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Application of Competitive Elisa (Compelisa) Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT) for Detection of Antibodies to *Brucella* Infection in Slaughter Cattle in Sokoto, Nigeria

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ABSTRACT
The application of Rose Bengal Plate Test, serum agglutination and competitive Elisa was employed for the detection of *Brucella* antibodies in slaughtered cattle in Sokoto metropolis. It was observed that of the 1711 screened serum samples, 383 (22.38%) 376 (21.97%) and 395 (23.08%) were positive for *Brucella* using RBPT, SAT and competitive Elisa, respectively. The females had a higher prevalence than the males in each of the three tests (23.63) RBPT, (23.05%) SAT and (24.49) Competitive Elisa. On age, the highest prevalence was recorded within the age of 24 months and above for all the three tests. The breed with the highest prevalence was Arzuwaq. Of the 3 seasons, dry season recorded the highest prevalence. Based on these results, it was observed that application of more than one test method might ensure more sensitivity and specificity of the diagnosis of brucellosis.

Key words: Application, SAT, RBPT Compelisa, slaughter cattle

INTRODUCTION
Several serological tests have been developed and used as screening methods for the detection of *Brucella* infection in animals and humans. The application of both RBPT and SAT as screening methods has been previously reported to be standard and accurate (Alton *et. al.,* 1975). However, a single serological test may have the limitation of not detecting all the relevant antibodies at different stages of infection, thus necessitating the application of more than one test method to enhanced sensitivity and specificity of diagnosis (Alton *et. al.,* 1975). The office of the International Epizootics (OLE) recommended the Rose Bengal Plate test (RBPT) and ELISA as screening test and SAT as supplementary test.

Evidence of bovine brucellosis and its public health significance has been reported by several authors (Adams and Mckay, 1966; Esuruoso 1974, 1977; Ajogi 1977; Garba *et. al..,* 1997). It has also been reported in various species like sheep and goats (Falade 1974, Brisbe *et. al.,* 1993), humans (Alausa and Awoseyi, 1976). However, there has not been any comprehensive report on brucellosis in slaughtered cattle from Sokoto. The objective of this study was to investigate the seroprevalence of brucellosis in Sokoto abattoir through the application of RBPT, SAT and competitive Elisa so as to update and contribute to the existing epidemiological data on bovine brucellosis in Nigeria as well as assess the sensitivity and specificity of the three tests.

MATERIALS AND METHODS
One thousand seven hundred and eleven (1711) slaughtered cattle consisting of Azuwarq, Sokoto Gudale and White Fulani from the Sokoto metropolitan abattoir were used in this study. Blood from the severed jugular vein of cattle was collected into clean, sterile and clearly labeled universal bottles without any anticoagulant. The sera

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were separated from the clotted blood within twelve hours of collection by centrifuging it at 3000 rpm for 5 minutes using tube centrifuge.

The collected sera were screened by the RBPT as described by Morgan (1967) and Alton *et. al.* (1975). A drop of *Brucella abortus* stained antigen was stirred on a clean plate with one drop of the test serum using sterile tooth pick and rocked repeatedly. The formation of distinct pink granules after four minutes was recorded as positive evidence of *Brucella* antibodies in that sample.

Those samples with positive reaction to the RBPT were tested by SAT using Weybridge tube agglutination test antigen according to the methods approved by World Health Organization (Morgan 1967). For each of the samples that were positive with RBPT, a two fold serial dilution of test sera from 1:10 (0.05 ml) serum mixed with 0.45 ml phenolized saline-serum to 1:1280 was made. To each 0.5 ml tube of phenolized saline-serum mixture, an equal volume (0.5 ml) of the antigen was added. The tubes containing the antigen-serum mixture were covered, shaken and incubated at 37°C for at least 24 hours. A positive reaction was one in which the serum-antigen mixture was clear with precipitate at the bottom not disrupted by gentle agitation. Any serum with agglutination at dilution of 1:40 (titre of 40) 50 international units) and above was recorded as positive sample (Alton *et. al.,* 1975). Competitive Elisa kit was obtained from Veterinary Laboratories Agency, Weybridge, United Kingdom. The reagents in the kit were reconstituted as directed by the manufacturers. These included diluting buffer, washing solution, stopping, conjugated solution and control sera. The test procedure was carried out as instructed by the manufactures.

All the collected sample sera were subjected to Competitive Elisa following the manufacturer's instruction as briefly described below:

Test procedure

The microtitre plate used has 60 columns which serve as well twelve wells (1 - 12) are longitudinally located while eight (A - H) laterally located. Twenty millilitre of each test serum was added per each well of the microtitre plate. Well 11 and 12 were used for controls.

Thus 20 μl of the negative control was added to wells Al 1, A12, B11, /B12, C1l and Cl2, similarly 20μl of the positive control was added to wells F1l, F12, G1l, G2, H1l, and H12 the remaining wells had no serum added i.e. D11, D12, Eli, E12 and these served as conjugate controls. 100μ1, of the prepared conjugate was immediately dispersed in to all wells. This gives a serum dilution of 1/6. The plate was then shaken with hand for 2 minutes in order to mix the serum and conjugate solution. The microtitre plate was then covered with lid and incubated at room temperature for 30 minutes. The plate was then shaken for 30 seconds followed by 10 seconds shaking at 10 minutes interval for one hour. This was done carefully to keep the liquid within their respective wells. The contents of the plate was then discarded and rinsed 5 times with washing solution and then dried by tapping on an absorbent tissue paper. The substrate chromagen solution was immediately prepared by dissolving one tablets of urea (H_2 O_2) in 1 2 ml of distilled water. A tablet of dihydro-o-chlrophenydiamire (OPD) was then added.

The solution was then mixed thoroughly. 1 00~il of this solution was then added and dissolved. The solution was then mixed thoroughly. 100 μl of this solution was then added to all walls. The plate was left at room temperature for 10 minutes. The reaction was stopped by adding 100 μl of stopping solution to all wells.

Lack of colour development indicates the sample tested was positive. A strongly positive sample causes the well to have a clear appearance, whereas a negative sample will cause the well to have a coloured (orange) appearance. The colours of the test wells were then compared with negative and positive control wells.

RESULTS

Result indicated that out of the 1711 screened samples 383, 376 and 395 were positive for RBPT, SAT, and ELISA respectively. The SAT titre showed that 103 samples had a litre of 1:40, 140 had 1:80, while, 40 had a titre of 1:160.

On sex distribution out of the 1017 males, 219(21.23%), 216 (21.23%) and 225 (22.85%) were positive for RBPT, Sat and Compelisa, respectively. Out of 694 females screened, 164 (23.63%), 160 (23.05%) and 170 (24.49%) were positive for RBPT, SAT and Compelisa respectively. There was no association between *Brucella* infection and sex (p>0.00l) (Table 1).

On age distribution: Out of the 261 samples in the age bracket of 0-12 months, 24 (2.66%), 23(2.55%), and 26 (2.88%) were positive for RBPT, SAT and Compelisa respectively. Similarly of the 361 samples in the age range of 13-24 months, 70(2.66%), 68(11.72%), and 73(12.58%), were positive for RBPT, SAT and Compelisa, respectively. Of the 1089 within the age group above 24 months, 289 (26.53%), 284 (26.07%) and 296(27.18%) were found to be positive for RBPT, SAT and Compelisa, respectively. There was an association between *Brucella* infection and age (p<0.001).

On Breed distribution, out of 901 Azuwarq breed., 212(23.52%), 208(23.08%) and 220(24.41%) were positive for RBPT, SAT and Elisa respectively. There was no association between *Brucella* infection and breed (p>0.001) Table 3. Seasonal prevalence also showed that, of the 706 samples during the dry season, 215(30.45%), were also positive for RBPT, 212(30.02%) for SAT and 216 (30.59%) for competitive Elisa. For the dry (hot) season, of the 528 samples; 89(16.85%), 88(16.66%) and 94(17.80%) were positive for RBPT, SAT and Compelisa, respectively compared to 477 screened samples collected during the wet season in which 79(16.56%) were positive for RBPT, 76(15.93%) for SAT and 85(17.81%) for Compelisa, respectively.

Table 1. Number of samples positive for RBPT, SAT and Compelisa according to sex

Sex	No. screened	RBPT	SAT	COMPELISA
Male	1017	219 (21.53%)	216 (21.23%)	225 (22.88%)
Female	694	164 (23.63%)	160 (23.05%)	170 (24.49%)
Total	1711	383	376	395

Table 2. Number of samples positive for RBPT, SAT and Compelisa according to breed

Sex	No. screened	RBPT	SAT	COMPELISA
Azuwarq	901	212 (23.52%)	208 (23.08%)	220 (24.41%)
Sokoto Gudali	508	124 (21.37%)	122 (24.0%)	126 (24.80%)
White Fulani	230	47 (20.43%)	46 (20%)	49 (21.30%)
Total	1171	383	376	395

Table 3. Number of samples positive for RBPT, SAT and Compelisa according to age

Age (months)	No. screened	RBPT	SAT	COMPELISA
0 - 12 months	901	24 (2.66%)	23 (2.55%)	26 (2.88%)
13 - 24 months	580	70 (2.06%)	68 (11.72%)	73 (12.58%)
Above 24 months	1089	289 (26.53%)	284 (26.07%)	296 (27.18%)
Total	1171	383	376	395

Table 4. Number of samples positive for RBPT, SAT and Compelisa according to season

Season	No. screened	RBPT	SAT	COMPELISA
Dry	706	215(30.45%)	212(30.02%)	216(30.59%)
Hot	528	89(16.85%)	88(16.66%)	94(17.80%)
Wet	477	79(16.56%)	76(15.93%)	85(17.81%)
Total	1171	383	376	395

DISCUSSION

The primary objective of a screening test in epidemiological surveillance of brucellosis is to detect cattle previous and recent exposure of affected animals to *Brucella* infections. In the present study, all the samples that were positive to RBPT and SAT were found to be positive to Competitive Elisa. This is a further confirmation of the validity and accuracy of both RBPT and SAT as a serodiagnostic method of this disease. The Elisa has been recommended as a good screening test for unvaccinated herds because of its ability to eliminate cross-reacting antibodies (Dehoo *et. al.,* 1986, Weldmann 1991).

An overall prevalence of 22.30% recorded in this study was high compared to previous studies reported by Shehu *et. al.* (1999) carried out in Northern Nigeria, who reported a prevalence rate of 10.8% in Bauchi abattoir. Further reports showed that Atsanda and Agbede (2001) had a prevalence of 6.53% in Maiduguri abattoir, while Nuru and Dennis (1975) also reported a prevalence of 0.4%) in North Central State of Nigeria.

Though Esuruoso (1977) observed that Northern Nigeria falls within the low infection areas of the country, the present increase in prevalence observed after 30 years is a cause for alarm. It is suggestive of the fact that vaccination exercise has not being carried out regularly or other unidentified factors might have lead to this unexplained increase in the prevalence rate. The non significant difference between the sexes is in accordance with

the findings of Olayinka and Ogundipe (2002) and Shehu *et. al.* (1999).

The higher prevalence recorded within the age band of above 24 months was in agreement with the findings of Corbel (1986), who observed that sexually matured animals are much more susceptible to infection and that younger animals that tend to show resistance to infections.

It is therefore, important to note that infected animals constitute public health risk to butchers, meat inspectors, meat sellers and other meat handlers (Samuel, 2003).

These groups should not only ensure full protection but also undergo periodic screening to determine their health status. Control measure such as compulsory vaccination, movement restriction, quarantine, test and slaughter and other hygienic practices should be instituted.

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