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Research Paper

Brucella abortus infection in a multispecies livestock farm in Nigeria

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Abstract. Brucellosis screening was carried out in a farm in Nigeria comprising of cattle, sheep, goats and horses reared under semi-intensive management system. Vaginal swabs and milk were cultured for *Brucella* isolation while sera and part of the milk were tested serologically. *Brucella abortus* was isolated from 2 out of 6 vaginal swabs collected from horses and from 3 out of 12 milk samples obtained from lactating cows on the farm. No *Brucella* was isolated from all the 10 milk samples from sheep and the 10 milk samples from goats. All sera from the 7 horses tested positive by the Rose Bengal plate test (RBPT) and serum agglutination test (SAT). Nineteen (8.34%) out of 44 sera from cattle tested positive by SAT and RBPT while 4 (12.5%) out of 32 sheep sera were positive by SAT and RBPT. Out of 50 sera from goats, 5 (10%) were positive by SAT and RBPT. Four (33.3%) of the 12 milk samples from cattle tested positive by Milk ring test while all the 10 milk samples each from sheep and goats tested negative by Milk ring test. The possible source of infection in the farm could be the cattle. It is therefore necessary to consider all species of domestic animals in brucellosis surveillance and eradication. Immediate culling of all reactors, prevention of contact between the various animal species and improvement on hygienic practices on the farm were recommended.

Keywords: *Brucella abortus,* infection, multispecies, livestock, farm, Nigeria.

INTRODUCTION

Brucellosis is one of the most important zoonotic diseases worldwide and in developing countries in particular. It is caused by intracellular Gram-negative coccobacilli bacteria of the genus *Brucella*. The disease is a major cause of direct economic losses in the livestock industry and an impediment to trade and exportation (Lopez-Goni *et al*., 2008). The disease is characterized by abortion, retained placenta, neonatal deaths and reduction in milk production. Other signs are infertility, sterility, orchitis, epididymitis and hygroma. Fistulous withers and tenosynovitis are other signs of brucellosis in horses (Lephard and Hutchins, 1968). It is also a disease of great public health significance, being an important zoonosis. Previous reports of isolation from various parts of the country showed evidence of the disease in cattle, small ruminants, dogs and horses (Ocholi *et al.*, 2004a; Ocholi *et al.*, 2004b). Serological evidence of brucellosis has also been reported in various parts of Nigeria (Ajogi, 1997). One of the six well-known species of *Brucella* is *Brucella abortus*. Though the primary host of this species is cattle, it has been isolated from other animal species such as sheep and horses (Ocholi *et al.*, 2004b) due to its ability of cross infection. This was the case in this study in a multispecies livestock farm. There has been no recent history of abortion, hygroma, neonatal mortality or infertility which is suggestive

of brucellosis on this farm. However, brucellosis screening of lactating animals on the farm revealed presence of the infection.

The objective of this paper is to report the prevalence of Brucellosis and the isolation of the incriminating *Brucella* species in a farm that hosts mixed species of domestic animals.

MATERIALS AND METHODS

The farm

This farm has existed for over seventy years, rearing mainly cattle, sheep, goats and pigs. Horses were however introduced about twenty years ago. There has been regular introduction of new animals to maintain flock size over the years but without corresponding brucellosis screening. The farm has a total of 150 cattle, 50 sheep, 65 goats and 7 horses at the time of this study. It is located in the North central region of Nigeria, lying between Latitudes 8.50°N and 10.46°N and Longitudes 8.20°E and 10.36°E. The husbandry system is semi-intensive in nature. The animals spend most of their time confined within the paddocks where they are fed with hays, silages and concentrate with adequate provision of portable drinking water. They were however often taken out daily for grazing on fresh grasses by the farm attendants.

Sample collection

Five (5) ml of venous blood was collected from the jugular vein of 7 horses, 44 cattle, 32 sheep and 50 goats into 10 ml vacutainers tubes. The blood samples were allowed to clot by laying them down in a slanting position. Serum was then decanted into 5ml plastic tubes after centrifuging at 1,061 g (1000 rpm) for 5 min.

Vaginal swabs were collected from 6 mares, 12 cows, 10 sheep and 10 goats. Similarly, 5 ml of milk samples were collected from 12, 10 and 10 lactating cattle, sheep and goats respectively into sterile Bijou bottles and were transported on ice in a cold box to the laboratory for milk ring test and *Brucella* isolation. Milk samples were not collected from the mares because none of them was lactating.

Serological tests

Sera were tested for *Brucella* antibodies by Rose Bengal plate test (RBPT) and serum agglutination test (SAT) as described by Alton *et al.* (1988). Milk ring test was also conducted for milk samples collected from lactating cattle, sheep and goats as described by Alton *et al.* (1988).

Bacteriological examination

This was carried out as described by Alton *et al.* (1988). Samples were cultured on serum dextrose agar (SDA) with the addition of 2 ml of *Brucella* antibiotic supplement (Oxoid, England). Incubation was at 37°C for 72 h both aerobically and in atmosphere containing 10% CO_2. Culture plates were examined daily for three days. Tiny discrete circular and convex colonies were observed with smooth glistening surface, which had bluish-white translucent appearance in reflected light but were transparent honey-coloured on transmitted light. Colonies were stained by Gram reaction and observed under oil immersion lens of light Microscope.

Characterization and biotyping

The organisms were characterized as described by Alton *et al.* (1988) based on their growth on SDA in atmosphere containing 10% CO_2, their ability to produce hydrogen sulphide from a slant containing lead acetate paper. They were also tested with oxidase and urease reagents. Another characterization was done by testing the isolates with positive control serum for *Brucella* as well as with the negative control serum.

Biotyping of the isolates was based on their ability to produce hydrogen sulphide, growth in the presence of basic fuchsin and thionin dyes, lysis by Tbilisi, Weybridge, Izatnagar and Rough phages, and agglutination in polyclonal sera anti-A and anti-M.

RESULTS

All the 7 serum samples from the horses were positive to the two serological tests, Rose Bengal plate test (RBPT) and serum agglutination test (SAT). They were highly positive by SAT (+++) for *Brucella* antibodies at a serum dilution 1:160 (200 IU/ml). Out of 44 cattle sera tested by RBPT and SAT, 19 (8.34%) were positive. Similarly, 4 (12.5%) out of 32 sheep were positive while and 5 (10%) out of 50 samples from goats respectively were positive in the two tests (Table 1).

Out of the 12 milk samples from cattle, 4 (33.33%) were positive by MRT while all the 10 milk samples from sheep and 10 milk samples from goats were negative by MRT (Table 2).

Out of the twelve milk samples collected from cattle, 3 (25%) yielded *Brucella* isolates. *Brucella* was not isolated from all the milk samples collected from cattle, sheep and goats (Table 3).

Brucella was isolated from two out of the six vaginal swabs obtained from the horses. None was isolated from the vaginal swabs collected from either cattle, sheep or goats (Table 4).

Table 1. Rose Bengal plate test (RBPT) and serum agglutination test (SAT) from serum samples from the farm.

Animal species	No. sampled	No. of positive	% positive
Horses	7	7	100
Cattle	44	19	8.34
Sheep	32	4	12.5
Goats	50	5	10

Table 2. Milk ring test (MRT) from milk samples from the farm.

Animal species	No. sampled	No. of positive	% positive
Cattle	12	4	33.33
Sheep	10	0	0
Goats	10	0	0

Table 3. Isolation of *Brucella* from milk samples.

Animal species	No. sampled	Source	No. of positive	% positive
Cattle	12	Milk	3	25
Sheep	10	Milk	0	0
Goats	10	Milk	0	0

Table 4. *Brucella* isolation from vaginal swabs.

Animal species	No. sampled	No. of positive	% positive
Horses	6	2	33.3
Cattle	12	0	0
Sheep	10	0	0
Goats	10	0	0

The isolates grew on SDA without CO_2 and in atmosphere containing 10% CO_2. They appeared as tiny Gram-negative, coccobacilli organisms that were non-motile no bipolar characteristics and non-spore forming. They produced hydrogen sulphide and were all oxidase positive. They hydrolyzed urea and agglutinated in polyclonal sera anti-A and not anti-M. This shows that the organisms were *Brucella abortus*. Biotyping of the isolates showed that the organisms were lysed by Tbilisi (Tb), Weybridge (Wb) and Izatnagar (Iz) phages but were not lysed by Rough colonies (R/C) phage. They grew in the presence of basic fuchsin dye but not in thionin dye. The organisms were therefore identified as *Brucella abortus* biotype 1 (Table 5).

DISCUSSION

The finding of this study in which *Brucella abortus* was isolated from cattle and horses that do not show the typical signs of brucellosis is of very serious public health implications. This finding is of economic and public health significance. This is because of the exposure potentials to the farm workers who have close relationship with these animals and sometimes perform their duties without using any form of protective materials such as hand gloves. These are practices that expose the workers to the risk of contracting brucellosis. This finding is particularly important to horse owners in Nigeria as horses are being used for polo games, horse racing and also as ornamental animals, as these activities expose them to infection. The isolation of *B. abortus* from milk of lactating cows is also of great public health implication. This is because it is being sold to the public and some buyers prefer to take the milk fresh without boiling or pasteurization.

This finding agrees with the first report of equine brucellosis in Nigeria in1986 when *B. abortus* biotype 1 was isolated in an Arab barb stallion with fistulous withers (Oladosu *et al*., 1986). It has also been reported that

Table 5. Characterization and biotyping of *Brucella* isolates.

Isolates / reference strains	Other confirmatory tests				Monospecific antisera		Sensitivity to *Brucella* phages				Sensitivity to Thionin and Basic fuchsin	
	H_2S production	CO_2 req.	Oxidase test	Urease test	A	M	Tb	Wb	Iz	R/C	Thionin	Basic fuchsin
S19*	+	-					+	+	+	-	-	+
1h	+	-	+	+	+	-	+	+	+	-	-	+
2h	+	-	+	+	+	-	+	+	+	-	-	+
3c	+	-	+	+	+	-	+	+	+	-	-	+
4c	+	-	+	+	+	-	+	+	+	-	-	+
5c	+	-	+	+	+	-	+	+	+	-	-	+

H = isolate from horse
C = isolate from cattle
* positive control strain

osteoarthritis and Osteomyelitis are other signs associated with equine brucellosis (Collins *et al.*, 1971; Denny, 1972). *B. abortus* was observed as a possible cause of bursitis, fistulus withers and tenosynovitis in horses (Lephard and Hutchins, 1968). This is similar with the report by Ocholi *et al*. (2004a) who previously isolated *B. abortus* from a foal with carpal bursitis in the farm. There are other reports on isolation of *B. abortus* in Nigerian livestock especially cattle, sheep and goats (Eze, 1978; Bale and Kumi-Diaka, 1981; Poester *et al.*, 2002; Ocholi *et al.*, 2004b). These were associated with various clinical signs such as abortion, retained placenta, neonatal mortality infertility and hygroma which are typical signs of brucellosis, but differs from this finding because none of these signs were observed in the infected animals.

In a serological study carried out by Bale and Kwanashie (1984), *Brucella* antibodies were demonstrated in the sera of horses in Nigeria in which 14 (8.4%) out of 166 were positive. MacMillan (1985) also reported serological evidence of brucellosis in horses in a comprehensive retrospective study. In a recent study in horses in two states of Nigeria, 14.7% prevalence of brucellosis was reported (Ehizibolo *et al.*, 2011). These prevalence rates were lower compared to the 100% obtained in this study. The finding in cattle, in this study, is higher compared to the 6.2, 5.5 and 3.8% reported by Cadmus *et al.* (2006), Gusi *et al.* (2010) and Wungak *et al.* (2011); but was comparable to the 8.4% reported by Bertu *et al.* (2012). The prevalence of brucellosis in cattle in this study was higher compared to those reported in some African countries (Tolosa *et al.,* 2008; Sanogo, *et al.,* 2012). The prevalence rates in sheep and goats in this study were however lower compared to those previously reported (Bertu *et al.*, 2010). The high prevalence in horses and cattle in this study may be due to the very low sample sizes.

The animals sampled in this study were not showing any clinical signs of brucellosis. This is similar to the report by Ehizibolo *et al.* (2011) who recorded serological evidence of brucellosis in horses that were not showing clinical signs of brucellosis. The fact that these animals were infected with *B. abortus* without showing any clinical signs poses a great danger for farmers and veterinarians. It indicates therefore that suspicion of brucellosis in animals should not be restricted to only those showing clinical signs but also in-contact animals not showing signs should be routinely tested. This is because brucellosis is a zoonotic disease (Collard, 1962; Falade, 2002), capable of infecting humans, therefore all effort must be made to detect it in a farm. The unidentified infected animals may continue to shed the organisms thereby contaminating the environment and the handlers may be infected unknowingly. The isolation of *B. abortus* in these animals that showed no clinical signs of brucellosis opens a new dimension to the study of the epidemiology of brucellosis. Although it has been speculated that brucellosis could be asymptomatic in horse (Denny, 1973), there has been no report of isolation of the organism in such non-clinical cases. This study may therefore be the

first to confirm this speculation. Although no isolation was made from sheep and goats on the farm, they showed serological evidence of brucellosis. This is an indication that they might have been exposed to brucellosis previously but not shedding the organisms at the time of this study. The fact that all the animal species sampled on the farm showed serological evidence of infection is not surprising considering the husbandry practice in which all the animals on the farm mix and move freely in the grazing paddocks and have access to the same drinking water and feeding troughs. The source of the infection could not be easily ascertained; however it is most probable that the infection could have emanated from the cattle to the other animals (Edward, 2004; Ocholi *et al*., 2004a). This is because cattle are the primary host for *B. abortus* and are capable of shedding the organism copiously in the environment. Previous reports show that horse to horse or horse to cattle transmission is not likely to occur (Macmillan and Cockrem, 1985) since horses do not excrete the organisms in sufficient quantity to cause infection (Corbel and Henry, 1983). It also not likely that sheep and goats could shed the organisms in sufficient quantity to infect cattle. It is very evident that the herding of various species of animals together could favour the spread of brucellosis among the various species. This is so because infected species serve as sources of infection to the others.

CONCLUSION AND RECOMMENDATIONS

The practice of keeping multiple species of animals in a farm favours the spread of infectious diseases among the animals and should therefore be discouraged. The farm was advised to isolate and cull all infected animals. They were also advised to separate all the various species of animals into distinct confinements where they do not share feeding and drinking troughs. There is the need for the farm to design a regular screening programme for the animals in order to keep tract with the health status of the animals at all times. From this finding, it is important that the epidemiology of brucellosis in all domestic animals in Nigeria be seriously taken into consideration and their role in the transmission of Brucellosis established. This will form the baseline for the institution of control programme.

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