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Brucellosis in Local Chickens in North Western Nigeria

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Abstract: A study to determine the seroprevalence of brucellosis in local chickens in Sokoto State of Northwestern Nigeria was carried out. A total of one thousand serum samples were collected over a period of six months. The serum samples were subjected to Rose Bengal Plate test, serum agglutination test (SAT), Elisa (Compelisa) using both *Brucella abortus* and *B. melitensis* antigens. Results indicated that 30 (3.0%) 28 (2.8%) and 26 (2.6%) were positive for melitensis using compelisa and RBPT while 24 (2.4%) for SAT. It is recommended that rearing method employed where cattle, sheep and other animals are housed together with chickens should be discouraged. Public education and enlightenment campaign to poultry owners and handlers on the dangers of brucellosis should be intensified and future surveillance and control programmes on brucellosis should include local poultry.

Key words: Brucellosis, seroprevalence, local chickens, Northwest Nigeria

Introduction

Brucellosis is said to be endemic in Nigeria and evidence of infection as well as frank outbreaks have occurred in cattle (Ajogi, 1997) humans (Alausa and Awoseyi, 1976; Falade, 1974) sheep and goats (Falade *et al.*, 1975) and Human (Ocholi *et al.*, 1993). There are few reports of serological evidence of avian brucellosis in Nigeria (Bale and Nuru, 1982; Abdu *et al.*, 1984). Chickens could be important reservoirs of brucella organism for man and other animals (Abdu *et al.*, 1984). Since chickens are kept in this part of Nigeria due to their nutritional and economic importance, chicken rearers, handlers and consumers could be at risk of contracting this disease. There was no report on the occurrence of brucellosis in chickens in the area of this study. This study therefore is aimed at determining the prevalence of brucellosis in local chickens in this part of the country so as to add to the existing epidemiological data in the country as well as to know the status of the disease in the area.

Materials and Methods

Study area: Sokoto State is situated in the Northwestern part of Nigeria. It is within the Savannah zone, between latitude 12°N and 13°5'N and longitude 4° 8'E and 6° 54'E.

It covers a total land area of about 32,200 sq. Kms. and has a projected population of about 2.8 million people and is the second largest in terms of livestock population in Nigeria. The climate has a distinct dry and wet season. The total annual rainfall is about 707mm with a mean temperature of 38°C.

Sample collection: Three milliliters of blood sample (3mls) was collected from 1000 apparently healthy local chicken that were randomly sampled from the area of

study over a six months period. All clotted samples were subjected to centrifugation at 2,000 revolution per minute for 3 minutes. The clear sera was harvested using the Pasteur's pipette decantation method. The sera was then stored at 4°C prior to conducting the various serological tests.

Serological tests: The Rose Bengal Plate Test (RBPT) and the serum Agglutination test (SAT) as well as the Competitive Elisa (compelisa) were used in this study.

The rose bengal plate test: This was carried out using standard Rose Bengal Plate Test antigen obtained from Central Veterinary Laboratory, Weybridge U.K, according to the method of Alton *et al.* (1975). Equal volumes (0.03ml of antigen and test serum were mixed thoroughly on the glass plate of the test box using a tooth pick and the box was hand rocked for four minutes. Samples that showed signs of agglutination were recorded as positive while those with no sign of agglutination were recorded normal.

Serum agglutination test: This test was carried out as described by Alton *et al.* (1975). For each of the samples that was positive in the RBPT two fold dilution of test sera from 1:10 (0.05ml serum mixed with 0.45ml phenolized saline) to 1:1280 was made. To each 0.5ml tube of phenolized saline serum mixture was added an equal volume (0.5ml) of the diluted (1:10) antigen resulting in a doubling (two-fold) dilution. The tubes containing the antigen-serum mixture were covered, shaken and incubated at 37°C for 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. While a negative reaction is one in which the serum antigen mixture was turbid and gentle

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Table 1: Sex Distribution of brucellosis in Local chickens (*Brucella abortus*)

Sex	No. of samples collected	Compelisa positive	RBPT positive	SAT positive
Male	747 (74.7%)	23 (2.3%)	21 (2.1%)	20 (2.0%)
Female	253 (25.3%)	7 (0.7%)	7 (0.7%)	6 (0.6%)
Total	1000	30 (3.0%)	28 (2.8%)	26 (2.6%)

Table 2: Sex Distribution of brucellosis in Local chickens (*Brucella melitensis*)

Sex	No. of samples collected	Compelisa positive	RBPT positive	SAT positive
Male	747 (74.7%)	20 (2.0%)	20 (2.0%)	20 (2.0%)
Female	253 (25.3%)	6 (6.0%)	4 (4.0%)	6 (0.6%)
Total	1000	26 (2.6%)	24 (2.4%)	26 (2.6%)

shaking revealed no precipitate at the bottom (Anon 1981; Brisbe *et al.*, 1993). Any serum with agglutination at a dilution of 1:40 (titre of 40) (50 international units) (Alton *et al.*, 1975) and above was recorded as positive sample.

Competitive elisa: The competitive Enzyme linked immunosorbent Assay kit was obtained from Central Veterinary Laboratory Weybridge, United Kingdom. The test was conducted according to the manufacturers instructions. Initially the diluting buffer, wash solution, stopping solution, conjugated and controls were reconstituted as directed by the manufacturer. Test serum was added per each well of the microtitre plate which has 60 columns (wells). Wells 11 and 12 were used as control.

20ml of the negative control was added to well A11, A12, B11, B12, C11 and C12 while another 20ml of the positive control was added to wells F11, F12, G11, G12, H11 and H12.

D11, D12, E11 and E12 served as conjugated controls. 100µl of the prepared conjugated was then dispersed into all wells. The plate was then shaken for 2 minutes in order to mix the serum with the conjugate solution. The plate was then covered with the lid and incubated at room temperature for 3 minutes. The content of the plate was then discarded and rinsed 5 times with washing solution and then dried. 100µl of the substrate chromogen solution was added to all wells. The plate was kept at room temperature for 10 minutes. The colours of the test wells were compared with negative and positive controls and the results recorded.

Results

A total of 1000 chickens were sampled out of this number 253 (25.3%) were female while 747 (74.7%) were male. From the one thousand chickens, 28 (2.8%), 26 (2.6%) and 30 (3.0%) were found to be positive for RBPT, SAT and Compelisa respectively.

On sex distribution, out of the 30 Compelisa positive samples, 23 (2.3%) were from males; while 7 (0.7%) were from females. Out of the 28 RBPT positive samples 21 (2.1%) were from males while 7 (0.7%) were from females of the 26 SAT positive samples 20

(2.0%) and 6 (0.6%) were from females and male samples respectively (Table 1).

The SAT titre showed that there were 12 samples with a titre of 1:40, 10 had a titre of 1:80, 3 with a titre of 1:160 while 1 had a titre of 1:320.

Brucella melitensis results indicate that 26 (2.6%) samples were *brucella melitensis* positive for compelisa and SAT while 24 (2.4%) were RBPT positive. Out of the 26, 20 (2.0%) were male and 6 (6.0%) were female. Similarly out of the 24 SAT positive samples 20 (2.0%) and 4 (0.4%) were for male and female respectively. (Table 2).

Discussion

The result of this study suggest that avian brucellosis is present in the Northern part of Nigeria. Most local chickens are on free range and the system of the animal husbandry where they are kept with other animals such as cattle, sheep and goat disposed them to brucella infection through contact with these animals.

Since birds are not usually vaccinated against brucellosis, the high antibody obtained in some of these birds (1:160, and 1:1320) could be due to natural infection as observed by Chukwu and Boniface (1988). However, the low antibody titre of 1:40 obtained in most of the birds could be attributed to the presence of receding brucella antibodies or to cross-agglutination with other organism which may infect poultry such as *pasteurella* or *salmonella* (Bale and Nuru, 1982).

Brucella infection could spread from chicken to chicken and to other livestock through infected chicken faeces, thus the presence of brucella reactors in local chicken on free range is of great public importance in our local community where apartments and atimes cooking and watering utensils are shared between animals and humans. Public enlightenment campaign to poultry owners, handlers and members of the public on dangers of brucellosis and the need for self protection should be intensified. The system of animal husbandry practice in this part of the country where different livestock species including poultry are housed together with humans should be discouraged in order to minimize the chances of getting infected by brucellosis causing organism.

Future brucellosis control programme in Nigeria should take into consideration the local chickens as one of the reservoirs of brucella.

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