR 0.27; 95% CI: 0.09, 0.82 and OR 0.27; 95% CI: 0.08, 0.99, respectively] not to be factors associated with occurrence of zoonotic brucellosis. However, eating infected raw meat, and contact with infected placenta and vaginal discharges from aborted fetuses were more likely [OR 7.49; 95% CI: 2.06, 28.32 and OR 5.74; 95% CI: 1.78-18.47, respectively] to be risk factors associated with zoonotic brucellosis in nomadic pastoralists of Niger State in Nigeria (Table 8).

4. Discussion

Bovine brucellosis is one of the few relatively well surveyed cattle diseases in Nigeria, but to our knowledge, this study was the first to cover Fulani nomadic pastoral herds and many agroecological zones at a time. The study shows that the prevalence of

this disease was low (1.9%) at cattle-level with Lateral Flow Assay as confirmatory test. This is lower than the 27.9%, 20.6%, and 23.1% reported in Adamawa, Kaduna and Kano States in Nigeria, respectively (Mai et al., 2012), and 5.5% was reported in smallholder herds in Zimbabwe (Matope et al., 2011a,b) using c-ELISA confirmatory test. The low sero-prevalence in this study might be due to the use of very sensitive and specific tests, traditional practice of culling infected animals by the Fulani nomads in the region, and variable geographical conditions. However, the message was that bovine brucellosis still persists in these developing countries. Interstate movements and trade in cattle across the country, as well as the nomadic nature of the pastoral Fulani may have contributed to the current observed seroprevalence, since Niger State is located at the North-central Nigeria and serving as transit route for nomadic pastoralists on seasonal movements between northern and southern parts of the country. Similar factors have been earlier attributed to the spread of bovine brucellosis (Bertu et al., 2012; Adugna et al., 2013; Boukary et al., 2013).

At the cattle-level, the survey observed sex and agro-ecological zones to significantly predispose to B. abortus sero-positivity. The odds of brucellosis sero-positivity were more significant in cows than in bulls and also significant in agro-ecological zone C than in agro-ecological zones A, but not significant in agro-zone B. These were likely due to free movements of cattle in Zone C. with common international border with the Republic of Benin that is porous, which exacerbates influx of many herds from this neighboring country, especially during dry season. The dynamics and frequent migrations of pastoral herds increase the chance of apparently healthy and susceptible cattle herds coming into contact with other potentially infected herds and exposure to geographically limited or seasonally abundant diseases (Boukary et al., 2010). Cross-border movement has been implicated in the transmission of brucellosis in Nigeria (Cadmus et al., 2008). Considering the contagious nature of Brucella species, sharing grazing land and drinking water points facilitate transmission of the bovine brucellosis among animals (Mekonnen et al., 2010). Significant higher sero-positivity in cows than in bulls has also been reported (Junaidu et al., 2011; Bashitu et al., 2015). In contrast, Chimana et al., (2010) found significant higher sero-positivity to be more in bulls than in cows and Bayemi et al. (2009) reported no difference between sexes. Age has been earlier reported to be an important factor for bovine brucellosis in Nigeria (Cadmus et al., 2008; Junaidu et al., 2011) and elsewhere in Africa (Matope et al., 2010; Megersa

Table 7Univariate analysis of exposure factors associated with occurrence bovine brucellosis among pastoralists in Niger State, Nigeria.

Factors	Categories	Satisfactory knowledgeN (Col.%)	Poor knowledge N (%)	Chi-square and P-value
Inhalation of droplets from milk of infected cattle	Yes	13 (25.5)	15 (55.6)	6.94
	No	38	12	0.008
Drinking raw or unpasteurized milk	Yes	23 (39.0)	13 (68.4)	5.01
	No	36	6	0.03
Eating infected meat	Yes	49 (84.5)	9 (42.1)	13.37
	No	9	11	0.0003
Consumption of contaminated cheese, butter or yoghurt	Yes	33 (55.9)	8 (42.1)	1.10
	No	26	11	0.295
Contact with infected placenta and vaginal discharges	Yes	51 (83.6)	8 (47.1)	9.64
	No	10	9	0.002

N-number; Col-column; %--percentage.

 Table 8

 Multivariate logistic regression models of exposure factors associated with occurrence of bovine brucellosis among pastoralists in Niger State, Nigeria.

Factors	Satisfactory knowledgeN (Col.%)	Poor knowledge N (%)	Odds ratio (95% CI)	P-value
Inhalation of dro	oplets from milk of infected cattle			
Yes	13 (25.5)	15 (55.6)	0.27 (0.09-0.82)	0.008
No	38	12	1.00 (ref.)	
Drinking raw or	unpasteurized milk			
Yes	23 (39.0)	13 (68.4)	0.27 (0.08-0.99)	0.02
No	36	6	1.00 (ref.)	
Eating infected r	raw meat			
Yes	49 (84.5)	8 (42.1)	7.49 (2.06-28.32)	0.0002
No	9	11	1.00 (ref.)	
Contact with inf	ected placenta and vaginal discharges			
Yes	51 (83.6)	8 (47.1)	5.74 (1.78-18.47)	0.004
No	10	9	1.00 (ref.)	

N-number; Col-column; %-percentage.

et al., 2011a,b). However, we did not observed any association of age with sero-positivity in this study. This does not nullify bovine brucellosis importance as being associated with the aforesaid risk factor. Also, we did not observed significant association of breeds with the sero-positivity to the infection, though it has been an important factor for brucellosis occurrence in South-western Nigeria (Cadmus et al., 2008) where N'Dama breed is predominant, while Bunaji is the predominant breed in North-central Nigeria.

The study observed moderately high (9.7%) herd-level prevalence of the disease in North-central Nigeria, which was much lower than the reported 82.3%, 72.0% and 78.5% in Adamawa, Kaduna and Kano States, respectively in far northern parts of Nigeria (Mai et al., 2012) and reported 40.0% in Zimbabwe (Matope et al., 2011a,b). This might be due to the nomadic nature of Fulani pastoral herds. Alhaji and Wungak (2013) had earlier reported free movement of the pastoral Fulani herdsmen and interaction of cattle with those of other Fulani herdsmen to be major factors in spreading bovine brucellosis.

Among the studied risk factors at herd-level, abortion in herds was observed to significantly predispose to bovine brucellosis. This finding is consistent with earlier reports of some authors (Makita et al., 2011; Tesfaye et al., 2011; Anka et al., 2014) that reproductive disorders of abortion and still birth factors are associated with *B. abortus* spread in herds. Presence of other livestock, such as sheep and goats, in herds was observed as risk factor predisposing to the disease. Herding of different species together has been reported to an exposure factor for *Brucella* infection in Nigeria (Junaidu et al., 2008). Furthermore, introduction of new cattle bought at cattle market into herds, and socio-cultural factor of cattle gifts or using cattle to pay for dowries were factors observed to be predisposing determinants of bovine brucellosis in the nomadic cattle herds.

Purchase of infected cattle has been reported to be associated with *Brucella* infection in cattle herds (OIE, 2011; Asmare et al., 2013).

This study observed factors that significantly predisposed to the transmission of B. abortus infection among humans, especially the pastoralists, to include handling infected placenta and vaginal discharges from aborted fetuses. This is consistent with the reports of Swai and Schoonman (2009) in Tanzania and John et al. (2010) in Chad that brucellosis in humans was strongly associated with handling of aborted fetuses and placenta of infected animals. We observed consumption of raw meat from infected cattle to be determinant of Brucella infection in pastoralists. Aworh et al. (2013) reported eating raw meat to be important epidemiological factor in contracting brucellosis by humans. Brucellosis in humans have been reported in livestock farmers, milkers, butchers and veterinarians who have direct contact with animal and its products or who consume raw milk (Islam et al., 2013). The survey further identified drinking raw or unpasteurized milk to significantly predispose to bovine brucellosis in nomadic pastoralists. This is also consistent with the report that people acquire bovine brucellosis through consumption of contaminated raw milk, milk products, blood and undercooked meat (El Kholy et al., 2009). Nevertheless, this study did not find any significant association between consumption of cheese, butter or yoghurt with Brucella infection in nomadic pastoralists. This could be due to the application of much heat during processing of these products; but it would still be emphasized that these products are important in the transmission link of the disease to humans and therefore their consumption should be done with cautions. B. abortus transmission to pastoralists through inhalations of milk droplets was found to be significant during traditional task of milking cows. There are reports that humans in closed contacts with infected cattle during milk processing are at high risk of developing brucellosis (Hashim et al., 2007; Adugna et al., 2013).

A major limitation in this survey was that Fulani nomadic pastoralists, who were less than 30 years of age, were excluded despite the fact that they constituted a large part of the populations studied and could also, be cattle herd owners. Their non inclusion was because they were not likely to possess significant existing veterinary knowledge about brucellosis in humans because of their less period of nomadic contacts experience in the field.

In conclusion, bovine brucellosis is prevalent and widely distributed among Fulani nomadic cattle herds of Niger State. The findings are of both economic and public health significance to the pastoralists. The agro-ecological zones variations in brucellosis prevalence may be linked to geographical factors. In general, the observed results highlighted the important risk factors for the disease in Fulani herds. The lack of association of age and breed with Brucella seropositivity suggests that other causes largely outweighed in studied production system and may demand an investigation. Pastoralists' sensitization on disease reporting, integration of vaccination against brucellosis into the annual cattle vaccination campaign program, and movement control of cattle across borders are recommended. Health education aimed at highlighting the importance of brucellosis as a zoonotic disease to pastoralists and other risk groups is advocated. Also, the need for isolation and characterization of circulating B. abortus and collaboration between physicians and veterinarians on brucellosis surveillance in human and animals in the spirit of 'One Health' for effective control of the disease in all populations are suggested.

Summary

Bovine brucellosis seroprevalence in Fulani nomadic cattle herds of Niger State agro-ecological zones using the Rose Bengal Plate Test and the Lateral flow Assay.

Conflict of interest

The authors declare that they have no conflict of interest.

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References

- Abdoel, T., Travassos, D.I., Cardoso, R., Smits, H.L., 2008. Simple and rapid field test for brucellosis in livestock. Vet. Microbiol. 130, 312–319.
- Adugna, K.E., Agga, G.E., Zewde, G., 2013. Seroepidemiological survey of bovine brucellosis in cattle under a traditional production system in western Ethiopia. Rev. Sci. Tech. Int. Off. Epizoot. 32 (3), 765–773.
- Alhaji, N.B., Wungak, Y., 2013. Epizootiological survey of bovine brucellosis in nomadic pastoral camps in Niger State, Nigeria. Niger. Vet. J. 34 (2), 795–800.
- Anka, M.S., Hassan, L., Adzhar, A., Khairani-Bejo, S., Mohamad, R.B., Salleh, A., Adzhar, A., 2013. Bovine brucellosis trends in Malaysia between 2000 and 2008. BMC Vet. Res. 9, 230–241.
- Anka, M.S., Hassan, L., Khairani-Bejo, S., Zainal, M.A., Mohamad, R.B., Salleh, A., Adzhar, A., 2014. A Case-control study of risk factors for bovine brucellosis seropositivity in Peninsular Malaysia. PLoS One 9 (9), e108673, http://dx.doi. org/10.1371/journal.pone.0108673.
- Apan, T.Z., Yildirim, M., Istanbulluoglu, E., 2007. Seroprevalence of brucellosis in human sheep, and cattle populations in Kirikkale (Turkey). Turk J. Vet. Anim. Sci. 31, 75, 78
- Asmare, K., Sibhat, B., Molla, W., Ayelet, J., Shiferaw, J., Martin, E., Skjerve, E., Godfroid, J., 2013. The status of bovine brucellosis in Ethiopia with special emphasis on exotic and cross bred cattle in dairy and breeding farms. Acta Trop. 126, 186–192.

- Aworh, M.K., Okolocha, E., Kwaga, J., Fasina, F., David Lazarus, D., Suleman, I., Poggensee, G., Nguku, P., Nsubuga, P., 2013. Human brucellosis: seroprevalence and associated exposure factors among abattoir workers in Abuja, Nigeria. Pan Afr. Med. J. 16, 103–113, http://dx.doi.org/10.11604/pamj.2013.16.103.2143.
- Baddour, M.M., 2012. Diagnosis of brucellosis in humans. J. Vet. Adv. 2 (4), 149–156.
 Bashitu, L., Afera, B., Tuli, G., Aklilu, F., 2015. Sero-prevalence study of bovine brucellosis and its associated risk factors in Debrebirhan and Ambo Towns. J. Adv. Dairy Res. 3, 131–134, http://dx.doi.org/10.4172/2329-888X.1000131.
- Bayemi, P.H., Webb, E.C., Nsongka, M.V., Unger, H., Njakoi, H., 2009. Prevalence of Brucella abortus antibodies in serum of Holstein cattle in Cameroon. Trop. Anim. Health Prod. 41, 141–144.
- Bertu, W.J., Gusi, A.M., Hassan, M., Mwankon, E., Ocholi, R.A., Ior, D.D., Husseini, B.A., Ibrahim, G., Abdoel, T.H., Smits, H.L., 2012. Serological evidence for brucellosis in *Bos indicus* in Nigeria. Trop. Anim. Health Prod. 44, 253–258.
- Boukary, A.R., Saegerman, C., Rigouts, L., Matthys, F., Berkvens, D., et al., 2010. Preliminary results of the study on zoonotic brucellosis and tuberculosis in Niamey. In: Globalization of Tropical Animal Diseases and Public Health Concerns; Proceedings of 13th AITVM 2010 International Conference, 23–26 August 2010, Bangkok, Thailand, pp. 22–24 [Chulalongkorn University; Utrecht: Association of Institutions for Tropical Veterinary Medicine (AITVM)].
- Boukary, A.R., Saegerman, C., Abatih, E., Fretin, D., Alambeídji Bada, R., et al., 2013. Seroprevalence and potential risk factors for *Brucella* spp. infection in traditional cattle, sheep and goats reared in urban, periurban and rural areas of Niger. PLoS One 8 (12), e83175.
- 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. J. S. Afr. Vet. Assoc. 79, 113–115.
- 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 to 2006. J. S. Afr. Vet. Assoc. 81, 50–53.
- Cadmus, S.I.B., Alabi, P.I., Adesokan, H.K., Dale, E.J., Stack, J.A., 2013. Serological investigation of bovine brucellosis in three cattle production systems in Yewa Division, south-western Nigeria. J. S. Afr. Vet. Assoc. 84 (1).
- Chimana, H.M., Muma, J.B., Samui, K.L., Hangombe, B.M., Munyeme, M., et al., 2010. A comparative study ofseroprevalence of brucellosis in commercial and small scale mixed dairy-beef cattle enterprises of Lusaka province and Chibombo district, Zambia. Trop. Anim. Health Prod. 42, 1541–1545.
- Christopher, S., Umapathy, B.L., Ravikuma, K.L., 2010. Brucellosis: review on the recent trends in pathogenicity and laboratory diagnosis. J. Lab. Physicians 2, 55–60.
- Dean, A.G., Sullivan, K.M., Soe, M.M., 2009. OpenEpi: Open Source Epidemiologic Statistics for Public Health, Version 2.3.1, http://www.openepi.com/OE2.3/ Menu/OpenEpiMenu.htm. Retrieved 2014-05-27.
- Dean, A.S., Crump, L., Greter, H., Schelling, E., Zinsstag, J., 2012. Global burden of human brucellosis: a systematic review of disease frequency. PLoS Negl. Trop. Dis. 6, e1865.
- FAO, 2009. FAOSTAT, Food and Agricultural Organization Statistic Division.

 Available at: http://faostat.fao.org/site/573/DesktopDefault.

 aspx?PageID=573#ancorr (accessed on 12.10.13.).
- FAO, 2009. The State of Food and Agriculture. Food and Agriculture Organization, Rome, 89 pp. ISSN 0081-4539.
- Godfroid, J., Cloeckaert, A., Liatutard, P.J., Kohler, S., Fretin, D., Walravens, K., Garin-Bastuji, B., Jacques, L.J., 2005. From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis: review article. Vet. Res. 36, 313–326.
- Gwida, M., Dahouk, S.A., Melzer, F., Rösler, U., Neubauer, H., et al., 2010. Brucellosis—regionally emerging zoonotic disease? Croatian Med. J. 51, 289–295.
- Holt, H.R., Eltholth, M.M., Hegazy, M.Y., El-Tras, W.F., Tayel, A.A., et al., 2011. *Brucella* spp. infection in large ruminants in an endemic area of Egypt: cross-sectional study investigating seroprevalence, risk factors and livestock owner's knowledge, attitudes and practices (KAPs). BMC Public Health 11, 341, http://dx.doi.org/10.1186/1471-2458-11-341.
- Hashim, N., Hassabo, A., Yagoub, S., 2007. Serological detection of brucellosis in cattle and human. Res. J. Microbiol. 2 (11), 861–865.
- Islam, M.A., Khatun, M.M., Were, S.R., Sriranganathan, N., Boyle, S.M., 2013. A review of *Brucella* seroprevalence among humans and animals in Bangladesh with special emphasis on epidemiology, risk factors and control opportunities. Vet. Microbiol. 166 (3–4), 317–326, http://dx.doi.org/10.1016/j.vetmic.2013. 06 014
- John, K., Fitzpatrick, J., French, N., Kazwala, R., et al., 2010. Quantifying risk factors for human brucellosis in rural northern Tanzania. PLoS One 5 (4), e9968.
- Junaidu, A.U., Obooegbulem, S.I., Salihu, M.D., 2008. Seroprevalence of brucellosis in prison farm in Sokoto, Nigeria. Asian J. Epidemiol. 1 (1), 24–28.
- Junaidu, A.U., Oboegbulem, S.I., Salihu, M.D., 2011. Serological survey of Brucella antibodies in breeding herds. J. Microbiol. Biotechnol. Res. 1 (1), 60–65.
- El Kholy, A.A., Gomaa, H.E., El Anany, M.G., Abd, E.L., Rasheed, E., 2009. Diagnosis of human brucellosis in Egypt by polymerase chain reaction. East Mediterr. Health J. 15 (5), 1068–1074.
- Mai, H.M., Irons, P.C., Kabir, J., Thompson, P.N., 2012. A large seroprevalence survey of brucellosis in cattle herds under diverse production systems in northern Nigeria. BMC Vet. Res. 8, 144–157.
- Makita, K.E., Fèvre, M., Waiswa, C., Kaboyo, W., De Clare Bronsvoort, B.M., Eisler, M.C., Welburn, S.C., 2008. Human brucellosis in urban and peri-urban areas of Kampala, Uganda. Ann. N. Y. Acad. Sci. 1149, 309–311.

- Makita, K., Fe'vre, M.E., Waiswa, C., Eisler, M., Thrusfield, M., Welbum, S.C., 2011. Herd prevalence of bovine brucellosis and analysis of risk factors in cattle in urban and peri-urban areas of the Kampala economic zone, Uganda. BMC Vet. Res. 7, 60–68.
- Matope, G., Bhebhe, E., Muma, J.B., Lund, A., Skjerve, E., 2010. Herd-level factors for Brucella seropositivity in cattle reared in smallholder dairy farms of Zimbabwe. Prev. Vet. Med. 94, 213–221.
- Matope, G., Bhebhe, E., Muma, J.B., Oloya, J., Madekurozwa, R.L., Lund, A., Skjerve, E., 2011a. Seroprevalence of brucellosis and its associated risk factors in cattle from small holder dairy farms in Zimbabwe. Trop. Anim. Health Prod. 43, 975–982, http://dx.doi.org/10.1007/s11250-011-9794-4.
- Matope, G., Bhebhe, E., Muma, J.B., Lund, A., Skjerve, E., 2011b. Risk factors for *Brucella* spp. infection in smallholder household herds. Epidemiol. Infect. 139, 157–164, http://dx.doi.org/10.1017/s0950268810000968.
- McDermott, J.J., Arimi, S.M., 2002. Brucellosis in sub-Saharan Africa; epidemiology, control and impact. Vet. Microbiol. 90, 111–134.
- McDermott, J., Grace, D., Zinsstag, J., 2013. Economics of brucellosis impact and control in low-income countries. Rev. Sci. Tech. Off. Int. Epizoot. 32, 249–261
- Megersa, B., Biffa, D., Abunna, F., Regassa, A., Godfroid, J., Skjerve, E., 2011a. Seroprevalence of brucellosis and its contribution to abortion in cattle, camel, and goat kept under pastoral management in Borana, Ethiopia. Trop. Anim. Health Prod. 43, 651–656.
- Megersa, B., Biffa, D., Niguse, F., Rufael, T., Asmare, K., Skjerve, E., 2011b. Cattle brucellosis in traditional livestock husbandry practice in Southern and Eastern Ethiopia, and its zoonotic implication. Acta Vet. Scand. 53, 24–53.
- Mekonnen, H., Kalayou, S., Kyule, M., 2010. Serological survey of bovine brucellosis in Barka and Arado breeds (*Bos indicus*) of Western Tigray, Ethiopia. Prev. Vet. Med. 94, 28–35.
- MLFD, 2013. Estimated livestock population in Niger State. 2012 Annual Livestock Report of the Ministry of Livestock and Fisheries Development (MLFD), Minna, Niger State, Nigeria, February, p. 49.
- Mohammed, F.U., Ibrahim, S., Ajogi, I., Olaniyi, B.J.O., 2011. Prevalence of BovineBrucellosis and Risk Factors Assessment in Cattle Herds in Jigawa State. ISRN Vet. Sci. 2011, Article ID 132897, p. 4. doi:10.5402/2011/132897.
- Nielsen, K., Yu, W.L., 2010. Serological Diagnosis of Brucellosis. Ottawa Laboratories (Fallow field), Canadian Food Inspection Agency, Nepean, Ontario, Canada.

- OIE, 2009. Bovine Brucellosis In: Terrestrial Manual; Chapter 2.4.3. Available at http://www.oie.int. Accessed on August 2014.
- OIE, 2011. Bovine brucellosis. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. World Organization for Animal Health Report, Paris, France. Series 1–35. Available at: http://www.oie.int/fileadmin/home/eng/health.standards/tahm/2.04.03.bovine.brucell.pdf (accessed on 30.09.14.).
- OIE, 2012. Bovine brucellosis. In: Manual of the Diagnostic Tests and Vaccines for Terrestrial Animals, vol. 1, 6th ed., Office International Des Epizooties, Paris, France
- Radostits, O.M., Gay, C.C., Hinchcliff, K.W., Constable, P.D., 2007. Veterinary Medicine. A Text book of Diseases of Cattle, Sheep, Pigs, Goats and Horses, 10th ed. W.B. Saunders, London, pp. 963–985.
- Sanogo, M., Abatih, E., Thys, E., Fretin, D., Berkvens, D., et al., 2012. Risk factors associated with brucellosis seropositivity among cattle in the central savannah forest area of Ivory Coast, Prev. Vet. Med. 107, 51–56.
- Seleem, M.N., Boyle, S.M., Sriranganathan, N., 2010. Brucellosis: a re-emerging zoonosis. Vet. Microbiol. 140, 392–398.
- Smits, H.L., Abdoel, T.H., Solera, J., Clavijo, E., Diaz, R., 2003. Immunochromatographic *Brucella*-specific immunoglobulin M and G lateral flow assays for the serodiagnosis of human brucellosis. Clin. Diagn. Lab. Immunol. 10, 1141–1146.
- Swai, E., Schoonman, L., 2009. Human brucellosis: seroprevalence and risk factors related in Tanzania. Zoonoses Public Health 56 (4), 183–187.
- Tesfaye, G., Tsegaye, W., Chanie, M., Abinet, F., 2011. Seroprevalence and associated risk factors of bovine brucellosis in Addis Ababa dairy farms. Trop. Anim. Health Prod., http://dx.doi.org/10.1007/s11250-011-9798-0.
- Thrusfield, M., 2009. Veterinary Epidemiology, 3rd ed. Blackwell Science, Ltd., Oxford, UK.
- WHO, 2006. The control of neglected zoonotic diseases: a route to poverty alleviation. Report of a Joint WHO/DFID-AHP Meeting with the participation of FAO and OIE Geneva, 20–21 September 2005.
- WHO, 2009. Integrated Control of Neglected Zoonotic Diseases in Africa: Applying the One Health Concept. WHO Document Production Services, Geneva, Switzerland.
- WMADH, 2001. World medical association declaration of Helsinki, Ethical principles for medical research involving human subjects. Bull. World Health Organ. 79, 373–374.