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RESEARCH ARTICLE

Pathobiological and Immunohistochemical Findings in Broiler Chickens Naturally Infected with Salmonella Enterica Serotype *Gallinarum* Biotype *Gallinarum*

Gulbeena Saleem[1]*, Umar Farooq[1], Muhammad Tariq javed[2], Rahat Naseer[3], Hassaan Bin Aslam[4], Ghulam Mustafa[1], M Ovais Omar[5] and Iram Liaqat[6]

[1]Department of Pathology, University of Veterinary and Animal Sciences, Lahore, Pakistan; [2]Department of Pathology, University of Agriculture Faisalabad, Pakistan; [3]Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Science, Lahore, Pakistan; [4]Institute of Microbiology, University of Veterinary and Animal Science, Lahore, Pakistan; [5]Department of Pharmacology and University of Veterinary and Animal Sciences, Lahore, Pakistan
[6]Department of Zoology, GC University Lahore, Pakistan
*Corresponding author: gulbeena.saleem@uvas.edu.pk

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A B S T R A C T

The present study evaluated the effect of *Salmonella enterica* serotype *Gallinarum* biotype *Gallinarum* (*Salmonella gallinarum*) on broiler chickens. A total of one hundred and fifty broiler chickens (25 of each flock) were selected from six outbreaks of Salmonellosis from commercial poultry farms showing typical signs of the disease. The biological, serological and microbiological investigations on various samples from the liver, spleen, lungs, intestine, kidney, caeca and heart were carried out. Investigations showed that overall, 12% of the samples were found positive for *Salmonella gallinarum.* The liver had the highest count ($P<0.001$), followed by spleen, lungs, intestine and caeca and least in heart and kidney. Histopathological findings showed sinusoidal congestion, hepatic cord necrosis, intestinal degeneration, nephritis and tubular necrosis in kidneys. All the organs showed localization of bacteria except in the heart. It may be concluded that the liver was affected significantly by *Salmonella gallinarum* as it harbored the highest number of *Salmonella gallinarum*. It is further concluded that immunohistochemistry may be a precise, sensible, and prudent tool to locate *Salmonella gallinarum* in different body organs.

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INTRODUCTION

Poultry is an accessible and economical source of protein for millions of peoples to overcome the malnutrition (Marangoni *et al.,* 2015; Sulieman *et al.,* 2020) and contributing a major share in economy of the nations (Folajinmi and Peter, 2020; Nasir *et al.,* 2020). In Pakistan this exciting and important agriculture segment contributes a major share in national gross domestic product (1.3%) and delivered major part of protein to Pakistani nation (Hussain *et al*., 2015). Salmonellosis is contributing a major share to loss in the poultry industry by causing systemic and host specific diseases primarily in chickens and other galliforms such as fowl. *Salmonella gallinarum* is a fastidious pathogen which can cause disease in mature and growing fowls resulting in high mortality and morbidity (Berchieri *et al*., 2001). Unpredictable morbidity and elevated death rate are associated with *Salmonella gallinarum* infection in adult birds, which is septicemic in nature. Anorexia, diarrhea, dehydration, weakness, along with gross and microscopic lesions in organs such as the spleen, liver, ovary and heart, are the main manifestations of fowl typhoid (Nazir *et al.,* 2012).

Salmonella gallinarum results in huge loses to the farmer and food producers as well. In spite of continuous strategies to control Salmonella infection in Pakistan, it has been difficult to eliminate Salmonella infection because of poor biosecurity practices, antibiotic and variability in disease producing ability of the pathogen (Zishiri *et al*., 2016). Effective treatment of salmonella subtypes requires early and exact diagnosis and detection. Mainly diagnostic techniques are based on post-mortem examination. This failure of diagnosis leads to unnecessary use of antibiotics (Swar and Shnawa, 2021).

Antibiotics resistance may encounter as previous exposure make *Salmonella* species resistant. So a more effective diagnosis of *Salmonella* has become an urgent priority for the poultry industry (Arora *et al*., 2015). In order to better understanding and deeper insight of pathological processes associated with *Salmonella gallinarum* infection correct diagnosis and detection of causative agent is necessary for successful eradication of *Salmonella gallinarum* (Aslam *et al*., 2012; Rajagopal and Mini, 2013). Therefore, present study was aimed to determine *Salmonella gallinarum* in naturally affected broiler chickens through pathomicrobial and immunohisto-chemical techniques.

MATERIALS AND METHODS

Sample collection: One hundred and fifty broiler chickens were selected from six outbreaks of Salmonellosis reported in different commercial poultry farms around Lahore. Each flock was composed of about thirty thousand birds. Birds were selected showing typical signs of disease: high fever, watery bright yellow diarrhea to mucoid green-yellow droppings, rapid respiration, pale and shrunken combs were selected. Following post-mortem examination, samples were collected from different organs (liver, spleen, lungs, intestine, kidney, caeca and heart). Samples were particularly taken from the organs showing gross lesions such as enlarged, congested and bronze colored liver with necrotic foci, swollen spleen, enlarged kidneys, intestine with catarrhal inflammation along with ulceration, pinhead to pea-size grey nodules on the heart and edematous lungs. Twenty-five birds were selected from each flock and tissue samples from liver, spleen, intestine, lungs, caeca heart and kidney were collected in duplicates: one in a polythene bag, the second in 10% neutral buffered formalin.

Isolation and identification of bacteria: For isolation and identification of *Salmonella gallinarum* pre-enrichment method was used according to the standard methods as described in the literature with some modifications (Ozbey and Ertas, 2006). Twenty-four grams of affected organs (liver, spleen, intestine, caeca, lungs, heart and kidneys) were cut into small pieces with sterile scalpel blade and suspended in 250 ml sterile buffered peptone water Following incubation for 18 hours at 37°C, 1 ml of buffered peptone water culture was added into Selenite broth and incubated at 42°C for 24 hours. The Selenite broth culture was then streaked onto plates of Salmonella Shigella agar and finally incubated at 37°C for 24 hours. The plates were observed for the characteristic colorless, whitish and slightly grey colored colonies of *Salmonella gallinarum* on Salmonella Shigella agar. Confirmation of suspected colonies on Salmonella Shigella agar was carried out by Gram-staining, biochemically and serologically. Serotyping of all isolates of *Salmonella* performed rendering to the WKL (White-Kauffmann-LeMinor scheme) as described by Grimont and Weill (2007), based on quick agglutination test with salmonella antiserum (BIO-RAD, France).

Viable counts of *Salmonella gallinarum* in different organs: Viable counts of bacteria in organs (liver, spleen, lungs, intestine, kidney, caeca and heart) were carried out. These organs were put in sterile stomacher bags and weighed. One gram from each organ was homogenized with 9 ml of phosphate-buffered saline (pH 7) using a stomacher laboratory blender for 3 minutes. One ml from homogenate was serially ten-fold diluted in phosphate-buffered saline. One ml from 5th to 7th dilution of each organ was spread on Salmonella Shigella agar (Oxoid, UK) plates and incubated at 37°C for 24 hours (OIE, 2012) and confirmation of *Salmonella gallinarum* was done biochemically (Christensen *et al*., 1992) and serologically (Majchrzak *et al*., 2014).

Histopathological examination of organs: Following post-mortem examination, 1-1.5cm of tissue samples from different organs (liver, spleen, lungs, intestine, caeca, heart and kidney) were preserved in 10% neutral buffered formalin. These were used for histopathological examination, applying the standard technique for fixation, dehydration, clearing and infiltration. (Bancroft and Gammable, 2008).

Immunohistochemistry: The primary antibodies were raised, rendering the technique defined in previous literature with modifications (Beyaz and Kutsal, 2003). Histostain®-Plus Kit (UK) was used to conduct immunohistochemistry. Avidin-biotine-peroxidase complex method was used in staining of tissues, and all the protocol used during staining was as per the standard protocol described by the manufacturer.

Statistical analysis: The data was analyzed using the statistical package Genstat 11 for windows (VSN International Ltd, Hemel, Hemstead, UK). One-way analysis of variance (ANOVA) was performed. In the case of significant differences Tukey's paired comparison procedure was applied. Effects were reported as significant at $P<0.05$.

RESULTS

One hundred and fifty birds were selected from six outbreaks of Salmonellosis reported in different commercial farms around Lahore. The majority of selected birds showed typical signs of the disease, including high fever, watery bright yellow diarrhea to mucoid green-yellow droppings, rapid respiration, pale and shrunken comb.

Post-mortem examination: Post-mortem examination revealed different lesions typical of fowl typhoid. Out of 150 broiler chickens, 80% showed enlarged, hemorrhagic and bronze colored liver with necrotic foci (Fig. 1A, 1B). Blackish and swollen spleen (Fig. 1F) was seen in 65% of broiler chickens. About 46% of broiler chickens showed edematous lungs (Fig. 1E). Hemorrhagic enteritis (Fig. 1D) and enlarged kidneys (Fig. 1C) was observed in 38.6% and 30.6% of broiler chickens, respectively. In contrast, only 0.66% showed grey nodules on the heart.

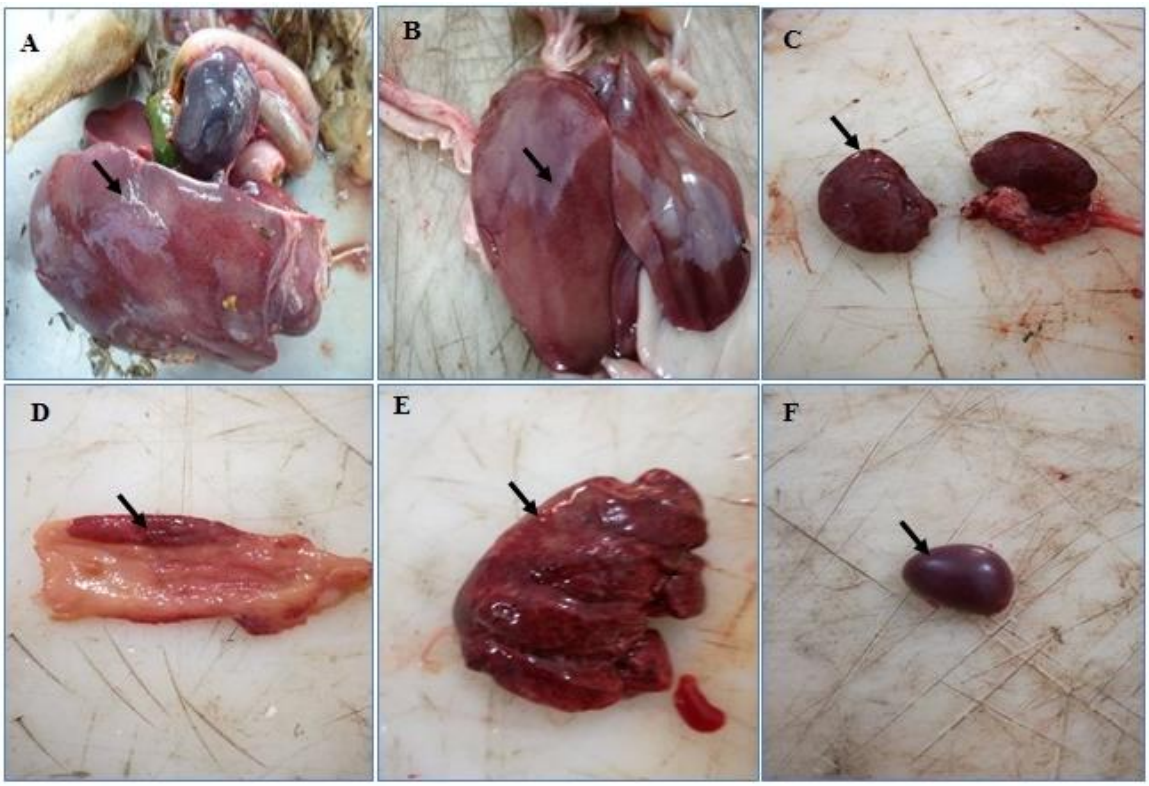

Fig. 1: Post-mortem findings in broiler chickens naturally infected with *S.gallinarum*. A-B: Enlarged and haemorrhagic liver. C:Enlarged kidney. D: Intestine showing enteritis. E: Congested and oedematous lungs. F: Blackish and enlarged spleen.

Isolation and identification of *Salmonella gallinarum*: Following a post-mortem, samples from the liver, spleen, intestine, lungs, caeca heart and kidney were collected to enumerate *Salmonella gallinarum* and its location in different body organs. In the current study, microbiological investigations showed that 12% of broiler chickens were positive for *Salmonella gallinarum* (18 out of 150 samples). *Salmonella gallinarum* was recovered from the liver, intestine, caeca, kidney, lung, heart and spleen. None of the bird had *Salmonella gallinarum* in all of the organs investigated.

The liver had the highest counts ($P<0.001$) of *Salmonella gallinarum* (2×10^{10}cfu/g) followed by spleen (1×10^{10}cfu/g), lungs (9×10^{9}cfu/g), intestine (7×10^{9}cfu/g), and caeca (5×10^{9}cfu/g) while the count was least in the kidney (3×10^{9}cfu/g) and heart (9×10^{8}cfu/g) (Fig. 2).

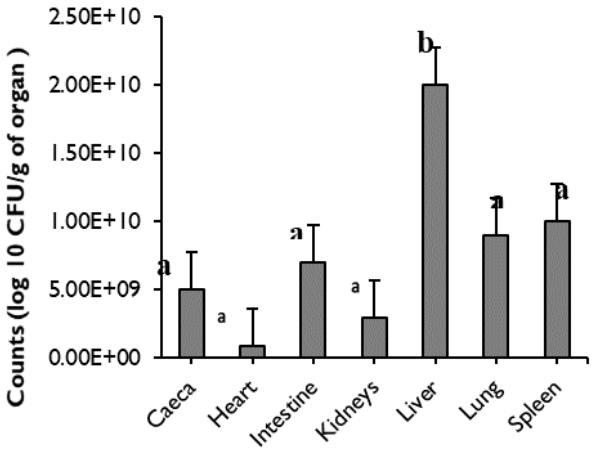

Fig. 2: Counts of S. *gallinarum* in different organs of broiler chickens showed signs of fowl typhoid. The error bars are the standard error of the mean (SEM).

Histopathological findings: Histopathological findings in different organs (liver, kidney, caeca, intestine, lungs, spleen and heart) from birds in which *Salmonella gallinarum* was isolated are summarized (Table 1). In most cases, necrosis in the hepatic cord and congestion in the central vein was observed in the liver with congestion in the sinusoid (Fig. 3A, B). Moderate necrosis was detected in proximal and distal tubules of the kidneys. In a few cases, peritubular congestion, tubular necrosis, interstitial nephritis and aggregation of inflammatory cells (Fig.3D) were also observed. The spleen showed degeneration (Fig. 3C). Microscopically the lungs showed congestion with an accumulation of blood cells. In many cases, intestinal histopathological sections showed degeneration and congestion (Fig. 3E). Intestinal caeca showed degeneration and necrosis as (Fig. 3F).

Immunohistochemical findings: Immunohistochemistry was performed on tissue samples from which *Salmonella gallinarum* was retrieved. Immunohistochemistry revealed histopathological lesions and the localization of bacteria in these tissues. Dark stained areas of immunoreactions between antigen and antibody showed *Salmonella gallinarum* in positive slides. In the liver, positive staining was present in necrotic areas and cytoplasm of hepatocytes (Fig. 4A). Immunoreactivity was detected in degenerated renal tubules. Necrotic material and cytoplasm of inflammatory cells in the kidney were also ladled with immunopositive stain (Fig. 4B). Strong immunoreactivity was detected in lungs where exudates were presented (Fig. 4C). Positive staining was present in the cytoplasm of the macrophage in the spleen (Fig. 4D),

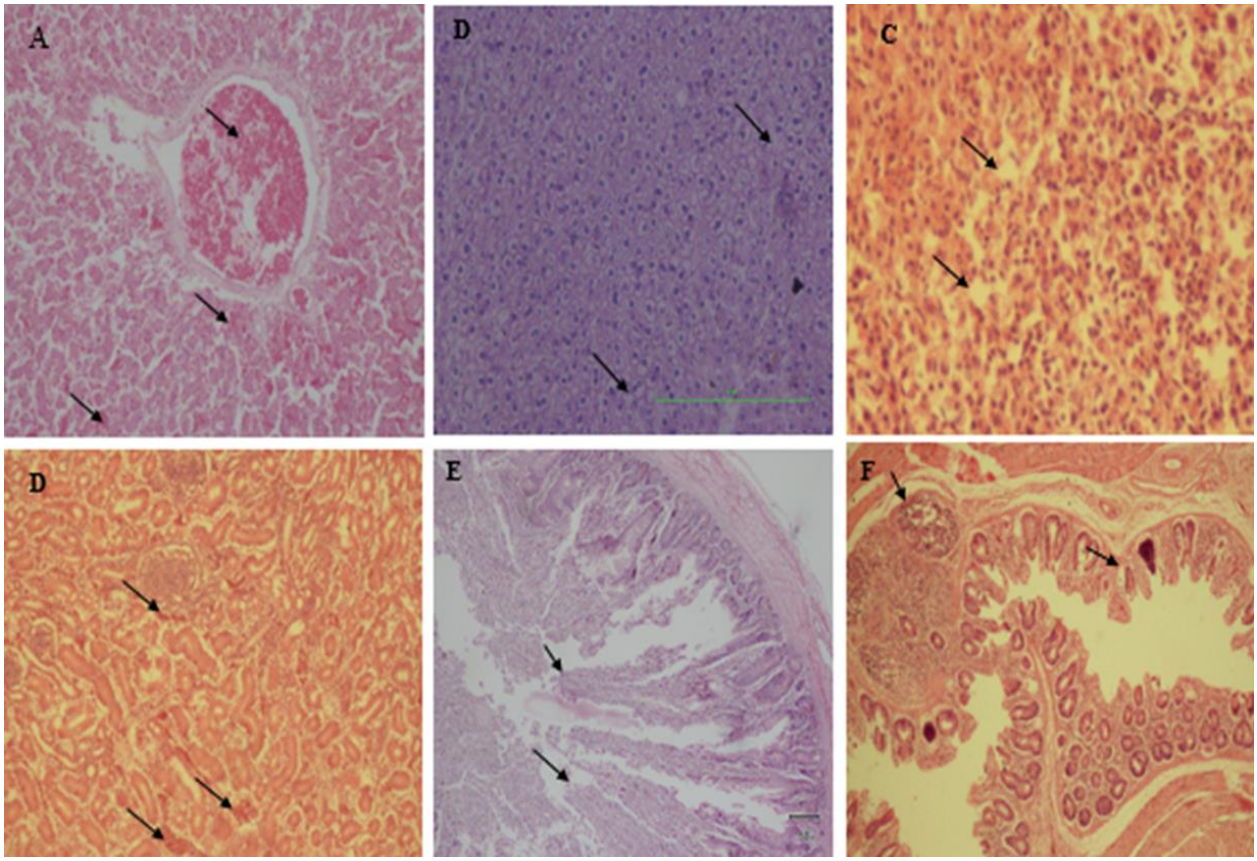

Fig. 3: Photomicrograph of broiler chickens naturally infected with *Salmonella gallinarum* showing A:Sinusoidal, central vein congestion and hepatic cord necrosis in the liver. B: Cellular swelling in liver. C: Congestion in the spleen. D: Tubular necrosis and interstitial nephritis in the kidney E: Degeneration in intestinal villi. F: Degeneration and necrosis in intestinal caeca.

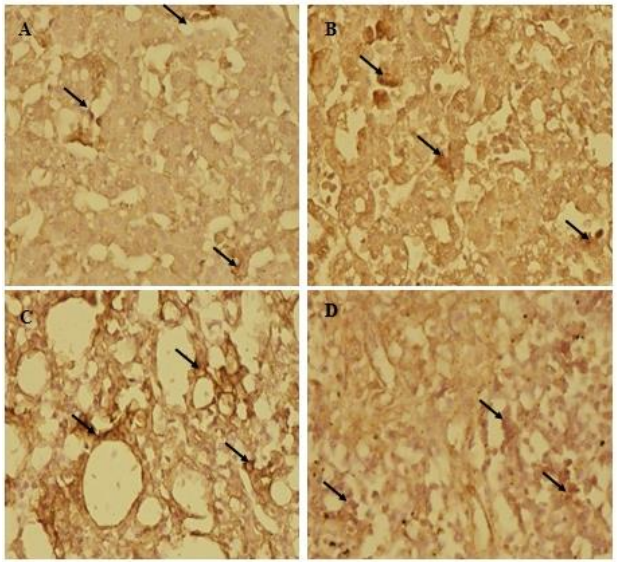

Fig. 4: A: Photomicrograph of liver of broiler showing Immunopositive reaction (arrow), (immunohistochemistry 40×). B: Photomicrograph of the kidney of broiler chicken showing *Salmonellagallinarum* positive reaction (arrow), (immunohistochemistry 40×). C: Photomicrograph of spleen showing immunoreaction (arrow), (immunohistochemistry 40×). D: Photomicrograph of spleen showing immunoreaction (arrow), (immunohistochemistry 40×).

indicating antigen-antibody reaction. Dark stained areas were detected in the mucosa of both intestine and caeca. However, no immunoreactivities were observed in any of the heart samples taken from *Salmonella gallinarum.*

Table 1: Histopathological lesions (%age) in organs of broiler chicken naturally infected with *S. gallinarum*

Organ	Histopathological lesions	Lesions (%)
Liver	sinusoidal congestion	72
	Hepatic cord necrosis	55.5
	infiltration of inflammatory cell	44
Kidney	Peritubular congestion	33
	Tubular necrosis	50
	Interstitial nephritis	8.8
Lungs	Congestion	77.7
Spleen	Degeneration	16
Intestine	Degeneration	40
Caeca	Degeneration	27
	Necrosis	38.8
Heart	Degeneration of muscles	5.5

DISCUSSION

Salmonella is a significant zoonotic pathogen of poultry delivering a major threat to poultry consumers through the infected birds and a primary reason for morbidity and mortality in developing countries due to poor sanitation and an open rearing system (Stevens *et al.*, 2009). Transmission of the disease occurs through vertical as well as horizontal routes (Tadele *et al.*, 2014). Control of *Salmonella gallinarum* is difficult because of improper and misleading diagnosis and development of multidrug resistance strains (Filho *et al.*, 2016) The present study was conducted to investigate the pathomicrobial study of naturally infected broiler chickens. The status of various organs in the liver, lungs, caeca, spleen and kidneys after *Salmonella gallinarum* infection was also investigated through histology and immunohistopathological techniques.

In the present study, *Salmonella gallinarum* was isolated and confirmed from the liver, spleen, intestine, caeca, lungs, kidney and heart of broiler chickens through culture and serological methods. On Salmonella Shigella agar, whitish-grey colonies were obtained, and all the biochemical tests were positive for *Salmonella gallinarum* as described previously (Ahmed *et al*., 2008; Tunca *et al*., 2012). In the current study overall prevalence of *Salmonella gallinarum* in broiler chickens was 12%. Previous studies have shown variable results in cultural prevalence. Compared to the present study (12%) higher prevalence 22.3% in layer and 47.7% in commercial broiler chicken were observed in some studies (Ahmed *et al.*, 2008; Kwon *et al*., 2010) Similar prevalence of *Salmonella gallinarum*, 22.3% through culture techniques, has also been reported in layers (Ahmed *et al*., 2008). In the present study,12% cultural prevalence corresponds with the 8.8% prevalence rate of *Salmonella gallinarum* in commercial poultry farms around Srinagar, India (Shah *et al*., 2012). Although these variations in cultural prevalence might be due to breed differences and the age of the birds as they considered 6-77-week-old birds. Kwon *et al.* (2010) described 47.7% prevalence of *Salmonella gallinarum* during year 2000-2008 in commercial broiler chickens indicating much higher prevalence than current study. This high prevalence may be due to a different geographical location, longer research duration by these researchers and strict policy of control and eradication in these areas.

In the present study *Salmonella gallinarum* was isolated from samples of liver, spleen, intestine, caeca, lungs, kidney, and heart of broiler chickens. The highest frequency of *Salmonella gallinarum* recovery was from the liver, followed by the spleen. The liver had the highest *Salmonella gallinarum* counts ($P<0.05$) while it was the least in the heart. These results are in line with the findings of research conducted by Rocha-e-Silva *et al*. (2013). Who recovered *Salmonella gallinarum* from different organs (liver, spleen, lungs, caecum and reproductive tract) following experimentally infected Japanese's quail. *Salmonella gallinarum* is known to be host specific with specific systemic infection sites for its growth (Chadfield *et al*., 2003). Similar to our findings higher concentrations of *Salmonella gallinarum* were recovered from the liver (Haider *et al*. 2014). Like the present study, the maximum recovery of *Salmonella gallinarum* has been reported from liver and spleen following experimental challenge in broiler chickens through oral and intra-peritoneal routes (Nazir *et al*., 2012).

The main gross findings at the time of post-mortem in broiler chickens were bronze discoloration of the liver, which was enlarged and congested with prominent necrotic foci. The spleen and kidney were also enlarged, the intestine and caeca showed inflammation. In majority of the broiler chickens both lungs were congested and edematous. Similar gross lesions like splenomegaly, congestion of lungs, bronze discoloration, of liver with hepatomegaly has been in broiler chickens have been reported in a natural outbreak of *Salmonella gallinarum* (Nazir *et al.,* 2012). Bronze discoloration of the liver is a common manifestation of *Salmonella gallinarum* infection. Bile canaliculi is the predilection site of *Salmonella gallinarum* that causes stasis of bile resulting in bronze discoloration of liver (Basnet *et al*. 2008; Chishti *et al.,* 1985). Shivaprasad, (2000) reported that *Salmonella gallinarum* mostly affects broiler chicken at 2-3 week of age. The gross lesions in kidneys, lungs and spleen of birds affected with *Salmonella gallinarum* in the current investigation agree with the observation of other studies (Nazir *et al.,* 2012; Kumari *et al.,* 2013). Like the present study, Majid *et al.* (2000) described similar thickened and catarrhal inflammatory changes in the intestine and caeca of birds infected with *Salmonella gallinarum*.

Microscopically sinusoidal congestion and coagulative necrosis in the hepatic cord were the main gross findings observed in the liver of broiler chickens. Congestion in the liver and necrosis had been reported in earlier research in acute cases of the disease (Shivaprasad, 2000). Dutta *et al.* (2015) described similar findings, including congestion and necrosis in the liver in layer chickens affected with *Salmonella gallinarum*. Mild congestion of blood capillaries and veins in the lungs was observed in 77.7% of the positive samples from broiler. Accumulation of exudates in alveoli with moderate pneumonia was observed in some of the broilers chickens. Similar microscopic changes in the lungs of broiler chickens have been reported earlier by Shivaprasad (2000) and Kumari *et al*. (2013), who observed serofibrinous pneumonia and congestion in lungs.

Nazir *et al*. (2012) conducted a study on spontaneously occurring salmonellosis in commercially broiler chicken. They reported mild to severe congestion, hemorrhage, mononuclear cells and heterophilic infiltration in interstitial tissues of kidney produced by *Salmonella gallinarum* in commercial broiler chickens. Peritubular congestion, tubular necrosis and interstitial nephritis in the kidney were also detected in the present study. Histological lesions like interstitial nephritis and peritubular congestion observed in the present study were similar to those described previously (Nazir *et al*., 2012). Vascular congestion in the kidney in acute cases was reported by Shivaprasad (2000). In the present study, most of the cases showed degeneration in the spleen. Beyaz *et al*. (2010) also reported the necrosis in spleen. In the present study, intestinal villi showed degeneration. Caeca showed degeneration, necrosis and infiltration of heterophils and mononuclear cells. These histopathological lesions in broiler chicken were corresponding to the observation described by Kumari *et al*. (2013).

Immunohistochemistry is effectively used to diagnose many poultry diseases (Singh *et al*., 2015), including Salmonellosis (Beyaz *et al*., 2010). It is highly particular and responsive in the infected tissues (Ahmed *et al*., 2018). As an intracellular organism, Salmonella can be located in the cytoplasm of the host cells (Castanheira and Garcia-del Portillo, 2017). It can proliferate within the cytoplasm of phagocytes and notorious for continuing to exist phagocytosis (Jones *et al.,* 2007). In the present study, antigenic localization of the agent was used. Immunoreactivity observed in the different samples from a variety of organs. Strong positive immunoreactivity was detected in the liver, lungs, spleen and kidney with immunoperoxidase. Strong immunoreactivity was

detected in the areas of necrosis as well as in the nearby cytoplasm of hepatocytes in the liver. The heart was the only organ that showed no positive stained area. This absence of immunoreactivity in the heart may be due to a clearance of the pathogen by the immune system so the tissue was in a repairing condition. Positive staining was observed in the spleen. Mostly immunoreactivity was noticed in the cytoplasm of inflammatory cells. Immunostaining was observed in necrosed renal tubules and within necrotic materials. Strong immunoreactivity was detected in the inflammatory cells of the lungs. Low uptake and low cytotoxicity of the host macrophages with *S. gallinarum* results in mild induction of pro-inflammatory responses that may be the possible reason for long-term persistence *S. gallinarum* in the intracellular environment (Huang *et al*., 2020). Similar immunohisto-chemical findings were reported by Tunca *et al*. (2012) in young and adult budgerigars (Melopsittacus undulates). Coincide with our immunohistochemical findings in broiler chickens Mshelbwala *et al*. (2020) detected localized immunoreactions to *Salmonella* as well as lesions in visceral organs of the chicken. Immunohisto-chemistry significantly emergent diagnostic precisions as it permits localization of antigen within lesions (Headley *et al*., 2020).

Conclusions: It can be concluded from the present study that the liver was the principal organ that harbored the highest number of *Salmonella gallinarum*. Furthermore, *Salmonella gallinarum* was not revived from all the birds showing fowl typhoid signs. Immunohistochemistry can be a specific and precise tool to locate *Salmonella gallinarum* in different body organs. Further studies are needed to explore the economic implications of *Salmonella gallinarum* infection under field conditions.

Authors contribution: GS gave the ideas and designed the research project. UF collected samples and conducted research. All authors were involved in the interpretation of research, statistical analysis, and manuscript write up. All authors declare no conflict of interest.

REFERENCES

Ahmed AKM, Islam M, Haider G, *et al*., 2008. Seroprevalence and pathology of naturally infected Salmonellosis in poultry with isolation and identification of causal agents. J Bangladesh Agri Uni 6:327-34.

Ahmed H, Mays J, Kiupel M, *et al*., 2018. Development of reliable techniques for the differential diagnosis of avian tumour viruses by immunohistochemistryand polymerase chain reaction from formalin-fixed paraffin-embedded tissue sections. Avian Pathol 47:364-74.

Arora D, Kumar S, Jindal N, *et al*., 2015. Prevalence and epidemiology of Salmonella enteric serovar Gallinarum from poultry in some parts of Haryana, India. Vet World 8:1300.

Bancroft JD, and Gamble M, 2008.Theory and Practice of Histological Techniques. 6th Edition, Churchill Livingstone, Elsevier, China.

Berchieri JA, Murphy C, Marston K, *et al*., 2001. Observations on the persistence and vertical transmission of *Salmonella enteric serovars pullorum* and *gallinarum* in chickens: effect of bacterial and host genetic background. Avian Pathol 30:221.

Beyaz L, Atasever A, Aydin F, *et al*., 2010. Pathological and clinical findings and tissue distribution of *Salmonella Gallinarum* infection in turkey poults. Turkish J Vet Ani Sci 34:101-10.

Beyaz L and Kutsal O, 2003. Pathological and immunohistochemical studies in Experimental *Salmonella gallinarum* infection (Fowl typhoid) in chickens. Ankara Univ Vet Fak Derg 50:219-27.

Castanheira S and García-del Portillo F, 2017. Salmonella populations inside host cells. Front Cell Infect Microbiol 7:432.

Chadfield MS, Brown DJ, Aabo S, *et al*., 2003. Comparison of intestinal invasion and macrophage response of Salmonella Gallinarum and other host-adapted Salmonella enterica serovars in the avian host. Vet Microbiol. 20:49-64.

Christensen JP, Olsen JE, Hansen HC *et al.,* 1992. Characterization of Salmonella enterica serovar gallinarum biovars gallinarum and pullorum by plasmid profiling and biochemical analysis. Avian Pathol 21:461-70.

Dutta P, Borah M, Gangil R, *et al*., 2015. Gross/Histopathologic impact of *Salmonella gallinarum* isolated from layer chickens in Jaipur and their antibiogram assay. Int J Adv Vet Sci Tech 4:153-9.

Filho RAC, Ferreira JC, Kanashiro AMI, *et al*., 2016. Antimicrobial susceptibility of *Salmonella* Gallinarum and *Salmonella* Pullorum isolated from ill poultry in Brazil. Cien Rural 46:513-8.

Folajinmi AF and Peter AO, 2020. Financial Management Practices and Performance of Small and Medium Scale Poultry Industry in Ogun State, Nigeria. J Bus Finance Account 8:90.

Grimont PAD and Weill FX, 2007. WHO collaborating centre for reference and research on Salmonella-antigenic formulae of the Salmonella serovars, 9th ED. Institute pasture., France.

Headley S, Oliveira T, Li H, *et al.,* 2020. Immunohistochemical detection of intralesional antigens of ovine gammaherpesvirus-2 in cattle with sheep-associated malignant catarrhal fever. J Comp Pathol 174:86-98.

Huang K, Fresno AH, Skov *et al.,* 2020. Dynamics and outcome of macrophage interaction between salmonella gallinarum, salmonella typhimurium, and salmonella dublin and macrophages from chicken and cattle. Front Cell Infec Microbiol 9:420.

Hussain J, Rabbani I, Aslam S, *et al*., 2015. An overview of poultry industry in Pakistan. Worlds Poult Sci J 71:689-700.

Jones MA, Hulme SD, Barrow PA, *et al.,* 2007. The Salmonella pathogenicity Island 2 type 111 secretion systems play a major role in pathogenesis of systemic disease and gastrointestinal tract colonization of Salmonella enteric serovar Typhimurium in the chicken. Avian Pathol 36:199-203.

Kumari D, Mishra SK and Deepika L, 2013.Pathomicrobial studies on *Salmonella gallinarum* infection in broiler chickens. Vet World 6:725-9.

Kwon YK, Kim A, Kang MS, *et al*., 2010. Prevalence and characterization of *Salmonella gallinarum* in the chicken in Korea during 2000 to 2008. Poult Sci 89:236-42.

Majchrzak M, Krzyzanowska, A, Kubiak, AB *et al.,* 2014. TRS-based PCR as a potential tool for inter-serovar discrimination of Salmonella Enteritidis, S. Typhimurium, S. Infantis, S. Virchow, S. Hadar, S. Newport and S. Anatum. Mol Biol Rep 41:7121-32.

Majid A, Siddique M and Khan A, *et al.,* 2000. Avian Salmonellosis: Gross and histopathological lesions. Pak Vet J 20:183-6.

Marangoni F, Corsello G, Cricelli C, *et al.,* 2015. Role of poultry meat in a balanced diet aimed at maintaining health and wellbeing: an Italian consensus document. Food Nutr Res 59:27606.

Mshelbwala FM, Ibrahim NDG, Saidu SN, *at al.,* 2020. Quantitative distribution and interaction of Salmonella Zega with host cells in visceral organs of chickens infected orally,intraperitoneally and per cloaca. Heliyon 6:03180.

Nasir AA, Ashraf MU, Kausar A, *et al.,* 2020. Detection of Clostridium perfringens Alpha, Epsilon and Clostridium chauvoei A Toxin Genes in Blackleg. Asian J Agric Biol 8:519-23. DOI: https://doi.org/10.35495/ajab.2020.02.120

Nazir S, Kamil SA, Darzi MD, *et al*., 2012. Pathology of spontaneously occurring Salmonellosis in commercial broiler Chickens of Kashmir Valley. J World's Poult Res 2:63-9.

OIE, 2012. Fowl typhoid and Pullorum disease Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 7th ed. Paris: Office International Des Epizooties pp:1-14.

Ozbey G and Ertas H, 2006. Salmonella spp. isolation from chicken samples and identification by polymerase chain reaction. Bulg J Vet Med 9:67-73.

Rajagopal R and Mini M, 2013. Outbreaks of salmonellosis in three different poultry farms of Kerala, India. Asian Pac j Tropi Biomed 3:496-500.

Rocha-e-Silva R, Cardoso W, Teixeira R, *et al*., 2013. *Salmonella Gallinarum* virulence in experimentally-infected Japanese quails (Coturnix japonica). Braz J Poult Sci 15:39-45.

Swar SO and Shnawa BH, 2021. Recent advances in molecular characterization of Sarcocystis species in some meat producing animals: An updated review. Asian J Agric Biol 2021. DOI: https://doi.org/10.35495/ajab.2020.09.502

Shah S, Kamil S and Shahnawaz M, 2012. Prevalence and Seasonality of Fowl Typhoid in Kashmir Valley. J Pure Appl Microbiol 6:479-82.

Shivaprasad HL, 2000. Fowl typhoid and pullorum disease. Rev Sci Tech 19:405-24.

Singh J, Banga H, Brar R, *et al*., 2015. histopathological and immuno-histochemical diagnosis of infectious bursal disease in poultry birds. Vet world 8:1331.

Stevens MP, Humphrey TJ and Maskell DJ, 2009. Molecular insights into farm animal and zoonotic Salmonella infections. Philos Trans R Soc Lond *B* Biol Sci 364: 2709-23.

Sulieman AME, Dafallah FE, Rahman EHA, *et al*., 2020. Isolation, Identification and Characterization of Salmonella spp. from Chicken purchased at Wad Madani City, Gezira State, Sudan. Adv Life Sci 8:98-102.

Tadele G, Asrade B, Bayleyegn G, *et al*., 2014. Sero-prevalence of Fowl Typhoid and Pullorum disease from apparently healthy chickens in Eastern Ethiopia. J Vet Sci Technol 12:123-35.

Tunca R, Toplu N, Kırkan Ş, *et al*., 2012. Pathomorphological, immunohistochemical and bacteriological findings in budgerigars (Melopsittacusundulatus) naturally infected with *Salmonella gallinarum*. Avian Pathol 4:203-9.

Zishiri, OT, Mkhize N and Mukaratirwa S., 2016. Prevalence of virulence and antimicrobial resistance genes in Salmonella spp. isolated from commercial chickens and human clinical isolates from South Africa and Brazil, Onderstepoort J Vet Res 83:1067.