

Seroprevalence and pathology of naturally infected Salmonellosis in poultry with isolation and identification of causal agents

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Abstract

The present study was conducted to determine the seroprevalence of *Salmonella* infections in poultry. This study covered cultural prevalence with isolation and identification of the causal agent and pathological lesions in different organs produced by *Salmonella* in layer farms of Mymensingh district during July-December 2007. The materials were blood samples, liver and cloacal swabs from live and dead birds. The used methods were whole blood agglutination test by commercially available *Salmonella* antigen kit, culture in different media, Gram's staining, motility test, basic 5 sugar fermentation test and histopathology. The overall seroprevalence was 45.9% in live bird and the rate of seroprevalence decreased with advancement of age of birds. The cultural prevalence in seropositive group was 71% and in seronegative group was 59%. In dead bird, the cultural prevalence in liver was 64% and from cloaca 57%. A total 160 isolates were characterized, among them 64.2% were *Salmonella* Pullorum, 22.3% were *Salmonella* Gallinarum and 13.5% were Paratyphoid group of *Salmonellae*. Pathologically friable, bronze color liver with focal necrosis, various grades of enteritis, and hemorrhagic and congested egg follicles with stalk formation were found. Microscopically, focal necrosis and degeneration with leukocytic infiltration in liver, inflammatory cells in the mucosa and submucosa of intestine were found. In egg follicles, congestion and hemorrhages with leukocytic infiltration were found. The isolated *Salmonella* organisms may be used for further research such as serotyping, vaccine production and antibiotic sensitivity test.

Keywords: Salmonellosis, Seroprevalence, Agglutination test, Histopathology, Poultry

Introduction

There are several constraints in the development of poultry industry in Bangladesh (Das *et al.*, 2005). Infections with bacteria of the genus *Salmonella* are responsible for major problem of poultry farming in Bangladesh (Kamaruddin and Giasuddin, 2003). In recent days, the prevalence of Salmonellosis in both breeder flock, commercial broiler and layer flocks is increasing day by day. For the control and proper treatment of Salmonellosis in a particular commercial poultry farm, the Salmonellosis status of that commercial farm should be determined considering age, sex, breed of poultry and management of the farm. Keeping this in consideration this study was undertaken.

Materials and Methods

The study was conducted during the period from July to December 2007, at Krishibid farm under Thrishal Upazilla of Mymensingh district. There were 10 layer flocks containing 60,582 birds where there was no history of using *Salmonella* vaccine.

Seroprevalence study

Collection of blood: At the rate of 0.8% (conventional method of seroprevalence determination practice), 481 birds were tested randomly from 60,582 birds. 3 ml sterile disposable syringe and needle was used and blood was aseptically collected from the wing veins. During this period, the birds were classified into group according to their age as starter (0-8 wks), grower (8-20 wks), and layer (20 wks onward) (Table1).

Whole blood agglutination (WBA) test

Whole blood agglutination test was performed according to the instruction of OIE (2004). To perform the test, 0.02 ml of blood was rapidly placed into the glass slide and 0.02 ml of *Salmonella* color antigen (Nobillis[®] SP antigen, Intervet, Holland) was poured on the blood and mixed thoroughly by using tooth picks followed by rocking. Grading of positive case as +++, ++, + (Figs. 2 & 3) was done on the basis of granule formation. In negative case, no such granules were found within 2 minutes. Granules found after 2 minutes was considered as negative.

Cultural prevalence study with isolation and identification of *Salmonella* organism

Sample collection: Total 192 cloacal swabs from live bird in which 96 from seropositive and 96 from seronegative birds were collected from the same flock at the same time of seroprevalence study. Postmortem examinations of 28 dead birds were performed and 28 liver and 28 cloacal swabs of dead birds were also collected from the flock throughout the experimental period.

Cultural media: After overnight incubation in TTB, all the samples were subcultured in the *Salmonella-Shigella* (SS) agar, Triple sugar iron (TSI) agar, Brilliant green agar (BGA), Blood agar (BA), Eosine methylene blue (EMB) agar.

Morphological characterization: The presumptive colonies of *Salmonella* in different media were characterized microscopically using Gram's stain (Merchant and Packer, 1967).

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 included MR test, VP test, Indole test and dulcitol fermentation test.

Motility test: For separation of motile and non-motile *Salmonellae* motility test was done according to Cown (1985).

Pathological studies

During the whole period of seroprevalence study in the representative flock, necropsy of dead bird was done routinely. Gross pathological changes at necropsy were carefully observed and recorded and tissue samples were preserved in 10% neutral buffered formalin for histopathological studies. For histopathological studies the tissues were trimmed, washed in water, dehydration in alcohol, cleaning in chloroform, blocking in paraffin and sectioned with microtome. The sections were stained with routine hematoxylin and eosin staining (Luna, 1968) and finally the section were studied with light microscope.

Results and Discussion

Seroprevalence study

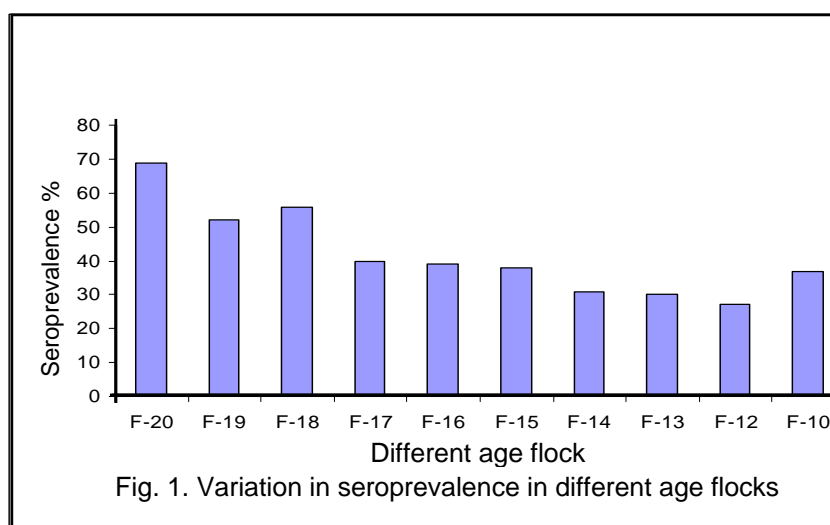
In this study, the overall seroprevalence of *Salmonella* infection in chicken was 45.9% which was supported by Arsenault *et al.* (2007), Islam *et al.* (2006). However, Jai-Sundar *et al.* (2007), Habib-ur-Rehman *et al.* (2003), Ashenafi *et al.* (2003) and Hoop and Pospischil (1993) reported 61.68%, 63.5%, 64.2% and 64.9 % seroprevalence, respectively which were higher than the present findings. The variation might be due to the differences in environmental, managemental and geographical distribution. Mdegela *et al.* (2000) reported 2.6%, Robinson *et al.* (2000) reported 18.4%, Jha *et al.* (1995) reported 21.3% and 12% seroprevalence in two village flock which was much lower than the present study. This might be due to as all the prevalence study was taken from village chickens where the prevalence of *Salmonella* infection was generally lower than the commercial chickens (Mdegela *et al.*, 2000; Robinson *et al.*, 2000) (Table 1).

Table 1. Seroprevalence of *Salmonella* infection in chicken of different age groups and flocks

| Flock Type | Flock No. | Age (day) | No. of blood test | Seropositive | | | | Prevalence % | Flock Sero-Prevalence % |
|------------|-----------|-----------|-------------------|--------------|----|----|-------|--------------|-------------------------|
| | | | | +++ | ++ | + | Total | | |
| Starter | F-20 | 6 | 97 | 13 | 13 | 41 | 67 | 69 | 63.4 |
| | F-19 | 52 | 48 | 5 | 8 | 12 | 25 | 52 | |
| Grower | F-18 | 112 | 48 | 3 | 7 | 17 | 27 | 56 | 56.2 |
| Layer | F-17 | 157 | 40 | 1 | 4 | 11 | 16 | 40 | 35.4 |
| | F-16 | 196 | 48 | - | 5 | 14 | 19 | 39 | |
| | F-15 | 201 | 52 | - | 4 | 16 | 20 | 38 | |
| | F-14 | 247 | 54 | 1 | 3 | 13 | 17 | 31 | |
| | F-13 | 266 | 40 | - | - | 12 | 12 | 30 | |
| | F-12 | 320 | 22 | - | - | 6 | 6 | 27 | |
| | F-10 | 543 | 32 | - | - | 12 | 12 | 37 | |
| Total | | | 481 | | | | 221 | | 45.9 |

F = Flock

Within the different age groups, starter showed the maximum seroprevalence of 63.4 %, grower 56.2 % and layer 35.4%. Among the different flocks, 6 days old chicks (F-20) showed the maximum 69 % seroprevalence and F-19, F-18, F-17, F-16, F-15, F-14, F-13, F-12 and F-10 showed 52%, 56%, 40%, 39%, 38%, 31%, 30%, 27% and 37% seroprevalence, respectively. 320 days old layer (F-12) showed the minimum 27% of seroprevalence. In the present study, the highest seroprevalence was observed in the starter group (63.4%) and lowest in the layer group (35.4%). Within the different aged flocks, one of the flocks showed the maximum 69% at the age of 6 days. The seroprevalence % declined with the advancement of age in the present study (Fig. 1). However, Islam *et al.*, (2006) found increased rate of seroprevalence with the advancement of age. In this study the increase rate of seroprevalence in young age may be due to 1). Infection received from parent stock through vertical transmission 2). Infection received from environment i.e. horizontal transmission. The decrease rate of seroprevalence with the advancement of age may be due to the continuous antibiotic treatment from day old chicks and upto post laying period.



In another two flocks with 112 and 543 days showed slightly higher seroprevalence 56% and 37%, irrespective to age. This might be due to the repeated infection of *Salmonella* organisms in that flocks.

Cultural prevalence with isolation and identification of *Salmonella* organism

Isolation and cultural character of *Salmonella* in different media: Isolated *Salmonella* organisms showed different cultural characteristics in different media. These were turbidity in TTB, pink white color colonies in BGA, whitish or slightly grayish color colonies in SS agar, black color colony in TSI agar, pale color colonies in Mac Conkey's agar, well defined glistening colonies in blood agar and pinkish colonies in EMB agar (Rybolt *et al.*, 2005; Perez *et al.*, 2004).

Morphological characterization: From pure culture of different media, Gram's staining was performed to observe *Salmonella* organism. Under compound light microscope the organism was identified as Gram-negative, rod shaped and arranged in single. Chains of more than two bacilli were normally absent (Freeman, 1985; Khan *et al.*, 1998).

Carbohydrate fermentation tests and biochemical tests: Different biochemical tests were used for identification of *Salmonella* organism in this study. All *Salmonella* were MR positive and VP and indole negative. In the dulcitol fermentation test, *Salmonella Pullorum* and *Salmonella Gallinarum* was dulcitol non-fermenter but paratyphoid causing *Salmonellae* fermented dulcitol (Haider *et al.*, 2003;; Buxton and Fraser, 1977; Merchant and Packer, 1967). Different carbohydrate fermentation tests were used to differentiate the isolates. All *Salmonella* isolates were lactose and sucrose negative but fermented glucose and mannitol with acid and gas production. *Salmonella Pullorum* did not ferment maltose while others fermented maltose (Sujatha *et al.*, 2003; Haider *et al.*, 2003) (Table 2)

Table 2. Carbohydrate fermentation tests and biochemical tests of isolated *Salmonella* from live and dead bird

| SL# | Lac | Suc | Mal | Glu | Man | Dul | MR | VP | Ind | Motility | Isolates | No. of isolates |
|-----|-----|-----|-----|------|-----|-----|----|----|-----|----------|--|-----------------|
| 1 | - | - | - | A/AG | AG | - | + | - | - | - | S. Pullorum | 103 |
| 2 | - | - | A | A | A | + | + | - | - | - | S. Gallinarum | 35 |
| 3 | - | - | AG | AG | AG | + | + | - | - | + | Paratyphoid causing <i>Salmonellae</i> | 22 |

Lac = Lactose, Suc = Sucrose, Mal = Maltose, Glu = Glucose, Man = Mannitol, Dul=Dulcitol, MR= Methyl Red, VP= Voges Proskauer, Ind= Indole, A =Acid, AG= Acid and Gas.

Motility test: 138 *Salmonella* isolates were identified as non-motile and 22 *Salmonella* were identified as motile (Buxton and Fraser, 1977; Islam *et al.*, 2006)

Cultural prevalence of *Salmonella* isolated from live birds

The cultural prevalence from cloacal swabs was 71% in seropositive birds and 59% in seronegative birds. From these findings it might be speculated that the presence or absence of antibody in the blood were not directly related with the presence of bacteria in the digestive tract. In seropositive birds, there was a higher cultural prevalence (71%) than the seronegative birds which might be due to repeated exposure of *Salmonella* infection and

continuous antibiotic treatment in all ages of birds. Similar result was described by Truong and Tieuquang, (2003) where they obtained upto 66% of *Salmonella* in seropositive group but they did not explained the mechanism why the isolation rate in seropositive group was higher. In seronegative group, *Salmonella* was also recovered at a prevalence rate of 59%. This might be assumed that after *Salmonella* infection, time did not elapse for antibody production (Table 3).

Table 3 Cultural prevalence of *Salmonella* isolated from live birds

| Seropositive group | | | Seronegative group | | |
|---------------------|---------------------|-----------------------|---------------------|---------------------|----------------------|
| Cloacal swab tested | Positive in culture | Cultural prevalence % | Cloacal swab tested | Positive in culture | Cultural prevalence% |
| 96 | 69 | 71 | 96 | 57 | 59 |

Cultural prevalence of *Salmonella* isolated from dead bird

The average cultural prevalence in the dead bird was 60.5% in which 64% cultural prevalence in the liver and 57% in the cloaca (Table 4). Habib-ur-Rehman *et al.* (2003) described 34.5% cultural prevalence of *Salmonella* infection in liver while Lee *et al.* (2001) described 47.6%.

Table 4 Cultural prevalence of *Salmonella* isolated from dead bird

| Liver | | | Cloaca | | | Average Prevalence % |
|-------------|---------------------|-----------------------|-------------|---------------------|-----------------------|----------------------|
| Swab tested | Positive in culture | Cultural prevalence % | Swab tested | Positive in culture | Cultural prevalence % | |
| 28 | 18 | 64 | 28 | 16 | 57 | 60.5 |

In the present study, the higher prevalence of *Salmonella* in dead bird may be due to the case fatality. Similar findings were supported by other investigators (Tibaijuka *et al.*, 2003; Lee *et al.*, 2001).

Distribution of *Salmonella* from live and dead birds

Among the 160 isolates, 64.2% (103) was identified as *Salmonella Pullorum*, 22.3% (35) *Salmonella Gallinarum* and 13.5% (22) paratyphoid causing *Salmonellae* (table 5). This result was similar to Islam *et al.* (2006) with a small variation which might be due to the difference in managerial condition (Table 5).

Table 5. Isolation and distribution of *Salmonella* from live and dead birds

| Source of Samples | No. of sample | Total | No. of Isolates | Prevalence % | Total isolates | Distribution | Species | Percentage % |
|-------------------|---------------|-------|-----------------|--------------|----------------|--------------|--|--------------|
| Live bird | 192 | 248 | 126 | 65 | 160 | 103 | <i>S. Pullorum</i> | 64.2 |
| Dead bird | 56 | | 34 | 60.5 | | 35 | <i>S. Gallinarum</i> | 22.3 |
| | | | | | | 22 | Paratyphoid causing <i>Salmonellae</i> | 13.5 |



Fig. 2. Shows the positive reaction (+++) of *Salmonella* antigen (Nobilis® SP) with whole blood of chickens

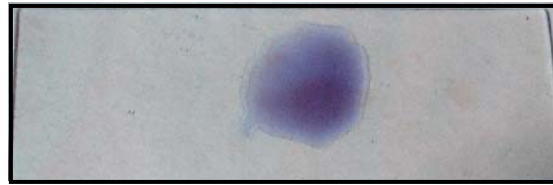


Fig. 3. Shows the negative (-) reaction of *Salmonella* antigen (Nobilis® SP) with whole blood of chickens



Fig. 4. *Salmonella* affected ovarian follicles of chicken shows congestion, discoloration and stalk formation

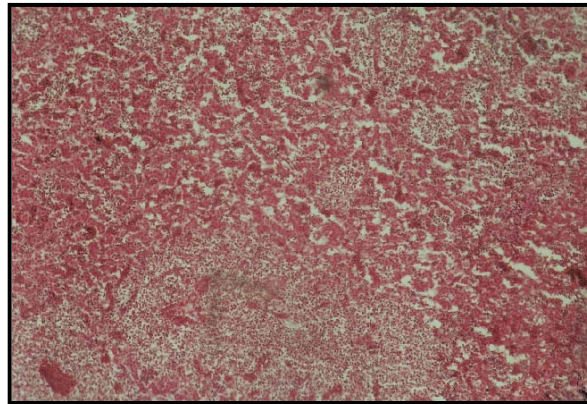


Fig. 5. *Salmonella* infected liver shows hemorrhages, focal degeneration and necrosis (H&E staining 83X)

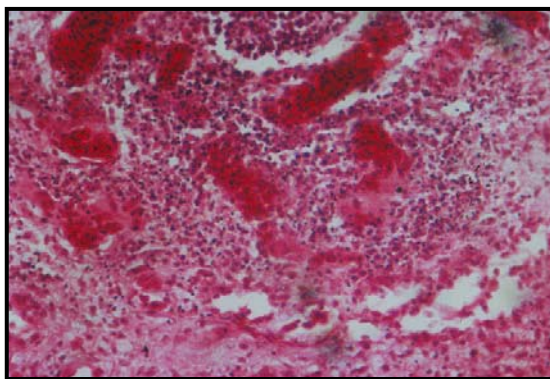


Fig. 6. *Salmonella* infected lungs shows inflammatory cells in the alveoli and bronchus that causes bronchopneumonia (H&E staining 330 X)

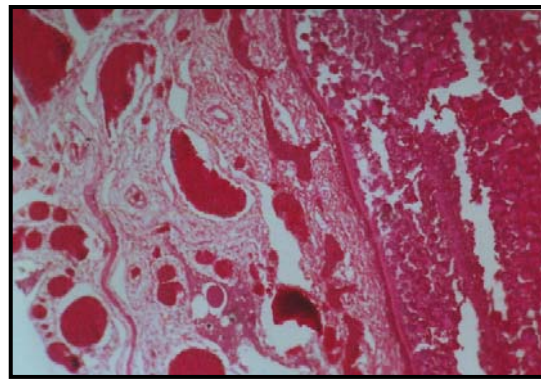


Fig. 7. *Salmonella* affected egg follicles shows marked congestion and leukocytic infiltration in the egg yolk (H&E staining 83X)

Pathological studies

The gross lesions in the liver showed variable changes including friable and bronze discoloration with white necrotic foci. Lungs were congested and pneumonic. Spleen was enlarged with discoloration. Intestine showed various grades of enteritis with mucous in the lumen. Ovary showed hemorrhagic lesions including stalk formation or pedunculated eggs (Fig. 4). Degenerated and discolored eggs were found. Yolk sac with hemorrhage and congestion was observed. These findings were supported by Deshmukh *et al.* (2007); Msoffe *et al.* (2006); Islam *et al.* (2006); Calnek *et al.*, (1991). Microscopically liver showed congestion, hemorrhages, focal degeneration and necrosis (Fig. 5). There was accumulation of leukocytes which replaced the degenerated or necrotic liver cells. In intestine there were inflammatory cells in the mucosa and submucosa, and the infiltrated reactive cells were heterophils and lymphocytes. *Salmonella* affected lungs of chickens showed pneumonia and congestion (Fig. 6). Egg follicles showed marked congestion with leukocytic infiltration in the yolk (Fig.7). Yolk sacs showed infiltration of leukocytes and marked congestion. The microscopic lesions recorded in the present investigation were almost similar to the lesions described by Deshmukh *et al.* (2007); Holt *et al.* (2006); Msoffe *et al.* (2006); Khan *et al.* (1998); and Calnek *et al.* (1991).

From the above discussion, it may be concluded that seroprevalence study is the way of choice of *Salmonella* antibody determination in serum. But the presence or absence of antibody in the blood is not directly related with the presence or absence of organisms in the digestive tract. Repeated exposure of *Salmonella* infection and time elapsed for antibody productions are also the factors for serological presence or absence of *Salmonella* antibody. In the present study, seroprevalence, cultural prevalence and pathology of *Salmonella* affected organs have been studied well. However further studies with serotyping, vaccine production and antibiotic sensitivity determination with locally isolated *Salmonellae* from poultry should be elucidated in future.

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