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Sero-prevalence of camel brucellosis in three abbatoirs of Nothern Nigeria

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A sero prevalence study of camel brucellosis was carried out in three abattoirs of Northern Nigeria during the period of October to December, 2013. A total of three hundred and eleven (311) serum samples were collected from Kano, Sokoto and Maiduguri municipal abattoirs. The serum samples were screened using the Rose Bengal plate test with positive samples further tested with the lateral flow immmnoaassay. Out of 180 camel sera collected from Kano Municipal abattoir, 4 (2.2%) were positive for *Brucella* antibodies by Rose Bengal plate test of which one was confirmed by lateral flow immunoassay. Moreover, on sex distribution 3 (3.5%) female camels and one (1.1%) male camel were positive. There was no significant association with sex and prevalence of *Brucella* antibodies (P>0.05). On age distribution, 3 (12.5%) adult camels were positive by only one young camel (0.6%) was positive. The result was statically significant (P<0.05). From the 32 camel sera collected from Sokoto Municipal abattoir, 2 (6.3%) were positive for *Brucella* antibodies by Rose Bengal plate test of which one was confirmed by lateral flow immunoassay. All positive samples were adult (16.7%) female (11.8%) camels. There was no significant association with sex and age of camel (P>0.05). None of the 99 serum samples collected from Maiduguri Municipal abattoir tested positive by Rose Bengal plate test. The study concluded that *Brucella* antibodies are present in camel and they were probably infected due to contact with infected cattle. The importance of these finding are discussed.

Key words: Sero-prevalence, brucellosis, camel, Nigeria.

INTRODUCTION

The world's camel population was estimated to be 19 million (FAO, 2010) with 80% found in Africa. The estimated camel population in Nigeria varied from 25, 000 to 90,000 (Adamu and Ajogi, 1999) with most animals kept around Borno, Kano and Sokoto States in the Northern part of the country. Most camels in Nigeria

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are imported from neighbouring Chad and Niger Republics where camel breeding is very common (FDLPCS,1992; Ducrotoy et al., 2014). Due to their physiological features, camels have the ability to survive under harsh environmental conditions. They are important sources of milk, meat; leather and wool in many parts of the world (Gwida et al., 2012). They have been used as a source of investment and long-time saving, in sports, transportation and tourism (Wilson 1984; Rollefson, 2000). As a result of their unique qualities, camels were in the past thought to be resistant to diseases affecting livestock (Gauthier-Pilters and Dagg, 1981; Zaki, 1948; Bitter, 1986, Dalling et al., 1988). However, recent studies have shown that camels are susceptible to many diseases affecting livestock species including brucellosis (Abbas and Tilley, 1990; Abbas and Agab, 2002; Musa, 2008; Gwida et al., 2012).

Brucellosis is one of the most important zoonotic diseases in the world. It is an economically important disease in livestock resulting in abortion, still births, retained placenta, reduced milk yield and infertility (Godfroid et al., 2011). Although the disease has been eradicated in some developed countries, it is still a problem in developing countries. There are also reports of its re-emergence in some developed countries where the disease has been previously eradicated (Seleem et al., 2011). The disease is caused by bacteria belonging to the genus *Brucella*. Eleven species are currently recognised belonging to the genus *Brucella*; with seven species found in terrestrial animals; *Brucella abortus* (in cattle and buffalos*), Brucella melitensis* (in sheep and goats), *Brucella ovis* (in sheep), *Brucella suis* (in pigs), *Brucella canis* (in dogs), *Brucella neotomae* (in desert wood rats) and *Brucella microti* found in the common vole (Verger et al., 1987). Two species that affect marine mammals are *Brucella pinnipedialis* found in pinnepeds and *Brucella ceti* in cetaceans (Foster et al., 2007); a novel *Brucella* species *Brucella inopinata,* has been isolated in a breast implant infection *(*Scholz *et al*., 2010*)*. Recently, *Brucella* species has been isolated from baboons and was named *Brucella papionis* (Whatmore et al., 2014). Brucellae are usually host specific meaning a particular species infect specific animal host; however, cross infection have been reported where infection can occur in animals that are not the primary host of a particular species. *B. abortus* has also been reported in sheep, goats, dogs, horses and camels (FAO, 2004) and *B. melitensis* in cattle, dogs, camels and pigs (Godfroid*,* 2004).

The earliest report of Brucellosis in camels was in 1931 (Solonitsuin, 1949). Since then the disease has been reported in camels in the middle East (Radwan et al., 1992; Dawood, 2008; Yawoz et al., *2012*), North Africa (Musa and Shigidi, 2011; Sisay and Mekonnen, 2012) and East Africa(Wanjohi *et al.,* 2001). In Nigeria, Brucellosis was first reported in camels by Okoh (1979) who carried out a serological survey on animals brought to slaughter house by showing 1.0% prevalence of *Brucella* antibodies. Subsequent studies reported prevalence rates of 2% (Zaria et al.,1990) and 7.5% (Kudi et al., 1997) in an abattoir surveys carried in Maiduguri and Kano respectively and in a very recent study carried out in Sokoto municipal abattoira prevalence rate of 19.5 % was found (Junaidu et al., 2006). This study examined sera from camels slaughtered in abattoirs in three cities of Northern Nigeria for presence of *Brucella* antibodies using the Rose Bengal plate test (RBPT) as a screening test, and also used lateral flow immunoassay (LFiA) for confirmation test.

MATERIALS AND METHODS

Study areas and period.

The studies were carried out in 2013 between October and December in Kano, Maiduguri and Sokoto Municipal abattoirs in Northern Nigeria (Figure 1)

Sample collection

Camels brought for slaughter were sampled based on consent and co-operation from butchers. Blood (5 ml) was collected from the jugular vein at the time of slaughter using sterile sample tubes. The tubes were labeled with the animal's sex and age (young 6 to 9 months and adult one year and above). The blood samples were allowed to clot in a slanting position, then transported to the laboratory in a leak-proof container with ice packs. They were centrifuged at 1000 rpm for 5 mins. Sera were then decanted into 5 ml plastic tubes and stored in the refrigerator at –20°C until required for testing. The distribution of samples collected from the three abattoirs is presented in Table 1.

Serological examination

Serum samples were tested for *Brucella* antibodies by RBPT as described by Alton et al. (1988). Samples positive by RBPT were further tested by *Brucella* LFiA done as described by Abdoel et al. (2008)

Rose Bengal plate test

Briefly, 30 µL of RBPT antigen (Procured from Veterinary Laboratory, United Kingdom) were added to an equal volume of serum on a ceramic tile. The sera and the antigen were mixed with an applicator stick and rocked gently. It was observed for four minutes for agglutination. Result was graded as +1, +2 or + 3 based on degree of agglutination.

Brucella lateral flow immuno assay

Lateral flow immunoassay was performed as described by Abdoel et al. (2008) using bovine *Brucella abortus* kit (Royal Tropical Institute, the Netherlands). The flow kit was removed from the packaging and placed on a bench top with the test window facing upwards. 5 uLof test serum were pipetted into the round sample port on the sample pad. Immediately, 130 ul of running fluid were added to the sample port. The sample pad was allowed standing for

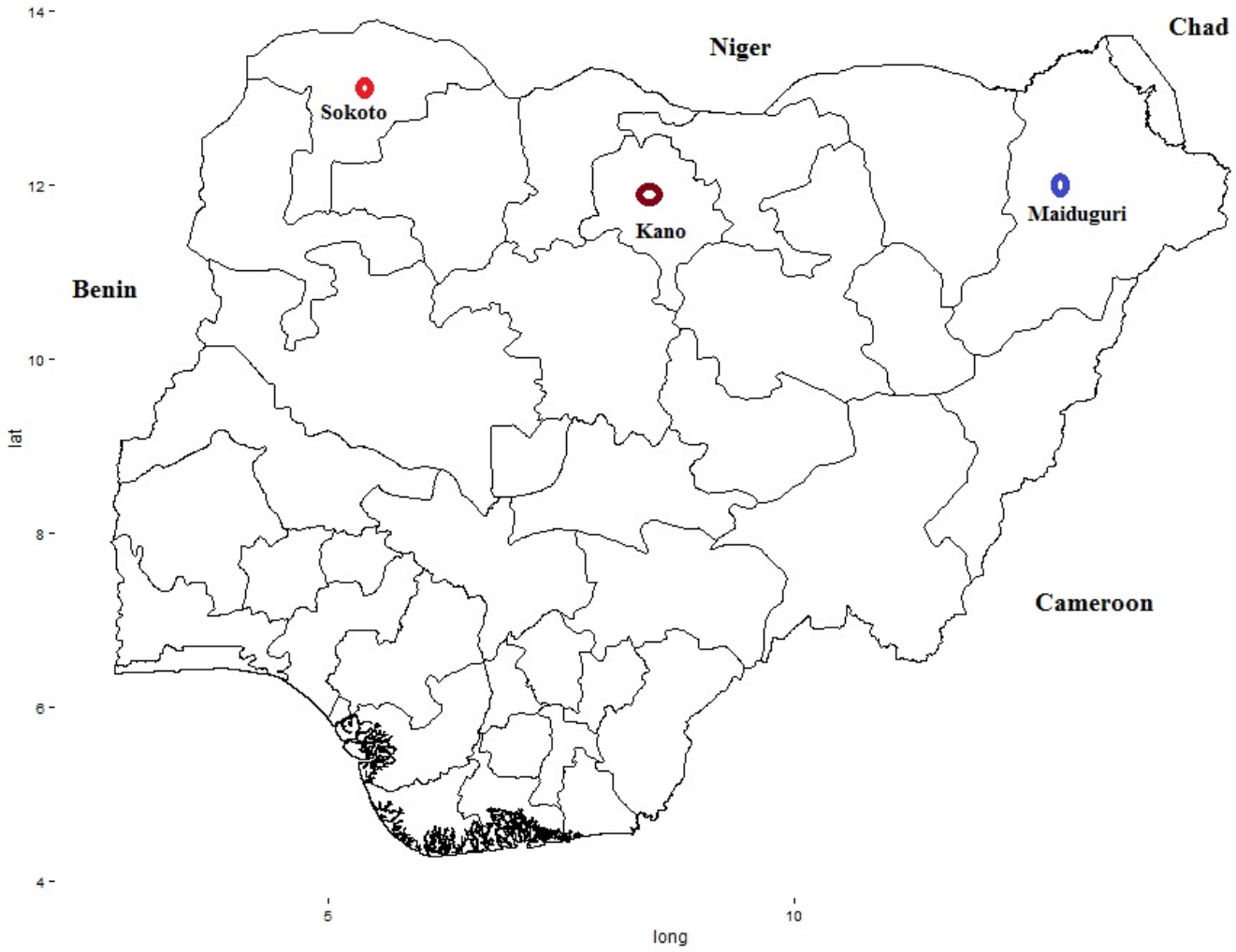

Figure 1. Map of Nigeria showing the cities where the study was carried out.

Table 1. Distribution of samples collected from camel in three abbatoirs of Northern Nigeria.

Variable	Total	Males	Females	Young	Adults
Maiduguri	99	39	60	85	14
Kano	180	94	86	156	24
Sokoto	32	15	17	20	12
Total	311	148	163	261	50

10 min before results were read. A positive result was indicated by the presence of a line at the test zone and a line at the control zone. A negative result is indicated by absence of a line in the test zone and the presence of a line in the control zone. Results were graded as +1or +2 based on intensity of the red line

Statistical analysis

Descriptive statistics was calculated using open Epi version 3.03 to determine the level of *Brucella* sero -prevalence in relation to age and sex of camels. P-value lower than 0.05, was considered statistically significant.

RESULTS

The sero-prevalence of *Brucella* antibodies in camel sera collected from abattoirs in the three cities by RBPT is presented in Table 2. Out of 180 camel sera collected from Kano Municipal abattoir, 4 (2.2%) were positive for *Brucella* antibodies by RBPT of which one was confirmed by LFiA. However, on sex distribution 3 (3.5%) female camels and one (1.1%) male camel were positive. There was no significant association with sex and prevalence of *Brucella* antibodies (P>0.05). On age distribution, 3 (12.5%) adult camels were positive by only one young camel (0.6%) was positive. The result was statically significant (P<0.05). From the 32 camel sera collected from Sokoto Municipal abattoir, 2 (6.3%) were positive for *Brucella* antibodies by RBPT of which one was confirmed

Table 2. Sero-prevalance of brucellosis in Camel in three abbatoirs of Northern Nigeria.

Variable	No	RBPT	Females	Males	Adults	Young	LFA*
Kano	180	4 (2.2%)	3 (3.5%)	1 (1.1%)	3 (12.5%)	1 (0.6%)	1
Sokoto	32	2 (6.3%)	2 (11.8%)	0 (0)	2 (16.7%)	0 (0)	1
Maiduguri	99	0	0	0	0	0	0
Total	311	6	5	1	5	1	2

*All sample positive by LFA were adult female camels.

by LFiA. All positive samples were adult (16.7%) female (11.8%) camels. There was no significant association with sex and age of camel (P>0.05). None of the 99 serum samples collected from Maiduguri Municipal abattoir tested positive by RBPT.

DISCUSSION

The 2.2% sero-prevalence recorded in camel from samples collected from Kano Municipal abattoir compares with that by Okoh (1979) who reported a prevalence rate of 1% in camel slaughtered in the same area. Warsame et al. (2012) also reported a sero-prevalence rate of 2% in a study carried out in and around Dire Dawa, District of Ethiopia. However, the result was lower than 7.5% prevalence reported by Kudi et al. (1997), who carried out a similar study realating to this study in Kano. The variation in the prevalence rates could be due to the fact that Kudi et al. (1997) used micro serum agglutination test (MSAT) while this study used the RBPT. The MSAT has been reported to be a poor test in camel sera with a lot of false positive results (Abbas and Agab, 2002)

A sero-prevalence rate of 6.3% was recorded in this study in camels slaughtered at Sokoto municipal abattoir. This is agrees with studies carried out by Junaidu et al. (2006) who reported a sero-prevalence rate of 11.42% in the same area. This shows that the prevalence rate has remained high in the area. This may probably be due to the fact that sick camels intended for slaughter may be easily imported into Nigeria through the Sokoto border due to its proximity to camels rearing region of Niger. This study is also in accord with Sisay and Mekonen (2012) who reported a sero-prevalence rate of 11.9% in a study carried out in Afar region of Ethiopia.

A zero percent sero-prevalence was reported in camel slaughtered in Maiduguri Municipal abattoir. The study result is quite surprising as sero-prevalence rates of 9.5 and 25.5% have been reported in the same area by Zaria et al. (1990).

The variations in the sero-prevalance rates recorded in the three cities when compared to other similar studies in the same areas could also be due to the fact that being an abattoir survey, the possibility of sampling an infected animal will depend on it being brought for slaughter. Some infected camels may be slaughtered without being taken to the abattoir or sold out.

In Nigeria, the RBPT is widely used as a screening test for brucellosis because of its simplicity, rapidity and field suitability. However, result of RBPT requires a confirmation by compliment fixation test (CFT) or enzyme linked immunosorbent assay (ELISA) as recommended by OIE (2009). These tests (CFT and ELISA), require specialize training and expensive laboratory equipment and reagents. The LFA has several practical advantages which include the fact that the use of the LFA does not require specific training, expertise, electricity or expensive equipment, and the test devices may be stored without the need for refrigeration. The test results are obtained almost instantaneously and by visual inspection with the unaided eye (Abdoel et al., 2008). The test has been compared to Competitive ELISA (cELISA), and was reported to be even more sensitive and specific than cELISA (Bronsvoort et al., 2009). Its sensitivity and specificity was reported to be 87 and 97%, respectively (Bronsvoort et al., 2009). It was suggested to be used as a confirmatory test.

In this study, the RBPT was used as a screening test, and LFiA as a confirmatory test. The result of the RBPT that were graded as +2 and above were all confirm by LFiA. Since the Kit had bovine specific conjugates, it is likely that the camels were infected with as a result of contact with cattle. The contact with infected cattle could be from countries where the camels were imported or with cattle within Nigeria, since camels and other livestock are usually reared together (Ducrotoy et al., 2014). Camels are not known to be primary host to *Brucell*a species but infection has been reported due to contact with infected cattle or sheep (FAO, 2004). Protocol for bovine brucellosis has been used to screen camel sera used (Gwida et al., 2012). Although the LFiA produces specifically cattle, sheep, pigs and goats using host specific conjugates. It has not been used to test camel sera. The study recommends a large scale study using bovine LFiA kit in camel sera.

A high prevalence rates recorded in females in Kano (3.5%)) and Sokoto (11.8%) than in males (1.1 and 0%). The results were not statistically significant in both cases (P< 0.5). The study findings is in accord with Sadiq et al. (2011) who reported a higher prevalence rate (5.5%) in females camels than (3.9%) in males in a study carried

out in Lake Chad region of Borno State, North Easthern, Nigeria and Warsame et al. (2012) who also reported a higher prevalence rate in females (1.7%) than males (1.4%) camels in another study carried out in and around Dire Dawa, Ethiopia. Dawood (2008) also reported a high prevalence rate in female (13.8%) than males (7.5%) camels in South province of Jordan. Female animals are known to be the main source and foci of *Brucella* infection (Godfroid et al., 2010). Brucellae are also known to have a high affinity to the alcohol D-erythritol found in higher volume in the gravid uterus than the seminal vesicles, making the infection more common in female than male cattle (Walker, 2004).

A higher prevalence rates were also recorded in adult camels in Kano (12.5%)) and Sokoto (16.7%) than in young camels (0.6 and 0%). The results were statistically significant in both cases (P< 0.5). This is in agreement with Dawood (2008) who reported a higher prevalence rate (64.8%) in adult than (35.2%) in young camels in southern province of Jordan, and Sisay and Mekonnen (2012) also reported a higher prevalence rate (13.8%) in adult than (0) in young camels in selected district of Afar region in Ethiopia. Musa and Shigidi (2001) also reported higher prevalence range of 6.8 to 9.2% in camels 21/2 to 4 years than 4.2% in camels of 6 to 9 months old. This is not surprising since Brucellosis is mainly a disease of the reproductive tract where the organisms have a higher affinity to the reproductive organs (Acha and Szyfres, 2003). Young animals are not sexually mature and may be resistant to *Brucella* infection.

Conclusion

The study showed that *Brucella* antibodies are present in camels in Nigeria with the infection possibly as a result of contact with infected cattle. Although there were variations in the prevalence rates when compare to similar studies in the same area, the reason could due to the fact that the present study is an abattoir survey. The LFiA kit, though produced specifically for cattle, it was able to detect the presence of *Brucella* antibodies in camel sera. The LFiA may be a good test in camels. The study recommends a large evaluation of the test in camel sera. More studies need to be carried out especially in mixed herds (camels and other livestock) to highlight the risk factor of the disease in camel. The study also recommends an officially coordinated control programmes for brucellosis in Livestock as a means of controlling the diseases in camels, since camels are spill over host

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Conflict of interest

The authors have none to declare.

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