The Bangladesh Veterinarian (2011) 28(1) : 8 – 18

Prevalence of *Salmonella* infection in naturally infected layer birds in Bangladesh

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Abstract

The seroprevalence, cultural prevalence and pathological study of *Salmonella* infections in chickens of selected layer farms of Birgonj Upazila (Sub-district), Dinajpur were determined. A total of 175 blood samples were tested randomly by locally prepared *Salmonella* coloured antigen for seroprevalence study. Out of 96 cloacal swabs, 80 samples from live birds (36 from seropositive and 44 from seronegative) and 16 samples from dead birds were collected to determine the cultural prevalence of *Salmonella* organisms. Post-mortem examination was done in 16 dead birds. Using whole blood agglutination test (WBA) with locally prepared *Salmonella* Pullorum coloured antigen, the overall seropositive prevalence was 46.2%. The seroprevalence decreased with age of birds. The cultural prevalence in seropositive was 33.3% and in seronegative 22.7%. In dead birds, the cultural prevalence using cloacal swab was 25%. A total 26 *Salmonella* were isolated, 27% *Salmonella* Pullorum, 58% *Salmonella* Gallinarum and 15% paratyphoid group of *Salmonella*. Isolation rate of *Salmonella* from cloacal swabs was significantly higher in seropositive than seronegative group. Grossly, the livers were friable, with bronze discolouration and necrotic foci, there was severe congestion in the lung, congested haemorrhagic egg follicles with stalk formation and enlarged discoloured spleen. Microscopically, there was focal necrosis and degeneration with leukocytic infiltration in liver, congestion and pneumonic lesions in the lung and various degrees of catarrhal to haemorrhagic enteritis in the intestine. In the egg follicles, congestion and haemorrhage with leukocytic infiltration and enlarged spleen with white necrotic foci were detected. In future, isolated *Salmonella* organisms may be used for vaccine production, serotyping and antibiotic sensitivity test. (*Bangl. vet*. 2011. Vol. 28, No. 1, 8 – 18)

Introduction

Salmonella infections are major problems for poultry industry in Bangladesh, and have public health importance (Islam *et al.,* 2007; Haider *et al.,* 2008). *Salmonella* Pullorum causes the disease pullorum, which is transmitted vertically from parent to offspring. Fowl typhoid, caused by *Salmonella* Gallinarum, is an acute or chronic disease that most often affects mature birds, and is a serious problem resulting in mortality and lowered egg production and hatchability (Christensen *et al*., 1996; Khan

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et al., 1998). *Salmonella* Gallinarum can produce lesions in chicks, indistinguishable from those associated with pullorum disease.

The seroprevalence of *Salmonella* infection is 45.9% in layer birds at Mymensingh district (Ahmed *et. al.,* 2008). Village chickens can act as a reservoir of salmonellosis (Bouzoubaa *et al*., 1992). Transmission is primarily through the egg but also via direct or indirect contact with infected birds. Infection transmitted via egg or hatchery contamination usually results in death up to 2-3 weeks of age (Wigley *et al.,* 2001; World Poultry VIV, 2008). The birds that survive clinical disease when infected at a young age may show few signs of infection but can act as carriers (Berchieri *et al.,* 2001). Environmental factors such as air, dirty litter and unclean facilities, and vectors, such as insects, humans, and rodents are responsible for *Salmonella* contamination in poultry farms (Jones *et al*., 1991; Hoover *et al*., 1997; Amick-Morris, 1998). The prevalence of salmonellosis in breeder flocks and specially layer flocks is increasing in Bangladesh. This study was undertaken (a) to determine the seroprevalence of *Salmonella* infection using locally prepared *Salmonella* Pullorum coloured antigen in layer birds at Dinajpur district in Bangladesh, (b) to determine correlation between cultural prevalence and seroprevalence and (c) to study the pathological lesions of organs of *Salmonella*-infected birds.

Materials and Methods

Poultry farms

Eight layer poultry farms (4 grower, age 13-18 weeks; 4 layer, age 22-52 weeks) of Birgonj Upazila under Dinajpur District, Bangladesh were selected.

Seroprevalence study by whole blood agglutination (WBA) test

The study was conducted from November 2009 to April 2010. Blood samples were collected from chickens of 8 layer farms, which contained 30,500 birds and had no history of using *Salmonella* vaccine. At the rate of 0.8% (conventional method of seroprevalence determination), 175 birds were tested randomly. Sterile disposable syringe was used for collection of 3 ml blood from wing vein under aseptic condition. Birds were divided into two groups, grower (9-20 weeks) and layer (21-80 weeks). One drop of rapidly collected blood and one drop of *Salmonella* coloured antigen were taken in glass slide and mixed well with toothpick and rocking. In positive cases granules observed within two minutes and graded +++ highly positive response, ++ moderate, + lower positive response and – without any reaction (Fig. 1; Muktaruzzaman *et al*., 2010). However, all the reactions were graded together for considering as positive case.

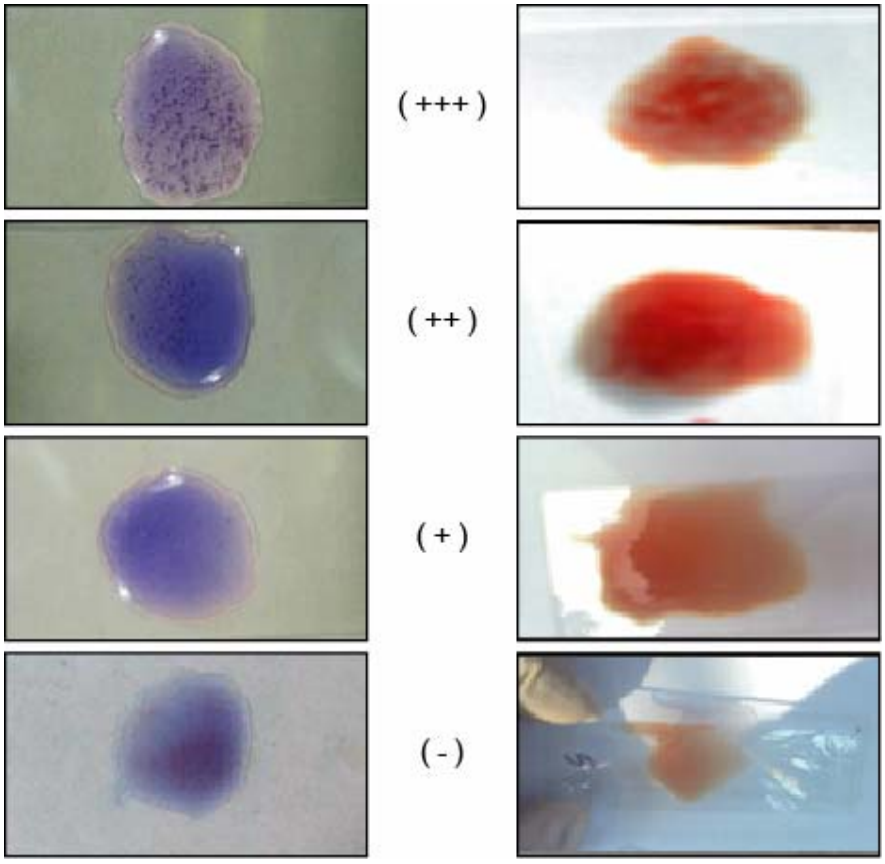

Fig. 1. Whole blood agglutination test (A) Serum, (B) Blood

Isolation and identification of Salmonella organisms

A total 80 cloacal swabs (36 from seropositive; 44 from seronegative birds) were collected from the same flock at the same time as the seroprevalence study. Post-mortem examinations of 16 dead birds were performed from 8 poultry farms. All the cloacal swabs were collected in test tubes containing 10 ml tetrathionate broth. Iodine-iodide solution (200 µl) was added in each test tube just before the swab was mixed in tetrathionate broth. For isolation and identification of *Salmonella* organisms, the samples were stored in icepack with sunlight-protected black box and carried to the laboratory. Tetrathionate broth (TTB), nutrient broth (NB), *Salmonella-Shigella* (SS) agar, brilliant green agar (BGA), blood agar (BA), Mac Conkey agar, eosin methylene blue (EMB) agar, triple sugar iron (TSI) agar, nutrient agar (NA), bacteriological peptone, methyl red-Voges Proskauer (MR-VP) medium, Gram's stain and motility test were used for the isolation and characterization of organisms (Merchant and Packer, 1967; OIE Manual, 2004).

Maintenance of stock culture

To preserve the isolated organisms for further studies, the organisms from pure culture were inoculated into tubes containing nutrient agar slant and incubated at 37°C for 24 hours. After the growth of organisms, the tubes were sealed with light liquid paraffin and kept at 4°C.

Gross pathology

During the seroprevalence study, necropsy of dead birds was done routinely. Gross tissue changes at necropsy were recorded and representative tissue samples were preserved in 10% neutral buffered formalin for histopathological studies.

Histopathology

The tissues were trimmed to 1.5 × 1 cm size, then kept in running tap water overnight to wash out formalin. Dehydration was done in ascending grades of alcohol, 50, 70, 80, 95% and three changes of absolute alcohol for one hour in each. Sections were cleaned in two changes of chloroform, one and half an hours for each. Tissues were embedded in two changes of melted paraffin wax at 56°C, one and half an hours for each. Blocks were sectioned at 5µm thickness. The sections were allowed to spread on warm water bath (45°C) and taken on grease-free glass slides. A small amount of gelatin was added to the water bath for better adhesion of the section to the slide. The slides were air-dried and kept in cool place, and stained routinely (Luna, 1968).

Photomicrography

Photomicrography was taken using compound microscope with "Sony" digital camera.

Results and Discussion

Birds (n = 175) were randomly tested for seroprevalence using whole blood agglutination test (WBA) with locally prepared *Salmonella* Pullorum coloured antigen in the selected eight layer farms. Samples (n = 80) of cloacal swabs (36 seropositive; 44 seronegative birds) were taken for culture. These samples were taken from four growers (non-production stage) and four layer (production stage) farms. Post-mortem examination of 16 laying birds was performed from the same layer farms.

Seroprevalence of Salmonella infection by whole blood agglutination (WBA) test

The results of the seroprevalence study by WBA test are shown in Table 1. The overall seroprevalence was 52.6% (Table 1) in grower birds. In layer chickens the overall seroprevalence was 38.7% (Table 1) in layers birds. The average overall seroprevalence of both growers and layers was 46.2% (Table 1).

Table 1. Overall seroprevalence of *Salmonella* infection in both grower and layer groups of poultry

Farm No.	Farm type	Age of birds (Week)	No. of tested blood samples	Average seronegative	Average seronegative prevalence	Average seropositive				Average seropositive prevalence %
						+++	++	+	Total	
F-1 to F-4	Grower	13-18	95	45	47.3	4	16	30	50	52.6
F-5 to F-8	Layer	22.52	80	49	61.2	2	12	17	31	38.7
Total			175	94	53.7	6	28	47	81	46.2

Isolation and identification of Salmonella organisms

A total of 96 (16 dead; 80 live) cloacal swabs were cultured (Figs. 2-9). In live birds, 36 from seropositive and 44 from seronegative birds were examined. From the 96 swabs, 26 isolates of bacteria were suspected as *Salmonella* (Table 2).

Fig. 2. Locally prepared *Salmonella* colored antigen

Fig. 3. Culture of *Salmonella* isolate No. 19 in TSI agar shows black color colonies

Fig. 4. Culture of *Salmonella* isolate No. 02 shows white colony on BGA agar

Fig. 5. Culture of *Salmonella* isolate No. 07 in SS agar shows whitish black color colonies

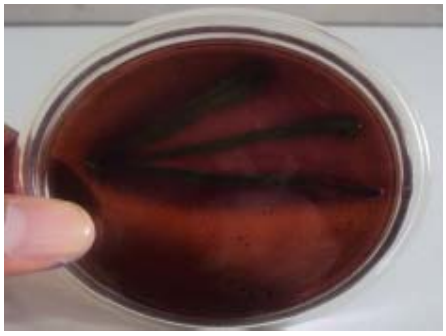

Fig. 6. Culture of *Salmonella* isolate No. 09 agar shows pink red colony on EMB

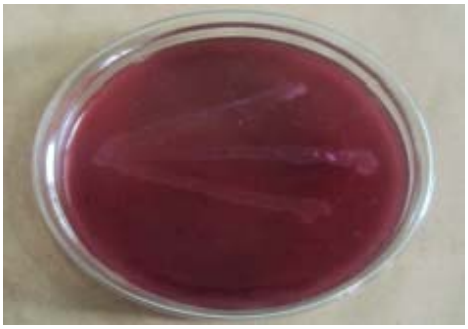

Fig. 7. Culture of *Salmonella* isolate No. 04 shows pale color colony on Mc. Conkey' sagar

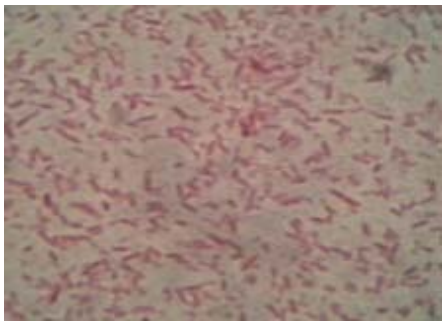

Fig. 8. *Salmonella* organism shows Gram negative, rod shaped, pink color bacilli in Gram's staining from isolate No. 19. 830X

Fig. 9. Culture of *Salmonella* isolate No. 02 shows whitish creamy colony on nutrient agar

Table 2. Cultural prevalence of *Salmonella* infection of live and dead birds

Type of birds	No. of samples	Total No. of samples	Culture in media	Suspected *Salmonella* isolate
Live birds (G+L)	80	96	*Salmonella–Shigella* (SS) agar Nutrient agar (NA) Brilliant green agar (BGA) Triple sugariron (TSI) agar	26*
Dead birds (G+L)	16		Blood agar (BA Mac Conkey'sagar Eosin methyleneblue (EMB)	

(G+L = Grower + Layer) * All 26 isolates showed characteristic growth pattern in 7 culture media

Correlation between seroprevalence and cultural prevalence of Salmonella isolates

The total cultural prevalence was 33.3% in seropositive birds (Table 3) and 22.7% in seronegative birds (Table 4). A total of 26 *Salmonella* were isolated, among them 27% were *Salmonella* Pullorum, 58% *Salmonella* Gallinarum and 15% paratyphoid group of *Salmonella* (Table 5).

Table 3. Correlation between seropositive and cultural prevalence of *Salmonella* isolates

Farm No.	Farm type	Age of birds (Weeks)	No. of tested cloacal swab	*Salmonella* isolated in culture	Cultural prevalence %	Seropositive prevalence %
F-1 to F-4	Grower	13-18	18	7	38.8	52.6 (50/95)*
F-5 to F-8	Layer	22.52	18	5	27.7	38.7 (31/80)*
Total			36	12	33.3	46.2 (81/175)*

F = Farm, * Seropositivecase/Total samples

Table 4. Correlation between seronegative and cultural prevalence of *Salmonella* isolates

Farm No.	Farm type	Age of birds (Weeks)	No. of tested cloacal swab	*Salmonella* isolated in culture	Cultural prevalence %	Seropositive prevalence %
F-1 to F-4	Grower	13-18	22	4	18	47.3 (45/95)*
F-5 to F-8	Layer	22.52	22	6	27	61.2 (49/80)*
Total			44	10	22.7	53.2 (94/175)*

F = Farm, * Seropositivecase/Total samples

Table 5. Species identification of *Salmonella* from cloacal swab by biochemical test

Sl. No.	Species	No. of isolates	Total isolates	Prevalence (%)
01	*S.* Pullorum	7		27
02	*S.* Gallinarum	15	26	58
03	Paratyphoid group	4		15

Gross and histopathological study

On the basis of gross and histopathological lesions four birds out of 16 were diagnosed as having *Salmonella* infection, with the liver friable and bronze with necrotic foci (Fig. 10), severe congestion in the lung, congested haemorrhagic egg follicles with stalk formation (Fig. 11) and enlarged discoloured spleen. Microscopically, there was focal necrosis and degeneration with leukocytic infiltration in the liver (Figs. 12 and 13), congestion and pneumonic lesions in the lung and various degrees of catarrhal to haemorrhagic enteritis in the intestine. In the egg

follicles, there was congestion and haemorrhage with leukocytic infiltration, and the spleen showed necrosis of lymphocytes.

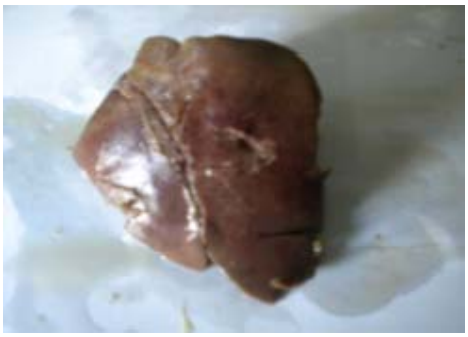

Fig. 10. Liver of *Salmonella* affected layer bird shows bronze discoloration and whitenecrotic foci from necropsied case No. 11

Fig. 11. *Salmonella* affected ovarian follicles of layer bird shows congestion, discoloration and stalk formation in case No. 1

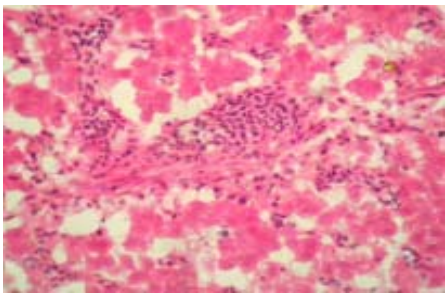

Fig. 12. *Salmonella* affected liver exhibits multifocal aggregation of histiocytes in liver parenchyma bird case No. 11 (H & E staining 330X)

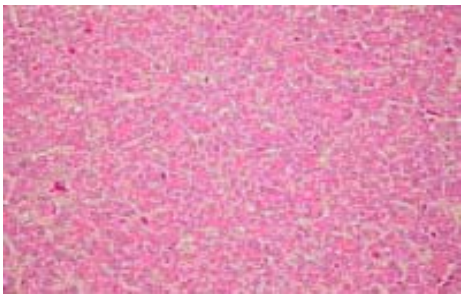

Fig. 13. *Salmonella* affected liver exhibits focal necrosis in hepatocytes in a bird case No. 1 (H & E staining 83X)

During recent years poultry industry has expanded rapidly in Bangladesh and salmonellosis is a common problem causing reduced production with high mortality. The overall seroprevalence (46.2%) is similar to that reported by Islam *et al*. (2006); Ahmed *et al*. (2008). However, Ashenafi *et al.* (2003); Habib-ur-Rehman *et al.* (2003); Jai-Sundar *et al*. (2007) reported 64.2%, 63.5% and 61.7%, seroprevalence, respectively, higher than the present findings.

The seroprevalence was higher in the grower (52.6%) and lower in the layer groups (38.7%). In grower birds, the highest seroprevalence was at 16 weeks and lowest at 13 weeks. In contrast, Islam *et al*. (2007) reported increased seroprevalence with age. In this study, the variation of seroprevalence in young age might be due to vertical transmission (infection from parent stock through eggs) and horizontal transmission (infection from environment).

The isolated *Salmonella* organisms were Gram-negative; rod shaped, pink and short to long chains and single or paired, similar to other investigators (Goswami *et al.,* 2003; Haider *et al.,* 2003).

All isolated *Salmonella* were MR positive and VP and indole negative. The isolated *Salmonella* Pullorum and *Salmonella* Gallinarum were dulcitol non-fermenters, but paratyphoid-causing *Salmonella* fermented dulcitol (Batabyal *et al.,* 2003; Haider *et al.,* 2003; Sujatha *et al*., 2003). 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 (Batabyal *et al*., 2003; Haider *et al.,* 2003; Sujatha *et al*., 2003).

Twenty-two isolates (*Salmonella* Pullorum and *Salmonella* Gallinarum) were non-motile and four isolates (paratyphoid-causing *Salmonella*) were motile (Christensen *et al*., 1996; Islam *et al*., 2007). Among the 26 isolates, 27% (n = 7) were identified as *Salmonella* Pullorum, 58% (15) *Salmonella* Gallinarum and 15% (n = 4) were paratyphoid-causing *Salmonella.* The overall seroprevalence of *Salmonella* was 46.2% (52.6% in growers; 38.7% in layers). Higher isolation rate in seropositive birds (46.2%) may be due to repeated exposure of *Salmonella* infection. Truong and Tieuquang (2003) obtained 66% *Salmonella* seropositive but they did not explain the mechanism of obtaining this higher rate. In seronegative birds the isolation rate was 53.2%. It might be assumed that after *Salmonella* infection, time did not elapse for antibody production. The cultural prevalence of *Salmonella* in seropositive birds was 33.3% (38.8% in growers; 27.7% in layers) and in seronegative birds the cultural prevalence was 22.7% (18% in growers; 7% in layers) but in dead birds the prevalence was 25% from the cloacal and liver sample. Habib-ur-Rehman *et al*. (2003) described 34.5% cultural prevalence of *Salmonella* infection in liver while Lee *et al*. (2003) described 47.6%. In the present study, the prevalence of *Salmonella* in dead birds is supported by other investigators (Lee *et al*., 2003).

Similar pathological findings were described by many investigators (Syed-Habib-ur-Rehman *et al.,* 2004; Msoffe *et al.,* 2006; Islam *et al*., 2007; Deshmukh *et al*., 2007). The microscopic lesions in present study were similar to those described by other authors (Msoffe *et al.,* 2006; Deshmukh *et al*., 2007; Ahmed *et al*., 2008).

Conclusions

The presence of antibody was not directly related with the presence of organisms in the digestive tract. Seroprevalence, culture and pathological examination should be performed simultaneously for confirmation of *Salmonella* infections in a poultry farm. Further studies with serotyping, vaccine production and antibiotic sensitivity determination with isolated *Salmonella* from poultry may be performed in the near future.

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