

SEROPREVALENCE AND PATHOLOGICAL STUDY OF SALMONELLA INFECTIONS IN LAYER CHICKENS AND ISOLATION AND IDENTIFICATION OF CAUSAL AGENTS

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ABSTRACT

The present research work was conducted to study about the seroprevalence and pathology of Salmonella infections in layer chickens of Dhaka and Gazipur regions of Bangladesh and to isolate and characterize Salmonellae from layer chickens during the period from January to May 2006. The used materials were blood sample, cloacal and liver swabs from live and dead birds respectively, and visceral organs (liver, lungs, spleen and intestine). The used methods were serum plate agglutination (SPA) test; necropsy and histopathology; cultural, morphological and biochemical test. The overall seroprevalence was 43.4%. During necropsy, congested and enlarged liver with focal necrosis, haemorrhagic and discoloured ovary with stalk formation and mild haemorrhagic to catarrhal enteritis in intestine were detected. Microscopically, the liver showed congestion, focal necrosis with multifocal infiltration of histiocytes in liver parenchyma. In ovum, infiltration of leukocytes, bacterial colony, RE cell proliferation and fibrinoid necrosis were recorded. The intestinal mucosa exhibited infiltration of mononuclear cells in mucosa, submucosa as well as muscularis mucosa. A total of 33 (21.02%) Salmonellae from live and dead birds were isolated. The isolation rate of Salmonellae was higher in seronegative (31.6%) group than seropositive (3.2%) group. Out of 33 Salmonella isolates, 25 were *S. pullorum*, 3 were *S. gallinarum* and the rest 5 were motile Salmonellae. The isolates obtained in the study may be investigated for serotyping, pathogenesis study, antibiogram and vaccine production in future.

Key words: Seroprevalence, histopathology, isolation, identification, Salmonella, chickens

INTRODUCTION

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.*, 1979). In Bangladesh, the Salmonella infections in chicks and layer chickens must be evaluated for effective control measures of the diseases. Therefore, the present study was undertaken to determine the seroprevalence and pathological study of Salmonella infections in layer chickens and to isolate and characterize Salmonellae from those chickens.

MATERIALS AND METHODS

The present research work was undertaken in the Department of Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh. Samples were collected from different layer farms of Dhaka and Gazipur regions of Bangladesh in the period of January to May 2006.

Seroprevalence study

Sample collection and preparation

A total of 362 blood samples (Table 1) were collected from layer chickens of Dhaka and Gazipur regions and where no Salmonella vaccine was used previously. Blood of about 1 ml from each bird was collected aseptically, allowed for clotting in the syringe and then sera were separated and centrifuged (where necessary) at 1000 rpm for 10 minutes for clarification. During the study according to age and status of growth, the birds were divided into 4 groups: starter (up to 8 wks), grower/pre-layer (9-20 wks), layer (21-60 wks) and post-layer (> 60 wks).

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Serum plate agglutination (SPA) test

Standard *Salmonella pullorum* (Nobilis® SP, Intervet International, Holland) antigen was used for SPA test. Antigen of 0.02 ml and chicken sera of 0.02 ml were placed side by side with a micropipette on ceramic tiles and mixed thoroughly by stirring with tooth pick followed by rocking. Results of positive reaction (clumping) on mixing (Plate 1) were read within 2 minutes.

Pathological study

Gross pathology

A total number of 37 dead layer chickens was examined to detect *Salmonella* infections. At necropsy, gross tissue changes were observed and recorded carefully and representative tissue samples (liver, lungs, spleen & intestine) containing lesions were preserved in 10 % buffered formalin for histopathological studies.

Histopathology

The tissues were trimmed, washed, processed in ascending grades of alcohol, cleared in chloroform, embedded in paraffin, sectioned using a microtome and stained with hematoxylin and eosin (H&E) stain as per standard procedure (Luna, 1968). Photomicrography was taken using photomicrographic camera (Olympus PM-C 35 Model).

Isolation and identification of Salmonellae

Collection of samples

A total of 120 cloacal swabs (63 from seronegative and 57 from seropositive birds) were collected after seroprevalence study. Along with these 120 samples, 37 liver swabs were collected from the birds at necropsy.

Culture

All the samples were collected on tetrathionate broth. Then subculture was performed on different selective and enriched media of *Salmonella* such as *Salmonella*-*Shigella* (SS) agar, lysine iron agar (LIA), triple sugar iron (TSI) agar, and brilliant green agar (BGA) to get pure culture and to study cultural characteristics (Cown, 1985; Merchant and Packer, 1967; OIE, 2004).

Morphological characterization

The representative *Salmonellae* colonies on BGA were characterized microscopically using Gram's stain according to the method described by Merchant and Packer (1967). The motility test was performed according to the method described by Cown (1985) to differentiate motile *Salmonellae* from the non-motile.

Biochemical test

Five basic sugars such as dextrose, sucrose, lactose, maltose, and mannitol were used for sugar fermentation test. Several types of biochemical tests such as TSI agar slant reaction, methyl red (MR) test, Voges Proskauer (VP) test, indole test and dulcitol fermentation test were also performed in this study according to the methods described by Cown (1985), Merchant and Packer (1967) and OIE (2004).

RESULTS AND DISCUSSION

Seroprevalence study

In this study, the overall seroprevalence of *Salmonellosis* was recorded as 43.4% (Table 1). Yang *et al.* (1996) reported relatively similar findings (39.02%). Ashenafi *et al.* (2003) and Habib-ur-Rahman *et al.* (2003) reported 64.2% and 63.5 % respectively, that was much higher than that of the present study. The variation of seroprevalence might be speculated due to geographical variation or difference of management. But the present finding (43.4%) in commercial farms was higher than the seroprevalence (23.46%) recorded by Sikder *et al.* (2005) in local chickens. The difference with Sikder *et al.* (2005) was corresponded with the findings of Jha *et al.* (1995) and Robinson *et al.* (2000), who recorded seroprevalence rate higher in commercial flock than local chickens.

Table 1. Seroprevalence of *Salmonella* infections in different groups of chickens

Groups	No. of sera samples tested	No. of seropositive samples	Seroprevalence
Starter (up to 8 wks)	024	001	04.2%
Grower/pre-layer (9 - 20 wks)	094	018	19.1%
Layer (21 - 60 wks)	196	103	52.6%
Post-layer (> 60 wks)	048	035	72.9%
Total	362	157	43.4%

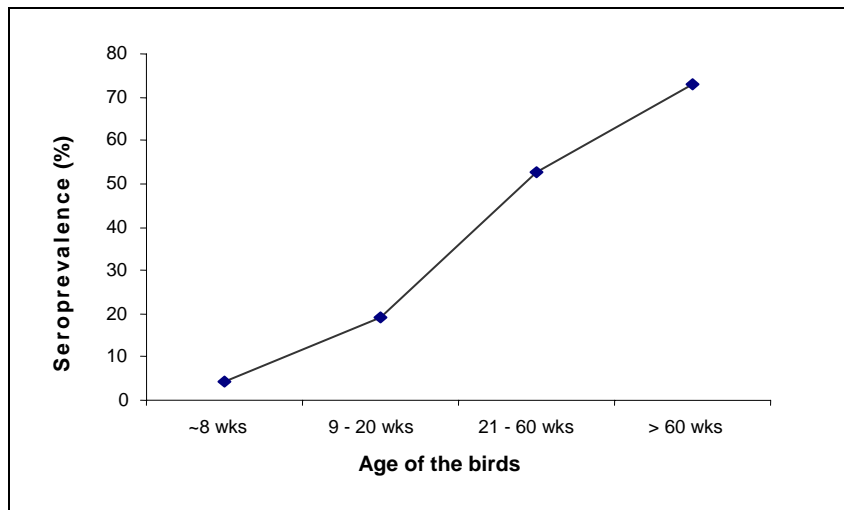


Fig. 1. Seroprevalence of *Salmonella* infections in terms of age of the birds

It was found that seroprevalence increased with the increase of age of the birds (Table 1 and Fig.1). This findings corresponded with the reports of Sikder *et al.* (2005) and Truong and Tieuquang (2003).

Pathological study

Gross pathology

Enlarged and congested liver with focal necrosis; haemorrhagic and discoloured ovary with stalk formation (Plate 2) and mild haemorrhagic to catarrhal enteritis in intestine and caecum were recorded during necropsy. These types of necropsy findings were supported by Calnek *et al.* (1997), Chauhan and Roy (1996), Syed-Habib-ur-Rahman *et al.* (2004), Hossain *et al.* (2003) and Khan *et al.* (1998).

Histopathology

Microscopically, the liver showed congestion, focal necrosis with multifocal infiltration of histiocytes in liver parenchyma (Plate 3). In ovum, infiltration of leukocyte, bacterial colony, RE cell proliferation and fibrinoid necrosis were recorded (Plate 4). The spleen showed severe congestion, mild hyperplasia of RE cells and fibrinoid necrosis. The intestinal and caecal mucosa exhibited infiltration of mononuclear cells in the mucosa and submucosa (Plate 5). These types of histological lesions were supported for *Salmonella* infection by different investigators (Calnek *et al.*, 1997; Chauhan and Roy, 1996; Syed-Habib-ur-Rahman *et al.*, 2004; Refsum *et al.*, 2002).

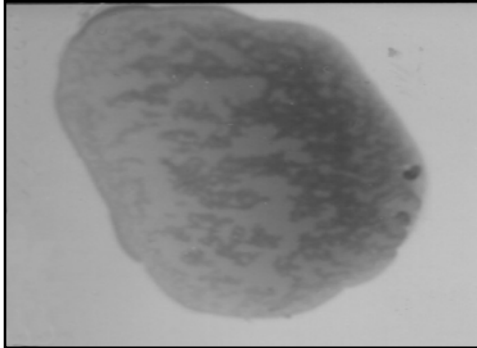


Plate 1. Serum plate agglutination test (positive reaction).

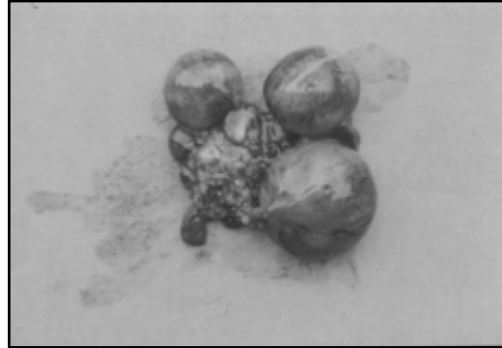


Plate 2. Ovarian follicles of Salmonella infected chicken showing haemorrhagic lesion, discolouration and stalk (peduncle) formation.

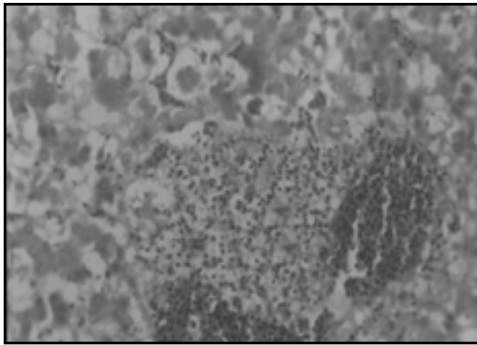


Plate 3. Histopathology of Salmonella infected liver showing congested blood vessel, infiltration of heterophils, lymphocyte and histiocytes around the blood vessel (H & E stain, X 184).

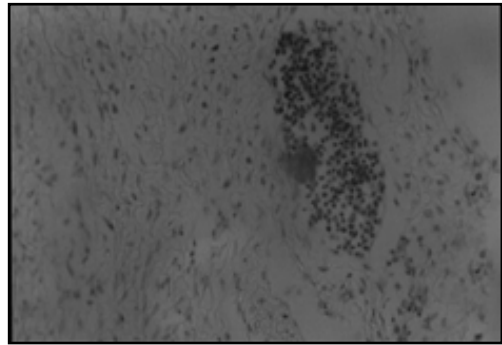


Plate 4. Histopathology of Salmonella infected ovary showing infiltration of leukocyte, congestion with pink colour bacterial colonies in blood vessel (H & E stain, X 184).

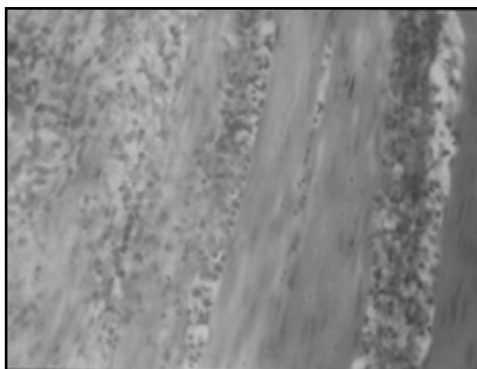


Plate 5. Histopathology of Salmonella infected caecum shows severe infiltration of heterophils, lymphocytes and plasma cells in mucosa and submucosa as well as in muscularis mucosa (H & E stain, X 186).

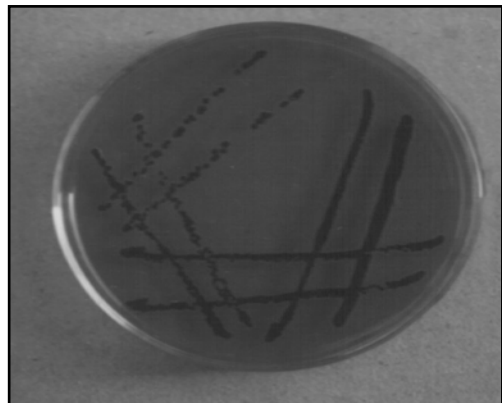


Plate 6. Culture of Salmonellae in TSI agar shows black colour colonies.

Isolation and characterization of Salmonellae

The colony characters of Salmonellae, in SS agar was whitish or slight grayish colonies with dark central spot reflecting production of hydrogen sulfide on the media; in LIA, slight blackish colour colonies; in TSI agar, black colour colonies (Plate 6) and in BGA, pink white colour colonies which corresponds with Perez *et al.* (2004), Sharma and Katok (1996), Yuno *et al.* (1995) and Old (1990). On the basis of colony characters, from 120 cloacal swabs and 37 liver swabs, 33 were identified as Salmonella spp (Table 2). In Gram's staining, the morphology of the isolated bacteria was small rod shape, gram negative, single or paired in arrangement which is supported by Freeman (1985).

Table 2. Salmonella isolated from live and dead birds

Source of sample	No. of sample	No. of isolates	Prevalence
Live bird (Cloacal swab)	120	20	16.7%
Dead bird (liver swab)	37	13	35.1%
Total	157	33	21.02%

The overall cultural prevalence in live and dead birds was recorded 21.02% (Table 2). The cultural prevalence in live birds was 16.7%, whereas in dead birds, it was 35.1%. It should be noted that the live birds were not clinically diseased; on the other hand the dead birds were due to case fatality. The higher cultural prevalence in dead bird than live bird was related with the report of Haider *et al.* (2003).

A total of 18 isolates (31.6%) was found from 57 seronegative birds and 2 (3.2%) from 63 seropositive birds (Table 3). The rate of isolation from seropositive birds was very lower than that of the seronegative birds. Higher rate of isolation from seronegative birds than seropositive birds were also found by Lee *et al.* (2001) and Hoque *et al.* (1996).

Table 3. Correlation between seroprevalence and Salmonella isolates

Agglutination test result group	Cloacal swab collected	Salmonella isolated
Seronegative	57	18 (31.6%)
Seropositive	63	2 (3.2%)
Total	120	20 (16.7%)

Table 4. Biochemical activities of different isolates of Salmonellae

Carbohydrate fermentation test					Indole	MR	VP	Motility	Dulcitol	Name of the isolated bacteria	No. of the isolated bacteria
Glu	Man	Lac	Suc	Mal							
A	AG	-	-	-	-	+	-	-	-	<i>S. pullorum</i>	25
AG	A	-	-	A	-	+	-	-	AG	<i>S. gallinarum</i>	3
AG	AG	-	-	AG	-	+	-	+	AG	Other motile salmonellae	5

Glu = Glucose, Man = Mannitol, Lac = Lactose, Suc = Sucrose, Mal = Maltose, MR = Methyl red test, VP = Voges-proskauer test, AG = Acid and Gas, A=Acid, + = Positive, - = Negative.

Motility test is fundamental basis for the detection of motile and non-motile Salmonellae. Non-motile organisms were considered to be either *S. pullorum* or *S. gallinarum*. The motile organisms were considered as other species of Salmonella (OIE, 2004; Calnek *et al.*, 1997; Chauhan and Roy, 1996). In the present study, 28 isolates were non-motile and 5 were motile (Table 4).

The ability or inability of Salmonellae to ferment different carbohydrates are used as fundamental basis for their identification (Freeman, 1985). In the present study, all isolates fermented glucose and mannitol but did not ferment lactose and sucrose. Some Salmonellae fermented maltose, some did not. All of the isolates were MR positive but VP and indole negative (Table 4). These findings were previously suggested by a number of scientists (Merchant and Packer, 1967; Buxton and Fraser, 1977; Sujatha *et al.*, 2003; Khan *et al.*, 1998).

The dulcitol fermentation test is performed to confirm the differentiation of *S. pullorum* and *S. gallinarum* (Robinson *et al.*, 2000). In the present study, 3 out of 28 isolated nonmotile Salmonellae, fermented dulcitol (Table 4). On the basis of this test, these three isolated bacteria were grouped into *Salmonella gallinarum* (OIE, 2004). Other 25 nonmotile isolates, produced acid and gas in glucose and mannitol but did not ferment maltose (or fermentation occurred in later stage) and dulcitol, so considered as *S. pullorum* (Shivaprasad, 1997 and OIE Manual, 2004). Rest five isolates were motile and fermented glucose, maltose, mannitol and dulcitol. So these motile Salmonellae were identified as paratyphoid Salmonellae. Among the total (33) isolates, 3 (9.1%) were *Salmonella gallinarum*, 25 (75.8%) were *Salmonella pullorum* and other 5 (15.2%) were paratyphoid Salmonella. These findings of isolation were also supported by Girao *et al.* (1985), Hoque *et al.* (1996).

However, for useful application of the present research findings further studies should be focused on serotyping and molecular characterization and identification of immunogenic variation and development of vaccine with isolated Salmonellae.

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