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Isolation and Identification of *Salmonella gallinarum* from Poultry Droppings Used as Manure in Jos East Local Government Area, Plateau State

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Abstract

Fowl typhoid caused by *Salmonella gallinarum* is recognised worldwide as a disease of social and economic significance. This study was aimed at the isolation and identification of *Salmonella gallinarum* from poultry droppings used as manure in selected areas of Jos East Local Government Area of Plateau State. One hundred [100] samples of poultry droppings made up of 10 fresh and 10 old samples each from poultry farms in five [5] villages in Jos East were collected and screened using standard microbiological techniques and biochemical tests. One [1] sample contained *Salmonella gallinarum* representing 1% of the samples examined. Other bacteria isolated were *Proteus species* [4%] and *Escherichia coli* [3%]. This work confirmed the presence of *Salmonella gallinarum* in poultry droppings thus it is recommended that poultry droppings should be used carefully on poultry farms so as to prevent poultry birds from being infected.

1.0 Introduction

Poultry farming is one of the major sources of meat protein in Nigeria, where large-scale farms with thousands of birds exist while others have a few hundred birds [1]. Poultry serves as a major source of animal protein either as meat or egg. According to Agbaje et al. [2], poultry is an essential component of the Nigerian economy and a good source of high-quality protein for the ever-growing population in Nigeria.

The poultry industry in Nigeria has been facing many problems such as avian influenza, the global financial crisis and inadequate credit [3]. Despite the enormous potentials the poultry industry holds, it regularly suffers from major limitation including diseases, poor husbandry, low egg production, poor chick quality among others [2]. Poultry birds are a very important source of essential proteins in developing countries but also play an important role in transmission of *Salmonella* to humans and non-humans [1]. Exposure to this pathogen also occurs through the use of poultry droppings as manure for crop and vegetable production.

Oyedeji et al. [4], described poultry wastes as consisting of droppings (faeces), wasted feed, broken eggs, feathers and sometimes sawdust from poultry floor, it also includes dead birds and hatchery waste, all of which are high in protein and contain substantial amount of calcium and phosphorus due to high level of mineral supplement in their diet. In Nigeria, poultry droppings are extensively used as manure for the cultivation of crops. The application of poultry droppings to land provides nutrients for the crop's growth as well as organic matter for soil conditioning and this can pose a danger to public health especially when such crops are eaten raw [5]. While poultry droppings are very useful as manure for crop and vegetable production, they can also serve as vehicle of spread of infectious disease to humans [1].

The genus *Salmonella* is one of the most common causes of food borne infectious diseases in the world [5]. A characteristic feature of this organism is its wide host range which comprises most animal species including mammals, birds and humans. *Salmonella* is a ubiquitous and hardy bacterium which is capable of surviving in both dry and wet environments. It is a prominent member of Enterobacteriaceae that consists of more than 2,500 strains [1]. According to Mamman et al. [6] *Salmonella gallinarum* causes fowl typhoid in chickens which results in huge economic losses as infected chickens have a high mortality rate, succumbing to septicaemia, enteritis and haemolytic anaemia. *Salmonella gallinarum* is the causative organism of fowl typhoid which is a highly contagious disease of poultry. The disease is spread through the droppings of infected birds and has a very high mortality rate among poultry. The bacteria may also be spread through contaminated food, water, clothing and equipment. Fowl typhoid is not the same as typhoid fever in humans and so it is not a public health threat [7]. Fowl typhoid is host-adapted to avian species and is considered to pose a minimal zoonotic risk throughout the world [8].

The expansion of poultry rearing and farming has made Salmonellosis to become an important public health problem in Nigeria and other parts of the world, causing heavy economic loss [9]. *Salmonella* is considered to be one of the major bacterial disease problems in the poultry industry worldwide [10]. In a study carried out by Agbaje et al. [2] in Ogun State in 2010, there were five [5] isolates cultured and all of them were positive for *Salmonella*. Only one of the isolates was obtained from the intestine while the other four were obtained from other internal

organs. All the isolates showed the same antimicrobial sensitivity pattern.

In a study by Ibrahim et al. [9] in Nassarawa State 150 samples of poultry droppings were collected and analysed. Two [1.3%] samples were positive for *Salmonella gallinarum*. Some other bacteria that were isolated include *Proteus* species, *Escherichia coli* and *Pseudomonas* species among others. Another study on the carrier rate of *Salmonella gallinarum* in free range chickens in Nasarawa State was investigated by Salihu et al. [11]. A total of 1870 visceral organ samples were collected from free range chickens in 12 selected village/towns across the 3 senatorial zones of Nasarawa State. The samples were cultured for isolation of *S. gallinarum* and the isolates were identified using standard biochemical and serological tests and the overall prevalence was 49 (2.5%). The isolation of *S. gallinarum* in apparently healthy free-range chickens shows their carrier status and confirms infection of fowl typhoid in the free range chickens in Nasarawa State.

Babatunde et al. [1] investigated the prevalence of *Salmonella* isolates from poultry farms in Ilorin Nigeria. Out of 170 samples collected and examined, 8 (4.7%) gave biochemical characteristics that resembled *Salmonella* but only 6 (3.5%) were confirmed as *Salmonella* by polyvalent antisera. In a study on the isolation and characterization of *Salmonella gallinarum* from outbreaks of fowl typhoid in Kaduna State by Mamman et al. [6] seventy (70) isolates of *Salmonella gallinarum* were recovered from culturing visceral organs and cloacal swabs from thirty (30) different outbreaks affecting 29 poultry farms in 8 parts of Kaduna State. Dasat et al. [12] investigated the prevalence of *Salmonella gallinarum* from chicken cloacal swabs as well as their antibacterial susceptibility in Jos South LGA of Plateau State. They got a prevalence of 4% out of the 100 samples investigated. Also, all isolates were 100% sensitive to 2 antibiotics [ciprofloxacin and gentamycin] and resistant to Oxytetracycline, Erythromycin and Ampicillin.

The occurrence and antimicrobial susceptibility patterns of *Salmonella* species from poultry farms in Ibadan, Nigeria was investigated by Orum et al. [13]. Cloacal swabs were obtained from 360 chickens randomly from 10 poultry farms in 5 local government areas of Ibadan, Oyo State. The overall performance was 21.4%. They reported statistically significant associations between *Salmonella* prevalence and the farm location, age of chickens and health status of chickens. All the *Salmonella* isolates were resistant to cefuroxime. Most of the isolates were resistant to five other antibiotics.

The overall objective of this study was to isolate *Salmonella gallinarum* from poultry droppings and to relate the presence of *Salmonella gallinarum*, the cause of fowl typhoid, to both dry and fresh poultry droppings in the study area. The study is important because the use of poultry droppings as manure in the study area as well as in other parts of Plateau State is a common occurrence mostly because the droppings are cheaper and more accessible than commercially produced fertilizer.

2. Materials and Methods

2.1 Study Area

The study was conducted in Jos East Local Government Area of Plateau State, North Central Nigeria. One poultry farm each from five different villages (namely Fobur, Rizek, Kerker, Kwang and Zarazon) was used.

Jos East Local Government Area has its headquarters at Angware and it has an area of 1,020km^2 and a population of 85,602 as at the 2006 census.

2.2 Sample Collection

A total of one hundred samples made up of 10 fresh and 10 old (dry) samples each from one poultry farm in 5 different villages in Jos

East Local Government Area were collected using sterile spatulas into sterile universal bottles and immediately transported to the laboratory for analysis.

2.3 Sample Processing

The method described by Orji et al. [5] was used. Each sample was thoroughly mixed and about 2 gram was aseptically inoculated into buffered peptone water which is a pre-enrichment medium then incubated at 37^0C for 24 hours. 0.1ml of the overnight broth was transferred in 15 mL of Rappaport medium then incubated at 37^0C for 24 hours after which it was inoculated on dry and sterile Xylose Lysine Deoxycholate Agar (XLD)and MacConkey Agar (MCA) using sterile inoculation loop. Flaming was done at intervals to obtain discrete colonies.

2.4 Identification

All colonial forms on the XLD and MCA plates were recorded as pure or mixed. The colonies were further examined macroscopically and identified by their colour, size, consistency, lactose fermentation on MCA as well as Hydrogen Sulphide Production on XLD.

Gram staining was carried out on colonial forms that resembled *Salmonella* species, were non-lactose fermenters and produced Hydrogen Sulphide.

2.5 Biochemical Tests

In order to confirm the presence of *Salmonella gallinarum*, some biochemical tests were carried out as described by Owuama (2015) [14]. These include:

2.5.1 Oxidase Test

Oxidase test using wet filter paper was done. Filter paper was soaked with 2 drops of 1% solution of Tetramethyl-phenylene diamine dihydrochloride. Using an inoculation loop, a colony was picked and smeared on the filter paper. If positive, a deep purple colour was seen in 10 seconds.

2.5.2 Catalase Test

A colony of the test organism was put on a drop of 3% Hydrogen peroxide in the centre of a clean glass slide. If positive, active or immediate bubbling occurs, if negative, there were no gas bubbles.

2.5.3 Motility Test

A bottle of peptone water was inoculated with a colony of test organism and incubated at 37^0C for 2 to 3 hours. A ring of plasticine was made on a clean glass slide and inverted over a drop of the peptone water culture containing the test organism on the centre of a cover slip. This slide was then inverted swiftly and examined under a microscope using x10 and x40 objectives.

2.5.4 Indole Test

1 mL of the Test organism was put in a test tube and 2 drops of Kovac's reagent was added. This was allowed to stand for a few minutes and observed for a colour development. A positive result was indicated by the formation of a red colour ring.

2.5.5 Urease Test

Using urease base Agar slants, a colony of the test organism was inoculated and incubated at 37^0C for 24 hours after which it was observed for colour changes. Pinkish colour formation indicated a positive urease reaction while yellowish colour showed negative.

2.5.6 Citrate Test

A colony of the test organism was inoculated into citrate Agar Slant and incubated at 37^0C for 24 hours. A positive citrate reaction was indicated by bluish colour formation.

2.5.7 Triple Sugar Iron (TSI) Test

The test organism was used to make a stab on the Triple Sugar Iron Agar slant in a test tube and incubated at 37^0C for 24 hours. A positive reaction was indicated by production of Hydrogen Sulphide and gas production (butt formation).

2.5.8 Lactose fermentation

Using a sterile inoculation loop, a test organism was inoculated into Lactose broth and incubated at 37^0C for 24 hours. Lactose fermentation was indicated by yellow colour formation while a negative result had no colour change.

2.6 Statistical Analysis

Pearson Chi-square Analysis at $p>0.05$ was calculated to determine significance levels.

1. Results

1.1 Microbial contamination of poultry droppings examined in poultry farms in Jos East LGA of Plateau State

Table 1 shows the analysis of poultry droppings sampled according to villages. The village with the highest occurrence of microbial contamination was Kerker where 3 out of 20 samples (15%) were contaminated. This was followed by Rizek and Zaruzon (10% each) and then Fobur had the least (5%). There was no microbial contamination in samples collected from Kwanga. Overall microbial contamination was 8%.

Here the p value was greater than 0.05 (0.473); this shows that there was no significant difference in microbial contamination.

Table 1: Analysis of microbial contamination of Poultry Droppings According to Villages in Jos East LGA of Plateau State

Location	Number Examined	Number Positive (%)	Number Negative (%)
Fobur	20	1 (5%)	19 (95%)
Rizek	20	2 (10%)	18 (90%)
Kerker	20	3 (15%)	17 (85%)
Kwanga	20	0	20 (100%)
Zarazon	20	2 (10%)	18 (90%)
TOTAL	**100**	**8 (8%)**	**92 (92%)**

3. 2 Biochemical tests results for identification of Bacterial Isolates from Poultry droppings examined in Jos East LGA, Plateau State

Salmonella gallinarum had the following biochemical test results. It was catalase positive, citrate positive, indole negative. It was non-lactose fermenting. It was motile and negative for oxidase, Hydrogen Sulphide production and urease.

Table 2: Biochemical tests results for identification of Bacterial Isolates from Poultry droppings examined in Jos East LGA, Plateau State

Isolate	Catalase Test	Citrate Test	Indole Test	Lactose fermentation	Motility Test	Oxidase Test	H_2S production	Urease Test
Escherichia coli	+	-	+	+	+	-	-	-
Proteus species	+	+	-	-	+	-	+	+
Salmonella gallinarum	+	+	-	-	+	-	-	-

Key: + = Positive - = Negative H_2S = Hydrogen Sulphide

3.3 Distribution of Organisms Isolated from both fresh and old poultry dropping in Jos East LGA of Plateau State

Table 3 shows the distribution of organisms isolated from both old and fresh poultry droppings in Jos East LGA. Three (3) organisms namely: *Salmonella gallinarum*, *Escherichia coli* and *Proteus species* were isolated from both fresh and old poultry droppings. *Proteus species* were isolated from both fresh and old poultry droppings. *Proteus species* occurred in 3 different villages, and had an overall occurrence of 4%, followed by *Escherichia coli* (3%) while the least was *Salmonella gallinarum* with 1% occurrence.

The p value was greater than 0.05 (0.273), which shows that there was no significant difference in distribution of organisms in villages examined.

Table 3: Distribution of Organisms Isolated from both fresh and old poultry dropping in Jos East LGA of Plateau State

Location	Number Examined	Organisms Isolated		
		Proteus sp	***Escherichia coli***	***Salmonella gallinarum***
Fobur	20	1 (5%)	0	0
Rizek	20	0	1 (5%)	1 (5%)
Kerker	20	1 (5%)	2 (10%)	0
Kwanga	20	0	0	0
Zarazon	20	2 (10%)	0	0
TOTAL	**100**	**4 (4%)**	**3 (3%)**	**1 (1%)**

3.4 Analysis of Microbial Contamination of Fresh and Old Poultry Dropping According to Villages in Jos East LGA of Plateau State

Table 4 shows the analysis of microbial contamination of fresh and old poultry dropping according to villages in Jos East LGA. Only in 1 village (Kerker) was there contamination of fresh poultry droppings (10%). However, in 4 villages namely Fobur, Rizek, Kerber and Kwanga, there was microbial contamination of old poultry droppings giving an overall prevalence of 7%.

The p value was less than 0.05(0.027) and this shows that there was a significant difference in contamination of fresh and old poultry droppings.

Table 4: Analysis of Microbial Contamination of Fresh and Old Poultry Dropping According to Villages in Jos East LGA of Plateau State

Location	Fresh Droppings		Old Droppings	
	Number Examined	Number Positive (%)	Number Examined	Number Positive (%)
Fobur	10	0	10	1 (10%)
Rizek	10	0	10	2 (20%)
Kerker	10	1(10%)	10	2 (20%)
Kwanga	10	0	10	2 (20%)
Zarazon	10	0	10	0
TOTAL	**50**	**1 (2%)**	**50**	**7 (14%)**

4 Discussion

In this study, the overall contamination of 1% by *Salmonella gallinarum* in poultry droppings is similar to that observed and reported by Okwori et al. [8] who isolated *Salmonella gallinarum* from poultry droppings in Jos metropolis. Out of 150 samples from 50 poultry houses, they had an overall contamination of 1.3% by *Salmonella gallinarum.* Ugwuzor [15] also conducted a study in Jos area on poultry droppings used as manure. Out of 150 samples, 2 were positive for *Salmonella gallinarum* giving a contamination of 1.3% which is similar to that obtained in this study. Similarly, Ibrahim et al. [9] got a prevalance of 1.3% for *Salmonella gallinarum* in Nassarawa State which is similar to that obtained in this study. In another study carried out by Orji et al. [5] in Awka, the contamination of 6.7% by *Salmonella gallinarum* was significantly higher than that reported in this study. The difference in the prevalence maybe due to the environment. Orji et al. [5] carried out his research in the south-eastern part of the country while this study was conducted in the north-central part. Thus, the climate may be a contributory factor.

In this study, *Proteus species* (4%) and *Escherichia coli* (3%) were also isolated. This is also in line with works done by authors such as Okwori et al. [8] who also isolated *Proteus species*, *Escherichia coli* as well as some other pathogenic bacteria. The use of poultry droppings as manure in Jos East as well as other local government areas of Plateau State is common. The main reason for this is that the droppings are relatively cheaper. However, the addition of the droppings directly to soil without proper treatment is dangerous because they contained pathogenic microorganisms as seen from the study carried out and become a source of infection to birds. Another disadvantage of using the poultry droppings as manure is that water sources such as streams and wells can become contaminated as a result of run-off leading to incidence of water borne infections. Difference in contamination in fresh and dry poultry droppings is similar to results obtained by Wilkinson et al. (2011) [16] who observed that high temperature reduced *Salmonella* count in poultry counts considerably.

In this present study, *Salmonella gallinanum* was catalase positive, citrate positive, indole negative, motile, oxidase negative, did not produce Hydrogen Sulphide and was urease negative. *Salmonella* species Isolates were catalase positive, oxidase negative, urease negative, produced Hydrogen Sulphide, indole negative, and were motile as seen in the study conducted by Omeike et al. [17] in Idi Ayunre community, Oyo State. In the study carried out by Orum et al. [13] *Salmonella* isolates were indole negative, citrate negative, catalase positive, oxidase negative, urease negative. The result obtained in the present study were similar to those reported by the authors above. The presence of *Salmonella* specie in different environments shows that it is ubiquitous in nature.

5. Conclusion

The presence of *Salmonella gallinarum* in poultry droppings could pose serious health hazard to other uninfected birds especially free range birds [that may feed on farms in which such droppings are applied] as well as humans who eat crops that are fertilized using infected poultry droppings. It is important for regulations to be laid down and enforced in commercial poultry production in order to ensure that there is an effective monitoring system aimed at detecting *Salmonella gallinarum* and instituting appropriate control measures. Poultry farmers in Jos East

should improve hygiene practices within their environment.

Declarations

Ethics approval: Not applicable

Consent for publication: Not applicable

Competing interest: The authors declare that they have no competing interests.

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Availability of data and materials: The data that supports the findings of this study are available from the corresponding Author upon reasonable request.

Author's contributions: FOK, DO, DG and AUM were all involved in samples collection. FOK and AUM carried out the laboratory analysis. FOK, DO and AUM were major contributors in writing the manuscript. All authors read and approved the final manuscripts.

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