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Avian Salmonellosis and Evaluation of Haematological Changes in Indigenous Birds at Bukuru Live Bird Market

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Abstract: *The study was conducted to investigate the occurrence Salmonella species and haematological parameters of indigenous birds slaughtered at Bukuru live bird market of Jos South Local Government Area of Plateau State, Nigeria. A total of 50 commercial indigenous birds were used for the evaluation. The sampling was done at point of slaughter, collecting both cloaca swab and blood in EDTA containers. Microbiological procedures including culture, Gram staining and biochemical sugar test were conducted on the samples, providing 4 samples positive with frequency of 0.396 in the sampled population. The haematology evaluation was done on the positive birds. The resultant parameters of red blood cell 0.4-1.7 x 10^{12}, hemoglobin 2.3 – 6.3 g/dl and packed cell volume 7-19%. All these values where below the normal range. This signifies that not all indigenous birds slaughtered in the study are apparently healthy and fit for consumption. Also as these birds are sometimes subjected to very strenuous condition before slaughter which includes overcrowding in creates during transportation and deprivation of feed and water , we recommended strict adherence to biosafety and biosecurity in places birds are kept, routine vaccination against salmonella and cross breeding with hybrid cockerel which may enhance disease resistance tendency as well as productivity.*

1. Introduction

Avian salmonellosis (sal-moh-neh-loh-sis) refers to the infection of birds with a bacterium called Salmonella, which has more than 2,500 types. These bacteria are typically found in the intestines of a variety of vertebrates. The bacteria are transmitted to birds either from direct contact with other infected birds, through exposure to contaminated surfaces (e.g. bird feeder platforms contaminated with faeces) or ingestion of contaminated food or water. Salmonella organisms are widely distributed in nature and survive well in a variety of food and contamination and can occur at multiple steps along the food chain (Barde *et al.,* 2021). In wild birds, outbreaks can kill many birds, especially during winter. When a free-ranging bird is infected with Salmonella, it can either become a carrier, appearing healthy but spreading the pathogen in the environment, or it can become sick and die. Sick birds appear lethargic, fluffed, and emaciated. They may or may not have diarrhea and are often unaware of human presence and can be picked up by people or predated by cats with ease. Pathogenic microorganisms in the food chain are transmitted to humans through a variety of foods including beef, poultry and eggs (Ximenes *et al.,* 2019). In spite of this large population of domestic animals and poultry, the animal protein requirements for the country is still very low and inadequate. Therefore, there is need for great increase in livestock and poultry production. Though in Nigeria, there is slight increase in hen egg production as of 2011 which was total at 636,000 metric tons (MT) and valued at N8, 527.49 million ranking 19th in the world and first in Africa (FAO, 2015). Despite the rise in egg production, avian Salmonellosis remains a major constraint to poultry production in all parts of Nigeria (Mbuko *et al.,* 2009).

Salmonella was first isolated and described by Daniel E Salmon and can be described as a

rod shape gram negative facultative non spore forming bacilli which belongs to the family Enterobacteriaceace (Popoff *et al.,* 2003). The genus salmonella is divided into two species Salmonella enterica and S. bongori. Salmonella enterica itself consists of six sub species which are S. enterica sub specie enterica, sub specie arizona's, sub specie diarizone, sub specie indica and sub specie houtane or i, ii, iiia, iiib, iv and vi respectively (Barde *et al.,* 2015). All salmonella are actively motile (Cheesbrough *et al.,* 2000). Salmonellosis is one of the most common infectious diseases in the world in human and animals and also the most frequently isolated food borne pathogen and causes a wide range of disease such as enteric fever, gastroenteritis and bacteremia (Shivaprasad, 2000; FAO, 2015). Infection with salmonella can occur through inadequate cleaning and disinfection of poultry house, presence of carrier rodents and insects, litters, water, dust, equipment and feed (Shivaprasad 1997; Shivaprasad, 2000). Infection in day old chicks could be vertical from infected breeder flocks or horizontally transmitted during hatching, loading and transporting to the farm. Salmonella can be found in poultry egg and dairy product (Silva *et al.,* 2015; Benson *et al.,* 2021). Outbreaks of salmonellosis has also been linked to wide varieties of fresh fruit and vegetables such as apple, cantaloupe, alfalfa, sprout, mangos, cilantro, unpasteurized orange juice, tomato, melon, celery and parsley (FAO, 2015).The alimentary tract is the normal route of Salmonella infection, and the mechanisms underlying this infection route have been well established by studies on Salmonella species (Bliska *et al.,* 1993). Alternate routes via intraperitoneal and respiratory routes have also been reported for inducing experimental fowl typhoid (Barrow *et al.,* 1987; Basnet *et al.,* 2008). There is continuing interest in finding ways of preventing flock infection and hence contamination of poultry products with Salmonella enterica. Control measures are difficult to use effectively because numerous potential sources of Salmonella infection and product contamination exist in an integrated poultry enterprise. Control of Salmonella infections in poultry farms needs to begin with good farming practices and appropriate management associated with strict sanitary measures. Preventive and curative strategies have been widely applied to reduce the incidence of Salmonella colonization in chickens at the farm level (Revolledo *et al.,* 2006; Vandeplas *et al.,* 2010). Various prophylactic measures have been used to prevent and control Salmonella infection in poultry production and vaccination is the most practical measure to avoid contamination of poultry products and by-products and to prevent the disease in humans. Killed and live attenuated products have been used for controlling Salmonella in poultry production and vaccination with live attenuated products has proved to be effective (Cerquetti *et al.,* 2006).

2. Statement of Research Problem

The poultry industry in Nigeria cannot thrive well due to problems such as disease and poor quality of feed which affects the cost of production. the major problem is disease which accounts for great losses in poultry production (Emenna *et al.,* 2019).The Food and Agricultural Organization (FAO) estimated the population of poultry in Nigeria to be 150 million which is three times the population of all domestic animals combined (FAO, 2015). In spite of this large population of domestic animals and poultry, the animal protein requirements for the country is still very low and inadequate. The salmonella are potentially responsible for various pathogenic processes in man and animal including poultry. This study is designed to isolate avian Salmonella and to evaluate the haematological changes in indigenous birds at Bukuru live bird market Jos, Nigeria.

3. Justification

Despite the rise in egg and meat production, avian Salmonellosis remains a major constraint to poultry production in all parts of Nigeria (Mbuko *et al.,* 2009). Infection with salmonella can occur through inadequate cleaning and disinfection of poultry houses, presence of carrier rodents and insects, litters, water, dust, equipment and feed (Shivaprasad 1997; Shivaprasad, 2000).The finding of this study will therefore provide information on the occurrence of salmonellosis in the study area which will serve as a base line data for further research work. The aims of study was to isolate avian Salmonella and evaluate haematological changes in local birds in the Bukuru live bids market, Jos south local government area of plateau state, Nigeria. The objectives of the study were to isolate salmonella in indigenous

birds in relation to sex and to evaluate the haematological changes of avian salmonellosis.

4. Materials And Method

4.1. Study Area

The study was carried out in Bukuru live birds market of Jos South Local Government Area, Plateau State, Nigeria located at latitude of 9.7051N and longitude of 8.8142E. The local government headquarter is Bukuru town, which is 15 kilometers south of the state (Talbot *et al.,* 2006).

Study Design

Sampling was done using simple random sampling method. A total of 50 indigenous chickens were sampled at slaughter at Bukuru live birds market of Jos south Local Government Area of Plateau State.

4.2. Sample Collection and Handling

The cloacal swab samples were collected before slaughter for isolation and identification and the blood samples were collected immediately after slaughter of the live bird and the blood were transferred into EDTA bottles for haematological analyses. The samples were placed in a sample container labeled and carried in a flask (cooler) with ice packs and taken to microbiology laboratory and haematology laboratory of the Central Diagnosis Laboratory of National Veterinary Research Institute, VOM for analysis.

4.3. Culture and Isolation

Cloacal swabs collected were incubated; this was done by pre-enrichment of the swab samples in buffered peptone water and incubated for 24hours at 37°C followed by plating on Mac Conkey agar (MCA) and blood agar (BA) and incubated for 24hours at 37°C using standard laboratory methods . The resulting colonies were examined for their features, colour and morphology and tested for Gram-reaction (Gram-negative) using gram staining technique (Parmer and Davies, 2007).

4.4. Biochemistry Testing

Biochemistry testing involving various biochemical sugars (lactose, mannose, sucrose, maltose, glucose) for fermentation.

4.5. Hematological Analysis

Packed cell volume

Procedure: Micro-hematocrit method

Whole blood sample was mixed in EDTA anticoagulant gently by rocking or repeated inversion to ensure proper mixing of the sample and the anticoagulant. Using a clean micro-hematocrit capillary tube, the sample was allowed to flow by capillary attraction through negative gradient up to ¾ capacities. The lower end of the tube was sealed with plastacine or a burning flame. It was centrifuged at a predetermined speed for 5 minutes using the hematocrit centrifuge The PCV was read using the hematocrit Reader. The Result was read and reported in percentage (%) (Jones, 2015).

4.6. Red Blood Cell Count

Procedure: Hemocytometer method

Test tubes for the test samples were labeled. The blood sample was mixed gently by repeated inversion. 4000µl of formal citrate solution was Pipette into the test tubes. It was allowed to stand on the bench for about 5 minutes. The chamber was filled by means of a Pasteur pipette and allows to settle for 2 minutes. The number of cells in the central squares was counted using a low power objective, (5 squares).

Calculation: N × DF × 109/A × DDF = Dilution factor;

A = Area of chamber; D = Depth of chamber; 106 = Conversion factor (Mitchell and Johns, 2008).

4.7. Hemoglobin Estimation

Procedure: cyanmethemoglobin method

20 microliter of blood + Drabkin 4 mL = 1: 200 dilution, mixed well. Read after 10minutes of mixing the blood with Drabkin's solution and allowing to settle. Read on a spectrophotometer at filter 540. Read against the blank of Drabkin's solution (Drabkin solution can be used as blank). Also, read the standard solution (12 G/dL) with the same dilution as the test sample. Read by the spectrophotometer; reading is called optical density (OD).

Calculation= OD of test/ OD of standard x concentration of standard= haemoglobin of test in g/dl (Campbell, 2015).

Total White Blood Cell

Procedure: Hemo-cytometer method sample

Test tube for test(s) was labeled. The blood was mixed gently by repeated inversion.380µl of Turk's solution was Pipette into test tubes. 20µl

of the sample was Pipette into the test tubes and mix gently. By means of a Pasteur pipette the counting chamber was filled and allows to settle for about 2 minutes. It was viewed using the low power objective (× 10) of microscope in all the 16 small square boxes of the 4 large outer squares

Calculation: N × DF × 106/ A × D

Where: N = Number of cells counted; DF = Dilution factor; A = Area of chamber; D = Depth of chamber; 106 = Conversion factor (Mitchell and Johns, 2008).

4.8. Data Analysis

The generated was analysis using simple descriptive statistic (percentage and table)

5. Results

Table1. *Laboratory results of cloaca swab samples from Bukuru live bird market*

Number of samples	Male	Female	*Escherichia coli*	Klebsiella spp.	Proteus spp.	Bacillus spp.	Salmonella spp.	Nill
50	22	28	23	7	5	2	4	9

Table2. *The bacteria identification on solid media and biochemical test of isolates from the swab samples*

Isolates	MacConkey agar	Blood agar	Gram stain	Dositol	Citrate	Indole	Mannitol	Triple sugar iron	Sucrose	Maltose	Trehalose	Salicin	Urease
Escherichia coli	LF	SGC	-rod	.	-	+	+	AG	+	+	+	D	-
Salmonella Gallinarum	NLF	TSC	-rod	+	D	-	+	.	.	+	+	-	_
Proteus spp	NLF	Swarming	- rod	.	+	-	+	+	+	-	+	D	+
Klebsiella spp.	LF	SGC	- rod	.	+	-	+	-	+	+	+	+	+
	MacConkey agar	Blood agar	Gram stian	Xylose	Urease	Gelatin	Indole	Citrate	Glucose	Arabinose			
Bacillus spp	LLF	DDC	+ Rod	+	-	+	-	+	+	-			

Key: *LF = lactose farmentase; NLF = Non lactose farmentase; LLF = Late lactose farmentase; SGC = Small gray colony; TSC = Tiny shiny colony; DDC = Dark dry colony; D = Delayed; AG = Acid and gas; + = Positive; - = Negative*

Table3. *Result of full blood count for positive samples*

Sample identification Number	PCV %	HB g/dl	RBC x 10^{12}	WBC x 10^{9}	N %	L %	M %	E %	B %	Blood pictures
C7	19	6.3	1.7	4.7	72	25	-	03	-	HYP++
C9	11	3.6	1.3	4.1	64	36	-	-	-	HYP++
C10	12	4.0	1.3	6.7	67	30	-	03	-	HYP++
C19	7	2.3	0.4	4.5	50	50	-	-	-	HYP++

Key: *HYP = Hypereosinophilic syndrome; RBC = Red blood cell; PCV = Packed cell volume; WBC = White blood cell; HB = Haemoglobin; H = Heterophile (Neutrophil); L = Lymphocyte; M = Monocyte; E = Eosinophil; B = Basophil;*

Normal Range for avian; PCV = 27 - 42%; RBC = 2.2 - 4.0 x 10^{12}; WBC = 1.0 - 9.5 x 10^{9}; HB = 7.0 - 11.0 g/dl; H = 50 - 65%; L = 20 - 50%; E = 0 - 4%; B = 0 - 2%

Table4. *Showing the occurrence of Salmonella infection in sampled birds in relation to sex of the birds*

Sex	Sample Population	+Positive	%
Male	22	2	9.1
Female	28	2	7.1
Total	50	4	16.2

X2 = 0.720 *df = 1* *p = 0.396*

6. Discussion

This study investigated 50 indigenous birds from Bukuru live bird market, with 4 birds positive for Salmonella species. Table 1 showed that out of 4 positive for salmonellosis, 2 (7.1%) females and 2 (9.1%) males with frequency of 0.396 among the sampled population. Other microbes isolated includes Klebsiella spp., Proteus spp., Bacillus spp and *Escherichia coli.* The hematological parameters of the positive birds are below the Normal range for the total red blood cells, packed cell volume and hemoglobin concentration indicating anemia. The above mentioned values were lower(red blood cell 0.4-1.7 x 10^{12}, hemoglobin 2.3 – 6.3 g/dl, packed cell volume 7-19 %) than that obtained in the work of (Ahmed., 2018) who reportedred blood cells 4.80- 4.95 x10^{6} /µl, hemoglobin 8.70-11.80g/dl, packed cell volume 25-45%,. Generally, the decreased PCV, haemoglobin and Total red blood cell is associated with anaemia. Lower values of total erythrocyte count, haemoglobin and packed cell volume in indigenous chicken also corresponded with the acute phase of disease in which anaemia had been reported in chickens (Assoku and Penhale 1978; Prasanna and Paliwal, 2002). Assoku and Penhale (1978) have suggested that the anaemia associated with acute fowl typhoid may be a direct result of the increased ability of the reticuloendothelial system to take up modified erythrocytes, it also results to reduced amount of oxygen in blood circulation. The haematological result's blood pictures shows that all the samples indicated Hypereosin-ophilic syndrome (HYP++) which shows double increase in number eosinophils. This condition can be initiated by the body as immune response against bacteria and other infections. Table shows the haematological changes in the four Salmonella positive with mean PCV values of 13% (female) and 11.5% (male). This is in agreement with the findings of Daramola *et al.,* (2005) who reported that age and sex of farm animals affect their haematological parameters, similarly Schalm, (1975) reported that blood pictures of animals might be influenced by certain factors such as nutrition, management, breeds of animal, sex, age, diseases and stress factors. Also Dukes (1955) and Afolabi (2010) posted that haematological values of farm animals are influenced by age, sex, breed, climate, geographical location, season, day length, time of day, nutritional status, life habit of species, present status of individual and other factors. Similarly Barde *et al.,* (2015) reported significant changes ($P<0.05$) in haematological parameters such as red blood cells (RBC), haemoglobin (Hb) concencentrations, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), heterophil and lymphocyte counts between the infected groups when compared with the control group. There were also significant changes ($P<0.05$) in these haematological parameters post-infection compared to before infection within each infected group.

The other microorganisms isolated included *Escherichia coli*, Proteus spp, Bacillus spp and Klebsiella spp with attention on salmonella spp. Although very similar antigenic features are shared by the two Salmonella biovars, some biochemical reactions such as glucose, maltose, and dulcitol fermentation, have been established and used to differentiate them with dulcitol reactive to only *Salmonella Gallinarum* among the Salmonella Spp (Crichton and Old, 1990). However, it is difficult to differentiate between S. Gallinarum and S. Pullorum since they share the same 1, 9, and 12 O antigens (Christensen *et al.,* 1993). Therefore, development of a rapid and reliable method for the accurate identification and discrimination of biovars Gallinarum and Pullorum would allow for earlier confirmation of the pathogens and subsequently a more effective elimination of the diseases from flocks. In correspondence to Ribeiro *et al.,* (2009) who said *Salmonella Gallinarum* can infect birds of any age and is highly pathogenic, causing fowl typhoid, which usually results in systemic infection, and may cause 40–80% mortality in the flock, so diagnosis and timely removal of infected birds are crucial to prevent the spread and control the prevalence of S. Gallinarum and S. Pullorum in poultry.

7. Conclusions

The findings of this study indicate 8 % Salmonella prevalence in the study area with other bacteria species such as *Escherichia coli*, Proteus spp, Bacillus spp and Klebsiella spp were also isolated. Anemia seen in this study was very severe due to the haemolytic effect of the Salmonella endotoxins.

8. Recommendation

Strict biosafety and biosecurity measures should be adhered to in the place where this indigenous

birds are kept. Routine vaccination can be administered to the birds to prevent Salmonella infection. Cross breeding the hens with hybrid cockerel may enhance their productivity, meat yield and disease resistant tendency.

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