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Original Article

Serological Survey Of *Salmonella gallinarum* Antibody In Chickens Around Jos, Plateau State, Nigeria

Authors
Okwori AEJ,
Department of Medical Microbiology, Federal College of Veterinary and Medical Laboratory Technology, Vom
Hashimu GA,
Bacterial Vaccine Department, National Veterinary research Institute, Vom
Adetunji JA,
Department of chemical Pathology, Jos University Teaching Hospital, Jos.
Okeke IO,
Department of Medical Microbiology, Federal College of Veterinary and Medical Laboratory Technology, Vom
Junaid SA
Department of Medical Microbiology, Federal College of Veterinary and Medical Laboratory Technology, Vom

Address For Correspondence
A.E.J. Okwori
Department of Medical Microbiology,
Federal College of Veterinary and Medical Laboratory Technology,
Vom
E-mail: Okwori2001@yahoo.com

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Abstract:
A serological survey of the prevalence of antibodies to *Salmonella gallinarum* among chickens under two different management systems around Jos, Plateau State, Nigeria was carried out using the standard plate agglutination test. The objective of this study was to determine serologically the prevalence of antibodies against *Salmonella gallinarum* among apparently healthy chickens around Jos. A total of 700 serum samples made up of 450 exotic and 250 local breed of chickens were used for this study with 37.9% seropositvity. In the free range system (19.3%) of the flocks sampled were seropositive for *Salmonella gallinarium* antibodies while in the semi intensive, 18.6% of the flock tested positive. The serum agglutination test (SAT) was adapted to the microtitre format used to determine somatic and flagella titres. The antigen used for this study was specific for *S. gallinarum*, hence differentiation between species infection was assessed in this study. Perhaps the most feasible way to eradicate the disease is to encourage farmers (both small and large scale) to break the disease cycle at their levels by embarking on prompt and regular vaccination programmes. It is thus concluded that S*almonella gallinarum* (fowl typhoid) is present in the area investigated. Fowl typhoid may continue to have a negative effect on the economy of poultry production in Nigeria if not controlled. A statistical analysis was precluded due to inadequate data sets.
Key Words: Fowl typhoid, Antibodies, Chickens, Jos, Nigeria

Introduction:
Fowl typhoid is caused by the bacterium *Salmonella gallinarum*, a member of the family *Enterobacteriaceae* widely distributed throughout the world, but have been eradicated from commercial poultry in many developed countries of western Europe, the United States of America (USA), Canada, Australia and Japan with an intensive poultry industry.(1) The disease (Fowl typhoid) is of particular economic importance in those countries which are beginning to intensify their industry, eg countries in Latin America, south America, the Middle East, the Indian subcontinent and parts of Africa.(2) In Africa for example, fowl typhoid has been reported in many countries including Nigeria (3), Tanzania (4), Uganda (5), Zambia (6), Libya (7), Senegal (8) and Morocco.(9) Salmonella has been of great concern to the poultry industry ever since man began raising poultry in a concentrated fashion.

Salmonella gallinarum is highly host adapted and seldom causes significant problems in host other than chickens, turkeys and pheasants.(10) It was formerly known as *Shigella gallinarum* when first isolated in 1989 by Klein in England.(2)

Fowl typhoid infection usually follows the ingestion of food or water contaminated by the excreta of clinically infected birds or carriers and can also be transmitted by attendants through hands, feet and clothes.(11)

Fowl typhoid seriously threatened the poultry industry in the early 1900s due to widespread outbreaks accompanied by high mortality.(2)

The majority of the strains of *S. gallinarum* is very similar at the chromosomal level.(12)

A definitive diagnoses of fowl typhoid requires the isolation and identification of *S. gallinarum.* However, a tentative diagnoses can be made based on the flock history, clinical signs mortality and lesions. Positive serological findings are of great value in detecting infection.(13)

In Nigeria, various serotypes of Salmonella species have been isolated from apparently healthy chickens with a carrier rate of 1.3% in 300 broilers as reported by Falade and Elizokhale.(14)

The aim of this study was to determine the prevalence of *Salmonella gallinarum* antibodies in vaccinated and non vaccinated birds and to advice farmers where asymptomatic infection in unvaccinated birds are identified.

Materials and Methods:

Reference Sera

Adult chickens with no signs of clinical Salmonellosis were inoculated intra-muscularly with 0.5ml of inactivated cultures of *Salmonella gallinarum* (10^8 cfu/ml) to obtain immunological responses specific to *Salmonella gallinarum*. Sera samples were there after collected after 5-14 days and stored as positive control. Negative serum samples to Salmonella was also obtained from the Poultry Department, National Veterinary Research Institute, Vom. All serum samples were stored at –20^oC, until required for use. Seven hundred (700) randomly selected flocks from free-range and semi-intensive poultry systems were used for this study. The two poultry management systems were defined as free range flock and semi intensive flock (Back yard).

Free Range Flock

These are not under any form of confinement and usually roam about the environment only to return to house hold at dusk, predominantly owned by families or household consisting of local breed of chickens.

Semi Intensive Flock (Back Yard)

Chickens in this group consist of those under confinement in either deep litter or cages. A maximum population of 1500-3000 chickens were allowed into this class.

Serum Samples

Blood samples were collected from both local and exotic chickens in Vom and Bukuru. Approximately 2ml of blood was drawn from the wing vein aseptically into sterile bijou bottles. Blood samples were kept in slant position and allowed clot at room temperature, stored overnight at 4^oC and spun at 3,000 rpm for 10 minutes.

Serum samples were collected using sterile Pasteur pipettes, labeled and stored frozen at –20^oC in bijou bottles and small vials until required.

Preparation And Standardization Of Stained Antigen

Lyophilized reference strains of *S. gallinarum* was obtained from the Fowl Typhoid Vaccine Department, National Veterinary Research Institute, Vom. The strains were inoculated into trytose broth, incubated at 37^0C and subsequently subcultured onto nutrient agar containing $^1/_{800}$ phenol. This inactivates the Vi antigen (if present) which could mask O antigen and to obtain much growth. The cultures were incubated at 37^oC for 24 hours. Bacteria growth on the surfaces of the plates were harvested into 10ml of nutrient broth, emulsified thoroughly and 20 times the volume of absolute alcohol was added. Heated for 30minutes at 56^oC, centrifuged hard, and re-suspended deposit in 0.2% formol saline solution. Tinged with crystal violet. Standardized by an opacity method to 750 x 10^6 organisms/ml using 0.2% formal saline and controlled using fowl typhoid vaccine as the standard reference antigen.

Serological Test

The standard plate agglutination test was used (15). Reagents used were the prepared *S. gallinarum* stained antigen. The reagent was allowed to warm up to room temperature prior to use.

Using a sterile Pasteur pipette, 0.02ml of the sera was dispensed on a tile and 0.02ml of the antigen was added using separate sterile pipette. Antigen and sera were properly mixed with wooden applicator sticks, the tile plate was gently rocked for a few seconds and reaction read within 2 minutes. Known positive and negative sera were tested simultaneously on each day of testing. Test serum samples giving visible agglutination were considered positive. While those that does not give visible agglutination were considered negative.

Results

Of the seven hundred (700) birds screened from Vom and Bukuru, sixty six (66) (9.4%) of the flocks tested positive to *S. gallinarum* antibodies. Both the exotic and local birds (250) screened from Vom area gave 28 (19%) positive reaction. While those from Bukuru area (450) similarly gave 34 (18.9%) positive reaction. In the free range system 9.6% of the flock sampled were seropositive for *S. gallinarum* antibodies. 9.3% of the semi intensive samples tested positive to *S. gallinarum* antibodies (Table 2).

Table 1: Distribution of serum samples among exotic and local birds screened

Location	Exotic birds	Local birds	Total
Vom	200	100	300
Bukuru	250	150	400
Total	**450**	**250**	**700**

Table 2: *Salmonella gallinarum* antibody in chickens raised under two different management systems

Location	Type of birds	Number of birds tested	Number positive
Vom	Exotic	200	18
	Local	100	10
Bukuru	Exotic	250	24
	Local	150	14
Total		**700**	**66**

Table 3: Percentage prevalence of *Salmonella gallinarum* antibodies in chickens from the two different management systems

Location	Exotic birds (%)	Local birds (%)	Total (%)
Vom	9.0	10	19
Bukuru	9.6	9.3	18.9
Total	**18.6**	**19.3**	**37.9**

Discussion

In most African countries, the chicken has no regular health control programme, may or may not have shelter and scavenge for most of their nutritional needs throughout life. These may of course account for persistent outbreak of poultry diseases documented to be easily transmitted through contact and aerosol.(16) The major diseases of poultry in Africa that have been prominently identified in commercial poultry flocks are Newcastle disease, Infectious Bursal Disease (IBD) or Gumboro, Marek's disease (MD), fowl typhoid, cholera, mycoplasmosis and coccidiosis.(17) Unlike avian parathyphiod Salmonella serotypes, Salmonella enterica serotypes *gallinarum* and *pullorum* are not frequently excreted in chicken faeces, but infected birds tend to produce humoral response.(13) Breeding flocks must be free of *Salmonella gallinarum* and pullorum, therefore, it is very important to detect them as soon as possible to prevent both disease and dissemination. Serological diagnosis of Fowl typhoid disease in poultry industry among other measures has utilized macroscopic tube agglutination test which predominantly detects Igm antibody to cell wall LPS antigen. (13,17) This can be adapted to the microtitre format and can be readily used to determine somatic and flagellar titres as demonstrated in this study.(19) Besides being a very simple method, this test has also been very useful for the control and eradication of Salmonellosis.(20) In order to specifically determine fowl typhoid disease, it could be essential to serologically differentiate these diseases from infections caused by other invasive Salmonella serotypes, including typhimurium and enteritidis and some other serotypes in group D, which might induce cross reactivity among circulating IgG antibodies following oral infection.(13,21)

Relatively high percentage of flocks from the two poultry farms located in Vom and Bukuru management systems had chickens with *Salmonella gallinarum* agglutinins and should be of concern. This is because the flocks had widespread distribution despite the fact that the birds were not vaccinated. This is suggestive of a high exposure rate of chickens to *Salmonella gallinarum* around Vom and Bukuru. Similarly Falade and Ehizokhale (14) have isolated different *Salmonella* serotypes from animal species including poultry in Nigeria.

The low prevalence rate documented in this study could be a reflection of intensification coupled with the deep litter form of management practiced currently in Jos and its environ. Interestingly, the semi-intensive system recorded a lower prevalence of *S. gallinarum* antibody in chickens than the free-range system, despite relative intensification in the former. This could be explained, in part, by the fact that chickens in the free-range system move about more freely thus increasing exposure rate to *S. gallinarum*. Chickens in the semi-intensive flocks however, remain under confinement in deep litters or cages, and with good management. Hence could be free of *S. gallinarum* disease if the day-old chicks were from disease free farms. Incidentally, a great proportion of semi-intensive poultry farmers in Vom and Bukuru area acquired their day-old chicks from where *S. gallinarum* disease eradication programmes have been successful.(22)

These results are comparable with other surveys in Africa, although knowledge on the prevalence of disease in Africa poultry seems to be rather limited.(23) Chrysostome *et al*, (24) in a similar study documented the presence of antibodies to *S. gallinarum* among chickens in Benin City, Nigeria. The overall prevalence of 9.4% found in all chickens sampled in Vom and Bukuru appears very high when compared with other reports from different parts of Nigeria. Onunkwo and Onoviran (25) reported *S. gallinarum* antibody prevalence in Plateaus State to be 3.2%. This variation could be due to differences in environmental contamination and management systems. Long term cohort studies examining the causes of poultry mortality have not been carried out in the free range production systems.

Unlike the findings of Oliveira *et al*, (26) which assessed the ability of an immunoenzymatic assay performed with either peroxidase or alkaline phosphatase conjugates to investigate serological response to a soluble protein antigen from *S. gallinarum*, the present study has addressed the simple antibody detection as the antigen was capable of promoting reliable serological reaction as suggested by Barrow *et al*, (13) and Iba *et al.*(27) This study is further buttressed by the findings of Bauzoubaa *et al*, (9) who in Morocco revealed that up to 58% of the village chickens had antibodies against *S. gallinarum* and *S. pollorum*. Similar findings were reported in Nigeria by Adesiyun *et al.*(28) There is no doubt that the disease play an important role in village poultry and semi-intensive poultry in Nigeria.

In conclusion regular blood testing and depopulation of infected flocks have enormously reduced the prevalence of *S. gallinarum* in other countries, but this approach is seldom practicable in Nigeria.

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