Pathology of experimental *Salmonella gallinarum* infection in turkeys (*Meleagris gallopavo*)

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Abstract

The present study investigated the pathology of a local Nigerian *Salmonella gallinarum* strain in turkeys. Fifty white Nicholas day-old poults were randomly assigned into two groups of 25 each: A/SGI – *Salmonella gallinarum* infected and B/SGU – *Salmonella gallinarum* uninfected. At fourteen-week-old, each bird in group A/SGI turkeys was inoculated with 0.2mL of 1×10^8 cfu of the *Salmonella gallinarum* orally into the crop by oral gavage, while each bird in group B/SGU received 0.2 mL of phosphate buffered saline through the same route as placebo. They were examined at different days post-challenge. A/SGI turkeys showed inappetence, depression, and yellow-green diarrhoea. The body weights were significantly (P <0.05) lower than those of B/SGU turkeys. Mortality in A/SGI was 60%. A/SGI turkeys initially had swollen and congested visceral organs with mahogany and bronze sheen liver and spleen, followed by atrophy of the pancreas and heart with thickened pericardium. The histopathological changes were fibrinoheterophilic exudate in the hepatic parenchyma with necrosis of the hepatocytes and epithelium of the bile duct, followed at the later stage by fibrosis and vacuolar degeneration. Severe lymphoid depletion was observed in the spleen**.** There was marked necrosis followed by pancreatic fibrosis. The heart showed marked congestion, inflammatory oedema with fibrinoheterophilic exudate, myocardial necrosis and myocardial fibrosis. These findings suggest that initial swelling, congestion of visceral organs and distinctive coppery bronze sheen of the liver and spleen, atrophy of the heart and pancreas, and fibrinoheterophilic exudates in the liver, spleen, heart and pancreas, hepatic and pancreatic fibrosis and hepatocellular vacuolar degeneration were lesions of Fowl typhoid in turkeys observed in this study.

Introduction

Fowl typhoid (FT) is an important peracute, acute or chronic septicaemic bacterial disease of primarily growing and adult chickens and turkeys, producing high morbidity and mortality rates with reductions in

productivity, although other birds, such as pheasants, quail, ducks, guinea-fowl and peafowl, are also susceptible (Shivaprasad & Barrow, 2013; Barde *et al*., 2017). The causative agent is *Salmonella enterica* subsp. *enterica* serovar Gallinarum biovar Gallinarum (*S. gallinarum*), a Gram-negative bacteria of the family *Enterobacteriaceae* which is non-motile, aerobic or facultative anaerobic, and highly host-specific for avian species (Shivaprasad & Barrow, 2013; Gast & Porter, 2020; Zhou *et al*., 2022; Chacón *et al*., 2023). The biovar can be spread by both horizontal and vertical transmissions (Chadfield *et al*., 2003; Shivaprasad & Barrow, 2013; Xu *et al*. 2021).

Due to the severe economic consequences of an outbreak of Fowl typhoid (FT) in commercial poultry, FT belongs to the list of notifiable diseases of the World and listed by *Office International des Epizooties* (OIE, 2018) because of the associated significant economic losses in the poultry industry (Shivaprasad, 2000; Shivaprasad & Barrow, 2013; Wigley, 2017; OIE, 2018). The disease has been eradicated from commercial poultry in economically developed countries such as the United States, Canada, Western Europe, and Australia, except in sporadic instances from backyard poultry flocks (Wunderwald & Hoop, 2002; Barrow & Freitas, 2011; Wigley, 2017; Gast & Porter, 2020; Zhou *et al.,* 2022). Although, in recent years there has been renewed interest in *S. gallinarum* as a model to be used for understanding host adaptation and pathogen evolution (Wigley, 2017). However, in Africa, Asia and Latin America, this organism and the diseases it causes remain highly prevalent in both subsistence and commercial flocks (Lee *et al*., 2013; Okwori *et al*., 2013; Wigley, 2017; Revolledo, 2018; Gast & Porter, 2020; Zhou *et al.,* 2022). Despite the availability of vaccines, FT remains enzootic in Nigeria. In places with developing intensive poultry production and where the ambient conditions are such that environmental infection is difficult to control through improvements in housing in Nigeria, there are cyclic or seasonal outbreaks of FT in chickens and turkeys despite established sanitary measures and official programs to prevent and control the disease (Ibrahim *et al*., 2003; Ezema *et al*., 2009; Agbaje *et al*., 2010). This scenario militates against successful operation of the poultry industry due to severe economic losses in both commercial production and backyard flocks. A survey report put the prevalence of FT in sampled flocks at 16.7% in turkeys (Mbuko *et al*., 2009; Agbaje *et al*., 2010). There have been many reports in various species of birds, not primarily involving chickens and turkeys alone, but also pheasants, quail, and guinea fowl in developed countries. However, descriptions of gross

and microscopic lesions associated with FT are sporadic (Shivaprasad, 2000; Shivaprasad & Barrow, 2013). In Nigeria, some natural and experimental infections in some avian species have been reported (Ibrahim *et al*., 2003; Ezema *et al*., 2009; Barde *et al*., 2017; Chiroma *et al*., 2018). However, there is paucity of information on the pathological changes in response to *S. gallinarum* infection in turkeys. Understanding the pathological response to FT infection in turkeys is important, as it will enhance development of effective vaccine strategies and provide a better control programme. In this study, we investigated the pathology of experimental FT in turkeys using a local Nigerian biovar of *S. gallinarum.*

Materials and Methods

Location of study
The study was carried out in Umudike, Abia State, which is located about 10 kilometers southeast of Umuahia, the state capital, within the Lowland Rain Forest Ecological Zone of Nigeria, between latitude 5°32'N, and longitude 7°29'E; the average rainfall of this zone is 3,500 mm per annum, and the average temperature ranges from 22^0C to 32^0C (FRoN-UNFCCC, 2019).

Experimental animals
Fifty white Nicholas turkey poults were purchased at day-old from a reputable local commercial hatchery and randomly assigned into two groups of 25 birds each as follows: *S. gallinarum*-challenged *(*SGI) and *S. gallinarum*-unchallenged (SGU). The poults were kept in isolation in the Poultry Experimental Unit of Department of Veterinary Pathology, MOUAU, under strict biosecurity measures. The poultry pens were previously cleaned, disinfected with formalin and fumigated with potassium permanganate ($KMNO_4$) before the arrival of the birds. Brooding was on deep litter. Feed and water were supplied *ad libitum* and the birds were not vaccinated against any disease. General care of the birds was provided in accordance with the Institutional Animal Care and Use Committee, as outlined in the Guide for the Care and Use of Agricultural Animals in Research and Teaching (Ag Guide, 2020).
Approval for the study was given by the Ethics Committee on Animal Use of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria with approval number MOUAU/CVM/REC/202320.

Bacteriological monitoring pre-infection
All the birds were examined to certify them being *Salmonella*-free by taking cloacal swab samples from

them before infection with *S. gallinarum*. The cloacal swabs were cultured in the Department of Veterinary Microbiology, College of Veterinary Medicine, MOUAU, which confirmed that they were *Salmonella*-free, when no growth of the organisms was observed from the culture after incubation at 37^0C for 48 hours. 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 (Mshelbwala *et al*., 2017; OIE, 2018).

Source of Salmonella gallinarum inoculum
A strain of *Salmonella enterica* serovar *gallinarum*, was obtained from the bacterial culture bank of the Central Diagnostic Laboratory, National Veterinary Research Institute (NVRI), Vom. The *Salmonella* strain was from day-old chicks that died of *S. gallinarum* infection.

Standardisation of the inoculation dose of Salmonella gallinarum
The lyophilized bacterium from the culture bank was re-activated by sub-culturing on blood agar (BA) and MacConkey agar (MCA) on an 18-hour plate culture. The inoculation dose (colony forming units [cfu]) was determined using Sensititre nephelometer (TREK Diagnostic Systems, UK) according to the method described by Waltman & Gast (2008) and Kohlerschmidt *et al.* (2021). The resulting colonies were examined for their features, colour and morphology and tested for Gram-reaction (Gram-negative). Sterile wire loop was used to pick two to three colonies of the *S*. *gallinarum* and emulsified in 50mL of sterile normal saline and incubated at 37°C for 10min to allow for bacterial growth. Two mL of the suspension containing the bacteria was put in a sterile tube and inserted into the Sensititre nephelometer after calibration, and the concentration of the bacteria was taken from the calibration. The procedure was repeated after 10 mins each of incubation at 37°C until a concentration of $1×10^8$ was obtained (Foley *et al*., 2011).

Salmonella gallinarum challenge
At fourteen weeks of age, after they were examined to certify being *Salmonella*-free, birds of group A/SGI were each inoculated with 0.2mL of $1×10^8$ cfu of the *S. gallinarum* orally into the crop by oral gavage, while each bird in group B/SGU received 0.2 mL of phosphate buffered saline (PBS) through the same route as placebo.

Clinical observation
After challenge at 14-week-old, the turkeys were observed twice daily for clinical signs of FT throughout the experimental period. The daily morbidity and mortality were recorded. Seven birds from each group were randomly selected and weighed individually on 0, 3, 7, 14, 21, 28 and 35 days post-challenge (dpc) using separate, as part of biosecurity measures, weighing balances (Camry® Kitchen Scale - 20kg). In the infected groups, those showing depression were picked before others.

Pathological examinations
On 3, 4, 6, 11 and 21 dpc, two to three turkeys in each group were humanely sacrificed and, together with the mortalities, examined for pathological changes. The distribution and consistency of the gross lesions were studied and recorded. Samples of the liver, spleen, heart, pancreas, intestine, kidney, caecal tonsils and bursa of Fabricius (which will later be called bursa for the sake of clarity) were fixed in 10% formal saline for 48 hours. The fixed tissues were trimmed and routinely processed before being embedded in paraffin wax. Sections of 5 µm thick were stained with haematoxylin and eosin (H&E) as described by Suvarna *et al*. (2018). The slides were studied under the light microscope.

Statistical analysis
Data generated for the study were subjected to Student t-test using SPSS Version 15 (SPSS, 2006). Variant means were separated post hoc using the least significant difference method (Okafor, 1992). Probabilities less or equal to 0.05 were accepted as significant.

Results
At 2–3 dpc, 80% of *S. gallinarum*-infected group (A/SGI) showed a drop in feed and water consumption. At 4 dpc, morbidity was 100%, and all turkeys in the infected group showed yellow–green diarrhoea which soiled the vent, ruffled feathers, depression, anorexia, dehydration and weakness. Clinical signs progressed to birds being droopy with watery to mucoid yellow–green diarrhoea at 5–11 dpc (Plate I and II). Weight loss was significant (P < 0.05) in the A/SGI turkeys from 3 to 35 dpc, when compared with the B/SGU group (Table 1). Weight loss was lowest at 3 to 14 dpc, but started increasing gradually at 21 dpc, although it did not normalize by 35 dpc when the last surviving turkeys were weighed. Mortality was first observed at 3 dpc and involved 12% of group A/SGI (3/25) turkeys and peaked at 4

dpc involving 41% (9/22) turkeys, followed by 15.4% (2/13) at 6 dpc. The last mortality was on 11 dpc and involved one bird, 9% (1/11). Total mortality was 60%, There were no clinical signs seen in the control group of turkeys (B/SGU).excluding the turkeys that were humanely sacrificed for pathological examination.

Birds that survived recovered spontaneously by 21 dpc. In the A/SGI turkeys, gross lesions included enlarged, mahogany, congested and friable liver with fibrinous exudate in the capsule at 3 dpc (Plate IB) and a distinctive coppery bronze sheen which only developed after exposure to air at 4 to 11 dpc (Plate IC). Lungs were markedly congested at 3 dpc (Plate IB; white arrow). Spleens were enlarged, congested with a distinctive coppery bronze sheen after exposure to air at 3 to 6 dpc (Plate ID), and later atrophic. Pancreas showed enlargement and congestion at 3 to 6 dpc
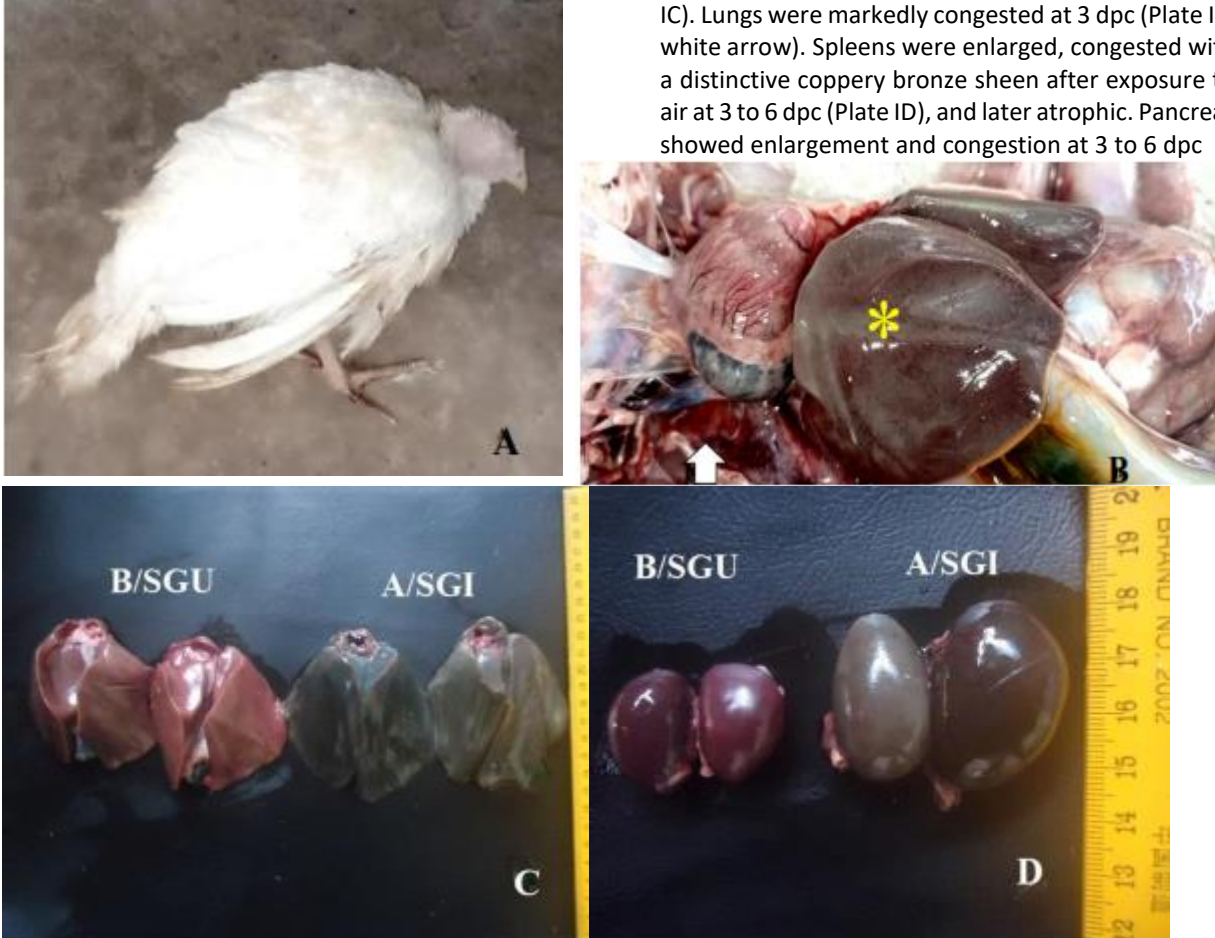

Plate I (A) Somnolescence, droopy appearance with ruffled feathers in A/SGI turkey at 7 dpc. (B) Enlarged mahogany or marked congestion of the liver with fibrinous exudate on the capsule of the liver (*) and congested lung (arrow) at 3 dpc (C) Liver with a distinctive coppery bronze sheen in A/SGI, compared with the normal, B/SGU, turkeys at 4 dpc. **(D)** Severely swollen, congested and coppery bronze sheen spleen in A/SGI turkey at 4 dpc

Table 1: The body weights of turkeys infected with *Salmonella gallinarum*, compared with uninfected controls, (Mean ± SD)

Experimental period (days post-challenge)	Mean body weight (kg) ± standard deviation		
	Group A (Infected group)	Group B (Uninfected Control)	P-value (Statistics)
0	6.11 ± 0.43	6.10 ± 0.42	0.958
3	5.36 ± 0.52 [b]	6.58 ± 0.46 [a]	0.000
7	5.07 ± 0.90 [b]	7.46 ± 0.54 [a]	0.000
14	5.40 ± 1.35 [b]	8.06 ± 0.62 [a]	0.001
21	6.44 ± 1.02 [b]	8.52 ± 0.68 [a]	0.007
28	6.80 ± 0.91 [b]	8.74 ± 0.80 [a]	0.007
35	7.94 ± 1.11 [b]	9.82 ± 1.09 [a]	0.027

[a, b] Different superscripts in a row indicate significant differences between the groups, $P < 0.05$

(Plate IIE), and later atrophic. Hearts showed severely swollen, haemorrhagic and hyperaemic lesions with dilated blood vessels by 3 dpc (Plate IIF), while the pericardium exhibited a translucency, and the pericardial fluid increased in volume, containing sero-fibrinous exudate and turbid at 4 to 6 dpc (Plate IIG). At 21 dpc, the heart became atrophic with thickening of the epicardium and pericardium, and obliteration of the pericardial cavity by adhesions making the pericardial sac opaque (Plate IIH). At 3 to 11 dpc, the kidneys were swollen, congested and haemorrhagic, while the intestine showed catarrhal enteritis with viscous, bile-stained, slimy intestinal contents and multifocal necrosis on the mucosal surface. The caecal tonsils were swollen and mildly haemorrhagic by 6 dpc.

There were no gross lesions in group B/SGU turkeys throughout the experimental period. The distribution and consistency of the lesions are shown in Table 2.

No histopathological changes were found in the B/SGU group.

In group A/SGI turkeys, the liver showed congestion, marked inflammatory oedema with multifocal fibrin deposition, diffuse vascular luminal exudation with or without infiltrates of polymorphonuclear leukocytes into the parenchyma accompanied by epithelial degeneration (Plates IIIA, B, C, D) and sloughing of the bile duct epithelia at 3 dpc compared with that of B/SGU, control (Plates IIIE, F). At 4 to 6 dpc, there was coagulative necrosis of the hepatocytes, vascular luminal exudation of severe infiltrates of polymorphonuclear leukocytes into the parenchyma (Plates IVA, B). At 11 dpc, there was fibroplasia in areas where many of the hepatocytes had been lost, accompanied by infiltration of many mononuclear inflammatory cells (Plate IVC), peri-portal hepatocellular degeneration with fibroplasia, mild interstitial fibrosis and mild mononuclear leukocytes
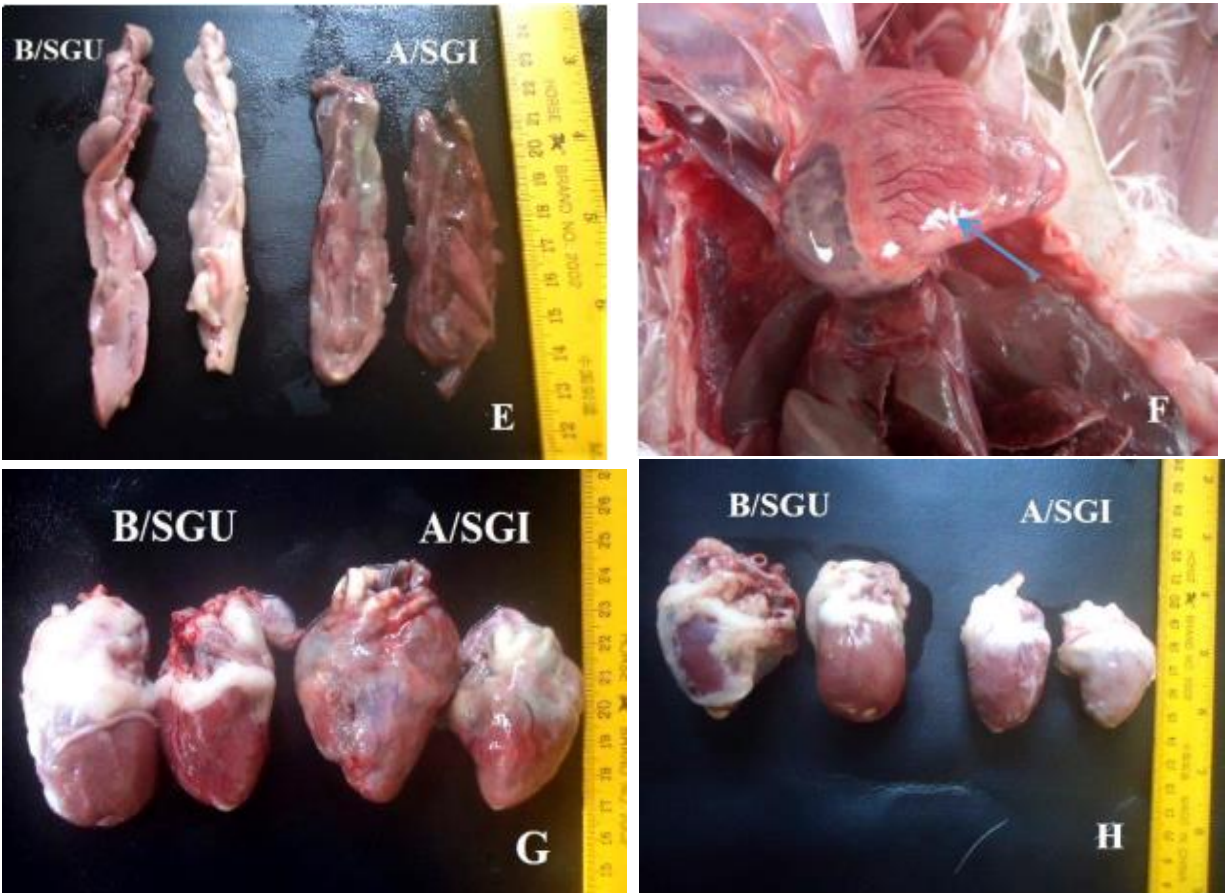

Plate II: (E) Severely swollen, congested and haemorrhagic pancreas in A/SGI turkey at 4 dpc compared with the normal pancreas in B/SGU turkeys. (F) Marked pericarditis with markedly congested coronary vessels (arrow) in A/SGI turkey at 3 dpc. (G) Mild hydropericardium or sero-fibrinous pericarditis, and serositis in A/SGI, compared with those of the B/SGU turkeys (left) at 4 dpc. (H) Atrophied and opaque heart with thickened pericardium of A/SGI turkeys, compared with those of the B/SGU turkeys at 21 dpc

Table 2: Distribution and persistence of gross lesions in turkeys challenged with *Salmonella gallinarum*, group A/SGI

Organ	Lesions	Days post-challenge					
		3	4	6	11	14	21
Liver	Congestion and Enlargement	3*/3†	9/9	2/2	2/2	0/2	0/2
	Parboiled/Friable with a distinctive bronze sheen	3/3	9/9	2/2	1/2	1/2	0/2
	Multiple necrotic foci	0/3	0/9	2/2	1/2	0/2	0/2
	Atrophy	0/3	0/9	1/2	1/2	0/2	1/2
Spleen	Congestion and enlargement	3/3	9/9	1/2	1/2	0/2	0/2
	Bronze sheen	3/3	9/9	1/2	1/2	0/2	0/2
	Mottling or white nodular lesion	0/3	0/9	1/2	1/2	0/2	0/2
	Atrophy	0/3	0/9	1/2	1/2	0/2	0/2
Heart	Hydropericardium	3/3	9/9	2/2	1/2	1/2	0/2
	Atrophy and opaque	0/3	0/9	0/2	0/2	1/2	2/2
Pancreas	Congestion and enlargement	3/3	9/9	2/2	1/2	0/2	0/2
	Multiple necrotic foci	0/3	0/9	1/2	1/2	0/2	0/2
Kidney	Congestion and enlargement	3/3	9/9	2/2	2/2	0/2	0/2
Bursa	Enlargement	1/3	5/9	1/2	0/2	0/2	0/2
	Atrophy	0/3	0/9	0/2	2/2	2/2	2/2
Thymus	Marked Atrophy	3/3	9/9	2/2	2/2	2/2	2/2
Intestine	Catarrhal enteritis	3/3	9/9	2/2	2/2	1/2	0/2
Caecal tonsils	Enlargement and mild haemorrhages	1/3	2/9	0/2	0/2	0/2	0/2
Trachea and Lungs	Congestion and enlargement	2/3	0/9	0/2	0/2	0/2	0/2

* Number of turkeys showing lesions.

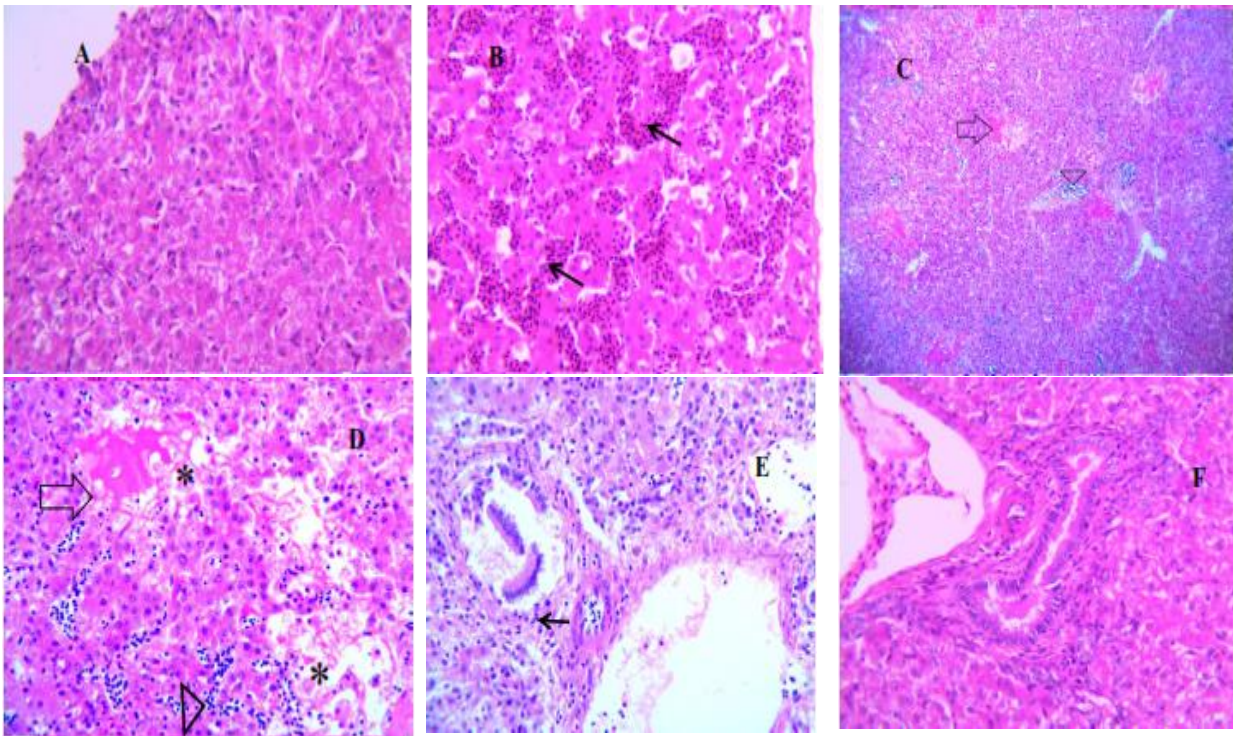**†** Total number of turkeys subjected to necropsy examination

Plate III: (A) Normal liver of group B/SGU turkeys. (B) Severe congestion of the hepatic sinusoids (arrows) in a A/SGI turkey, H&E x 200. (C) Multifocal necrotic foci and multifocal fibrin deposition (arrow) with diffuse infiltrates of polymorphonuclear leukocytes (arrow head), into the parenchyma in A/SGI turkeys at 3 dpc. H&E, x 50. (D) Higher magnification of hepatocellular necrosis with inflammatory oedema (*), fibrinous exudation (arrow) and diffuse infiltrates of polymorphonuclear leukocytes (arrow head), from (C). H &E, x 200. (E) Sloughing of the bile duct epithelia (arrow), compared with those of a B/SGU turkey (F) at 3 dpc. H&E, x 200

infiltration, and evidence of commencement of regeneration (hyperplasia of regeneration) of the bile duct was seen, with restoration of ductular epithelial cells, which were hyperplastic (Plates IVC, D). However, there were multifocal areas of vacuolar degeneration of the hepatocytes (Plate IVE) and mild interstitial fibrosis around the central vein at 21 dpc.

Lesions in the spleen were severe inflammatory oedema with deposition of fibrin, and depletion of lymphocytes at 3 dpc, compared with the control (Plates VA, B), progressing to diffuse and severe infiltration of inflammatory polymorphonuclear leukocytes at the lymphocytic depleted areas, extending to the serous membrane at 4 to 6 dpc (Plates VC). At 11 dpc there was multifocal infiltration of mononuclear phagocyte system cells with many mononuclear inflammatory cells. There was clear evidence of commencement of regeneration of the lymphoid tissues at 21 dpc.

The heart showed diffuse congestion of the blood vessels, inflammatory oedema in the epicardium, pericardium, myocardium and infiltrate of polymorphonuclear leukocytes into the interstices of the myofibres at 3 to 6 dpc. There was multifocal infiltration of mononuclear cells with many mononuclear inflammatory cells, compared with the control (Plates VA, B). Necrosis, hyaline degeneration of myofibers and multifocal severe infiltration of the atrophic inter-myofibre spaces by inflammatory

mononuclear cells were observed at 11 dpc. At 21 dpc, there was severe thickening of the pericardium and fibrosis in areas where many of the muscle fibres had been lost (Plate VC).

In the pancreas, there were marked congestion, moderate accumulations of polymorphonuclear inflammatory cells, severe necrosis, and depletion of acini and islets of Langerhan, fibroplasia extensive interstitial and peri-ductal fibrosis, and aggregates of lymphocytes that infiltrate through the acinar tissue, with complete collapse of the intralobular duct, resulting in obliteration of normal architecture at 3 to 4 dpc compared with the control (Plates VIA, B). In addition, haemorrhages and infiltration of the fibrotic area with mononuclear leukocytes were the most common change at 11 dpc (Plate VIC). By 21 dpc, there was hyperplasia of the regenerating intralobular duct epithelial cells (Plate VID). There was clear evidence of commencement of regeneration of the ductular epithelial cells.

Discussion

The Nigerian *S. gallinarum* strain obtained from the bacterial culture bank of the Central Diagnostic Laboratory, NVRI, Vom was used to study the pathology of experimental *S. gallinarum* infection in white Nicholas turkeys. Following oral inoculation, this strain caused rapid morbidity in turkeys with clinical signs of depression, yellow–green diarrhoea
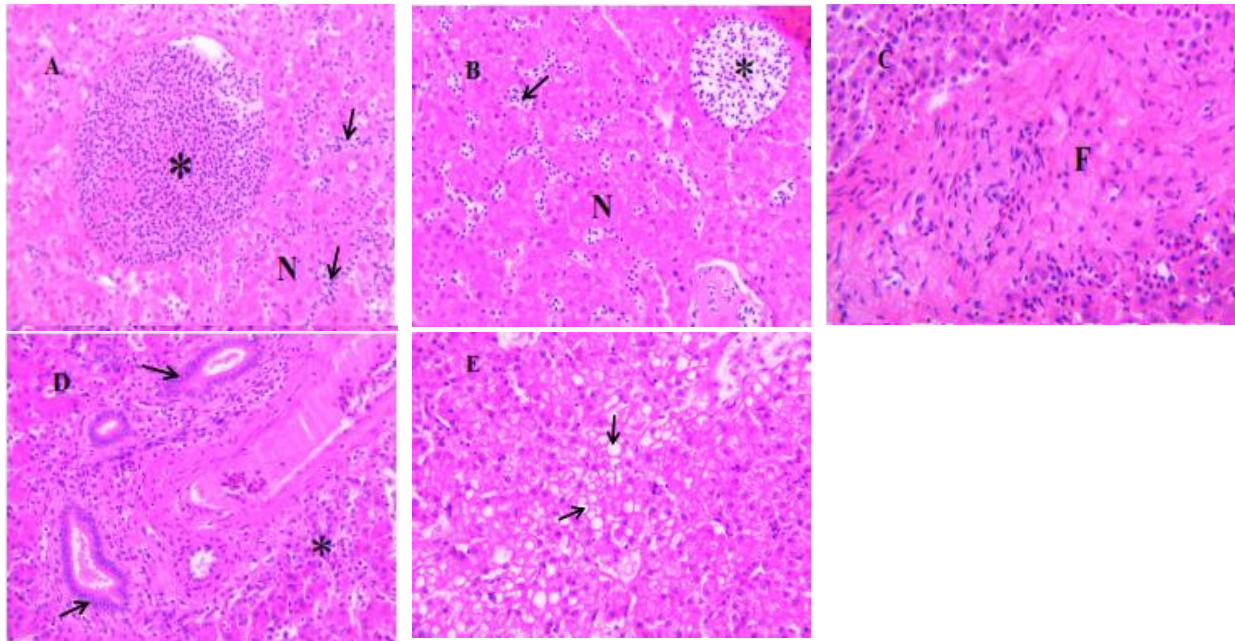

Plate IV: Liver of A/SGI turkeys showing **(A, B)** Necrosis of the hepatocytes (N), severe congestion of the central vein (*) and sinusoids (arrows) at 4 to 6 dpc. **(C)** Focally extensive necrosis and fibrosis (F) at 11 dpc. **(D)** Hyperplasia due to regeneration of bile ducts (arrows), and mononuclear inflammatory cells in the peri-portal area (*) at 11 dpc. **(E)** Multifocal vacuolar or fatty degeneration (arrows) at 21 dpc. H&E, x 200
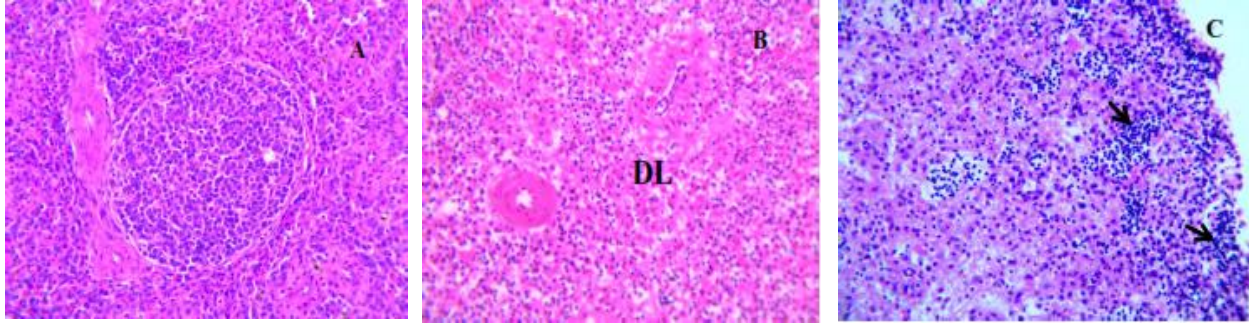
Plate V. (A) Normal spleen of group B/SGU turkeys, H&E x 200. **(B)** Spleen of group A/SGI turkeys showing severe necrosis (severe depletion of lymphocytes) (DL) on 4 dpc. **(C)** Spleen of A/SGI turkey showing diffuse infiltration of polymorphonuclear leukocytes in the parenchyma (arrows) at 4 dpc. H&E x 200

and weight loss, and mortality. Generally, the pathological changes were characterized by initial swelling, congestion, haemorrhages and distinctive coppery bronze sheen of the liver, and also by degeneration and infiltration of polymorphonuclear inflammatory cells in the parenchyma. Then, necrosis, atrophy, fibrosis and vacoular degeneration in the liver. Similar changes, except vacoular degeneration, were observed in the spleen, heart and pancreas. The lymphoid organs showed depletion of lymphocytes. These pathological changes observed in this study were due to acute fibrinoheterophilic inflammation followed by cellular necrosis and depletion, of the tissues. The changes observed may be due to the virulence factors of *S. gallinarum*. This is supported by the observation over time that the endotoxins from *S. gallinarum*, which is a Gram-negative bacterium, and the large virulence plasmids present in *S. gallinarum* are crucial in causing severe clinical signs and clinical disease in susceptible birds (Smith *et al.*, 1978; Barrow *et al.*, 1987; Rychlik *et al.*, 1998; Chacón *et al.*, 2023). Lipopolysaccharide (LPS), a bacterial endotoxin, constituent of the outer membrane of Gram-negative bacteria or a portion of *Salmonella* cell wall, can initiate systemic inflammatory response by activating a variety of polymorphonuclear leukocytes and monocytic cells, stimulating the release of proinflammatory cytokines (Garcia *et al.*, 2013; Gast & Porter, 2020).

The onset of clinical signs in *S. gallinarum* infected birds could vary depending on the species, age, breed, genetic endowment of the birds, dose and route of infection, genotype, host adaptability and pathogenicity of the strain of the organism (Berchieri *et al.*, 2000; Shivaprasad & Barrow, 2013; OIE, 2018). It has been reported that infection caused by *S. gallinarum* strain results in high morbidity and mortality that can range from 0% to 100% in susceptible birds (Shivaprasad, 2000). In turkeys, mortality may be as high as in chickens (Shivaprasad & Barrow, 2013). In the present study, clinical signs of *S. gallinarum* strain in 14-week-old turkeys started at 2 dpc, whereas a variable onset of clinical signs between 3–9 dpc was reported in day-old, 3-week-old, and 2-month-old turkeys by Beyaz *et al.*(2010). However, the 100% morbidity and 60% mortality recorded in the present study in the growing turkeys were not reported by the later authors in 3-week-old, and 2-month-old turkeys but only morbidity which was most evident in day-old poults, which suggests that the basis of virulence could be multifactorial. The differences observed reflected the possible differences in the virulence factors of *S. gallinarum* strain used in this study, which are essential for enteric and systemic colonization in experimental infection of the birds by *S. gallinarum*, and their involvement in *Salmonella* pathogenesis (Blondel *et al.*, 2010; 2013). In addition, since the period of greatest susceptibility to severe clinical disease has been reported to be primarily at the growing or adult stage of chickens and turkeys (Shivaprasad & Barrow, 2013; OIE, 2018), our study results suggest a potential bacteria virulence-breed and genetic-related susceptibility factor in the turkeys, which were not reported by Beyaz *et al*. (2010) in 2-month-old turkeys. The weight loss recorded in this study may be due to inappetence and dehydration, loss of villi and or villous atrophy of the small intestine and pancreatic atrophy or fibrosis resulting in poor absorption as recorded in this study. The observed diarrhoea may have been caused by the systemic infection adversely affecting normal intestinal function or may have resulted from microbial activity directly affecting the intestine. Respiratory distress was not observed in the infected group suggesting there was no lung contamination after oral inoculation of microorganisms into the crop.

The turkeys had marked gross lesions similar to those already reported by earlier scholars in chickens (Shivaprasad, 2000; Garcia *et al*., 2013), quails (Barde *et al*., 2015) and turkeys (Beyaz *et al*., 2010). However, the characteristic gross lesions of experimental *S. gallinarum* infection in this study which were septicaemic in nature were observed very early at 3-4 dpc in the infected turkeys. At necropsy, vascular changes like severe congestions, haemorrhages and swellings were evident at a very early stage in the liver, lungs, spleen, pancreas and heart, and on exposure to air the liver and spleen took on a "bronzed" appearance. Moreover, the liver showed peri-hepatitis at 3 dpc and moderate necrotic spots at 6-11 dpc. Pericarditis and pancreatitis were observed at 3-4 dpc which progressed to atrophy with adhesions making the pericardial sac opaque and pancreas atrophic by the end of the experiment. The organism was re-isolated from the livers of the dead birds. The production of septicaemic and systemic disease by the *S. gallinarum* strain used in the present study could be attributed to the important contribution that the large plasmid makes to the virulence of *S. gallinarum* and to the production of FT (Barrow *et al*., 1987; Barrow & Freitas, 2011; Shivaprasad & Barrow, 2013). The large plasmid including absence of flagella has a role in the pathogenesis of FT and/or bacterial invasion. Following oral infection of birds, *S. gallinarum* is able to invade the alimentary tract without provoking a strong inflammatory response, and favouring systemic infection, a specific adaptation to avian hosts (Barrow *et al*., 1987; Barrow & Freitas, 2011; Shivaprasad & Barrow, 2013). The absence of flagella in *S. gallinarum* is considered to be one of the factors related to the difference between the pathogenesis of FT and fowl paratyphoid provoked by *S. enterica* serovar Enteritidis (Barrow & Freitas, 2011). It has been postulated that *S. gallinarum* does not fully stimulate the innate immune system at the intestinal mucosa and that this facilitates the ability of the bacterium to cross the intestinal barrier, producing severe systemic disease (Lopes *et al*., 2016). Intracellular bacterial multiplication takes place, largely through the activities of SPI-2 genes (Shivaprasad & Barrow, 2013), with replication of the bacteria in spleen and liver, organs rich in reticuloendothelial tissue, because the large plasmid enables the strains to survive, grow and multiply in the cells of the reticuloendothelial system (Barrow *et al*., 1987), leading to hepato-splenomegaly with white spot lesions on the organ surface, diffuse bacteraemia, pericarditis and re-invasion of the gut leading to diarrhoea (Wigley, 2017).

Histopathologically, the *S. gallinarum* strain used in the present study caused histologic lesions characteristic of the peracute, acute and chronic stages of FT (Shivaprasad & Barrow, 2013) at the early and by the end of the experimental study especially in the liver, spleen, pancreas, kidney and heart, indicating primary predilection and bacteria replication of *S. gallinarum* to these sites. In the liver, the peracute stage was shown by severe vascular injury leading to congestion and haemorrhages in the liver, degeneration of hepatocytes and ductular epithelia, inflammatory oedema with diffuse infiltrates of polymorphonuclear leukocytes and fibrinous exudation at 3 dpc. These were followed by the acute stage characterized by marked extensive multifocal necrosis of the hepatocytes, and severe luminal exudation of inflammatory cells and fibrin in all the vascular lumen with infiltration to the parenchyma including peri-portal areas in the liver at 4-6 dpc. The chronic stage showed fibroplasia and or fibrosis in areas of hepatic necrosis at 11 dpc, and hepatocellular steatosis (vacuolar, lipidosis and fatty change) by 21 dpc. These microscopic lesions are similar to those already reported in liver in experimental FT in chickens and quails (Garcia *et al*., 2013; Barde *et al*., 2015) and in turkeys (Beyaz *et al*., 2010). However, marked fibrosis at the later stage of the study were not reported by them. Fibrosis is one the responses of liver to chronic injury (Cullen & Stalker, 2016). A possible explanation for this could be the hepatic stellate cells (Ito cells), the cell types found in the peri-sinusoidal space, store vitamin A; however, in pathologic conditions, they differentiate into myofibroblasts and synthesize collagen. These cells appear to play a significant role in hepatic fibrogenesis; they synthesize and deposit type I and type III collagen within the peri-sinusoidal space, resulting in hepatic fibrosis (Cullen & Stalker, 2016; Pawlina, 2016). The liver is involved in many other important metabolic pathways, and plays important roles in the uptake, storage, and distribution of both nutrients and vitamins from the bloodstream for animal growth or energy storage (Porter, 2015; Cullen & Stalker, 2016). It also maintains the blood glucose level and regulates circulating levels of very low-density lipoproteins (VLDLs). VLDLs are denser and smaller than chylomicrons; they are synthesized predominately in the liver. VLDLs are rich in triglycerides. Their function is to transport most of the triglycerides from the liver to other organs (Pawlina, 2016). Liver VLDLs are associated with

circulating apolipoprotein B-100, also synthesized in the liver, which aids in secretion of VLDLs. Hepatocellular steatosis can be physiologic or pathologic(Cullen & Stalker, 2016).In acute and chronic disorders, the liver is unable to produce apolipoprotein B-100, leading to blockage in the secretion of VLDLs, resulting in large lipid droplets occupying most of the hepatocyte cytoplasm, leading to hepatocellular vacuolar degeneration as observed in the liver of infected group A/SGI by 21 dpc.

Microscopic lesions characteristic of the peracute and acute stages were also observed in the spleen and gut associated lymphoid tissues of the intestine. The microscopic lesions in the spleen started at 3 dpc with severe extensive necrosis and depletion of lymphocytes with fibrin exudation of vascular lumen and accumulation of fibrin and infiltration of heterophils in the splenic parenchyma with fibrinoheterophilic serositis. The microscopic lesions in the spleen are highly indicative of septicaemic infection. *S*. *gallinarum* infect birds via the oral route where they invade the intestinal epithelial cells or lymphoid tissue localized mainly in the Peyer's patch and caecal tonsils. Infected phagocytes and free bacteria move to lymphoid tissues (spleen, bursa of Fabricius, thymus, conjunctival associated lymphoid tissues, Harderian gland, tracheal and bronchial associated lymphoid tissues), where bacterial multiplication takes place. They re-enter lymphoid tissue in the intestine by a completely unknown mechanism and are shed in the faeces. The damage to the barrier of the intestinal mucosa predisposes to systemic infection and consequently, to typical tissue lesions, especially in the liver, spleen, and heart (Barrow & Freitas, 2011).

The heart showed microscopic lesions of peracute, acute and chronic FT characterised by marked congestion, interstitial inflammatory oedema, fibrinoheterophilic infitrates and myofibrillar necrosis by 3–6 dpc. These are correlated with bacterial multiplication, and were followed by the chronic phase as the disease progresses, characterized by interstitial and myofiber fibrosis.

Histopathologic changes characteristic of the chronic stage were also observed in the pancreas. The pancreas showed extensive interstitial and peri-ductal fibrosis, and aggregates of mononuclear infiltrates. This is consistent with the reports of earlier authors in the heart and pancreas (Shivaprasad & Barrow, 2013), however, pancreatic fibrosis in turkeys was not reported by Beyaz *et al*. (2010). A possible explanation for this could be that the experimental-challenge with *S*. *gallinarum* injured the stellate cells.

Stellate cells akin to those of the liver have been identified in the normal pancreas in a peri-acinar location and appear to be the major mediators of pancreatic fibrogenesis. In the quiescent state, pancreatic stellate cells express desmin but not α-smooth-muscle actin. Following activation by injury, these cells acquire a myofibroblastic phenotype and synthesize fibrillar collagens, including collagen type I (Jubb & Stent, 2016). There is an important inter-dependence between the endocrine and exocrine elements of the organ, and the hormones produced by islets are important for the regulation of exocrine tissue. The complex structural and functional inter-relationship between the endocrine and exocrine pancreas is mediated most importantly by insulin and somatostatin (Jubb & Stent, 2016). In the present study, the histopathology showed that both the exocrine and the endocrine portions were also markedly necrotic with depletion of the cells and fibrotic. The implication is enhancement of multiple organ failure such as hepatic steatosis, and loss of body weight as a result of protein malabsorption as observed in the present study. Reduced insulin-dependent glucose uptake by cells leads to accelerated lipolysis from adipose tissue in much the same way as when energy intake is limiting. The liver is thus presented with a large load of fatty acids. Insulin deficiency alone will produce fatty liver complicated by concurrent exocrine pancreatic insufficiency and interfering with the digestive process such as protein malabsorption which can evolve into a dysmetabolic condition that resembles diabetic hepatic steatosis.

In conclusion, the local Nigerian *S. gallinarum* strain infection caused bronze-sheen and serofibrinous perihepatitis and splenomegaly, atrophied and opaque heart with thickened pericardium and pancreatitis, inflammatory oedema and severe infiltrate of polymorphonuclear leukocytes, hepatocellular and myocardial vacoular degeneration, hepatic and myocardial fibrosis in this study. The information derived from this study will be helpful in the accurate diagnosis of FT in turkeys.

Conflict of Interest
The authors declare that there is no conflict of interest.

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