Pathological Investigation of Salmonella Infection in Layer chicken at Nilphamari District of Bangladesh

A Thesis

By

MD. AKHTARUZZAMAN

Registration No. 1205100

Semester: July – December, 2013

Session: 2012-2013

MASTER OF SCIENCE (M.S.)

IN

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DEPARTMENT OF PATHOLOGY AND PARASITOLOGY

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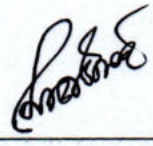

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DEPARTMENT OF PATHOLOGY AND PARASITOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY DINAJPUR-5200

DECEMBER, 2013

DEDICATED
TO MY
BELOVED PARENTS

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I express my profound gratitude to Almighty Allah for giving me the opportunity to carry on this research.

With great pleasure I would like to express my heartfelt gratitude and indebtedness to my honorable teacher and research supervisor Dr. Md. Nazrul Islam, Associate Professor, Department of Pathology and Parasitology, Hajee Mohammad Danesh Science and Technology University,Dinajpur-5200, for his valuable advice, suggestion's, guidance and keen interest that he took in preparing this thesis. His constructive criticism, painstaking endeavors in going through the manuscripts and his demands for throughness in caring out the present work will be remembered with gratitude. I am also grateful to him for his co-operation and skill for the photography and photo-micrography.

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I wish to acknowledge my debt to my Father and Mother for her constant help throughout the period of my study.

Author

December, 2013

ABSTRACT

The research work was conducted during the period from July to December 2013 at Nilphamari district to investigate the pathology of *Salmonella* infection in layer birds. The histopathological studies were performed at the pathology laboratory of Hajee Mohammad Danesh Science and Technology Unuversity, Dinajpur and Bacteriological test was performed at the Laboratory of Nilsagor Agro Industries, Nilphamari. *Salmonella* organisms were isolated and identified from ovaries of dead layer birds and from inner content of laid eggs of different poultry farms. 38 ovarian swabs for bacteriology, visceral organs (liver, lung, spleen, egg follicles and intestine) of 38 dead birds for pathological study from 16 layer farms and 45 laid eggs (5 eggs/farm) from reported 9 *Salmonella* infected farms constituted samples of the study. Samples were subjected to isolation and identification of the causal agent followed by gross and histopathological study of the affected visceral organs. 17 of 38 ovarian swabs (44.7%) were *Salmonella* positive. The percentage of *Salmonella gallinarum*, *Salmonella pullorum* and paratyphoid causing *Salmonella* were 70.6%, 17.6% & 11.8%, respectively. About 53% livers of *Salmonella* affected birds were enlarged, congested, friable and bronze coloured with white necrotic foci. About 59% egg follicles were congested, hemorrhagic, discoloured with stalk formation and 70.6% intestines showed hemorrhagic to catarrhal enteritis. At histopathology, 76.5% livers were congested with formation of multifocal nodules and 82.4% egg follicles were congested with huge leukocytic infiltration. Infiltration of heterophils in intestinal mucosa was found in 47.1% cases. 4 *Salmonella* isolates were found from 9 laid egg samples (5 eggs content comprised as 1 sample) and isolation rate was 44.4% reporting transovarian transmission in poultry

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LIST OF ABBREVIATIONS AND SYMBOLS

&	and
TTB	Tetrathionate broth
SS	Salmonella-Shigella
TSI	Triple sugar iron
BGA	Brilliant green agar
EMB	Eosine methylene blue
et al.	and other
FAO	Food and Agricultural Organization
Gm	Gram
H&E	Hematoxylin and Eosin
i.e.	that is
mm	Millimeter
MR	Methyl Red test
VP	Voges Proskauer Test
µm	Micrometer
BPW	Buffered Peptone Water
NO.	Number
Fig.	Figure
%	Percentage
^{o}C	Degree celsius
CC	Cubic centimeter
LSD	Least standard deviation
CV	Co-variance
µl	Micro litter

CHAPTER I

INTRODUCTION

CHAPTER I

INTRODUCTION

In Bangladesh chickens are playing a significant role in national economy and reducing poverty by supplying meat, egg and other by-products. Several constraints such as the diseases, poor husbandry, low productivity and shortage of feed affect the optimal performance of this industry in Bangladesh (Haque *et al.,* 1991). Salmonellosis in poultry causes heavy economic loss through mortality and reduced production (Khan *et al.*, 1998). With great expansion of poultry rearing and farming, pullorum disease and fowl typhoid have become wide spread problem in Bangladesh (Rahman *et al.*, 1997). Age wise prevalence of avian Salmonellosis showed highest infection rate in adult layers (53.25%) in comparison to brooding (14.55%), growing (16.10%) and pullet (16.10%) (Rahman *et al.*, 2004).

Salmonella are Gram negative, short plump shaped rods, non-sporeforming, non-capsulated, aerobic and facultative anaerobic organisms and classified under the family Enterobacteriaceae (OIE Manual, 2006). More than 2300 serotypes of *Salmonella* have been identified, only about 10% of these have been isolated from poultry (Gast, 1997). Chickens are the natural hosts for both *S. pullorum and S. gallinarum* (Snoeyenbos, 1991). Pullorum disease is usually confined to the first 2-3 weeks of age and occasionally occurs in adults (Shivaprashad, 1997). Fowl typhoid is frequently referred to as a disease of adult birds and there are also reports of high mortality in young chicks (Christensen *et al.,* 1992). The epidemiology of fowl typhoid and pullorum disease in poultry, particularly with regard to transmission from one generation to the next are known to be closely associated with infected eggs (Wigley *et al.,* 2001). Contaminated eggs produced by infected laying hens are thought to be one of the main sources of human infection with *Salmonella enteritidis* (Humphrey *et al.*, 1989). Eggs may become contaminated with *Salmonella* in two main ways: (i) *Salmonella* may silently infect the ovaries of apparently healthy hens and contaminate the eggs before the

shells are formed. (ii) *Salmonella* infected bird droppings contain *Salmonella* that can contaminate the outer egg shells and may penetrate when crack the shell (Deryck and Pattron, 2004).

Therefore, the present study was designed to investigate the pathology of *Salmonella* infection from layer birds of different poultry farms at Nilphamari district as well as to study the pathology of different organs of *Salmonella* infected layer birds.

It is hoped that the present investigation will be a base line for the study of *Salmonella* infection from layer birds, and also will provide valuable information to poultry immunologist, pathologist, researchers, cell biologist and anatomist.

So, the present study was carried out to investigate the pathology of *Salmonella* infection in layer birds at Nilphamari district.

In view of above consideration, the present research program was undertaken with following objectives-

- To study the clinical findings of avian salmonellosis
- To study the necropsy & histopathological findings in liver of affected birds
- Bacteriological study of the organisms isolated from the suspected birds

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

The purpose of this chapter is to provide a selective review of the previous research conducted so far in relation to the present study, the number of works directly related to the present study were scanty. Detailed Similar findings with intensity of the severity of lesions in different organs were described by many investigators (Chishti *et al.*, 1985; Khan *et al.*, 1998; Hafeji *et al.*, 2001; Habib-ur-Rehman *et al.*, 2003; Beyaz and Kutsal, 2003; Goswami *et al.*, 2003; Hossain *et al.*, 2003; Prasanna and Paliwal, 2003; Islam *et al.,* 2006; Deshmukh *et al.*, 2007).

2.1 Clinical findings of avian salmonellosis

Fowl typhoid is frequently occur in growing & adult birds characterized by whitish materials to the vent, ruffled feathers, depression & anorexia, loss of egg production. Pullorum disease principally occur in chicks under 3weeks characterized by adherence of chalky white material to the vent, droopy wings and gasping. Paratyphoid infection usually occur in chicks, pullets characterized by profuse watery diarrhea, pasting of the vent area, reluctant to move, ruffled feathers (Habib-ur-Rehman *et al.*, 2003).

2.2 Pathological studies

2.2.1 Necropsy

Habib-ur-Rehman *et al.*, (2003) reported that the gross lesions of 14 *Salmonella* infected layer birds were variable. During necropsy, 63% livers were friable, bronze discoloration with white focal necrosis. A total of 37.1% livers were congested and enlarged. About 49% egg follicles were congested, hemorrhagic and discolored with stalk formation while 51.2% egg follicles were mildly congested and hemorrhagic. A total of 60.6% intestines were hemorrhagic to catarrhal enteritis while 39.4% only hemorrhagic and congested. About 54.7%

lungs were severely congested and pneumonic while 45.3% lung showed mild congestion. About 47.1% spleens were enlarged and discolored.

Similar findings with intensity of the severity of lesions in different organs were described by many investigators (Chishti *et al.*, 1985; Khan *et al.*, 1998; Hafeji *et al.*, 2001; Beyaz and Kutsal, 2003; Goswami *et al.*, 2003; Hossain *et al.*, 2003; Prasanna and Paliwal, 2003; Islam *et al.,* 2006; Deshmukh *et al.*, 2007).

Mamun *et al.,* (2011) reported that the lesions in the liver showed enlargement, congestion, hemorrhages, congestion, friable with necrotic foci.

Nazir *et al.,* (2012) showed that the lesions in chicks affected with fowl typhoid were indistinguishable from those associated with pullorum disease. In typical cases of fowl typhoid, appearance of bronze coloured liver was characteristic and prominent lesion. The bronze discolouration of the liver was observed more frequently at 7 to 15 days of age. Livers of affected chicks of this age group also revealed numerous greyish necrotic foci or necrotic patches, reddish haemorrhagic foci which were distributed uniformly on their surfaces. The chicks which died in early age group also showed pale discolouration, enlargement and congestion of liver along with mild distension of gall bladder. In few of these cases diffused areas of necrosis were also observed. The proportionate distribution of gross lesions in fowl typhoid, pullorum disease and paratyphoid observed in naturally infected broiler chicken is given.

The heart generally revealed congestion and thickening of pericardium (4.6%) along with few indistinct necrotic foci in some cases. In a few birds of over 2 weeks of age, small elevated greyish white nodular areas were observed on the myocardium, which were more predominant on the ventricular region. Spleen revealed congestion and enlargement in chicks at early age (1 to 7 days). Presence of hemorrhagic or whitish diffused or multiple necrotic foci were the consistent lesions in the spleen of birds older than this age group. The lungs in general were congested in 11 percent of affected birds. At early age, there was mild congestion

in lungs whereas chicks older than 1 week, revealed moderate congestion and in a few birds focal areas of consolidation were also observed. The kidneys in general were congested and slightly swollen in 19.8 percent affected birds. Grossly, intestines appeared congested with hemorrhages mostly on the mucosal side in 24.2 percent of the affected birds. Dropping were thin and occasionally blood stained. Bursa showed mild to moderate atrophy in 8 percent of affected birds. In acute cases of Pullorum disease in birds having history of diarrhoea, pasting of vent with loose whitish faecal material, impaction of claoca was observed in 3.1 percent of affected birds. In addition to above changes in Pullorum disease, unabsorbed yolk and yolk sac infection were observed in 5.4 and 6.2 percent of affected birds respectively during first week of early age. The grossly observed lesions in Paratyphoid infection included necrotic foci on liver (26.4%), pericarditis with the presence of fibrinous exudates in pericardial sac (14.8%), perihepatitis (16.4%), presence of greyish white nodules on ventricular region of heart (very rare), congestion of intestines (21.4%) and kidneys (14.4%), yolk sac infection (18.3%). The lesions in caeca were more prominent and severe in cases of Paratyphoid where in the caeca were inflamed and swollen and in chronic cases revealed presence of cheesy, dry, necrotic material in their lumen (4.8%)

2.2.2 Histopathology

Habib-ur-Rehman *et al.*, (2003) stated that Only 14 *Salmonella* positive dead bird tissues of different organs were selected for histopathology and describes the histopathological findings of different organs. In histopathological investigation, all the tissues of different organs of 17 layer birds did not evoke similar kinds of lesions. A total of 66.5% livers were congested and formed multifocal nodules with coagulation necrosis while remaining 33.5% liver showed hepatitis. Besides, 66.5% lungs were severely congested and hemorrhagic and 33.5% lung showed inflammatory cells in alveoli and bronchi. Infiltration of heterophils and lymphocytes in the mucosa of intestines were found in 57.1% cases. Sever lymphocytic depletion and focal necrosis in the spleen was found in 43% birds.

Whereas, about 72.4% egg follicles was markedly congested and showed huge leukocytic infiltration.

The microscopic lesions recorded and described by (Chishti *et al.*, 1985; Calnek *et al.*, 1991; Khan *et al.*, 1998; Kinde *et al.*, 2000; Hafeji *et al.*, 2001; Prasanna and Paliwal, 2003; Holt *et al.*, 2006; Msoffe *et al.*, 2006). Infiltration of heterophils and lymphocytes in the mucosa of intestines were found, lymphocytic depletion in the spleen was found.

Mamun *et al.*, (2011) described in section of liver infiltration of histiocytes and accumulation of leucocytes which replace the degenerated or necrotic liver cells.

Nazir *et al.*, (2012) showed that microscopically severe vacuolar degeneration, fatty changes was frequently observed change in the liver in chicks of early age group. Congestion of blood vessels and haemorrhages were observed in chicks of all age groups. The chicks mostly of 7 to 15 days old showed isolated foci of necrosis in hepatic parenchyma along with infiltration of leucocytes predominantly mononuclear cells which were mostly centered around portal triads and perivascular areas. Generally adjacent to the necrotic areas there was infiltration with mononuclear cells and heterophils. In a few cases aggregations of mononuclear cell resulted were observed. Sometimes mild reticular cell hyperplasia was observed which resulted in disruption of hepatic cords. In heart thickening of pericardium along with the mono-nuclear cell infiltration was generally observed in cases of typhoid and paratyphoid infection. Congestion and haemorrhage especially below the epicardium was most frequently observed change in early age group. Necrosis was also observed focally along with infiltration by mononuclear cells. In a few isolated cases of over 2 weeks of age group with nodular appearance grossly, there was extensive infiltration of mononuclear cells along with few heterophils which at several occasions resulted in atrophy, necrosis and replacement by the infiltrating cells. In certain cases of paratyphoid there was complete replacement of muscle fibres by the infiltrating cells. In general the spleen showed congestion and thickening of blood vessels

and haemorrhages particularly below the spleenic capsule in early stages where as at later stages the changes generally included depletion of lymphocytes along focal areas of necrosis. Depletion of lymphocytes at times was accompanied by reticular cell proliferation. Lungs revealed congestion of the interlobular saptae and haemorrhages in the parabronchi. Besides congestion, haemorrhage and mild infiltration of mononuclear cells and suppurative bronchopneumonia characterised by the presence of exudates in the parabronchi comprising of infiltration of heterophils was also observed in few cases. Kidneys showed mild to severe congestion and haemorrhages in the interstitial tissue. In a few isolated cases focal mononuclear cell and hetrophilic infiltration was also observed in the interstitial tissue. The bursa generally revealed mild to moderate depletion of lymphocytes in bursal follicles and in few cases there was atrophy of bursal follicles. In a few birds only mild degenerative changes were noticed in the bursal follicles. The intestines in affected birds generally revealed congestion of mucosal vessels, marked goblet cell hyperplasia mild to moderate infiltration of heterophils and mononuclear cells in the lamina propria of the villi, In chronic cases of paratyphoid the lesions in caeca comprised of congestion and haemorrhage with degeneration and desquamation of lining epithelium and mononuclear cell infiltration in the mucosa and submucosa which resulted in atrophy of intestinal glands.

2.3 Bacteriological study

Salmonella are Gram negative, short plump shaped rods, non-sporeforming, non-capsulated, aerobic and facultative anaerobic organisms and classified under the family Enterobacteriaceae (OIE Manual, 2006). More than 2300 serotypes of *Salmonella* have been identified, only about 10% of these have been isolated from poultry (Gast, 1997). Chickens are the natural hosts for both *S. pullorum* and *S. gallinarum* (Snoeyenbos, 1991).

CHAPTER III

MATERIALS AND METHODS

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MATERIALS AND METHODS

3.1 Experimental animal/bird

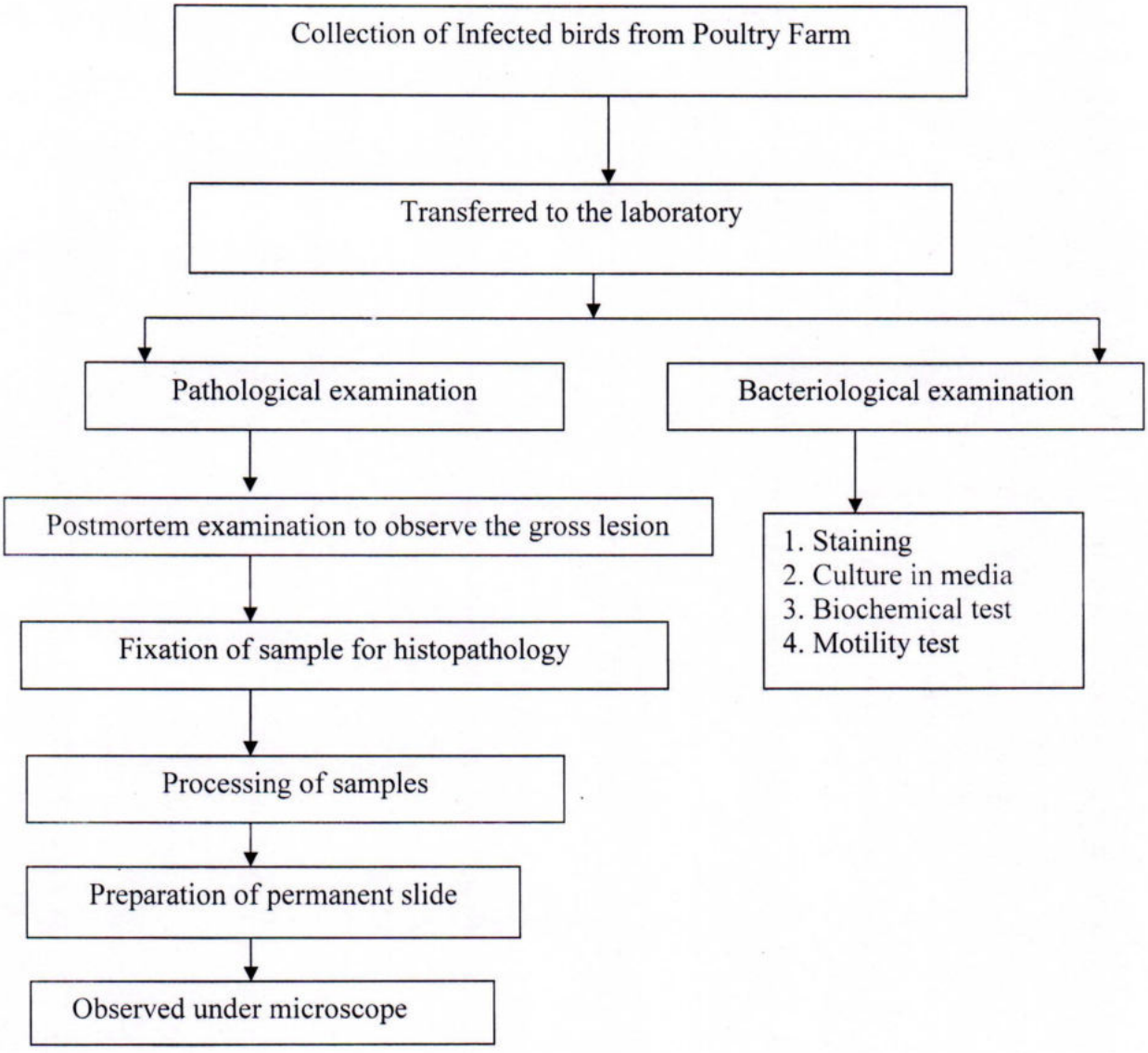
Layer birds

ISA brown: ISA brown is actually of French origin with Institute de Selection Animale. ISA brown chickens are prolific egg layer that can lay up to 300 eggs in a year of egg production. ISA brown was created by crossing Rhode Island Red (RIR) rooster and Rhode Island White hen. Feather color brown, shank light brown, comb red & egg shell brown in color.

3.2 Experimental area and research period

The study was conducted during the period from July to December 2013 at Nilphamari district. A total of 16 layer farms which contained 28,425 birds were considered as experimental birds. Sample birds were collected and having no history of *Salmonella* vaccine used in the selected farms. The histopathological studies were performed at the pathology laboratory of Hajee Mohammad Danesh Science and Technology University, Dinajpur and Bacteriological test was performed at the Laboratory of Nilsagor Agro Industries, Nilphamari.

3.3 Flow chart of the experiment



3.4 Clinical examination

History

Under this point the following data was collected from the farmers: Name of farmer, name of area, total number of birds in farm, total number of affected birds, daily mortality and total mortality. During the period present and previous history was taken from each farmer.

Examination of clinical findings

At first the external appearance of the bird was observed then general condition of the chicken, condition of vent, feathers. Presence or absence of diarrhea was also marked.

Postmortem examination

It was conducted with the help of rubber gloves, a pair of shears, scissors, knife, scalpel and forceps.

Technique

At first the chicken was laid on its back and each leg to expose the internal organs, in turn drawn outward away from the body while the skin was incised between the leg and abdomen on each side. Then the both legs were grasped firmly in the area of the femur and bent forward, downward and outward, until the head of both femurs were broken free of the acetabular attachment so that both legs lied flat on the table. The skin was cut between the two previous incisions at a point midway between keel and vent. The cut edge was then forcible reflected forward, cutting was necessary, until the entire ventral aspect of the body including the neck, was exposed.

For exposing of the viscera, knife was used to cut through the abdominal wall transversely mid-way between the keel and vent, then through the breast muscle on each side. Positioning shears were used to cut first the rib cage, the coracoids and clavicle on both side. With some care this was done without severing the large blood vessels

3.5 Histopathological studies

During the whole period of study, postmortem examinations of 38 dead birds were performed from the representative selected 16 layer farms. Gross pathological changes at necropsy were carefully observed and recorded.

For the purpose of histopathological studies the tissues of different organs were collected and fixed in the 10 % neutral buffered formalin for few days. 10 % formalin was prepared as follows-

40 % formaldehyde	10 ml
Distilled water	90 ml

Then tissues were taken from the formalin and were dehydrated by using ascending graded of alcohol (70%, 80%, 90%, 95%, 100% and 100%). For each grade of alcohol one hour was provided. After dehydrating the tissue it was transferred to the chloroform solution each for ninety minutes for clearing purpose. Then the tissues were infiltrated in the liquid paraffin at 60°C temperature for ninety minutes and it was repeated again. Finally the tissues were embedded in paraffin and paraffin blocks were made.

The paraffin blocks were cut at 6 µm thickness using microtome machine (Mu509, Euromex, Japan). After sectioning of paraffin block, the slices were floated on warm water in a water bath at 45°C for stretching. Then the sliced tissue was placed on greeze free clean glass slide using adhesive like gelatin. Then the glass slides were dried at 37° C temperature for 24 hours in an incubator. After drying the slides the sections were stained with Hematoxylin and Eosin (H & E) stain for general histological study.

Hematoxylin and Eosin (H & E) Stain

Preparation of Harris hematoxylin solution:

Hematoxylin crystal	5 gm
Alcohol, absolute	50 cc
Potassium alum	100 gm
Distilled water	1000 cc
Mercuric oxide (red)	2.5 gm

Hematoxylin was dissolved in alcohol, the alum in water by the aid of heat. After mixing the solutions it was brought to heat. After removal from heat, the mercuric oxide was added slowly. Reheated and was removed immediately and the vessel was plunged into basin of cold water until cool. 4cc glacial acetic acid per 100cc of solution was added. The solution was filtrated before use.

Preparation of counter stain for hematoxylin stain

Stock 1% aqueous eosin solution:

Eosin Y, water soluble	10 g
Distilled water	1000 cc
Glacial acetic acid	2 cc

Working eosin solution:

Eosin, 1% stock solution	1 part
Alcohol, 80 %	3 part

Staining procedure

Xylene-1	2 minutes
Xylene-2	2 minutes
Absolute alcohol-1	2 minutes
Absolute alcohol-2	2 minutes
70 % alcohol	2 minutes
Running water	2 minutes
Hematoxylin	5 minutes
Running water	15 minutes
Distilled water	5 minutes
Eosin	5 seconds
70 % alcohol	2 minutes
80 % alcohol	2 minutes
90 % alcohol	2 minutes
95 % alcohol	2 minutes
Absolute alcohol-1	2 minutes
Absolute alcohol-2	2 minutes
Xylene-1	2 minutes
Xylene-2	2 minutes

Finally the section were Mounted with cover slip using DPX

3.6 Bacteriological examination

Isolation and identification of *Salmonella* organism-

a) From ovarian swabs of dead birds

Collection of samples

A total of 38 ovarian swab samples were collected from the same flock along with the necropsy study. All the ovarian swabs were collected in test tubes containing 10 ml tetrathionate broth (TTB) and 200 µl of iodine-iodide solution was added in each test tube just before the collection of swab samples according to methods described elsewhere (Merchant and Packer, 1967and Haider *et al.*, 2003).

Cultural media

After overnight incubation in TTB, all the samples were primarily cultured in Nutrient agar and then subcultured in the *Salmonella-Shigella* (SS) agar, Triple sugar iron (TSI) agar, Brilliant green agar (BGA), Eosine methylene blue (EMB) agar, Mac Conkey's agar, Blood agar (Merchant and Packer, 1967 and Haider *et al.*, 2003).

Morphological characterization

The presumptive colonies of *Salmonella* in different media were characterized microscopically using Gram's stain.

Biochemical test

Five basic sugars such as dextrose, sucrose, lactose, mannitol and maltose were used for sugar fermentation test. MR test, VP test, indole test ornithine and dulcitol fermentation test (Merchant and Packer, 1967and Haider *et al.*, 2003).

Motility test

Motility test was performed for the separation of motile and non-motile *Salmonella* according to the method described elsewhere (Merchant and Packer, 1967and Haider *et al.*, 2003).

b) From inner content of laid eggs

This test was performed by following the method described by Poppe *et al.* (1992) and Haider (2009). Total 45 eggs were collected from 9 farms (5 eggs/9 farms) followed by washing and disinfection of egg surface by 70% ethanol. Manually homogenization of pooled egg contents was performed by using stirrer. Incubation of homogenized egg contents was done with pre-enrichment media (BPW) at 37°C temperature as a standard proportion of 1:10 fold dilution for 48 hours followed by incubation in selective enrichment media (SRV) at 37°C temperature as a proportion of 1:10 fold dilution for 24 hours. Then plating was performed into selective agar media (BGA, SS, TSI & EMB agar).

3.7 Statistical Analysis

Data were statistically analyzed using the (ANOVA) "Analysis of Variance" technique with the help of the computer package MSTAT-C. The mean differences were adjusted by the Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

3.8 Photography

The microphotographs made from the selected areas of tissues (with the help of digital camera connected with microscope) under 10x and 40x microscopic objectives were placed in this thesis for better illustration of the result.

CHAPTER IV

RESULTS

CHAPTER IV

RESULTS

4.1Clinical findings of avian salmonellosis

In the present study the following clinical findings were observed; fowl typhoid is characterized by whitish materials to the vent, ruffled feathers, depression, anorexia, loss of egg production. Pullorum disease is characterized by adherence of chalky white material to the vent, droopy wings, gasping. Paratyphoid infection is characterized by profuse watery diarrhea, pasting of the vent area, reluctant to move, ruffled feathers and finally death of affected bird

Fig. 1: Infected bird showing depression

Fig. 2 Chalky white material to the vent

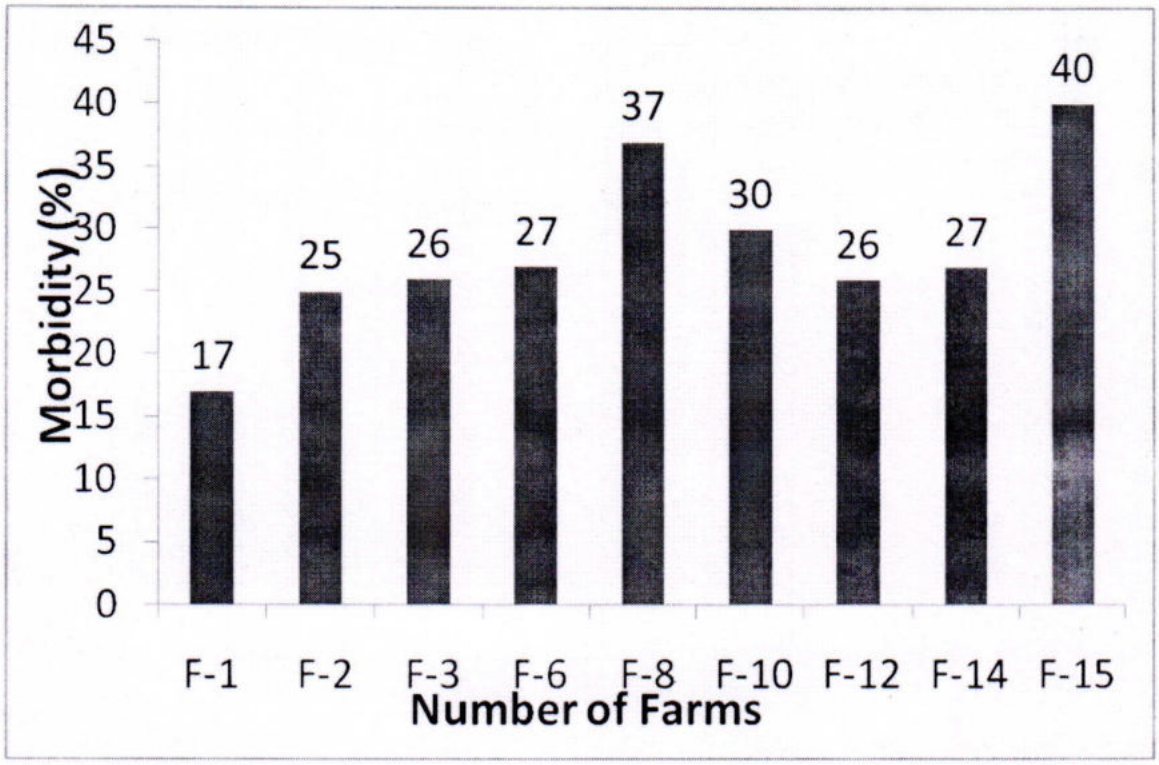
Table-1 Morbidity and Mortality of different layer farm

Farm No.	Total bird	No. of Infected bird	No. of dead bird	Percentage of morbidity	Percentage of mortality
F 1	1520	260	70	17	5
F 2	2000	500	120	25	6
F 3	1700	440	90	26	5
F 4	1650	0	0	0	0
F 5	2215	0	0	0	0
F 6	1570	420	112	27	7
F 7	1200	0	0	0	0
F 8	1460	595	106	37	7
F 9	1600	0	0	0	0
F 10	2100	625	130	30	6
F 11	2300	0	0	0	0
F 12	2020	540	92	27	5
F 13	1070	0	0	0	0
F 14	2150	580	150	27	7
F 15	2070	828	269	40	13
F 16	1800	0	0	0	0

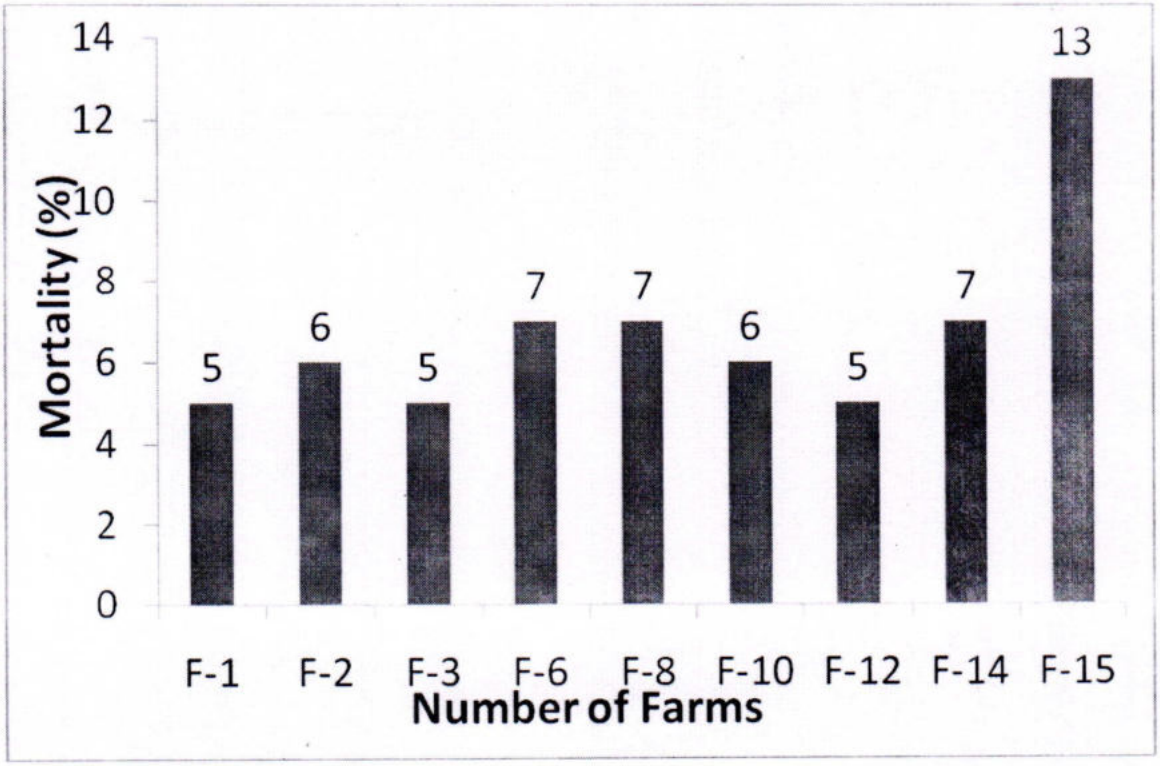
Table 2. Morbidity and mortality of different layer farms adjusted by DMRT

Farms	Morbidity (%)	Mortality (%)
F-1	17.45 e	4.633 e
F-2	25.43 d	6.267 c
F-3	25.99 d	5.330 d
F-6	26.75 d	7.413 b
F-8	37.23 b	5.533 d
F-10	29.85 c	6.230 c
F-12	26.91 d	4.887 e
F-14	27.21 d	7.093 b
F-15	40.03 a	13.30 a
Lsd (0.05%)	1.633	0.3792
CV%	1.04	3.26

The table 2 shows highest mortality and morbidity by a symbol and e posses lowest mortality and morbidity .The value of co-efficient of variation indicates the are more homogenous and more significant.



Graph 1. Graphical presentation of morbidity of different layer farms



Graph 2. Graphical presentation of mortality of different layer farms

4.2 Necropsy findings

In present study, the gross lesions of 17 *Salmonella* infected layer birds were variable that presented in table-3. During necropsy, 53% livers were friable, bronze discoloration with white focal necrosis (Fig. 7). A total of 47.1% livers were congested and enlarged with necrotic foci (Fig. 4). About 59% egg follicles were congested, hemorrhagic and discolored with stalk formation while 41.2% egg follicles were mildly congested and hemorrhagic (Fig. 5). A total of 70.6% intestines were hemorrhagic to catarrhal enteritis while 29.4% only hemorrhagic and congested (Fig. 6). About 64.7% lungs were severely congested and pneumonic while 35.3% lung showed mild congestion. About 47.1% spleens were enlarged and discolored.

Fig. 3: Fragile liver with necrotic foci

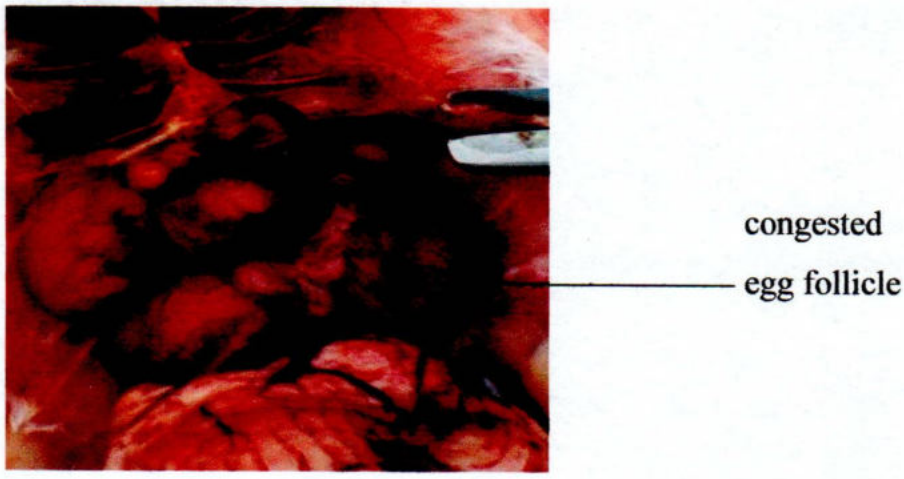


Fig. 4: *Salmonella* affected egg follicles (farm no.8, sample no.20) shows haemorrhagic, congested and discolored with stalk formation

Fig. 5 : Congested intestinal mucosa

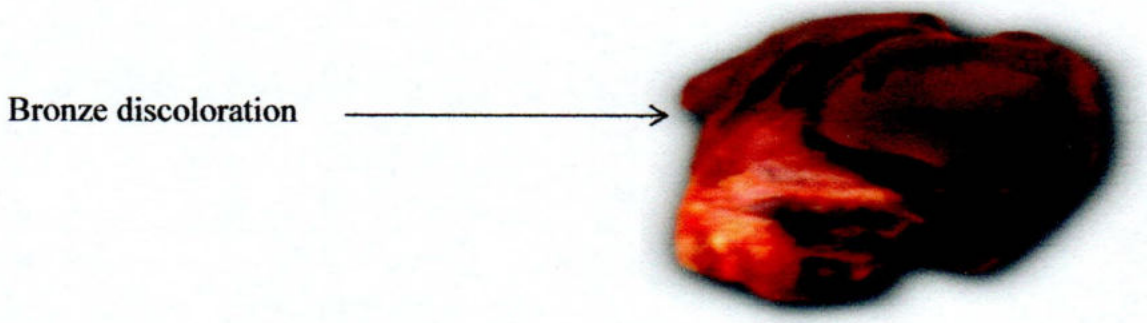


Fig. 6 Friable liver with bronze discoloration

4.3 Histopathology

Only 17 *Salmonella* positive dead bird tissues of different organs were selected for histopathology. Table 4 describes the histopathological findings of different organs. In histopathological investigation, all the tissues of different organs of 17 layer birds did not evoke similar kinds of lesions. A total of 76.5% livers were congested and formed multifocal nodules with coagulation necrosis (Fig. 8) while remaining 23.5% liver showed hepatitis (Fig. 12). Besides, 76.5% lungs were severely congested and hemorrhagic and 23.5% lung showed inflammatory cells in alveoli and bronchi (Fig. 8). Infiltration of heterophils and lymphocytes in the mucosa of intestines were found in 47.1% cases. Sever lymphocytic depletion and focal necrosis in the spleen was found in 53% birds (Fig. 10).

Whereas, about 82.4% egg follicles was markedly congested and showed huge leukocytic infiltration (Fig. 11).

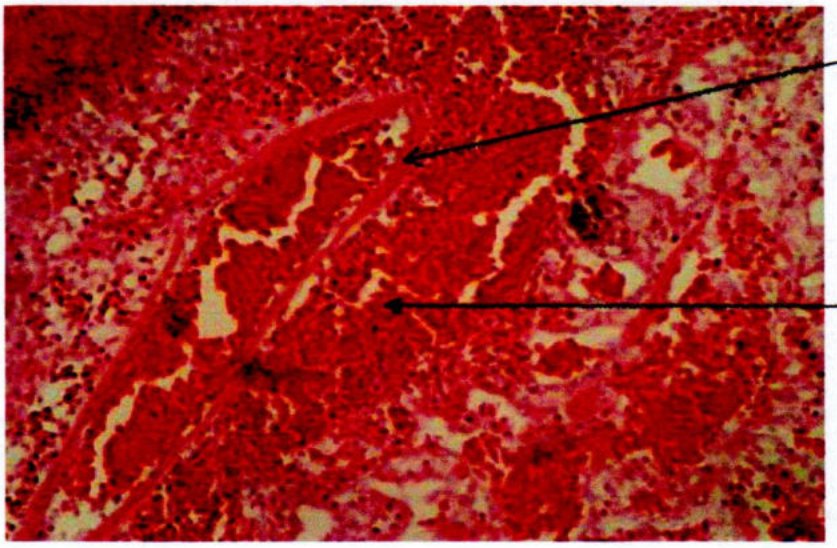


Fig. 7 The section of *Salmonella* infected lung (farm no.1, tissue of sample no.3) showing sever congestion and pneumonia (H &E staining)

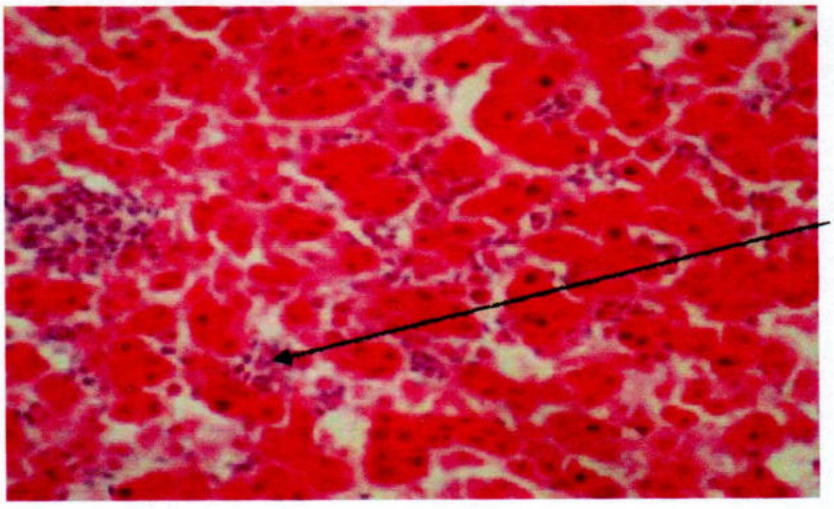


Fig. 8 The section of *Salmonella* infected liver (farm no.8, tissue of sample no.20) showing multifocal nodule formation and infiltration of inflammatory cells (H & E staining)

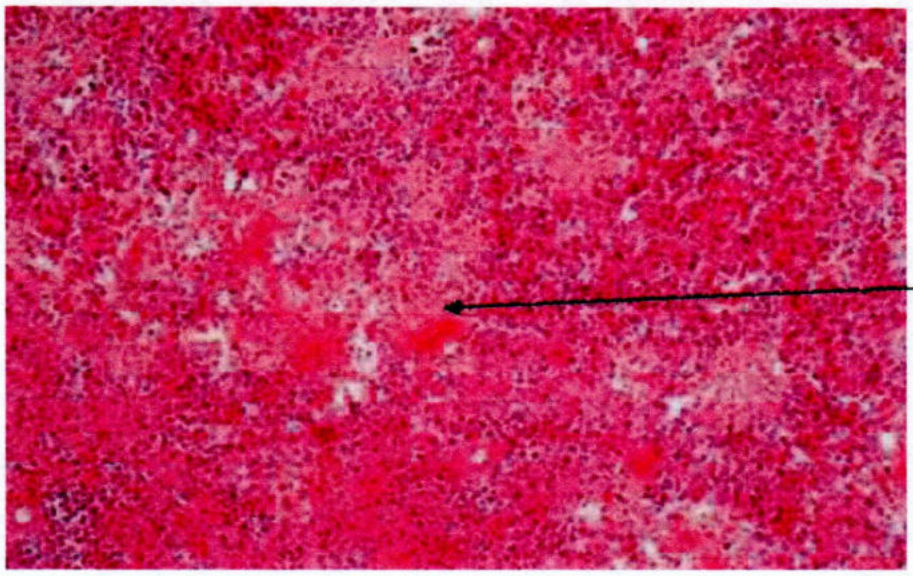


Fig. 9 The section of *Salmonella* infected spleen (farm no.14, tissue of sample no.33) showing sever lymphocytic depletion and marked reticuloendothelial cell hyperplasia (H &E staining)

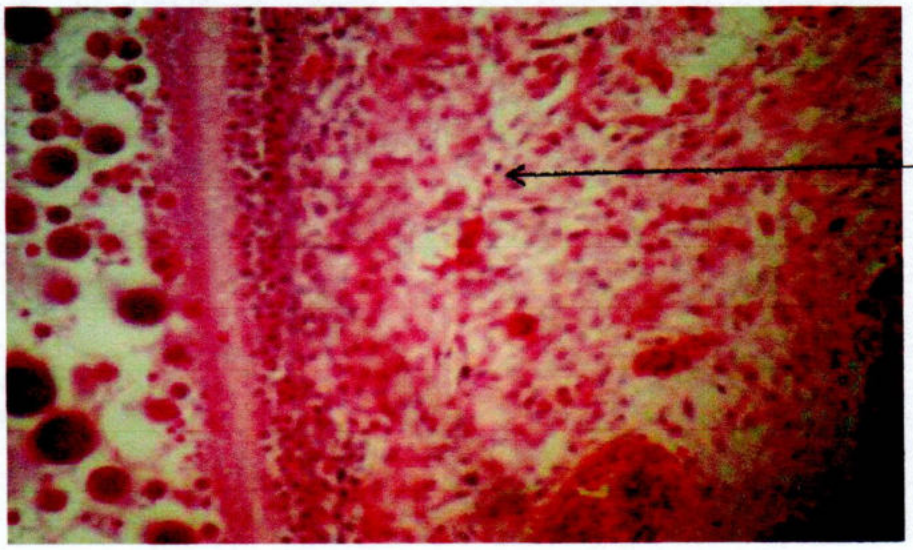


Fig. 10 The section of *Salmonella* infected egg follicles (farm no.2, tissue of sample no.6)showing marked congestion and leukocytic infiltration (H &E staining)

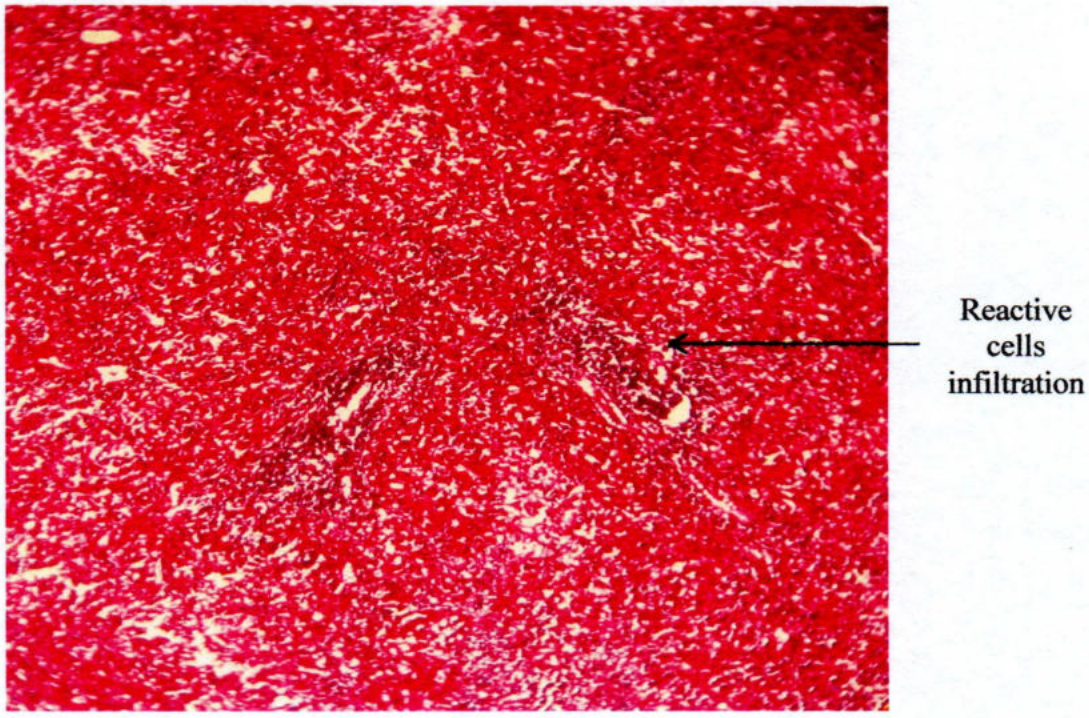


Fig. 11 The section of *Salmonella* infected liver showing infiltration of inflammatory cells (H & E staining)

Table 3. Necropsy findings of *Salmonella* affected birds of different layer farms

Lesions	Infected farm no.									Total N=17	%
	F-1 (n=3)	F-2 (n=2)	F-3 (n=3)	F-6 (n=2)	F-8 (n=1)	F-10 (n=1)	F-12 (n=2)	F-14 (n=1)	F-15 (n=2)		
Friable, bronze discoloration liverwith white focal necrosis	+	+	-	-	+	+	-	-	+	9	53
Congested and enlarged liver	-	-	+	+	-	-	+	+	-	8	47
Congested hemorrhagic, and discolored egg follicles with stalk formation	+	+	-	+	+	+	-	+	-	10	58
Mild congested and haemorrhagic egg follicles	-	-	+	-	-	-	+	-	+	7	41
Haemorrhagic to catarrhal enteritis	+	+	+	-	+	+	-	-	+	12	71
Congested and haemorrhagic intestine	-	-	-	+	-	+	+	+	+	5	29
Severely congested and pneumonic lung	+	+	+	-	-	+	-	-	+	11	65
Mild congested lung	-	-	-	+	+	+	+	+	+	6	35
Enlarged with discolored spleen	+	+	-	-	-	+	-	-	+	8	47

"+"present, "-"absent and "n"= No. of positive bird / farm and F= Farm

Table 4. Histopathological findings of *Salmonella* affected tissues from different farm

Lesions	Infected farm no.									Total N=17	%
	F-1 (n=3)	F-2 (n=2)	F-3 (n=3)	F-6 (n=2)	F-8 (n=1)	F-10 (n=1)	F-12 (n=2)	F-14 (n=1)	F-15 (n=2)		
Congestion and multifocal nodule formation in liver	+	+	+	+	-	+	-	-	+	13	77
Hepatitis and infiltration of inflammatory cells	-	-	-	-	+	-	+	+	-	4	24
Marked congestion and leukocytic infiltration in egg follicles	+	+	+	+	-	+	-	+	+	14	82
Infiltration of heterophils and lymphocyte in the mucosa of intestine	+	+	-	-	+	-	-	-	+	6	47
Severely congested and hemorrhagic lung	+	+	-	+	+	-	+	+	+	13	77
Inflammatory cells in the alveoli and bronchus		-	+	-		+				4	24
Severely lymphocytic depletion and focal necrosis in the spleen	+	+	+	+	+	-	-	+	-	9	53

"+"present, "-"absent and "n"= No. of positive bird / farm and F= Farm

4.4 Bacteriological findings

4.4.1Cultural properties

In the present investigation, all 17 isolates of *Salmonella* organisms showed different cultural characteristics in different media. These were turbidity in TTB broth and isolates showed slightly yellowish white color colonies in BGA, slightly grayish color colonies in SS agar, black color colony in TSI agar, gray white colony in nutrient agar, pinkish in EMB agar and pale color colonies in Mc Conkey's agar. *Salmonella gallinarum* produce defined, opaque, glistening colonies in blood agar media and pale colonies in Mc Conkey agar media. *Salmonella pullorum* produce small discrete, round, transparent glistening colonies in blood agar media. Paratyphoid Salmonellae produced translucent pale pink colonies in BGA media.

Isolation and cultural *Salmonella* characters of *Salmonella* sp in inner content of laid eggs on selective media:

A total 45 laid eggs (5 eggs/farm) samples were collected from *Salmonella* infected 9 layer farms. Out of 9 farms 4 were positive and isolation rate was 44.4%. The results corresponded with the findings of Haider (2009) while the author reported 95% isolation rate of *Salmonella* organism from outer shell, 45% from inner shell, 35% from egg albumin and 50% from egg yolk. It can be concluded that, laid eggs content received contamination by *Salmonella* organism as a vertical transmission of Salmonellosis or/and contamination with the droppings of *Salmonella* infected layer birds.

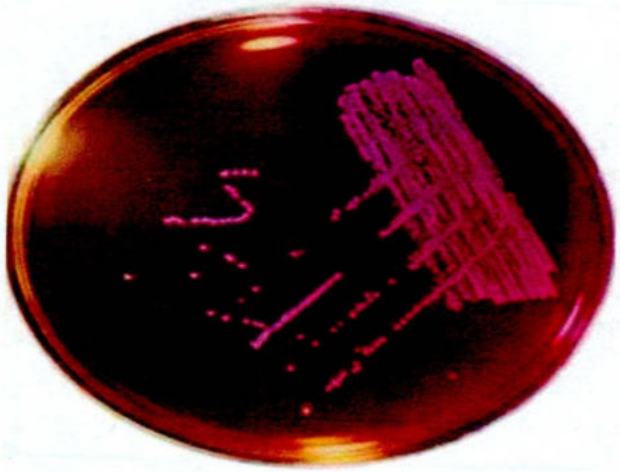


Fig. 12 Appearance of typical *Salmonella* colonies in Brilliant green agar

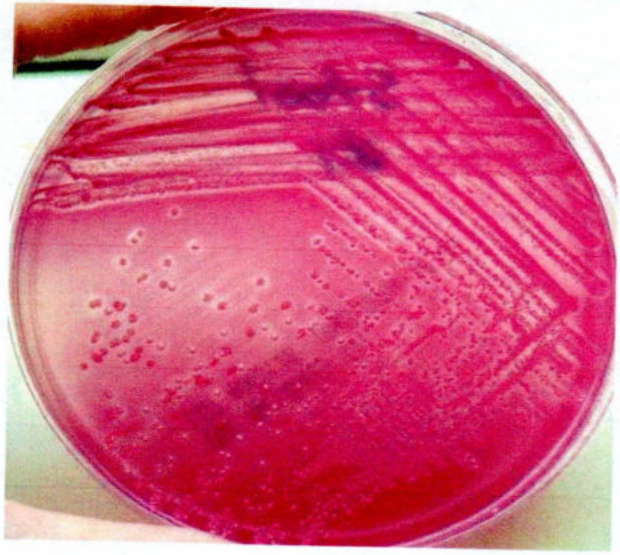


Fig. 13 Appearance of typical *Salmonella* colonies in Mac Conkey agar

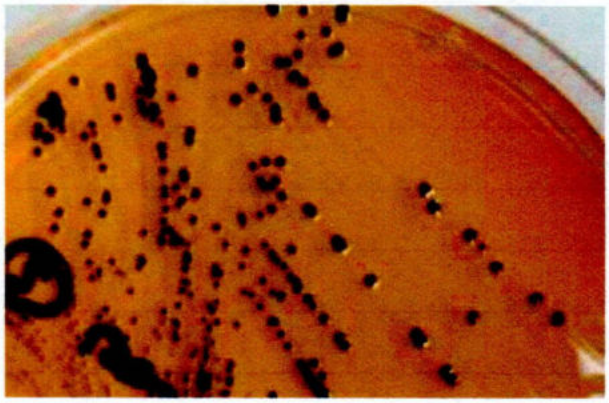


Fig:14 Appearance of typical *Salmonella* colonies in salmonella-shigella (ss) agar

4.4.2Staining character

In Gram's staining, all the isolates in the present investigation revealed gram-negative, rod shaped appearance and arranged in single. Chains of more than two bacilli were normally absent.

4.4.3 Motility test

In motility test, 15 *Salmonella* isolates were identified as non-motile and 2 *Salmonella* were identified as motile and percentage of motile *Salmonella* was 11.8%.

4.4.4 Findings of biochemical tests

In the present investigation, among 17 isolates, 70.6% (12) were *Salmonella gallinarum*, 17.6% (03) were *Salmonella pullorum* and 11.8% (02) isolates were identified as paratyphoid causing *Salmonella*. *Salmonella gallinarum* farment dextrose, maltose, dulcitol without gas but not farment ornithine. *Salmonella pullorum* farment ornithine, dextrose with gas production but not farment maltose and dulcitol. Paratyphoid *Salmonellae* can farment malonate but not farment dulcitol.

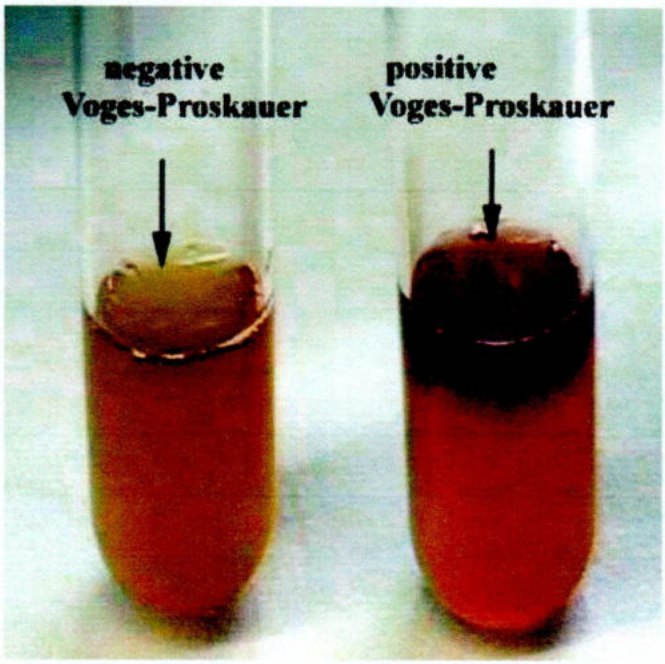


Fig. 15 Voges- Proskauer test

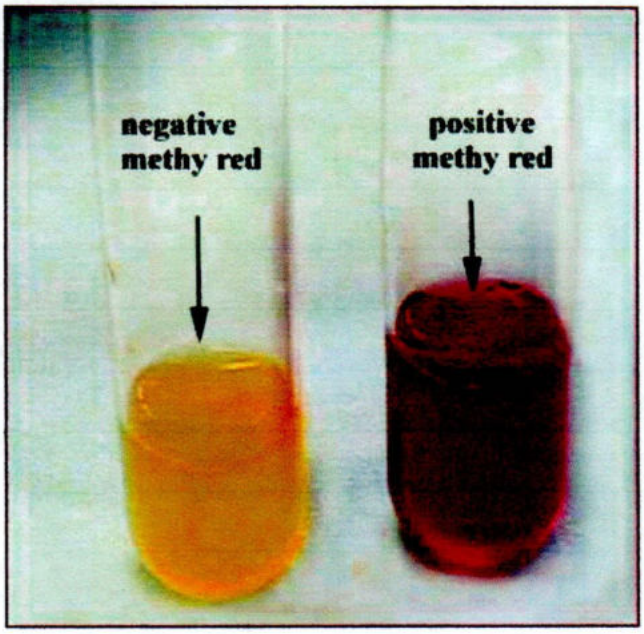


Fig. 16 Methyl red test

CHAPTER V

DISCUSSION

CHAPTER V

DISCUSSION

In the present investigation, observation of gross pathological lesion and histopathological lesion of different structures and isolation and identification of *salmonella* organisms of layer chicken. Similarities and dissimilarities has been observed in the different organs of layer chicken in comparison to other previous investigation related to my investigation and the results were discussed very briefly under the following sub-heading.

5.1Clinical findings

In the present study the following clinical findings were observed; fowl typhoid is characterized by whitish materials to the vent, ruffled feathers, depression, anorexia, loss of egg production. Pullorum disease is characterized by adherence of chalky white material to the vent, droopy wings, gasping. Paratyphoid infection is characterized by profuse watery diarrhea, pasting of the vent area, reluctant to move, ruffled feathers and finally death of affected bird. The percentage of morbidity was highest in the farm No. 15 (40 %) and the lowest was farm 1 (17 %). The percentage of mortality was highest in the farm No. 15 (13%) and lowest was farm No. 1 (5 %). Similar findings were described by many investigators (Chishti *et al.*, 1985; Khan *et al.*, 1998; Hafeji *et al.*, 2001; Habib-ur-Rehman *et al.*, 2003; Beyaz and Kutsal, 2003; Goswami *et al.*, 2003; Hossain *et al.*, 2003).

5.2 Necropsy findings

In present investigation, the gross lesions of 17 *Salmonella* infected layer birds were variable. During necropsy, 53% livers were friable, bronze discoloration with white focal necrosis (Fig. 7). A total of 47% livers were congested and enlarged with necrotic foci (Fig. 4). About 58% egg follicles were congested, hemorrhagic and discolored with stalk formation while 41% egg follicles were mild congested and hemorrhagic (Table 3; Fig. 5). A total of 71% intestines were

hemorrhagic to catarrhal enteritis while 29% only hemorrhagic and congested (Fig. 6). About 65% lungs were severely congested and pneumonic while 35% lung showed mild congestion. About 47% spleens were enlarged and discolored. Similar findings with intensity of the severity of lesions in different organs were described by many investigators (Chishti *et al.*, 1985; Khan *et al.*, 1998; Hafeji *et al.*, 2001; Habib-ur-Rehman *et al.*, 2003; Beyaz and Kutsal, 2003; Goswami *et al.*, 2003; Hossain *et al.*, 2003; Prasanna and Paliwal, 2003; Islam *et al.*, 2006; Deshmukh *et al.*, 2007).

5.3 Histopathology:

Only 17 *Salmonella* positive dead bird tissues of different organs were selected for histopathology. Table 4 describes the histopathological findings of different organs. In histopathological investigation, all the tissues of different organs of 17 layer birds did not evoke similar kinds of lesions. A total of 76.5% livers were congested and formed multifocal nodules with coagulation necrosis (Fig. 8) while remaining 23.5% liver showed hepatitis (Fig. 12). Besides, 76.5% lungs were severely congested and hemorrhagic and 23.5% lung showed inflammatory cells in alveoli and bronchi (Fig. 8). Infiltration of heterophils and lymphocytes in the mucosa of intestines were found in 47.1% cases. Sever lymphocytic depletion and focal necrosis in the spleen was found in 53% birds (Fig. 10). Whereas, about 82.4% egg follicles was markedly congested and showed huge leukocytic infiltration (Fig. 11). The microscopic lesions recorded in the present investigation were almost similar to the lesions described by other authors (Chishti *et al.*, 1985; Calnek *et al.*, 1991; Khan *et al.*, 1998; Kinde *et al.*, 2000; Hafeji *et al.*, 2001; Prasanna and Paliwal, 2003; Holt *et al.*, 2006; Msoffe *et al.*, 2006).

5. 4 Bacteriological examination

5.4.1Cultural properties

In the present analysis, all 17 isolates of *Salmonella* organisms showed different cultural characteristics in different media. These were turbidity in TTB broth and isolates showed slightly yellowish white color colonies in BGA, slightly grayish color colonies in SS agar, black color colony in TSI agar, gray white colony in nutrient agar, pinkish in EMB agar and pale color colonies in Mac Conkey's agar. These findings of present investigation corresponded with the results of Old (1990); Yuno *et al.* (1995); Sharma and Katok (1996); Sujatha *et al.* (2003); Perez *et al.* (2004); Rybolt *et al.* (2005) and Ahmed *et al.* (2008).

5.4.2 Identification (Gram's staining)

In Gram's staining, all the isolates in the present investigation revealed gram-negative, rod shaped appearance and arranged in single. Chains of more than two bacilli were normally absent. Freeman (1985) described the morphology of the isolated *Salmonella* bacteria as small rod shaped, gram negative, single or paired in arrangement by Gram's staining which supported the findings of the present investigation. Similar morphological findings of *Salmonella* were also supported by other investigators (Islam *et al.*, 2006; Ahmed *et al.*, 2008).

5.4.3 Motility test

In motility test, 15 *Salmonella* isolates were identified as non-motile and 2 *Salmonella* were identified as motile and percentage of motile *Salmonella* was 11.8%. These results were similar to Christensen *et al.* (1996) and they found 11.69% motile *Salmonella*. On the other hand, there was small variation with the results of Buxton and Fraser (1977) which may be due to the difference in managemental condition of farms. The results of the present investigation were also supported by other investigators (Shane, 1989; Pomeroy and Nagaraja, 1991;

Islam *et al.,* 2006; Ahmed *et al.,* 2008; who found 13.71%, 13.5%, 12.82%, and 13.5% motile *Salmonella*, respectively).

5.4.4 Different biochemical tests

In the present investigation, among 17 isolates, 70.6% (12) were *Salmonella* Gallinarum, 17.6% (03) were *Salmonella* Pullorum and 11.8% (02) isolates were identified as paratyphoid causing *Salmonella*. The findings of present investigation corresponded with Buxton and Fraser (1977); Shane (1989); Pomeroy and Nagaraja (1991) and Christensen *et al.* (1996).

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The gross lesions of 17 *Salmonella* infected layer birds were variable. During necropsy, 53% livers were friable, bronze discoloration with white focal necrosis. A total of 47.1% livers were congested and enlarged. About 58% egg follicles were congested, hemorrhagic and discolored with stalk formation while 41.2% egg follicles were mildly congested and hemorrhagic. A total of 70.6% intestines were hemorrhagic to catarrhal enteritis while 29.4% only hemorrhagic and congested. About 64.7% lungs were severely congested and pneumonic while 35.3% lung showed mild congestion. About 47.1% spleens were enlarged and discolored. In histopathological investigation, all the tissues of different organs of 17 layer birds did not evoke similar kinds of lesions. A total of 76.5% livers were congested and formed multifocal nodules with coagulation necrosis while remaining 23.5% liver showed hepatitis. A total 45 laid eggs (5 eggs/farm) samples were collected from *Salmonella* infected 9 layer farms. Out of 9 farms 4 were positive and isolation rate was 44.4%. In the present study, 4 *Salmonella* isolates were found from laid eggs (5 eggs/farm) content from respective 9 *Salmonella* infected farms and isolation rate was 44.4%. Histologically, 76.5% liver, 82.4% egg follicles and 47.1% intestines revealed tissue changes with variation in birds. *Salmonella* isolation rate was 44.4% in laid eggs of *Salmonella*-affected farms indicating transovarian transmission in poultry Salmonellosis.

From the above findings, it may be concluded that Salmonellosis has emerged as one of the most serious problems having adverse effects on poultry and human being (due to zoonotic impact). So the farmer should conscious during buying the day old chick, so that they should collect day old chick from those breeder farm in where salmonella vaccination schedule properly followed and maintained. Strong bio-security, hygienic

management both feed and water, proper vaccination, medication and finally the awareness of farmer can control avian salmonellosis. In future for the control of *Salmonella* infection in poultry, research should be conducted to develop specific vaccine against specific salmonella strain by using local isolate, prevalence and gene level study need to be performed in Bangladesh to save the poultry industry.

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