

Seroprevalence of *Salmonella* Infection in Commercial Layer Chickens in Cox's Bazar District, Bangladesh

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Abstract Salmonellosis is a zoonotic disease, usually transmitted from animal to human and vice versa and causes huge economic losses in poultry industry of Bangladesh. This study was aimed to estimate the seroprevalence of *Salmonella* infection in commercial layer farms of Cox's Bazar district of Bangladesh, during the period from February to April, 2016. A total of 200 blood samples were collected from 20 farms of 4 randomly selected upazillas of Cox's Bazar considering the age groups, and flock sizes. Rapid serum plate agglutination test was done and 42% chickens were found positive for *Salmonella* infection. With respect to age groups, the seroprevalence was higher (68%) in adults compared to young (20%) chickens. However, the seroprevalence of *Salmonella* infection was recorded remarkably (54.28%) in large flocks compared to small flocks. In conclusion, we can say that, *Salmonella* infection is prevalent in the commercial layer chickens of Cox's Bazar district, Bangladesh. Appropriate measures and strategies should be taken for successful prevention and control of this disease in Bangladesh.

Keywords: commercial layer, *Salmonella*, seroprevalence, age, flock-size

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1. Introduction

In Bangladesh, commercial poultry production has been developing quickly since early 1990 by utilizing enhanced hereditary traits, manufactured feeds and management. This dramatic development of poultry farms throughout the country has occurred without considering feasibility of the farm in the area. This change is happened principally in the private area as an additional tool of income and employment creation mainly in rural area. This process has been conducted by the project of various NGOs and the government [1].

There are a few limitations being developed of poultry industry in Bangladesh [2]. Among bacterial diseases of poultry, Salmonellosis is one of the major diseases as it can cause heavy financial loss due to increased mortality and decreased egg production [3]. The disease is highly significant because it can easily be transmitted vertically from parent to offspring. This disease in poultry caused by two non-motile types avian *Salmonella* namely *Salmonella gallinarum* responsible for causing fowl typhoid and *Salmonella pullorum* for pullorum disease respectively [4]. Pullorum disease is generally limited to

initial 2-3 weeks of age and merely occurs in adults. Fowl typhoid is frequently found in adults and there are also evident of high mortality in young chicks.

Environmental factors include air, dirty litter and unclean facilities, and vectors, such as insects, humans, and rodents are responsible for *Salmonella* contamination in poultry farm. There are several methods of diagnosis of *Salmonella* in field level such as an indirect enzyme linked immune sorbent assay (ELISA), double ELISA, rapid plate agglutination and whole blood agglutination test [5]. Rapid plate agglutination (RPA) test is commonly used in field condition to detect *Salmonella* as it can be performed easily and require less time as well as economic.

In recent time, *Salmonella* infections in commercial layer, broiler and breeder farms are increasing predominantly. Several number of research works has been performed on the prevalence of *Salmonella* infection by previous author in different districts of Bangladesh mainly focused on isolation, identification and serological tests [4,6,7,8]. However, more study on the seroprevalence of *Salmonella* disease covering wide geographical areas of Bangladesh is required to design effective control program. Due to be a zoonotic disease, the human health is always at risk of getting the resistant human pathogens through food channel. It is said that food from poultry origins are the

main source of causing human salmonellosis as salmonella remains as a reservoir in most of the poultry products. Keeping the above said fact, the study was conducted to determine the seroprevalence of *Salmonella* infection using purchased *Salmonella* colored antigen in commercial layer birds at Cox's Bazar district in Bangladesh.

2. Materials and Methods

2.1. Study Area

The study was conducted in the four randomly selected upazilla under Cox's Bazar districts where commercial layer farms were available. There are eight upazilla in Cox's Bazar district and among them Cox's Bazar Sadar, Ramu, Ukhiya and Chakaria were selected in this study conducted during the period of February to April 2016.

2.2. Sample Collection and Processing

A total of 200 blood samples comprising 50 samples from 5 farms of each upazilla were collected. Blood samples were collected aseptically from the wing vein using sterile 3 ml syringe and 23 G needles. From each bird 2 ml of blood was collected. The syringe containing blood was put in a standing position allowed to clot formation and serum was collected by decanting according to Hossain et al. [8]. 1.5 ml micro centrifuge tubes were used for transferring and shipping of the harvested sera into laboratory through cold box. Serum sample were stored at -20°C until perform the Rapid Serum Agglutination Test following the methods described by Selvam et al. [9].

2.3. Salmonella Antigens

Antigens are the killed and colored Salmonella. In this surveillance program, Salmonella O group D (Somatic 9, 12) antigens were used for pullorum disease and fowl typhoid [10]. The *Salmonella* antigen (Serotest® SP) used in this study were purchased from the S & A Reagents Lab Ltd., Part Thailand.

2.4. Rapid Plate Agglutination (RPA) Test

As indicated by the guidance of OIE Manual [11] the RPA test was performed. For this test 0.02 ml of antigen and 0.02 ml of chicken serum were set one next to the other with micropipettes on a glass slide. At that point test serum and antigen were mixed by using a tooth pick. The glass slide was lit up from underneath to observe the reaction, evading excessive warmth from the light source. In case of positive reaction, definite clumps was formed within 2 minutes after mixing of antigen (Figure 1) and test serum whereas it was mostly visible in peripheral

region of clumps and agglutination was absent in negative case (Figure 2).

2.5. Statistical Analysis

The data obtained from this study were analyzed using STATA/IC-13.0. Significant differences among the variables were calculated using Pearson's Chi-square test. p value less than 0.05 were considered as significant.



Figure 1. RPA showed the positive reaction of *Salmonella* antigen (Serotest®SP) with serum of birds



Figure 2. RPA showed the negative reaction of *Salmonella* antigen (Serotest®SP) with serum of birds

3. Results

3.1. Overall Prevalence of *Salmonella* Infection in Selected Farm

Descriptive study shows that, out of 200 samples, 82 samples were found seropositive which means the seroprevalence of Salmonellosis in the layer farms of our research areas were 42% and it was given in the Table 1.

Table 1. Overall seroprevalence of *Salmonella* infection in selected farm

Study area	No. of flocks	No of serum sample tested	Positive cases	Sero-positive %
Cox's Bazar district	20	200	84	42

3.2. Prevalence of *Salmonella* Infection in Different Ages

The Table 2 shows the seroprevalence of *Salmonella* infection in different age groups of layer birds. The estimated seroprevalence of *Salmonella* infections were 20% in 15-24 weeks, 25% in 25-34 weeks, 35% in 35-44 weeks, 50% in 45-54 weeks and 68% in 55-above week of ages and the differences were significantly varied ($p < 0.05$). According to different ages, the highest seroprevalence of *Salmonella* infections were reported 68% at above 55 weeks of age group whereas the lowest seroprevalence was reported 20% at less than 24 weeks of age group. The seroprevalence of *Salmonella* infection was significantly higher in adult in relation to young group of layer birds ($p < 0.05$).

Table 2. Seroprevalence of *Salmonella* infection in different ages

Age (Weeks)	No. of flocks	No of serum sample tested	Positive cases	Sero-positive %	P value
15-24	3	30	6	20	0.010
25-34	4	40	10	25	
35-44	4	40	14	35	
45-54	4	40	20	50	
Above 55	5	50	34	68	
Total	20	200	84	39.6	

3.3. Prevalence of *Salmonella* Infection according to Flock Sizes

Regarding to prevalence based on flock sizes, the detailed *Salmonella* infections in layer birds were consequently 20% within (≤ 1000 birds) flocks, 30% within (1001-1500 birds) flocks, 48% within (1501-2000 birds) flocks as well as 54.28% within (≥ 2000 birds) flock sizes. The Table 3 demonstrates that the seroprevalence associated with *Salmonella* infections were larger (54.28%) within larger flocks (≥ 2000 birds) within resemblance in order to in small (≤ 1000) flocks. Flock size was not significantly varied with *Salmonella* infections in layer birds ($p > 0.05$).

Table 3. Seroprevalence of *Salmonella* infection according to flock sizes

Flock size	No. of flocks	No of serum sample tested	Positive cases	Sero-positive %	P value
500-1000	2	20	4	20	0.098
1001-1500	6	60	18	30	
1501-2000	5	50	24	48	
Above 2000	7	70	38	54.28	
Total	20	200	84	38.07	

3.4. Prevalence of *Salmonella* Infection in Relation to Study Areas

The Table 4 presents the results of seroprevalence of *Salmonella* infection in layer birds based on different study areas. It was observed that the seroprevalence of *Salmonella* infections were 60% in Ramu, 36% in Cox's Bazar, 28% in Chakaria and 44% in Ukhyia upazilla. In the study it was shown that the prevalence of *Salmonella*

infections were highest 60% in Ramu upazilla in relation to others upazillas. Study areas were not significantly differ with *Salmonella* infection ($p > 0.05$).

Table 4. Seroprevalence of *Salmonella* infection based on study areas

Location	No. of flocks	No of serum sample tested	Positive cases	Sero-positive %	P value
Ramu	5	50	30	60	0.125
Cox's Bazar	5	50	18	36	
Chakaria	5	50	14	28	
Ukhyia	5	50	22	44	
Total	20	200	84	42	

4. Discussion

In our study area, the overall seroprevalence of *Salmonella* infections in layer farms was high. The present finding agrees with several previous reports such as Kindu and Addis [12] described seroprevalence 41.9% in chickens flocks in Jimma town, Ethiopia, Islam et al. [7] showed 43.4% sero-prevalence in layer farms of Dhaka and Gazipur regions of Bangladesh and Bhattacharya et al. [13] stated 37.7% seroprevalence in layer farms in India. The percentages of my investigation were higher than that of Alam et al. [14], Sikder et al. [6], Akter et al. [15], Hossain et al. [8] and Barua et al. [16] whose reported 23.8%, 23.46%, 23.11%, 25.3% and 18% sero-positive chickens for *Salmonella* infection in Dinajpur district, Patuakhali district, Gobindapur of Dinajpur district, Rajshahi and surrounding districts and Chittagong district of Bangladesh respectively. However, Nath et al. [17] found 60% seroprevalence in commercial layer farm of Chittagong district as well as Habib-Ur-Rehman et al. [18], Ashenafi et al. [19], Sundar et al. [20] and Jalil and Islam [21] noted 63.5%, 64.2%, 61.68% and 65.9% seroprevalence subsequently, which were comparatively higher than the present findings. The fluctuation might be due to differences in environmental, managerial and geographical location.

In present studies, within the different age groups, above 55 weeks of age showed highest sero-prevalence of *Salmonella* infection followed by others weeks of age and lowest prevalence showed in less than 15 weeks of age. Jalil and Islam [21] recorded higher seroprevalence (76.6%) in layer birds of 56 weeks of age than those of other age groups. Similarly, Hossain et al. [8] also found the highest seroprevalence of *Salmonella* infection was 37.6% at 64 weeks and above age group whereas the lowest seroprevalence was 16.6% at 16-23 weeks age group that supports our present findings. Suchlike report was noted by Sikder et al. [6] who reported 30.8% at 39 weeks of age and lowest was 13.3% at 32 weeks of age. Interestingly, Islam et al. [7], Akter et al. [15], Barua et al. [16] and Nath et al. [17] also found increased rate of seroprevalence of *Salmonella* infection with advancement of age. But Ahmed et al. [22] who reported highest seroprevalence in grower 63.4% stage in relation grower 56.2% and layer 35.4% respectively which was opposite to my study. Similar report also demonstrated by Rahman et al. [23] that reported highest *Salmonella*

infection in grower 52.6% groups and lower in layer 38.4% groups. In this study the increase rate of seroprevalence in adult age may be due to concurrent infection of *Salmonella*.

According to present investigation, highest seroprevalence of *Salmonella* infection in layer chickens showed in large flocks (≥ 2000) in comparison to small (≤ 1000) flocks. Similar results was also observed by Hossain *et al.* [8] who noted higher 34.2% seroprevalence of *Salmonella* infection in large flocks (≥ 5001 birds) in comparison to small (≤ 1000 birds) flocks 21.3%. Identical observation was studied by Jalil and Islam [21] at Khulna district of Bangladesh. The present data were higher than those in report of Skov *et al.* [24] who listed 16% *Salmonella* infection in a flock containing 30-40 thousand chickens in comparison to 11.9% in a flock containing 10-20 thousand and 9.7% in a flock containing less than 10 thousands chickens. Mdegela *et al.* [25] stated that infection rate increased with increase of flock size and also recorded higher prevalence of *Salmonella* infection in commercial flock 18.4% than in scavenging chickens 6.3%. The variation of infection rate in larger flock might be due to faulty management and bio-security as well as the horizontal transmission of the organisms.

In my study, serological investigation showed highest seroprevalence of *Salmonella* infection in layer chickens in Ramu upazilla followed by others upazillas. Sikder *et al.* [6] and Salihu *et al.* [26] observed same findings at two union of Patuakhali district of Bangladesh and various village and town of Nasarawa State, Nigeria respectively. An identical result was noted by Jalil and Islam [21] at six upazilla s of Khulna district of Bangladesh. Mdegela *et al.* [26] reported 2.6% and 18.4% sero-prevalence in two village flock which was much lower than the present study. This might be due to large number of flock, bio-security and geographical location.

5. Conclusion

From the above findings, it may be concluded that the seroprevalence of *Salmonella* infections were high in the present study areas. However, further studies with cultural prevalence, pathological findings, serotyping and antibody sensitivity determination with isolated *Salmonella* from poultry may be performed in near future. And also effort should be made towards educating farmers. Moreover, for effective control measures such as bio-security should be improved especially in commercial layer farm.

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Conflict of interest

The authors declare that there is no conflicting interest with regards to the publication of this manuscript.

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