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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 North-central 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 agro-ecological 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 18½" gage needle for each animal. The sera were transferred into sterile plain tubes and centrifuged at 3000 rpm for 10 min and then decanted into cryovials, identified before storage at −20 °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 °C. They were screened for antibodies against natural *Brucella* infection using Rose Bengal Plate-agglutination test (RBPT) and confirmed by use of IgM/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

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