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Review Article

Clinico-Pathological Lesions Associated with Experimental Infection of *Salmonella enterica* Subspecies *Enterica* Serovar Gallinarum in Cockerels

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Abstract

Salmonella enterica subspecies enterica serovar Gallinarum (SG) causes fowl typhoid (FT), a disease responsible for economic losses to the poultry industry worldwide. Experimental investigation of clinico-pathological lesions associated with experimental infection of *Salmonella* enterica subspecies enterica serovar Gallinarum in cockerels was evaluated base on clinical signs, haematological examination, serum biochemical assessment, gross and histological examination of the effect caused on the birds. Twenty cockerels were used after raring them from day old to four weeks before infection. Ten were subjected to the salmonella infection while other ten were used as control. This study revealed the severity of Salmonella enterica subspecies enterica serovar Gallinarum infection against multiple organs lesions to the birds which cause mortality as observed in the study after 5 days of infection with clinical signs of weakness, ruffled feathers, inappetence, difficulty in breathing, reluctance to move, loss of weight, increase temperature,watery and yellowish diarrhea. The haematology result indicates anemia, leucocytopenia, heterophilia and lymphocytopenia while the serum biochemical results showed that alanine transferase (ALT) and alkaline phosphatase (ALP) were statistically significant ($P < 0.05$), while aspartate aminotransferase (ASP), total protein (TP) and urea (U) were statistically insignificant ($P > 0.05$) in the infected group. The gross lesions observed include: pale carcasses, severely congested lungs, and whitish nodular lesions on the heart, haemorrhagic enteritis, and bloody intestinal content, bronze colouration of the liver, swollen kidney and enlarge spleen (splenomegaly). The histopathological lesions revealed neuronal necrosis, necrosis of the myocardium, sloughing of the intestinal villi, severe necrosis of the renal tubules, thickening of alveolar septae and bronchiolar epithelial lining, necrosis of the hepatocytes and congestion and splenic lymphocytic depletion. These findings showed the severity of Salmonella enterica subspecies enterica serovar Gallinarum on the organs of the cockerel birds (young birds) just as it does in adult birds.

Keywords: Pathological Lesions; Salmonella; Cockerel

Introduction

In highly developed poultry industries Fowl Typhoid and Pullorum Disease have largely been eradicated by control programmes implemented in the 1950s and 1960s. However occasional outbreaks of Fowl Typhoid, as seen in the UK in 2005 and 2012, may still occur [1]. Eradication of Fowl Typhoid and Pullorum Disease diverted much avian *Salmonella* research in Europe and North America away from *S. Gallinarum* and *S. Pullorum* towards studies that centered on the public health importance of the chicken as a major reservoir of food borne zoonotic infection through contaminated eggs and poultry meat [1].

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 [2,3]. *Salmonella* organisms are widely distributed in nature and survive well in a variety of food and contamination can occur at multiple steps along the food chain [3]. *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 Enterobacteriaceae [4]. According to Barde., *et al.* [5], 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, sub specie diarizona, sub specie indica and sub specie houtane or i, ii, iiia, iiib, iv and vi respectively. All *salmonella* are actively motile [6].

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 [3,7]. Infection in day old cockerel 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 [8,9]. Fowl typhoid is frequently referred to as a disease of adult birds caused by *Salmonella enterica* subspecies *enterica* serovar Gallinarum which is nowadays referred to simply as *Salmonella* Gallinarum [10].

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 [2]. Post mortem lesions in birds include unabsorbed yolk sac, congested lung, dark and swollen hemorrhagic liver, inflamed ceca and birds may die after one or two days of age. In hens, there is irregular ovary, cystic, deformed and pedunculated with prominent thickened stalks. Sometimes, the ovary is inactive with small pale and underdeveloped ova [5]. Over the years, attempts in controlling *salmonellosis* has not been very successful because it is widely distributed in nature and can survive in varieties of food [8].

Avian and mammalian species differ both in their physiology and immune system, reflecting more than 200 million years of divergent evolution. Yet there are more features of host response and pathogenic salmonellosis shared between birds and mammals than there are differences. These include an inflammatory response elicited by pathogen factors including the SPI1 Type III secretion system (T3SS) and the innate immune system recognizing key pathogen features, survival in macrophages mediated by virulence factors including the SPI2 T3SS [11] and the development of asymptomatic carriers following infection with host-restricted serovars [12]. As such the chicken is becoming adopted as a model that allows the study of adaption in not only *S. Gallinarum* and *S. Pullorum* but also in the study of newly emerging variants of S. Typhimurium. Olabode., *et al*., [13] carried out a study where in three Local Government Areas: Jos North, Jos South and Jos East. Twelve (12) samples each were collected from five (5) farms. A total of 360 samples were randomly collected consisting of equal number of quail and chicken eggs (180 each). A well-structured questionnaire was used to help analyze the results. They reported that low prevalence of salmonellosis in chicken and quail egg in the study area was observed, but they suggest the need for constant monitoring on regular basis to avert health risks associated with consuming Salmonellae infected poultry products in endemic areas.

Poultry farmers still experience great losses by way of mortality, morbidity due to *Salmonella* infection despite huge amounts spent on vaccination and medication. *Salmonellosis* in poultry causes heavy economic loss due to mortality and reduced production [14-17]. A three year (January 2007 – August 2009) retrospective study of the post mortem records of the Zonal Investigation Laboratory of the National Veterinary Research Institute, Yola was carried out. Of the 196 post-mortem poultry cases diagnosed, 80 (40.8%) were Salmonellosis [18]. In a study carried out by Barde., *et al*., [19], a total of 2512 cases of poultry diseases were documented for a period of five years from 2015 to 2019 with an average of 502.4 cases annually. A total of 436 of salmonellosis was documented during the period under review and an average of 87.2 cases annually. 24.4% prevalence was recorded in 2018 and 12.4% in 2015. 2019 had the highest prevalence of 28.4 with an average avian salmonellosis prevalence of 19%. Avian salmonellosis affects all types of birds. Out of 436 positive avian salmonellosis cases recorded in the period under review, 251 were layers while 156 were broilers with 29 being local birds and cocks. Salmonella *enterica* subspecies *enterica* serovar Gallinarum (SG) causes fowl typhoid (FT), a disease responsible for economic losses to the poultry industry worldwide [20].

The aim of the study is to evaluate the clinico-pathological lesions associated with experimental infection of Salmonella *enterica* subspecies *enterica* serovar Gallinarum in cockerel; and the objectives were: to evaluate the clinical signs associated with experimental infection of Salmonella *enterica* subspecies *enterica* se-

rovar Gallinarum in cockerel in Jos Plateau State, to evaluate gross and histopathological lesions associated with experimental infection of Salmonella *enterica* subspecies *enterica* serovar Gallinarum in cockerel in Jos Plateau State and to evaluate the haematological and serum biochemical changes associated with experimental infection of Salmonella *enterica* subspecies *enterica* serovar Gallinarum in cockerel in Jos Plateau State.

Materials and Method

Study area

The study was carried out at the Central Diagnostic Laboratory of the National Veterinary Research Institute, located in Vom, in the Jos South Local Government Area of Plateau State, Nigeria. The laboratory's geographical coordinates are latitude 9^0 30^0 to 10^0 N and longitude 8^0 30^0 E. Vom is situated in the northern part of Plateau State, with its headquarters at Bukuru, approximately 15 km from the state capital, Jos. Furthermore, Jos South is nearly at the geographical centre of Nigeria and is around 244 km from Abuja, the nation's capital [21,22].

Experimental animal

Twenty-day-old cockerels were obtained from the poultry division of National Veterinary Research Institute Vom and reared for four weeks before commencing the experiment. This cockerel were put under the required hygienic condition, fed on a balanced ration, supplied with clean water in sufficient quantities and not supplemented with antimicrobial agents or any vaccination until the end of the experimental period.

Bacteriological monitoring before infection

Before infection, cloacal swabs were collected from all the cockerels using sterile swabs in order to confirm if they were free from Salmonella organism or indeed any other bacterial pathogens. This was done by pre-enrichment of the swab samples in buffered peptone water, followed by plating on MacConkey agar (MCA) and blood agar (BA) using standard laboratory methods [23].

Experimental design

This experiment was carried out at Central Diagnostic Laboratory of the National Veterinary Research Institute, Vom. Twenty-day old cockerel were randomly divided into two groups. The first group (N = 10) were kept as a normal control and each one was inoculated with 0.2 ml of sterile saline through *per os*. The second group (N = 10) were infected with isolated *S.* Gallinarum strain at a dose 0.2 ml of sterile saline containing 1×10^8 CFU/ml through the same route. After inoculation, all experimental birds were kept under strict daily observation for recording clinical signs and mortality rates. Four blood samples were collected from each group as representative samples, for determination of hematological and serum biochemical parameters before and post infection. The cockerels were sacrificed for gross and histopathological examination.

Blood samples

Four blood samples were taken from wing vein from each group. Each blood sample was divided into two portions; the first one were anti-coagulated with tripotassium EDTA and were used for determination of hematological investigations. The second portion were placed in a plain centrifuge tube for serum separation and used for determination of serum biochemical parameters according to the method described by Patton and Crouch, [24].

Haematological evaluation

Blood for haematological analysis was collected into 2 ml labelled Bijou-bottles, containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant. Red blood cell count, packed cell volume and haemoglobin concentration were determined using Mindray auto haemo analyser, model BC-3000 PLUS, manufactured by Shenzhen Mindray Biomedical Electronics Ltd, China. The haemo analyser machine was switched on and it automatically flushed the fluidic lines, check the background and enter the count screen. The menu key was pressed to select the sample mode, and whole blood was selected. The blood in the sample tubes was mixed well using Stuart roller mixer machine model SRT9D, after bringing it to room temperature. It was then presented to the analyser probe tip making sure it submerges well into the sample. The aspirate key was pressed to start the count. The result was printed out in a hard copy automatically from the machine and recorded [25-27]. For differential leukocyte count, samples were mixed well using Stuart roller mixer machine model SRT9D. A drop of blood was placed one centimeter away from the edge of clean, grease free slide using pipette. A spreader was placed in front of the blood to allow the blood to spread across its edge. The spreader was moved forward quickly and smoothly. The smear was allowed to air dry, and then labelled. The air dried smear was then placed on a staining rack, flooded with filtered Leishman's stain and allowed to fix and stain for two minutes. The stained smears was then flooded with equal volume of buffered distilled water (pH 6.8) and allowed to rinse for 8 minutes. Excess stain was washed off with tap water and allowed to differentiate to salmon pink colour. The slides were air dried and view using oil immersion objective, starting from the thin end. A systematic meander system of slow, careful and detailed leucocytes count was made, and the result recorded [26,28].

Leukocyte count

Leukogram (Total leukocytes and its differential leukocyte counts) were determined using midrey auto-analyser model BC-300 PLUS manufactured by Shenzhen midrey by medical electronic Ltd, China. Samples were mixed well using Stuart roller mixer machine model SRT9D. A drop of blood was placed one centimetre away from the edge of clean, grease free slide using pipette. A spreader was placed in front of the blood to allow the blood to spread across its edge. The spreader was moved forward quickly and smoothly. The smear was allowed to air dry, and then labelled. The air dried smear was then placed on a staining rack, flooded with filtered Leishman's stain and allowed to fix and stain for two minutes. The stained smears were then flooded with equal volume of buffered distilled water (pH 6.8) and allowed to rinse for 8 minutes. Excess stain was washed off with tap water and allowed to differentiate to salmon pink colour. The slides were air dried and viewed using oil immersion objective, starting from the thin end. A systematic meander system of slow, careful and detailed haematological parameters was made, and the result recorded [26,29,30].

The serum-biochemical parameters was according to standard procedures [24,31-34]. Blood samples was collected via the wing vein, using 25 gauge sterile hypodermic needles and syringes and then kept in a slanted position for serum to settle. The blood was collected 5 days after challenge, so as to get the serum-biochemical picture within the incubation period of the disease in case of the experimental birds, similarly blood samples were taken from the control group too. Two millilitres of serum from each bird sample was used to measure the serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea and total protein levels in the Clinical Chemistry Laboratory of the Central Diagnostic Laboratory, National Veterinary Research Institute (N.V.R.I), Vom, Nigeria.

Assay for serum total protein

Biuret method was used to determine total protein, as described by Benjamin [35]. A blank test tube, standard and the test sample tubes was labelled. 1000 µl of biuret reagent was pipetted into each tube, and 200 µl of distilled water, standard and test samples were pipetted into the respective tubes. They were thoroughly mixed and incubated for 30 minutes at 25 ^{o}C. A spectrophotometer (model 6400/6405 manufactured by Jenway, Barloworld Scientific, England) was set at 546 nm and zero using blank test tube, the absorbance was read, and the total protein calculated using the formula.

A1 × C/A2

Where:

- A1 = Absorbance of the test sample,
- A2 = Absorbance of the standard,
- C = Standard concentration [33]

Assay for Serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)

Pippeted into tubes was 100 µl of sample and 500 µl of buffer were added, mix and incubated for 30 minutes at 37°C. Then 500 µl of dye reagent was added and allow to stand for 20 minutes at 25°C; after which 5 ml of sodium hydroxide was added, mix and the absorbance of the sample against sample blank was read after 5 minutes. Calculation for both AST and ALT were obtained from the activity table [36].

Assay for Serum Alkaline Phosphatase (ALP)

This was done using the method of Buttery., *et al.* [32]. 500 µl of blank, standard and sample was pipetted into test tube and incubated at 37°C for 10 minutes, after which 2500 µl of alkaline phosphatase colour developer was added into each of the test tubes, mixed and then the absorbance was read using a spectrophotometer at wavelength of 590 nm. The serum alkaline phosphatase was calculated using this formula.

Absorbance of test × Concentration of standard/Absorbance of standard.

Assay for serum urea

One microliter (1 µl) of buffer was pipette into 3 test tubes (blank, standard and sample) and 50 µl were added to the 3 test tubes, mix and incubated for 5 minutes at 37°C. Then 200 µl of hydrochloride was added and incubated for further 5 minutes at 37°C. The absorbance of the sample and standard were measured against reagent blank at 600 nm. The value was calculated using this formula.

Absorbance of test × Concentration of standard /Absorbance of standard [24].

Histopathological examination

Histopathological examination was made of killed cockerel. Samples of different organs (lung, heart, spleen, liver, kidney, brain and intestine) were fixed in 10% buffered formalin. Tissues were

processed and embedded in paraffin, sectioned, and stained with hematoxylin and eosin, and histopathological slides were evaluated using light microscope and the photomicrographs were documented accordingly [26,37].

Tissue processing method

Preservation using fixative (10% buffered formalin). Dehydration: removal of water using ascending grades of alcohol (70%, 80%, 90%, and 100%). Clearing: removal of alcohol from the tissue using agents such as xylene, benzene, and toluene. Impregnation: process of placing the tissue in molten paraffin wax to displace the clearing agent and fill in available spaces in the tissue. Embedding: process of burying the tissue inside molten paraffin wax in a mould. It is allowed to solidify in a cooling surface and blocked out. This gives the tissue an external support for proper sectioning. Sectioning: the embedded tissue is "sectioned" using a Microtome machine and placed on a slide.

Tissue staining procedure

The slides were dewaxed with xylene (2-3 changes). The slides were hydrated by passing through descending grades of alcohol from absolute to70% alcohol and then to water. The slides were stained with Hematoxylin for 5 minutes and then rinsed with water. The slides were differentiated in 1% acid alcohol briefly and then rinsed with water. The slides were blued with Scott's tap water substitute for 2-3 minutes. The slides were counterstained with eosin for 1 minute and then rinsed with water. The slides were dehydrated in ascending grades of alcohol (70). The slides were mounted with DPX and cover slip and allowed to dry ready for microscopy examination.

Gross examination

Gross lesions were examine and documented accordingly. The gross appearance of muscle the organs examine and photographs were taken. The abnormal organs were then examined following post- mortem standard procedure. The tissues of the lungs, heart, liver, spleen, Kidney, brain and intestine were taken from both the infected and control groups for comparison [37].

Statistical analysis

All data were subjected to statistical analysis including the calculation of the mean and standard error of the mean and descriptive analysis of gross and histopathology. Significance between data of groups were evaluated by student *t*-test at levels $P > 0.05$ using SPSS (Statistical Package for Social Sciences) version 15 Computer program.

Results

Parameters	Infected	control
Weight (Mean)	1.38 kg	1.52 kg
Temperature (Mean)	34.13 ºC	32 ºC
Mortality rate	50% mortality rate	Nil
Feed consumption	Off feed/ inappetence	normal
Other clinical signs	Weakness, ruffle feathers, reluctance to move, yellowish and bloody diarrhea	Nil

Table 1: Clinical signs of birds within 5-10 days of infection.

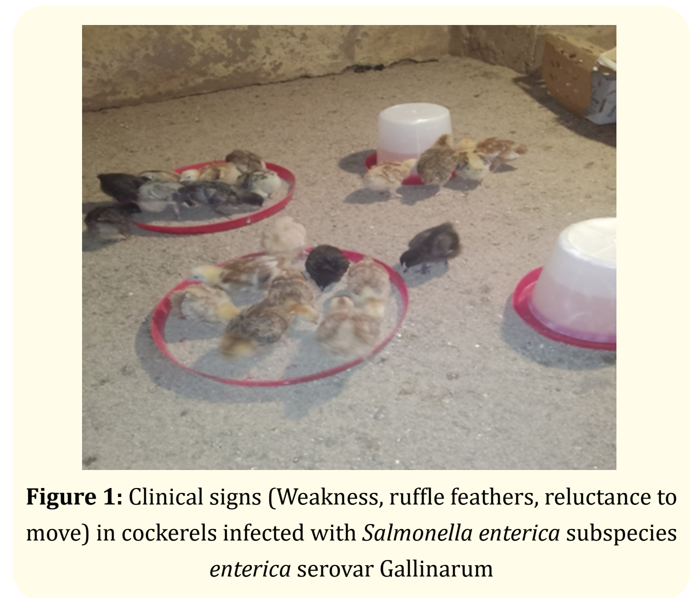

Figure 1: Clinical signs (Weakness, ruffle feathers, reluctance to move) in cockerels infected with *Salmonella enterica* subspecies *enterica* serovar Gallinarum

Discussion

Salmonella enterica subspecies enterica serovar *Gallinarum* infection, always cause economical lose in poultry farm based on the mortality rate and weight lost [38]. The organism causes fowl typhoid in birds and more often observed in growing and adult birds with clinical signs such as anorexia, diarrhoea, dehydration and ultimate death outcome [39]. Ten birds were purposefully infected with Salmonella Gallinarum and ten other birds were use as control aims to evaluate the clinical signs, gross/histopathological lesions, haematological and serum biochemical changes associated with the ten experimental infected birds against the ten non infected used as control. Clinical signs showed response of the infected birds with 50% mortality rate after five days of infection, weakness, ruffled feathers, inappetence, difficulty in breathing, reluctance to move, watery and yellowish diarrhea, was observed

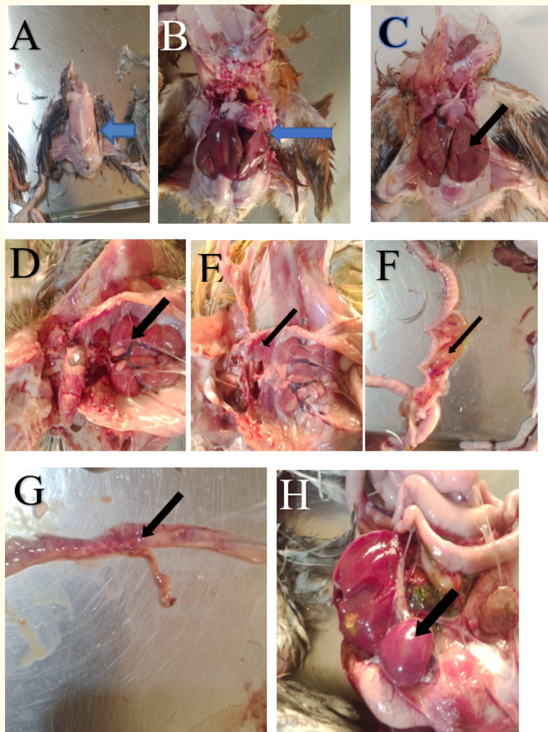

Figure 2: Gross lesions in cockerels infected with Salmonella enterica subspecies enterica serovar Gallinarum: A-Pale carcass; B-Whitish nodules on the heart; C-Bronze coloration of the liver; D- Swollen kidneys; E- Severe congestion of the lungs; F-Bloody intestinal contents; G- Haemorrhagic enteritis; H- Severe splenomegaly.

	Group (mean)			
Parameter	**Control**	**Infected**	**Mean ± SEM**	**P-value**
AST u/l	38.48	41.04	39.75 ± 0.88	0.160
ALT u/l	2.75	4.00	3.38 ± 0.33	0.042
ALP u/l	154.00	153.2	153.69 ± 21.90	0.044
TP g/L	40.78	39.54	39.16 ± 1.93	0.193
Urea mg/dl	15.21	14.13	14.17 ± 1.22	0.438

Table 2: Serum biochemical changes in cockerels infected with *Salmonella enterica* subspecies *enterica* serovar Gallinarum.

$P < 0.05$ within each row is statistically significant.

Key

AST: Aspartate Aminotransferase; ALT: Alaninetransferase; ALP: Alkaline phosphatase; TP: Total Protein

Urea.

	Group (mean)			
Parameter	**Control**	**Infected**	**Mean ± SEM**	**P-value**
PCV	23.50	20.00	22.18 ± 1.36	0.620
HB	7.83	7.35	7.59 ± 0.45	0.638
RBC	2.00	1.75	1.88 ± 0.20	0.576
WBC	5.45	3.50	4.48 ± 0.52	0.049
N (Heterophil)	66.00	77.50	71.75 ± 4.32	0.020
L	30.25	22.00	26.13 ± 3.40	0.025

Table 3: Haematological changes in cockerels infected with *Salmonella enterica* subspecies *enterica* serovar Gallinarum.

$P < 0.05$ within each row is statistically significant.

Key: PCV: Packed Cell Volume; HB: Haemoglobin; RBC: Red blood Cell; WBC- White Blood Cell; N: Neutrophils; L: Leukocyte.

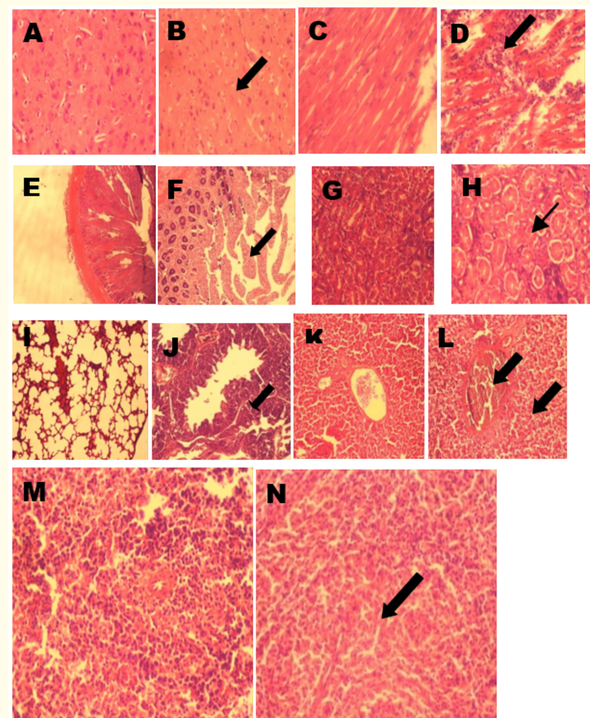

Figure 3: Histopathological lesions in cockerels infected with Salmonella enterica subspecies enterica serovar Gallinarum: A-Normal brain (control group); B-Neuronal necrosis (infected group); C-Normal myocardium (control group); D- Necrosis of the myocardium (infected group); E- Normal intestine (infected group); F- Sloughing of the intestinal villi (infected group); G- Normal renal tubules (control group); H- Severe necrosis of the renal tubules (infected group); I-Normal lung (control group); J-Thickening of alveolar septae and bronchiolar lining (infected group); K-Normal Hepatocytes (control group); L- Necrosis of the hepatocytes and congestion (infected group); M- Normal red and white pulps (control group); N-Depletion of the red and white pulps (infected group).

(Figure 1 and Table 1). This is in conformity with a report by Bradley., *et al*., [40] and Barde., *et al*., [17]. Gross lesions include: pale carcasses, severely congested lungs, and whitish nodular lesions on the heart, haemorrhagic enteritis, and bloody intestinal content, bronze colouration of the liver, swollen kidney and enlarge spleen, which confirmed the severity of the disease caused this organism (Figure 2). These findings are similar to those reported in quails experimentally infected with Salmonella enterica subspecies enterica serovar Gallinarum by Barde., *et al*., [5] and that of Khaton and Siddique, [41]. The mortality recorded in this work is 50% which agreed with previous researchers reports of 30- 100% mortality rate [42] and 30-35% mortality [5]. The serum biochemical assessment in table 2 showed that (ALT) alanine transferase, (ALP) alkaline phosphatase, were statistically significant ($P < 0.05$), among the row in table 2, while aspartate aminotransferase and total protein were statistically insignificant ($P > 0.05$). This is not in agreement with the findings in quails infected with the same organism where there were significant changes in serum glucose, total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and urea concentrations between infected groups and the control group [27]. This variation could be due to species difference. Haematological evaluation in table 3 showed that the WBC, N (heterophils) and L (lymphocytes) are statistically significant among the row in table 3, with the manifestation of leucocytopenia, heterophilia and lymphocytopenia respectively; while PCV, HB and RBC are statistically significant, showing low values compared with the control group. This is in agreement with the findings of Barde., *et al*., [34] who reported significant changes in haematological parameters such as red blood cells (RBC), haemoglobin (Hb) concentrations, 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 in quails. The histological description showed the significant clear view of the different organs or tissues staining of the cells cytoplasm. Figure 3A, 3C, 3E, 3G, 3I, 3K and 3M are the photomicrographs of the control group showing normal histology while Figure 3B, 3D, 3F, 3H, 3J, 3L and 3N are the photomicrographs of the infected group showing neuronal necrosis, necrosis of the myocardium, sloughing of the intestinal villi, severe necrosis of the renal tubules, thickening of alveolar septae and bronchiolar epithelial lining, necrosis of the hepatocytes and congestion and splenic lymphocytic depletion respectively on the organs of the birds. These findings are in conformity with the reports of Davya., *et al*., [43], Barde., *et al*., [5], Soufy and Tantawy, [44], Akhtaruzzaman., *et al*., [45] and Khaton and Siddique, [41].

Conclusion

This study revealed the severity of Salmonella enterica subspecies enterica serovar Gallinarum infection against multiple organ lesions to the birds which cause mortality as observed in the study after 5 days of infection with clinical signs of weakness, ruffled feathers, inappetence, difficulty in breathing, reluctance to move, watery and yellowish diarrhea. The gross lesions observed include: pale carcasses, severely congested lungs, and whitish nodular lesions on the heart, haemorrhagic enteritis, and bloody intestinal content, bronze colouration of the liver, swollen kidney and enlarge spleen. The haematology result indicates anemia, leucocytopenia, heterophilia and lymphocytopenia while the serum biochemical results showed that alanine transferase (ALT) and alkaline phosphatase (ALP) were statistically significant ($P < 0.05$), while Aspartate aminotransferase (ASP) and Total protein (TP) were statistically insignificant ($P > 0.05$) in the infected group. The histopathological lesions revealed neuronal necrosis, necrosis of the myocardium, sloughing of the intestinal villi, severe necrosis of the renal tubules, thickening of alveolar septae and bronchiolar epithelial lining, necrosis of the hepatocytes and congestion and splenic lymphocytic depletion. These findings showed the severity of Salmonella enterica subspecies enterica serovar Gallinarum on the organs of the cockerel birds.

Recommendation

Further studies are required to confirm the effect of Salmonella enterica subspecies enterica serovar Gallinarum at different doses of inoculation. Cockerels should be vaccinated accordingly. Poultry famers should institute and intensify good biosecurity measures in their farms to minimize horizontal transmission.

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