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Sequential Pathological Study of Fowl Typhoid in Experimentally Infected Chicks

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Abstract
This study was undertaken to examine the sequential pathological study of fowl typhoid disease in experimentally infected chicks. A total of 40 chicken feed samples was randomly collected from different shops in Ihiala market and screened for *Salmonella enterica* serovar Gallinarum using pour plate method. The isolate obtained was characterized and identified using the morphological and biochemical characteristics. The pathogenic potential of the isolate on day old chicks was investigated by challenging the day old chicks orally using 0.5ml of the inoculum (10^8cells/ml). All the chicks were kept under complete examination for 2 weeks for obvious pathological signs and lesions. *Salmonella enterica* serovar Gallinarum was detected in 13(32.5%) chicken feed samples analyzed. Obvious pathological signs and lesions were significantly ($P<0.5$) seen among the infected chicks whereas the non-infected chicks showed no obvious pathological signs. The internal organs (liver and spleen) of the infected chicks showed significant ($P<0.5$) viable counts of the challenged organism, which was mostly recorded in the spleen. This study has shown that *Salmonella enterica* serovar Gallinarum isolated from chicken feed samples exhibited reasonable pathological features among the infected chicks.

INTRODUCTION

Fowl typhoid is a septicemic disease of poultry that causes considerable economic losses through mortality and increased morbidity. The disease fowl typhoid was first recorded in the 18th century. However, it was not until 1880s that Louis Pasteur isolated and grew it in pure culture. It was formerly known as *Shigella gallinarum* and when isolated by Klein in England in 1889, it was called fowl typhoid in 1902 (Pattison, 2008). Originally as disease of fowl in Europe, it was first recorded in North America in 1943–1944. Since then the outbreaks have been recorded almost annually in chicken (Waldroup, 2002).

Salmonella enterica serovar Gallinarum is the causative agent of fowl typhoid (Waldroup, 2002). It is a non motile, non spore forming, gram negative rod-shaped bacteria belonging to the family of Enterobacteriaceae (sero group D). *Salmonella* Serovar GallinIarum causes a severe systemic disease of chickens and other galliform birds and along with the closely related *Salmonella enterica* Serovar Pullorum is host-specific for poultry but rarely presents a risk of zoonotic transmission to man (Barrow *et al*., 2002). Fowl typhoid infection usually follows the ingestion of food or water contaminated by faecal material. Infection in chickens may occur at all ages and is typified by severe hepatosplenomegaly accompanied by characteristic liver 'bronzing', anaemia and septicaemia (Barrow *et al*., 2002)

Salmonell aenterica serovar Gallinarum is primarily associated with the mononuclear phagocyte system and resides primarily within macrophages in the liver and spleen. It is only found in the gastrointestinal tract early in the infection, usually through faecal-oral transmission, and in the end stage of fowl typhoid where bacteria are shed into the intestines leading to substantial hemorrhaging of the intestinal wall (Cooper, 2001). Infection leads to high rates of morbidity and mortality with a recent study describing a mortality rate in excess of 60% in experimentally infected outbreed chickens. Fowl typhoid disease is an increasing concern in chickens. On several occasions, the disease has been reintroduced to commercial chicken farms and therefore it remains of high economic importance to developing poultry industries in developing countries. This study therefore evaluates the sequential pathological study of fowl typhoid disease in experimentally infected chicks.

MATERIALS AND METHODS

Sample collection: A total of 40 representative samples of different types of chicken feeds (Starter, Layer, Grower, Finisher) of different brands was collected from different shops at Ihiala market using sterile polyethene bags, and kept in disinfected cooler before culture. Sampling was performed manually from different bags such that the product was collected from different parts of the bags. The sample was pooled, mixed properly and formed one cup of the feed sample, then 10g of the mixture was taken for analysis. The samples were brought to the laboratory in a cooler maintaining low temperature (≤ 4℃) using ice blocks. The collected samples were processed within 6 h of its collection. The samples were collected randomly and each collected sample was marked with identification code with respect to the date and time of collection. Sampling criteria was limited to

one cup of one sample and the collection criteria were not limited to any specific part.

Isolation and identification of *Salmonella* Serovar Gallinarum: Tenfold serial dilution were carried out on each different samples and 1.0 ml was aseptically taken for the third test tube and pour plated into the *Salmonella Shigella* Agar and incubated at 37˚C for 48 h. After 48 h of incubation the grown colonies were sub-cultured, characterized and identified using their colony descriptions, microscopic and biochemical characteristics (Willey *et al.,* 2008).

Procurement of chicks: A total of (18) day old chicks of mixed sex was obtained from Mr. Eze poultry farm at Ihiala, Anambra State were used for this study. The birds were kept in separate, thoroughly cleaned, disinfected cages and provided with feeds and water frequently.

Preparation of test organism: The test organism used for inoculation of chicks was prepared and incubated for 48h for heavy growth.

Inoculation into chicks: This was carried out using the method of Wafaa *et al.* (2012). Broth culture of the isolate was centrifuged at 3000r.p.m for 1ominutes. The sediment was diluted with sterile phosphate buffer saline (PBS) and adjusted to the 10^8CFu/ml using 0.5 McFarland matching standard which is (0.6ml of 1% $BaCl_2.2H_2O$ + 99.4ml of 1% concentration of H_2SO_4). Then the chicks for infection (two in number) were inoculated orally with 0.5ml of the inoculums.

Examination of infected chicks: The infected chicks were carefully observed for the clinical manifestation of the organism for a period of 14 days. After fourteen (14) days, the infected chicks were sacrificed and gross examination of their internal organs morphologies was carried out.

Re-isolation of the organism from the infected organs: The internal organs of the infected chicks were harvested and portions were aseptically macerated in peptone water and serial diluted using three-fold serial dilution. Samples were inoculated into *Salmonella Shigella* Agar and incubated at 37˚C for 48h.

Statistical analysis: The data generated from this study were represented as mean ±standard deviation and then charts. The test for significance at 95% confidence interval was carried out using (Iheukwumere and Umedum, 2013).

RESULTS

The presence of *Salmonella enterica* serovar Gallinarum in chicken feed samples randomly collected from different shops in Ihiala market is shown in Table 1. The study revealed the presence of the isolates in the test samples. Out of 40 samples analyzed, 13(32.5%) samples were positive for the isolate. The morphological and biochemical characteristics of the isolate on Salmonella Shigella Agar (SSA) plate shown in Table 2.

The obvious pathological signs and symptoms of the challenge *Salmonella enterica* Serovar Gallinarum on the infected chickens are shown in the Table 3. Anorexia, diarrhoea, respiratory distress, weakness and cases of death were common to the infected chicks but none of these pathological signs and symptoms was observed in the control chicks. These obvious pathological signs were significant ($P<0.05$) among the infected chicks when compared to non infected chicks. The pathological features of *Salmonella enterica* Serovar Gallinarum in the visceral organs of the infected chicks is shown in Table 4. The study showed that the internal organs of the infected chicks were associated with air sacculitis, pericarditis, perihepatitis, splenomegaly, liver haemorrhage and congestion. These pathological features were significant ($p<0.05$) among the infected chicks when compared to non infected chicks (control).

The total mean plate counts (TMPCs) of enteric bacteria from the internal organs are shown in Table 5. The results showed that the total mean plate counts of *Salmonella enterica* Serovar Gallinarum were higher in the spleen. There was generally significant ($P<0.05$) decrease in the total mean plate counts of the chickens that are not infected with the isolate. The infected organs were able to show significant ($P<0.05$) growth of *Salmonella* serovar Gallinarum compared to the control organs from the chicks that had no visible growth.

Table 1: Presence *Salmonellaenterica*SerovarGallinarumin chicken feed samples

Type of Feed	Positive Sample (%)	Negative Sample (%)	Total (%)
Y	2(20)	8(80)	10(25)
O	2(20)	8(80)	10(25)
P	3(30)	7(70)	10(25)
X	6(60)	4(40)	10(25)
Total	13(32.5)	27(67.5)	40(100)

Table 2:Characteristics and identity of *Salmonella* serovar Gallinarum

Parameter	***Salmonella* serovar Gallinarum**
Appearance in SSA plate	colourless and dark at the centre
Elevation	raised
Edge	smooth
Gram reaction	-
Morphology	rod
Motility	-
Catalase	+
H_2S production	+/-
Indole	-
Citrate	-
VP	-
MR	+
Oxidase	-
Lactose	-
Galactose	-
Inositol	-
Xylitol	+
Mannitol	+
Dulcitol	-
Sorbitol	-
Maltose	+

VP - Vogesproskaur, MR - Methyl red, H2S - Hydrogen sulphide, SSA - Salmonella Shigella Agar

Table 3: Obvious pathological signs of *Salmonellaenterica* Serovar Gallinarumin infected chicks

Parameter	N=3 test	control
Diarrhea	2	0
Respiratory distress	0	0
Weakness	2	0
Anorexia	3	0
Dysentery	2	0
Alopesia	0	0
Death	2	0

Table 4: Morphological characteristics of the visceral organ of the infected chickens

Parameter	N=3 test	control
Perihepatitis	3	0
Pericarditis	0	0
Air sacanlitis	0	0
Haemorrhage of the liver	2	0
Congestion of the liver	3	0
Splenomegaly	2	0
Enterocolitis	1	0

Table 5: Total mean viable plate counts of *Salmonella enterica* serovar Gallinarum isolated from the internal organs of infected chicken

Organ	viable count (Cfu/g)
Liver	34.00 ± 0.41
Spleen	57.00 ± 0.31
Control	0.00 ± 0.14

DISCUSSION

The presence of *Salmonella* Serovar Gallinarum in the chicken feed samples could be traced from the feed ingredients, transportation of feeds, poor handling and sanitary conditions attributed to the feed samples. Similar findings were reported by many researchers (Immerseel *et al.,* 2002; Jones and Richardson, 2004; Alshawabkeh, 2006; Maciorowki *et al.,* 2007).

The significant pathological signs such as anorexia, weakness, diarrhoea, bloody diarrhoea, enterocolitis and respiratory distress collaborate with the findings of Watkins *et al.* (2003). The occurrence of air sacculitis, pericarditis and liver haemorrhage among those chickens infected by *Salmonella* serovar Gallinarum could be due to the organism's capability of invading the liver and spleen of the infected chickens. Severe hepatosplenomegaly accompanied by characteristic liver 'bronzing' suggests that the spleen is an important site for detection of *Salmonella* serovar Gallinarum. A similar finding was stated by Barrow *et al*. (2002).

The significant mean viable plate counts of *Salmonella* serovar Gallinarum recorded from the internal organs of the infected chickens supported the reports of many researchers (Waldroup, 2002;Watkins *et al.,* 2003; Dashe *et al.,* 2012). The presence of of *Salmonella* serovar Gallinarum in the liver and spleen suggests that the organ contains sufficient nutrients and favourable environment for the bacteria. The activities of the invaded organisms on the organ on the organs might cause degradation of nutrients, deterioration and deformation of the organs, thereby producing pathological lesions that can clinically manifest on the infected chickens. This is in agreement with the report of Dashe *et al.* (2013).

CONCLUSION

This study has revealed the presence of *Salmonella enterica* serovar Gallinarum in chicken feed sample randomly collected from different shops located in Ihiala commercial market in Ihiala Local Government Area of Anambra State.The studied isolate exhibited significant pathogenic potentials among the infected broiler.

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