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SEROLOGICAL SURVEY OF SALMONELLA INFECTION IN NON-VACCINATED COMMERCIAL LAYER BIRDS IN KHULNA DISTRICT OF BANGLADESH

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ABSTRACT

A cross sectional study was conducted on 164120 non-vaccinated layer birds of 96 farms in six upazilas (Sadar, Batiaghata, Dumuria, Dighulia, Rupsha and Fultala) of Khulna district to determine the seroprevalence of *Salmonella* infection (*S. pullorum* and *S. gallinarum*) during the period from August 2009 to July 2010. Sera samples were collected from 1268 layer birds of different ages and the birds were selected through a disproportionate stratified random sampling technique based on the flock size of each farm. Sera samples were tested by Serum plate agglutination (SPA) test applying commercial *Salmonella* antigen (Nobilis® SA, Intervet International B.V. Boxmeer- Holland) to detect the presence of antibodies against *Salmonella*. The overall seroprevalence of *Salmonella* infection was recorded as 65.9%. The significantly higher seroprevalence (76.6%) of *Salmonella* infection was recorded in layer birds of >56 weeks of age than those of other age groups. Seasons had significant influence on the seroprevalence of *Salmonella* infection. The seroprevalence was significantly higher in summer (82%) than that in rainy (66.8%) and winter (50%) seasons. The location of farms, i.e. upazilas also had significant association with the occurrence of *Salmonella* infection. The seroprevalence significantly differed between the different categories of flock size. The flock size of 5001 and above had the highest seroprevalence (81.4%) among other categories. It may be concluded that above 60% layer birds in 92 out of 96 farms are infected with *Salmonella* organism, which requires keeping of vigilant eye of the poultry farmers and the hatchery owners in the control of *Salmonella* infection in poultry farms.

Key words: Seroprevalence, *Salmonella* infection, serum plate agglutination (SPA), layer birds

INTRODUCTION

As in other poultry producing countries, salmonellosis is one of the important disease problems for poultry in Bangladesh, both for commercial exotic breeds and indigenous local breeds (Rahman *et al*., 2004; Saleque *et al*., 2003; Sikder *et al*., 2005). Infections of poultry with salmonellae are caused by two groups of serotypes, i) two nonmotile serotypes *S. pullorum* and *S. gallinarum* and ii) the numerous motile *Salmonella* serotypes (paratyphoid salmonellae). Infections with serotypes of first group have been responsible for serious economic losses to poultry producers and have been addressed by the implementation of extensive testing and eradication programs (Gast, 2003). Although in all types of poultry production, infection by *Salmonella* can occur during any part of the production cycle (Byrd *et al*., 1999; Bailey *et al*., 2002), it is likely that in both broilers and layers most of the initial infection takes place early post-hatch, as a result of hatchery contamination or persistent farm contamination. Infection of very young chicks results in high levels of environmental contamination and rapid transmission of pathogens as a result of litter contamination (Van Immerseel *et al*., 2005).

A definitive diagnosis of *Salmonella* infection (*S. pullorum* and *S. gallinarum*) requires the isolation and identification of the *Salmonella* organisms. However, a tentative diagnosis can be made based on the flock history, clinical signs, mortality and lesions. Positive serological findings can also be of great value in detecting infection (Shivaprasad, 2003). Serological tests such as the macroscopic tube agglutination (TA) test, whole blood agglutination test, serum plate agglutination (SPA) test, and enzyme-linked immunnosorbent assay (ELISA) have been used to detect *Salmonella* organism (Barrow, 1992; Gast, 1997; Feberwee *et al.*, 2001; Jalil and Islam, 2010). Of these tests, the TA and SPA tests are commonly used for detection and removal of reactor birds in the control programme of salmonellosis.

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Much work has been done on the prevalence of *Salmonella* infection in chickens in many countries of the world. However, there were wide variations in selecting target population, sample size, test procedure etc. In Bangladesh, a good number of works has been done on the prevalence of *Salmonella* infection based on isolation, identification and serological tests in Rajshahi, Gazipur, Patuakhali districts (Saleque *et al*., 2003; Rahman *et al*., 2004; Sikder *et al*., 2005; Hossain *et al*., 2006; Islam *et al*., 2006; Hossain *et al*., 2010). However, more survey on the seroprevalence of *Salmonella* infection covering wide geographical areas of Bangladesh is required to plan effective control programme. The objective of this study was to determine the seroprevalence of *Salmonella* infection (*S. pullorum* and *S. gallinarum*) in non-vaccinated layer birds in Khulna district.

MATERIALS AND METHODS

Study population and sampling

A cross sectional study was conducted on 164120 commercial layer birds of 96 farms in six upazilas (Sadar, Batiaghata, Dumuria, Dighulia, Rupsha and Fultala) of Khulna district during the period from August 2009 to July 2010. A total of 1268 layer birds of different ages were selected through a disproportionate stratified random sampling technique based on the flock size of each farm. Blood samples without any anticoagulant were collected aseptically from wing vein of the selected birds and sera were separated and stored at -20^{o}C until used.

Salmonella antigen

Salmonella antigen (Nobilis® SP, Intervet International) was used for rapid plate agglutination (RPA) test to detect antibodies due to infections caused by both standard and variant strains of *Salmonella pullorum* and *Salmonella gallinarum* in the sera samples.

Rapid plate agglutination (RPA) test

The RPA test was conducted according to the manufacturer's instruction. Briefly, 0.02 ml of antigen and 0.02 ml of chicken serum were placed side by side with micropipettes on a glass plate. After that antigen and serum sample were mixed thoroughly by stirring with a small tooth pick. Then the glass plate was illuminated from below so as to facilitate observing the reaction, avoiding excessive heat from the light source. Positive reaction was characterized by the formation of definite clumps within 2 minutes after mixing the test serum with antigen. The clumps usually started appearing and became concentrated at the periphery of the mixture. Negative reaction was judged by the absence of agglutination reaction. Care was taken so that no natural granulation of the antigen could be taken as a positive reaction.

Statistical analysis

In order to study the temporal distribution of the disease, the year was divided into four quarters viz. July-September (A), October-December (B), January- March (C) and April-June (D). Quarters B and C comprised of the winter season; D, the summer season and A, the rainy season. The data so collected were analyzed statistically using Z test for proportions to draw the inferences (Beri, 2005).

RESULTS AND DISCUSSION

Of the 1268 birds screened from 96 flocks, 835 (65.9%) birds tested positive to *Salmonella* antibodies, i.e. the overall seroprevalence of *Salmonella* infection was 65.9% (Table 1). The present finding is discordant with two inland reports of Islam *et al.* (2006) and Hossain *et al*. (2010) who observed 43.4% and 25.3% seroprevalence in Dhaka-Gazipur and Rajshahi, respectively. Geographical variation or difference in management practices might be the cause of variation in the seroprevalence (Sikder *et al*., 2005; Islam *et al*., 2006). However, the present finding is concordant with an overseas report of Ashenafi *et al.* (2003) who reported 64.2% seroprevalence in local chickens of Central Ethiopia.

Table 1. Seasonal influence on the seroprevalence of *Salmonella* infection in layer birds in six upazilas of Khulna district

Seasons	Population	No. of samples tested	No. of samples positive	Prevalence (%)
Summer	54650	383	314	82.0a
Rainy	55570	467	312	66.8b
Winter	53900	418	209	50.0c
Total	164120	1268	835	65.9

Values with different letters within a column differ significantly at $p<0.05$.

There was significant influence of seasons on the seroprevalence of *Salmonella* infection in layer birds. The seroprevalence of *Salmonella* infection was significantly higher in summer (82%) than that in rainy (66.8%) and winter (50%) seasons. Similar findings were reported by Hossain *et al.* (2010) who recorded higher seroprevalence in summer (30.4%) followed by rainy (25.0%) and winter (23.7%) seasons. The higher seroprevalence of *Salmonella* infection in summer (48.1%) than in winter (23.7%) was also reported by Rahman *et al.* (2004). The highest rate of *Salmonella* infection in summer season may be due to increase in bacterial growth in summer and the influence hot weather that might reduce the immune status of birds against infection (Sikder *at al.*, 2005; Hossain *et al*., 2010).

Table 2. Age-wise seroprevalence of *Salmonella* infection in layer birds in six upazilas under Khulna district

Upazilas	Seroprevalence of *Salmonella* infection							
	Pullet (8-20 wks)		Layer (21-56 wks)		Layer (>56 wks)		Overall	
	No. tested	Prevalence No. (%)	No. tested	Prevalence No. (%)	No. tested	Prevalence No. (%)	No. tested	Prevalence No. (%)
Sadar	137	74 (54.0)	333	179 (53.8)	163	99 (60.7)	633	352 (55.6)a
Batiaghata	27	24 (88.9)	269	190 (70.6)	43	41 (95.3)	339	255 (75.2)b
Dumuria	6	6 (100.0)	43	16 (37.2)	0	0 (0)	49	22 (44.9)a
Dighulia	29	23 (79.3)	45	35 (77.8)	44	44 (100.0)	118	102 (86.4)b
Rupsha	3	1 (33.3)	66	56 (84.9)	15	12 (80.0)	84	69 (82.1)b
Fultala	0	0 (0)	15	5 (33.3)	30	30 (100.0)	45	35 (77.8)b
Total	202	128 (63.4)a	771	481 (62.4)a	295	226 (76.6)b	1268	835 (65.9)

Values with different letters within a column as well as a row differ significantly at $p<0.05$.

Regarding the age of birds, significantly higher seroprevalence (76.6%) of *Salmonella* infection was recorded in layer birds of >56 weeks of age than those of other age groups. However, no significant difference was observed in seroprevalence between birds of 8-20 weeks old and 21-56 weeks old (Table 2). The increased seroprevalence of *Salmonella* infection in aged layer birds corresponds with the report of Islam *et al.* (2006) who reported higher prevalence (72.9%) of *Salmonella* infection in layer birds of >60 weeks old than those of any other age group. It has been reported that the prevalence of *Salmonella* infection increased with the increase of age of birds (Truong and Tieuquang, 2003; Hossain *et al.,* 2010). Rahman *et al*. (2004) reported highest infection rate with *Salmonella* in adult layers (53.25%) in comparison to brooding (14.55%), growing (16.10%) and pullet (16.10%) chickens.

In relation to study area, there was significant variation was recorded in the seroprevalence of *Salmonella* infection among farms of six upazilas ($p<0.05$). The significantly highest (86.4%) prevalence of *Salmonella* infection was in Dighulia upazila and lowest (44.9%) in Dumuria upazila (Table 2). The day-old chicks of different farms in six upazilas were purchased from different hatcheries and the management practices in those farms were also different, which might be the probable factors for variation in the seroprevalence.

Table 3. Influence of flock size on the seroprevalence of *Salmonella* infection in layer birds in six upazilas of Khulna district

Flock Size	No. of flocks	No. of samples tested	No. of samples positive	Prevalence (%)
500-2000	76	703	455	64.7a
2001-3500	13	297	196	66.0a
3501-5000	4	139	79	56.8a
5001- above	3	129	105	81.4b
Total	96	1268	835	65.9

Values with different letters within a column differ significantly at $p<0.05$.

The seroprevalence of *Salmonella* infection significantly ($p<0.05$) differed between the different categories of flock size (Table 3). The seroprevalence was significantly higher (81.4%) in the flock size of 5001 and above than any other category of flock size. However, no significant difference was found between the other three categories of flock size (500-2000, 2001-3500 and 3501-5001). This finding is in agreement with the findings of Hossain *et al*. (2010) who reported the higher (34.2%) seroprevalence of *Salmonella* infection in large flocks (≥5001 birds) in comparison to 21.3% in small flocks (≤1000 birds). The highest infection rate in large flocks may be due to the high flock density, which facilitate easy spread of any infection. Besides, error in management practices is not unlikely in large flocks that may contribute to the increase in seroprevalence of *Salmonella* infection (Hossain *et al*., 2010).

It may be concluded that above 60% layer birds in 92 out of 96 farms are infected with *Salmonella* organism, which requires keeping of vigilant eye of the poultry farmers and the hatchery owners in the control of *Salmonella* infection in poultry farms.

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