

## ORIGINAL RESEARCH ARTICLE

## Occurrence of *Salmonellae* in Chickens from some Selected Poultry Farms in Kano, Nigeria.

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### ABSTRACT

Infections with bacteria of the genus *Salmonella* are responsible for a variety of acute and chronic diseases in poultry. This study was carried out to determine the prevalence of *Salmonella species* in chickens from some selected poultry farms in Kano, Nigeria. Random sampling method was used in the study. One (1) farm was chosen from each of the eight (8) selected Local Government areas of the State. Thirteen (13) samples each from cloacae, egg, dust, drag and boots which is (65) from each farm) were collected from apparently healthy chickens (layers), transported immediately to the laboratory for analyses. The colonies were subsequently subjected to Gram-staining and microscopy. Eight *Salmonella* isolates were determined, three (3) were isolated from boot swabs, two (2) from cloacal swabs and one (1) each from egg, dust and drag swabs. All the isolates were confirmed by serological test; using *Salmonella* “O” antigen; group A-S (Oxoid). Asymptomatic *Salmonella* infection among chickens in the sampled farms is low with (1.54%). The low occurrence could be attributed to proper hygienic practices, biosecurity measure as well as application of biological control of rodents and other vehicles of *Salmonella*.

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### KEYWORDS

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### INTRODUCTION

There are more than 2,500 species or serotypes belonging to genus *Salmonella* (Breytenbach, 2004). Infections with bacteria of the genus *Salmonella* are responsible for a variety of acute and chronic diseases in poultry. These diseases continue to cause economically significant losses in many nations and absorb a large investment of resources in testing and control efforts. Infected poultry flocks are also among the most important reservoirs of *Salmonellae* that can be transmitted through the food chain to humans (Abd EL-Hamid *et al.*, 2004).

Salmonellosis (*salmonella* gastroenteritis) is caused by over 2000 *Salmonella* serovars (serological variation or strains). Based on DNA homology studies, all known salmonella are thought to belong to a single species, *S. enterica*, although the taxonomy of this bacterium remains controversial. The most frequently isolated serovars from Chickens are Gallinarum and Pullorum and the frequent ones isolated from humans are Typhimurium and Enteritidis.

The initial source of the bacterium is the intestinal tract of birds and other animals. Humans acquire the bacterium from contaminated foods such as beef products, poultry,

eggs, eggs products, or water. Around 45,000 cases a year are reported in the United States but there actually may be as many as 2-3 million cases annually. Once the bacteria are in the body, the incubation time is  $\approx$  8 to 48 hours. The disease results from a true food-borne infection because the bacteria multiply and invade the intestinal mucosa, where they produce an enterotoxins and cytotoxins that destroys the epithelial cells. Classical symptoms include abdominal pain, cramps, diarrhea, nausea, vomiting, and fever, which usually persist for 2 to 5 days but can also last for several weeks. During the acute phase of the disease, as many as 1 billion *Salmonella* can be found per gram of feces. Most adult patients recover, but the loss of fluids can cause problems in children and the elderly (Prescott *et al.*, 2005).

### MATERIALS AND METHODS

#### Sample Collection

Samples were collected according to the recommendations of the Office of International Epizootics (OIE, 2012). The samples were collected from

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apparently healthy chickens. All the farms were supervised by veterinary doctors during the study period.

#### Cloacal Swabs

Cloacal swabs ( $n=13$ ) for *Salmonella*-surveillance were collected from apparently healthy chickens, using sterile cotton swab sticks moistened with normal saline. The swabs were kept properly and transported immediately on ice to the Microbiology laboratory, where they were inoculated immediately in peptone water for non-selective enrichment.

#### Eggshell Swab

Surface swabs ( $n=13$ ) from egg shells were collected using sterile swab sticks moistened with normal saline and transported immediately on ice to the laboratory. The samples were inoculated in Peptone water for non-selective enrichment.

#### Drag Swab

Drag swabs ( $n=13$ ) were obtained by dragging the sterile swab sticks on feed containers used in providing feed and water to the chickens; the samples were transported aseptically to the laboratory for further analyses.

#### Dust Samples

Dust samples ( $n=13$ ) were collected from dust on surrounding materials and the poultry buildings. The samples were transported immediately and aseptically to the laboratory for analyses.

#### Boot Swab

Boot swab ( $n=13$ ) was obtained by walking throughout the poultry building and swabbing the bottom of the boot. The samples were transported immediately to the laboratory for analyses.

#### Isolation and Identification of *Salmonella*

Isolation and identification were carried out using the procedure outlined by Cheesbrough (2006) and OIE (2012). The samples were introduced into Peptone water and incubated at 37°C for 24h for pre-enrichment. After 24h of incubation, 1ml of the enriched sample was taken into 9ml of Selenite-F-broth and incubated at 37°C for 24h for selective enrichment. A loopful of the enriched sample was inoculated on MacConkey agar and incubated at 37°C for 24h. Suspected colonies were picked using sterile wire loop and streaked on Desoxycholate citrate agar and incubated at 37°C for 24h. Suspected *Salmonella* colonies were taken and streaked on *Salmonella* Shigella agar and incubated at 37°C for 24h. Suspected colonies were purified by repeated subcultures on *Salmonella*-Shigella agar. The purified isolates were streaked on slant bottles containing Nutrient agar and incubated at 37°C for

24h. The slants were kept in a refrigerator at 4°C as a stock for further tests.

#### Presumptive Isolation of *Salmonella*

The cultured plates of SSA were examined for the presence of typical colonies of *Salmonella* based on cultural and morphological characteristics; that is, transparent colonies with black or no black centre on SSA followed by Gram staining and motility test.

#### Purification of Isolates

Pure cultures were obtained by repeated cultures on SSA and the cultures were subsequently identified by biochemical tests.

#### Serological test.

An agglutination test was performed on a clean glass slide. The slide was marked using marker, small drop of the test isolate (suspected *Salmonella* isolate grown overnight in peptone water) was placed at the center of the glass slide using sterile disposable pipette and the equivalent quantity of the antisera (*Salmonella* antigen “O” group A-S (Oxoid)) was placed beside the drop of the suspected *salmonella* sample. A sterile tooth pick was used to stir the two drops together. The slide was rocked until the visible agglutination appeared clearly within 30 to 60 seconds. (Cheesbrough, 2002; Andrews *et al.*, 2005).

In this research all the suspected colonies subjected to serotyping were all positive for agglutination with antisera “O” group A-S.

## RESULTS

A total of 520 swab samples collected from eight selected poultry farms were subjected to *Salmonella species* analysis. Of this number, eight *Salmonella species* were isolated based on Cultural, Gram Staining, Microscopic and Biochemical characteristics (Table 1). The isolates were confirmed by Agglutination Test using commercial kit (*Salmonella* Polyvalent “O” antigens group A-S (Oxoid)). Overall prevalence of (1.54%) or 8/520 was obtained (Table 2).

#### Sites and number *Salmonella species* Isolated

Out of 65 samples each collected from Dala, Fagge and Tarauni, two *Salmonella species* were isolated. One *Salmonella species* each was isolated from Ungoggo and Nassarawa. No *Salmonella* was recovered from Gwale, Kumbotso, and Kano Municipal Samples (Table 2) (Fig. 1).

#### Growth in Liquid Media

Growth in peptone water was indicated by turbidity and slight white sediment at the bottom of the test-tube after incubation at 37°C for 24hours.

Growth in Selenite-F-Broth was indicated by brown precipitate in the medium after incubation at 37°C for 24hours.

*Growth on Solid Media*

The growth of *Salmonella species* on MacConkey agar was indicated by colourless, round, smooth, shiny colonies up to 3mm in diameter after incubation at 37°C for 24hours.

On Deoxycholate Citrate Agar (DCA), *Salmonella* colonies were characterized by slight opaque dome shaped colonies measuring (2-4mm) in diameter with characteristic black centers (hydrogen sulphide production), surrounded by clear zones after incubation at 37°C for 24-48hours.

On Salmonella Shigella Agar (SSA), the *Salmonella* species appeared colourless, with characteristic black centers after incubation at 37°C for 24hours.

On Triple sugar ion agar (TSI), *Salmonella* colonies produced hydrogen sulphide which was characterized by blackening of the medium (hydrogen sulphide production). Gas production causes bubbles or cracks or both in the medium and pH change was indicated by

production of red colour in the slant after incubation at 37°C for 24hours.

*Motility*

*Salmonella* colonies were seen motile under light microscope (at X10 and X40 objective lenses after 24hours incubation in peptone water at 37°C.

*Microscopic Appearance*

*Salmonella species* were observed as Gram-negative short rods, occurring singly or in groups.

*Biochemical Characteristics.*

*Salmonella species* were identified as urease and oxidase negative, and were mannitol positive, they produced gas from glucose fermentation on TSI medium, and hydrogen sulphide (H<sub>2</sub>S) is produced by *Salmonella species* (Table 1).

*Serological Confirmation of the Isolates*

All the isolates were confirmed as *Salmonella species* by the use of commercial kit (*Salmonella* agglutination test) using *Salmonella* polyvalent “O” antigens group A-S (Oxoid).

Table 1: Cultural and Biochemical Characteristics of the Salmonella Isolated

S/No.	Sample	Cultural Characteristics				Biochemical Characteristics											TSI	
		MCA	DCA	SSA	GXN	Mot	Ure	MR	VP	Man	Cit	Ind	Oxd	Slope	Butt	H <sub>2</sub> S	Gas	Inference
1	NSR Ft1	CSRS	SODB	CBC	GN SR	+	-	+	-	+	+	-	-	R	Y	+	+	<i>Salmonella spp.</i>
2	DAL Egl	CSRS	SODB	CBC	GN SR	+	-	+	-	+	+	-	-	R	Y	+	+	<i>Salmonella spp.</i>
3	FGG Dt2	CSRS	SODB	CBC	GN SR	+	-	+	-	+	+	-	-	R	Y	+	+	<i>Salmonella spp.</i>
4	FGG Dr2	CSRS	SODB	CBC	GN SR	+	-	+	-	+	+	-	-	R	Y	+	+	<i>Salmonella spp.</i>
5	DAL Ft4	CSRS	SODB	CBC	GN SR	+	-	+	-	+	+	-	-	R	Y	+	+	<i>Salmonella spp.</i>
6	TRN CI2	CSRS	SODB	CBC	GN SR	+	-	+	-	+	-	-	-	R	Y	+	+	<i>Salmonella spp.</i>
7	TRN CI1	CSRS	SODB	CBC	GN SR	+	-	+	-	+	-	-	-	R	Y	+	+	<i>Salmonella spp.</i>
8	UGG Ft9	CSRS	SODB	CBC	GN SR	+	-	+	-	+	-	-	-	R	Y	+	+	<i>Salmonella spp.</i>

**Legend:** MCA = MacConkey agar, DCA = Desoxycholate citrate agar, SSA = *Salmonella shigella* agar, MR = methyl red, VP = Vogesproskauer, CSRS = colourless small round and shiny, SODB = Slight opaque dome shaped with black center, CBC= colourless with black center, GNSR= Gram-negative short rod, Mot= motility, Ure=urease, MR=methyl Red, VP=Vogesproskauer, Man=Mannitol, Cit=Citrate, Ind= Indole, Oxd=Oxidase, TSI=Triple Sugar Ion, R= red, and Y= yellow

Table 2: Prevalence of the Salmonella species isolated

S/N	Sample site	No. of samples examined	No. of Salmonella species isolated	Source of the isolate/Sample type	Prevalence
1	Gwale	65	0	None	0.00%
2	Kumbotso	65	0	None	0.00%
3	Ungoggo	65	1	Boot swabs	1.54%
4	Fagge	65	2	Drag and dust swabs	3.08%
5	Kano Municipal	65	0	None	0.00%
6	Nassarawa	65	1	Boot swabs	1.54%
7	Dala	65	2	Egg and boot swabs	3.08%
8	Tarauni	65	2	Cloacal swabs	3.08%
Total	8	520	8		1.54%

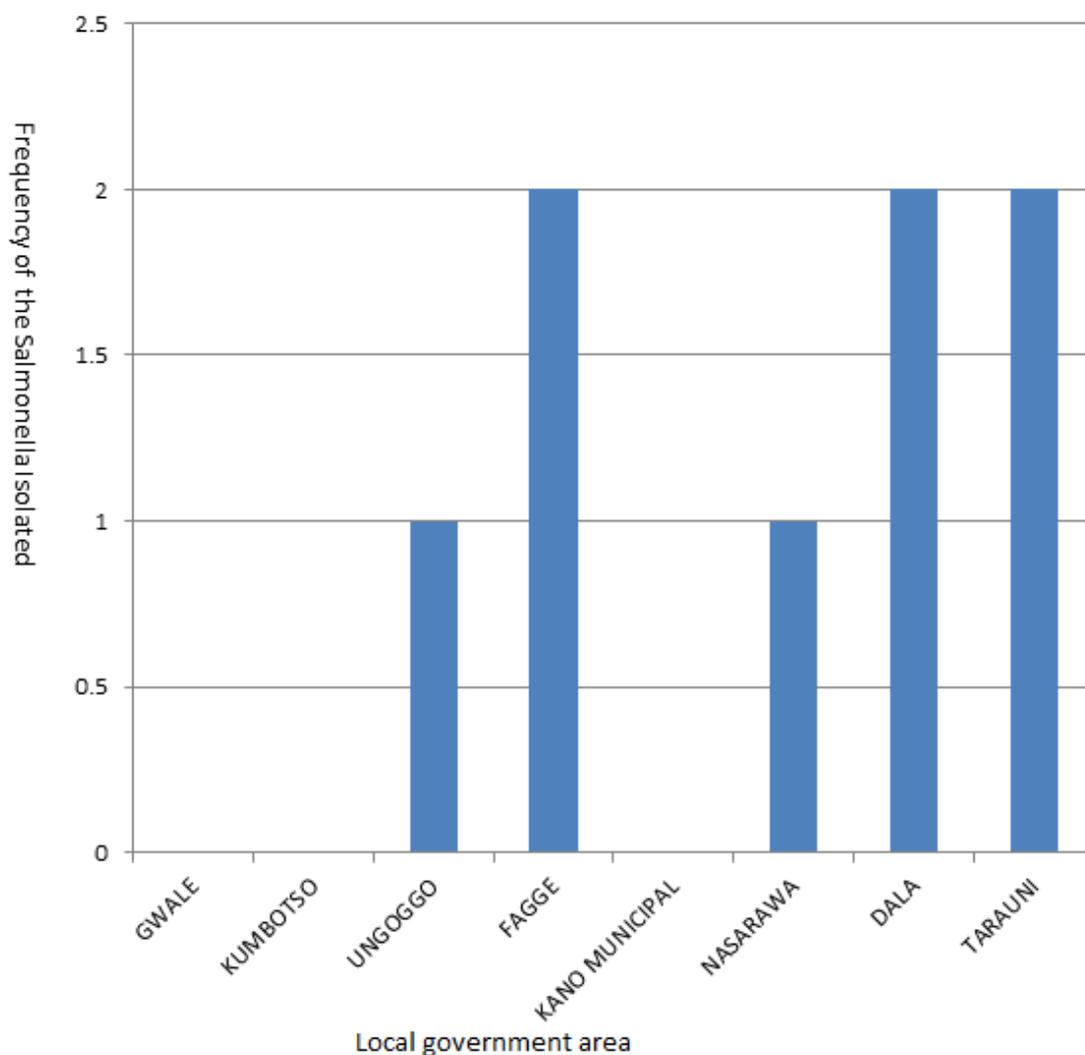


Figure 1: Number of Salmonella isolated from each farm

## DISCUSSION

The present study was conducted to investigate the occurrence of *Salmonella species* in apparently healthy chickens from selected poultry farms in Kano metropolis. *Salmonella species* were isolated based on cultural and biochemical characteristics and subsequently confirmed by serological test.

Several reports have shown that poultry is one of the major vehicles of *Salmonella* transmission through the food chain to humans, and is, therefore, a major public health concern. In the current Study, 1.54% (8/520) prevalence of *Salmonella species* was recorded. This prevalence rate is low compared to those reported from other parts of Nigeria and other Countries. [Obi et al., \(2015\)](#) recorded 8% (12/150) in a study in Nsukka, South-Eastern Nigeria. It disagrees with the current study; this may probably be as a result of proper sanitary measures and supervision of veterinary doctors to the farms under study.

In another study conducted by [Agada et al., \(2014\)](#) in Jos Plateau State, Nigeria, 10.9% *Salmonella* prevalence was obtained out of 450 samples analyzed. Similarly, [Mai et al. \(2013\)](#) reported 32.5% *Salmonella* prevalence in Jos.

The relatively low prevalence rate observed in the present study may be attributed to the intensive hygienic practices, medical personnel advice and other environmental conditions favourable for the chickens that may hinder the survival of *Salmonella species*. A number of environmental factors including the poultry house environment, untreated drinking water, old litter, other farm animals, domestic pets, rodents, insects, wild birds, farm handlers, equipment and transport vehicles have been suggested as sources of *Salmonella* infection in chicken flocks.

All the farms surveyed in this study were devoid of most of these risk factors because of the proper supervision of veterinary doctors to the farms. Proper hygienic practice is maintained in all the studied farms. In some of the farms, cats and dogs were kept to control rodents and other vehicles of *Salmonella* transmission. A number of studies had comparatively low prevalence. For instance, [Hiba, \(2007\)](#) obtained 2.9% prevalence in Khartoum, Sudan [Salihu et al., \(2014\)](#) obtained a *Salmonella* prevalence of 2.5% from free range chickens in Nassarawa State, Nigeria. Environmental conditions and proper hygienic practices were reported to account for the low prevalence.

In addition to the hygienic practices from the farms surveyed, there were strict measures taken by the farmers as advised by their veterinary doctors to ensure the safety of the populace and their chickens as an investment. These included: bio-security and prohibition of strangers. Litter management practice was adequate throughout the farms. Chickens droppings were kept far away from the poultry houses. Sanitation of the poultry houses was daily in most

of the poultry houses. Adherence to hygienic practices was enjoined by their veterinary Doctors. These practices have probably resulted in low occurrence of the *Salmonella species* in the studied farms. One of the alarming problems is that, some unhealthy chicks with evidence of watery diarrhea were kept close to the layers (this was observed at one farm in Kumbutso during sampling). This is potentially hazardous to the layers as cross infection may occur, since they were managed by the same workers and this may lead to zoonosis. Antibiotic usage in the farms is controlled by their doctors.

Moreover, most of the farms under this study were well ventilated. In Gwale L.G.A, the number of chickens was up to 1000 birds and they were in close proximity. The building provides inadequate aeration to the chickens. This is alarming as an outbreak may occur. Also, it was observed that all the farms buy their feeds from the market. The implication of purchasing feeds from the market is that, the chicken may be prone to consuming contaminated feeds.

however, most of the farm attendants were not using hand gloves, face masks and other protective gears while handling the chickens. This may lead to transmitting pathogens to the chickens or vice-versa, though, they disinfect themselves before getting into the chickens house.

## CONCLUSIONS

Asymptomatic *Salmonella* infection among chickens in the sampled farms Kano metropolis is low (1.54%). The low occurrence could be attributed to proper hygienic practices, and biosecurity measure as well as application of biological control of rodents and other vehicles of *Salmonella*, and supervision of the veterinary Doctors, who give proper advice and appropriate medication to the flocks.

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