

Salmonellosis in layer chickens: pathological features and isolation of bacteria from ovaries and inner content of laid eggs

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Abstract

We isolated and identified *Salmonella* organisms from ovaries of dead layer birds and from inner content of laid eggs of different poultry farms. The thirty eight ovarian swabs for bacteriology, visceral organs (liver, lung, spleen, egg follicles and intestine) of 38 dead birds for pathological study from 15 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. Seventeen 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. Four *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 Salmonellosis.

Keywords: Histopathology, Laid eggs, Layer, Salmonellosis

Introduction

Poultry is essential to the national economy of Bangladesh and the welfare of human beings. 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 isolate and identify *Salmonella* organism from ovaries of dead layer birds and inner content of laid eggs of different poultry farms at Mymensingh district as well as to study the pathology of different organs of *Salmonella* infected layer birds.

Materials and Methods

Experimental area: The study was conducted during the period from January to August 2010 at Mymensingh district. A total of 15 layer farms which contained 29,800 birds with no history of using *Salmonella* vaccine were selected for the study.

Isolation and identification of *Salmonella* organism 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 (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 (Haider *et al.*, 2003).

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

Carbohydrate fermentation test and biochemical test: Five basic sugars such as glucose, sucrose, lactose, mannitol and maltose were used for sugar fermentation test.

Biochemical test: It included MR test, VP test, indole test and dulcitol fermentation test (Merchant and Packer, 1967; 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 (Haider *et al.*, 2003).

Pathological studies: During the whole period of study, postmortem examinations of 38 dead birds were performed from the representative selected 15 layer farms. Gross pathological changes at necropsy were carefully observed and recorded. The tissues of different organs were preserved in 10% neutral buffered formalin for histopathological studies. The tissues were trimmed with 1.5 X 1 cm size, washed overnight in running tap water, dehydration in ascending grades of alcohol, cleaning in chloroform, blocking in paraffin and sectioned with microtome at 6 micron thickness. The sections were stained with routine hematoxylin and eosin staining (Luna, 1968) and finally the sections were studied with light microscope.

Isolation of *Salmonella* sp 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 onto selective agar media (BGA, SS, TSI & EMB agar).

Results and Discussion

Cultural prevalence with isolation and identification of *Salmonella* organism

In the present study, 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. These findings of present study 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).

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 study. Similar morphological findings of *Salmonella* were also supported by other investigators (Islam *et al.*, 2006; Ahmed *et al.*, 2008).

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 study 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).

Carbohydrate fermentation and 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 study corresponded with Buxton and Fraser (1977); Shane (1989); Pomeroy and Nagaraja (1991) and Christensen *et al.* (1996).

Pathological studies

Gross pathology: In present study, the gross lesions of 17 *Salmonella* infected layer birds were variable. During necropsy, 53% livers were friable, bronze discoloration with white focal necrosis (Fig. 1). A total of 47.1% livers were congested and enlarged. About 59% egg follicles were congested, hemorrhagic and discolored with stalk formation while 41.2% egg follicles were mildly congested and hemorrhagic (Table 1; Fig. 2). 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. 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).

Histopathology: Only 17 *Salmonella* positive dead bird tissues of different organs were selected for histopathology. Table 2 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 while remaining 23.5% liver showed hepatitis (Fig. 4). Besides, 76.5% lungs were severely congested and hemorrhagic and 23.5% lung showed inflammatory cells in alveoli and bronchi (Fig. 3). 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. 5). Whereas, about 82.4% egg follicles was markedly congested and showed huge leukocytic infiltration (Fig. 6). 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).

Table 1. Gross pathological 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-5 (n=3)	F-6 (n=2)	F-8 (n=1)	F-10 (n=1)	F-11 (n=2)	F-13 (n=1)	F-14 (n=2)		
Friable, bronze discoloration liver with white focal necrosis	+	+	-	-	+	+	-	-	+	9	53.0
Congested and enlarged liver	-	-	+	+	-	-	+	+	-	8	47.1
Congested haemorrhagic, and discolored egg follicles with stalk formation	+	+	-	+	+	+	-	+	-	10	59.0
Mild congested and haemorrhagic egg follicles	-	-	+	-	-	-	+	-	+	7	41.2
Haemorrhagic to catarrhal enteritis	+	+	+	-	+	+	-	-	+	12	70.6
Congested and haemorrhagic intestine	-	-	-	+	-	-	+	+	-	5	29.4
Severely congested and pneumonic lung	+	+	+	-	-	+	-	-	+	11	64.7
Mild congested lung	-	-	-	+	+	-	+	+	-	6	35.3
Enlarged with discolored spleen	+	+	-	-	-	+	-	-	+	8	47.1

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

Table 2. 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-5 (n=3)	F-6 (n=2)	F-8 (n=1)	F-10 (n=1)	F-11 (n=2)	F-13 (n=1)	F-14 (n=2)		
Congestion and multifocal nodule formation in liver	+	+	+	+	-	+	-	-	+	13	76.5
Hepatitis and infiltration of inflammatory cells	-	-	-	-	+	-	+	+	-	4	23.5
Marked congestion and leukocytic infiltration in egg follicles	+	+	+	+	-	+	-	+	+	14	82.4
Infiltration of heterophils and lymphocyte in the mucosa of intestine	+	+	-	-	+	-	-	-	+	8	47.1
Severely congested and hemorrhagic lung	+	+	-	+	+	-	+	+	+	13	76.5
Inflammatory cells in the alveoli and bronchus			+			+				4	23.5
Severely lymphocytic depletion and focal necrosis in the spleen	+	+	-	+	+	-	-	+	-	9	53.0

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

Isolation and cultural 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%. 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%. 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. 1. The liver (farm no.10, sample no. 25) shows friable congestion and bronze discoloration with focal necrosis



Fig. 2. *Salmonella* affected egg follicles (farm no.8, sample no.20) shows haemorrhagic, congested and discolored with stalk formation

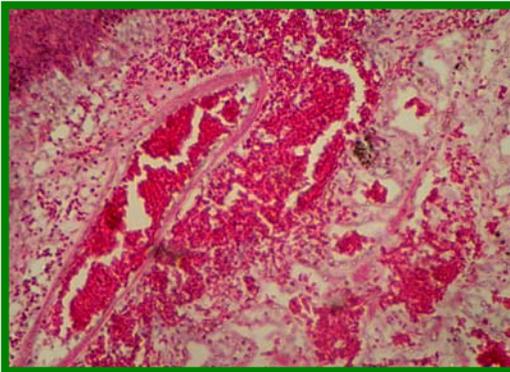


Fig. 3. *Salmonella* infected lung (farm no.1, tissue of sample no.3) shows severe congestion and pneumonia (H & E staining X 83)

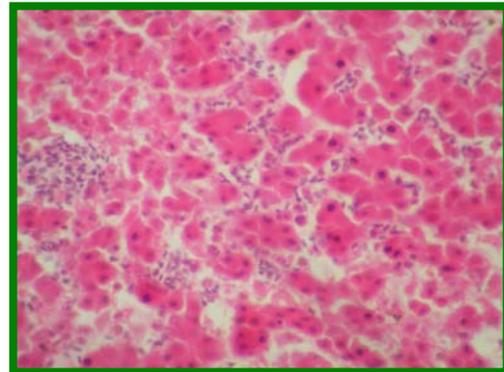


Fig. 4. *Salmonella* infected liver (farm no.8, tissue of sample no.20) shows multifocal nodule formation and infiltration of inflammatory cells (H & E staining X 333)

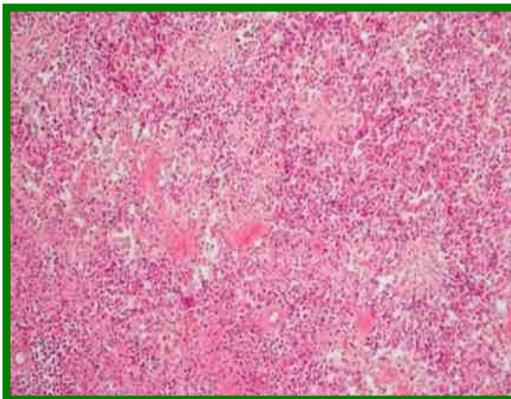


Fig. 5. *Salmonella* infected spleen (farm no.13, tissue of sample no.33) shows severe lymphocytic depletion and marked reticuloendothelial cell hyperplasia (H & E staining X 83)

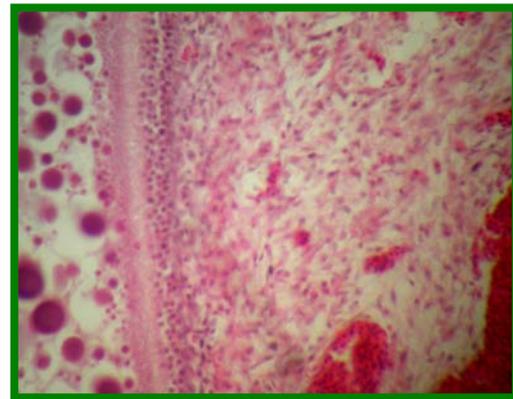


Fig. 6. *Salmonella* infected egg follicles (farm no.2, tissue of sample no.6) shows marked congestion and leukocytic infiltration (H & E staining X 83)

Briefly, in the present investigation, 44.7% *Salmonella* positive cases were recorded from ovarian swabs in which, *Salmonella* Gallinarum, *Salmonella* Pullorum and Paratyphoid causing *Salmonella* were 70.6%, 17.6% and 11.8%, respectively. Variable gross lesions were found in *Salmonella* affected organs of birds. A total of 53% liver, 59% egg follicles and 70.6% intestine were affected in this study. 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. In future for the control of *Salmonella* infection in poultry, vaccine production and gene level study need to be performed in Bangladesh to save the poultry industry.

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