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Serological survey of Brucella antibodies in breeding herds

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ABSTRACT

Background: *Brucellosis has continued to be a problem in Nigeria with little or no attention being paid towards its eradication. Sokoto state which is the study area for this work is second in terms of cattle population in Nigeria. Several studies on brucellosis in Nigeria are mainly in slaughter cattle and few farms. A serological survey was conducted in all the season to determine the prevalence of brucella antibodies in breeding herds Sokoto.* ***Results:*** *A total of 1547 serum samples and 99 milk samples from lactating cow collected from cattle in the selected herds over a period of one year. Only 305(19.5%) of the cattle were seropositive while 25(25.25%) of the milk samples were positive. Sex distribution of brucella infection in cattle indicates that 16.6% and 21.96% were recorded in males and females respectively. There was an association between brucella infection and sex of animals (χ^2=8.80; P=0.009). In the age distribution, 34(11.11%) of 305 at age band of 0-12 months were positive while cattle in the age band > 24 months recorded the highest prevalence of 188(23.82%) of 789. there was a significant association between brucella infection and age (χ^2=22.19; P< 0.0001). Sokoto gudali had the highest prevalence rate of 29.59% while Azuwarq and White Fulani recorded 13.23% and 6.45% respectively. There was an association between bruclla infection and breed (χ2=99.11; P< 0.0001).* ***Conclusion:*** *Detection of antibodies against brucella in the herds and detection of brucella in milk is of serious public health and economic importance. This study has shown that brucellosis is endemic in all parts of the state. The high prevalence recoded is a wake-up call to all stakeholders for a rapid response to arrest the spread to other animals within and neighboring states and neighboring countries.*

Keywords: Antibodies, Brucella, Breeding, Cattle, Seroprevalence,

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INTRODUCTION

Brucellosis is a world-wide problem of both Public health and economic importance. It is of public health significance not only because of direct and indirect transmission of the disease

from infected animals to man, but also because it causes serious diminution of much needed animal protein which are essential to human health.

The prevalence of *Brucella abortus* agglutinins in different parts of the country have been reported [1, 2, 5, 7, 8, 9, 13, 14].

Most of the studies carried out in the Northwestern Nigeria are based on abattoir reports. This paper reports on observation on seroprevalence of bovine brucella infection in breeding herds in Sokoto State, Nigeria.

MATERIAL AND METHODS

Study Area: Sokoto State is located between Longitude 11^o 30' and 13^o 50' East and Latitude 4^o and 6^o North. The State is divided into four agricultural zones namely Gwadabawa, Sokoto, Isa and Tambuwal. There is 5-6 local government area in each zone.

Sampling method
Multistage sampling technique was used in this study. Two local government areas were selected by simple random method in each of the four zones. The numbers of herds to be sampled were determined for each of the local government using systematic sampling techniques. Sampling was done for one year from January to December 2008

Sample collection:
Serum collection: Five milliliter (5ml) of uncoagulated blood was collected aspetically from the jugular vein. The samples were transported in vacuum containers to the laboratory and allowed to clot. Serum formed was then stored at -20^oC until required for use. The ages and sexes of the animals were being recorded as blood samples were collected.

Milk Samples: Milk samples were collected from lactating cows for brucella milk ring test. From each animal, 10 and 20ml of milk was collected mid stream into a clean sterile bottle. The samples were then kept at 4^oC until required for use.

A total of 1547 serum samples were collected from all the four Agricultural zones of the State. The Rose Bengal Plat Test (RBPT), serum agglutination test (SAT) and competitive Elisa (Compelisa were employed). All the antigens and Compelisa kit were obtained from Weybridge Laboratory London.

The RBPT and SAT were performed according to the Procedure described by Alton *et al.*, [3]. The RBPT was used as a screening test and the results interpreted as either positive. If there was agglutination or negative, if there was no agglutination only positive sera were re-tested by the SAT to quantify the antibody titres. A reaction with antibody titre of 50IU/ml and above was considered as evidence of infection [11]. The competitive enzyme linked immunosorbent Assay reagents and protocol were obtained from the Weybridge laboratories London.

Milk Ring Test:
The milk samples for Milk Ring Test (MRT) brucella abortus antigen were removed from refrigeration when required for use and kept at room temperature on the bench for 1 hour before the commencement of the test. The MRT was carried out as described by Morgan (1967) and Alton *et al* [3].

Each milk sample was gently mixed in the sample bottle to ensure even distribution of the cream. Two milliliter (2ml) of the test milk was placed in a test tube and one drop (0.03ml) of the Milk Ring Test (MRK) *Brucella abortus* antigen was added into it using a pipette. The content of the tube was mixed within 1 minute of adding the MRK antigen. The tube was then incubated at 37^{o}C for 2 hours. The tubes were then removed and observed for formation of blue ring colour at the cream layer of the milk.

Statistical Analysis: Data obtained from the study were subjected to Chi-square statistical test to determine the association between brucella infection and the test variables.

RESULTS

Out of a total of 1,547 cattle screened from 65 herds over the entire period of the study, 305 were found to be positive (19.71%) using Rose Bengal Plate Test. Out of the 272 samples that were positive for serum agglutination test, the titres were found to be in the range of 1:40 – 1:160. There were 22 samples with a titre of 1:40; 232 samples had a titre of 1:80 while 61 had a titre of 1:160.

Sex distribution: Out of the 1,547 cattle screened, 650 were males and 897 were females. A prevalence of 16.61% in males and 21.96% in females were recorded. There was an association between brucella infection and sex (χ^2 = 8.80, P = 0.0091) (Table 1).

Age distribution indicated that 34 (11.11%) out of the 305 cattle were positive in the age band of 0-12 months, 83(18.36%) out of 452 were positive for those within 13-24 months age band and 188 (23.82%) out of 789 for those above 24 months of age. There was a significant association between brucella infection and age (χ^2 = 22.19, P<0.0001) (Table 2).

Breed distribution: The three breeds identified during the study were Sokoto Gudali, Azuwarq and White Fulani. Result on breed showed that Sokoto Gudali had a prevalence of 29.59%. Azuwarq had a prevalence of 13.23% while a prevalence of 6.45% was recorded in the White Fulani. There was a strong association between brucella infection and breed (χ^2 = 99.11, P<0.0001) (Table 3).

Seasonal distribution: Result on seasonal distribution indicated that during the dry season, 137 (25%) out of 552 screened samples were positive, 82 (17.5%) out of 468 were positive in the wet season while during the hot season, 86 out of 527 screened samples (16.37%) were positive. There was a significant association between season and brucella infection (χ^2 = 14.02, P<0.007 (Table 4).

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Agricultural Zones distribution: The highest prevalence of 22.3% was recorded in Isa zone. Sokoto zone, a prevalence of 20.0% was observed while in Gwadabawa and Tambuwal zones a prevalence of 18.3% and 17.6% was recorded respectively (Table 5).

Milk Ring Test: Out of the 235 lactating cows recorded 99 cows were screened and from this number, 25 (25.25%) were found to be positive; out of this number 6 were posiive during the dry season, 9 during the hot season and 10 during the wet season (Table 6).
Competitive Elisa: All the positive samples RBPT and SAT were found to be positive for competitive Elisa. Similarly 100 negative samples were further tested using competitive Elisa and two were found to be positive.

Table 1: Sex Distribution of *Brucella* infection in Cattle (Breeding) herds in (RBPT)

Sex	Brucella infected	Non infected	Total
Male	108(16.61%)	542	650
Female	197(21.96%)	700	897
Total	305	1242	1547

$\chi^2 = 6.8069$, p = 0.0091

Table 2: Age Distribution of *Brucella* infection in Cattle Herds (RBPT)

Age (Months	Brucella infected	Non infected	Total
0-12	34(11.11%)	272	304
13-24	83(18.36%)	369	452
>24	188(23.82%)	601	789
Total	305	1242	1547

$\chi^2 = 22.902$, p< 0.0001

Table 3: Breed Distribution of *Brucella* infection in Cattle (Breeding) herds (RBPT)

Breed	Brucella infected	Non infected	Total
Sokoto Gudali	227(29.6%)	540	767
Azuwarq	54(13.2%)	354	408
White Fulani	24(6.5%)	348	372
Total	305(100%)	1242	1547

$\chi^2 = 199.11$, p<0.0001

Table 4: Seasonal prevalence of *Brucella* infection in Cattle (Breeding) Herds

Season	Brucella infected	Non infected	Total
Dry	137(25%)	415	552
Hot	86(16.5%)	441	527
Wet	82(17.5%)	386	468
Total	305	1242	1547

$\chi^2 = 14.02$, p = 0.0007

Table 5: Distribution of *Brucella* infection in the four Agric zones (RBPT)

Zone	Brucella infected	Non infected	Total
Isa	78(22.3%)	271	349
Sokoto	89(20.0%)	355	444
Gwadabawa	83(18.3%)	360	453
Tambuwal	55(17.6%)	256	311
Total	305	1242	1547

Table 6: Summary of Results on Milk Ring Test from Breeding Herds

S/No.	Season	No. of Herds	No. of Herds samples	No. of Animals in the Herds	No. of Cattle Screened	No. of Lactating cows in the Herds	No. of Lactating cows screened	No. of positive samples MRT
1.	Dry	15	3	603	121	10	4	2
2.	Dry	20	4	573	115	13	5	1
3.	Dry	30	6	1159	239	40	16	3
4.	Dry	21	4	424	85	9	4	-
Sub-Total		86	17	2759	560	62	29	6
5.	Hot	20	4	870	174	19	8	2
6.	Hot	19	4	626	126	11	4	-
7.	Hot	30	6	714	143	25	10	4
8.	Hot	16	3	420	84	27	11	3
Sub-Total		85	17	2630	527	82	33	9
9.	Wet	28	7	732	147	21	8	2
10.	Wet	22	5	541	108	13	5	1
11.	Wet	15	3	359	71	31	12	5
12.	Wet	27	6	796	142	31	12	3
Sub-Total		92	21	2428	468	96	27	11
Grand Total		263	55	7817	1051	235	99	25

DISCUSSION

Prevalence of 19.71% recorded in this study is in agreement with the findings of Esuruoso [8], who observed that the prevalence rate of brucellosis in Northern State falls within the range of 0-2% which is low compared with that in the South which is as high as 20-60%. The high prevalence rate recorded in this study may result from absence of annual vaccination programme, improper hygienic practices in disposing aborted fetuses, placenta and vaginal discharges which contain virulent *Brucella abortus* thus contaminating the pasture and water, serving as reservoirs of infection to other cattle [6]. Infected cows and bulls are not also normally isolated or culled from the herd thus making the dissemination of the infection in the herd a continuing cycle [16].

The higher prevalence recorded in the female than in the male may result from the fact that the foci of infection remain in females, which spread the infection from one animal to another as observed by Ogundipe *et al* [15). Similarly, it appears that female animals are generally more susceptible Brucella infection that the males because of D-erythritol, an alcohol normally found in higher volume in foetal tissues in pregnant females than in seminal vesicles and testes of the males [10] and is known to stimulate the growth of virulent strains of Brucella [17]. The higher prevalence recorded in the age band of above 24 months indicated that older cattle are more susceptible due to exposure. It was observed that younger animals tend to be more resistant to infection and frequently eliminate infections while sexually matured animals are much more susceptible to infection [5]. The higher prevalence recorded during the dry season could be due to the higher concentration of animals around water points during the period [13] and close contact in the night [12].

A prevalence of 25.25% in lactating cows further leads credence to the findings in the sera of the cattle in the herds. *Brucella abortus* has been isolated from raw and sour milk of Fulani cattle in Nigeria [4, 7]. The prevalence of *Brucella abortus* in the milk of lactating cows may suggests

that *Brucella abortus* is secreted in the milk and may serve as a source of infection in milk consumers.

The identification of brucella agglutinins in milk of infected lactating cows in the breeding herds is significant and further confirms the endemicity of brucellosis in cattle in the study area. This also indicate that *Brucella abortus* is being continually shed in lactating infected milk of cows thus constituting public health hazard to the consumers of raw or unpasteurised milk and milk products. The Fulani herdsmen and their families are at high risk of exposure and infection due to their closeness to the cattle and their food habits. The high prevalence recorded in the breeding herds portrayed serious economic losses in terms of death of adult cattle, abortion, and infertility and decreased milk production. Based on these there is the need for improved hygienic measures such as proper disposal of aborted foetal materials and protection of livestock farmers and veterinarians as well as periodic screening to determine their health status. Control measures such as compulsory vaccination, movement restriction, quarantine, test and slaughter should be instituted.

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