RESEARCH ARTICLE

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Seroprevalence of *Salmonella spp.* infection in different types of poultry and biosecurity measures associated with Salmonellosis

Mirza Mienur Meher[1], Md. Arman Sharif[2], Abdullah Al Bayazid[3]

[1] Bangabandhu Sheikh Mujibur Rahman Agricultural University, Faculty of Veterinary Medicine and Animal Science, Department of Microbiology and Public Health, Gazipur-1706, Bangladesh

[2] Bangabandhu Sheikh Mujibur Rahman Agricultural University, Faculty of Veterinary Medicine and Animal Science, Department of Anatomy and Histology, Gazipur-1706, Bangladesh

[3] Bangabandhu Sheikh Mujibur Rahman Agricultural University, Faculty of Veterinary Medicine and Animal Science, Gazipur-1706, Bangladesh

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Correspondence: Mirza Mienur Meher
E-mail: mienur@bsmrau.edu.bd

Abstract

Salmonella spp. infection is considered a crucial trouble of the poultry industry in Bangladesh. Hence, this study was aimed to estimate the seroprevalence of *Salmonella spp.* in poultry along with biosecurity practices that are associated with this *Salmonella spp.* infection. The study was conducted during the period from January to September, 2021 in Mymensingh and Gazipur district of Bangladesh. A total of 314 samples were considered to determine the seroprevalence. Seroprevalence was determined by performing the rapid serum plate agglutination test. The result revealed that the overall *Salmonella spp.* seroprevalence was 47.77% in the study area. The higher seroprevalence was in Mymensingh (51.59%) than Gazipur (45.21%) without significant ($p>0.05$) difference. The highest seroprevalence was in broiler (51.33%) where in layer and sonali was 32.67% and 16% respectively. Seroprevalence was significantly ($p<0.05$) higher in summer for layer (56.45%), broiler (60.64%) and sonali (51.22%) than the winter seasons. In layer farms, the flock size of >2000 to <2500 had significantly ($p<0.05$) higher (71.43%) seroprevalence. In broiler farms, 15 to 30 days old birds had significantly ($p<0.01$) higher (77.05%) seroprevalence than other age. Among the different categorical level of biosecurity practices, the poultry farms that used surface water (OR=0.182, 95% CI=0.106-0.314); disinfectant regularly (OR=0.296, 95% CI=0.171-0.511); having density of 8-10 birds/meter2 (OR=0.379, 95% CI=0.219-0.654); cleaned waterer and feeder regularly (OR=0.503, 95% CI=0.294-0.862); and having visitor restriction (OR=0.375, 95% CI=0.219-0.643) showed lower tendency ($p<0.001$) to seroprevalence. In brief, Strict farm hygienic practice and biosecurity measures are significantly linked to decrease the *Salmonella spp.* seroprevalence.

Keywords: *Salmonella spp.*, Seroprevalence, Biosecurity, Broiler, Layer, Sonali

INTRODUCTION

Poultry farming in Bangladesh is a fast-growing sub-sector which takes an important place in socio-profitable development especially pastoral livelihood development by generating employment prospects (Islam & Nishibori, 2009). This subsector plays an important part in narrowing the gap between demand and force of protein of animal origin (Islam & Nishibori, 2009). Particularly, this subsector contributes nearly 40% of the total meat supply and more than 25% of Bangladesh's total human protein demands (Abdullah et al., 2019; Hamid et al.,

2016). However, several factors reduce the growth rate of this sector. Among them, poultry diseases are the major constraints (Karim, 2003). About 30% of poultry birds die annually in Bangladesh due to outbreaks of several infectious diseases. The major infectious and contagious diseases are Avian influenza, Gumboro disease, Newcastle disease, Mycoplasmosis, Colibacillosis, Salmonellosis, and Fowl cholera frequently affecting marketable Poultry farm (Chanie et al., 2009). Among the bacterial diseases *Salmonella spp.* infection is one of the major problems for poultry in Bangladesh, which is considered a crucial trouble of the poultry assiduity (Rahman et al., 2016). In Bangladesh, the circumstance of *Salmonella spp.* infection is about 21-30% in layer and about 15% in broiler which is measured as the loftiest frequency among different types of poultry disease (Rahman et al., 2017), among which a variety of acute and habitual diseases in poultry are included (Laxman Bahadur et al., 2016). Salmonellosis in poultry causes significant profitable loss due to mortality and reduced product (Rahman et al., 2016). Salmonellosis in chickens caused by *Salmonella pullorum* and *Salmonella gallinarum* and is appertained to as pullorum complaint and fowl typhoid, independently. Pullorum disease occurs in chicks during their first many days of life and causes severe enteritis and bacteremia (Rahman et al., 2016). Whereas, fowl typhoid is a disease of mature chicken and causes either acute enteritis with greenish diarrhea or a habitual complaint of the genital tract that reduces egg product (Rahman et al., 2016). Chicks can be infected with *Salmonella spp.* by vertical transmission through infected parents or by horizontal transmission through hatcheries, sexing in defiled hatcheries, cloacal infection, and transportation of outfit and feed (Kabir, 2010). Motile Salmonella spp. (paratyphoid group) infection causes salmonellosis in chickens with zoonotic significance (Kabir, 2010). Basically, *Salmonella spp.* are short bacilli, 0.7-1.5×2.5 µm, Gram-negative, aerobic or facultative anaerobic, positive catalase, negative oxidase; they raise sugars with gas product, produce H2S, are non sporogenic, and are typically motile with peritrichous flagella, except for *Salmonella pullorum* and *Salmonella gallinarum*, which are immotile (Gantois et al., 2009; Rahman et al., 2016). The diseases frequencies in a particular area depends on several factors like geographical condition, immunization status of the ranch, quality and condition of the chicks, bio-security status of farm etc. Biosecurity measure commonly may be the implementation of policies, practices, and essential actions that enhance preparedness and prevent the introduction and rapid spread of diseases within the country and across national borders (Fathelrahman et al., 2020). The increasing preparedness against biosecurity threats has a tendency of reduction to disease outbreaks and also the poultry production systems need an increase drive for improved biosecurity practices (Maduka et al., 2016). In Bangladesh is particularly at risk of transmission of infectious diseases because of its high population density and widespread contact between people and animals. Hence the most important measure for sustainable and profitable product on a poultry point must be to have in place forward defenses similar as a biosecurity Program. Though, the several studies were conducted on seroprevalence of *Salmonella spp.* in poultry in different area of Bangladesh (Hossain et al., 2010; Jalil & Islam, 2012; Sabuj et al., 2019; Sikder et al., 2005). But the effect of different biosecurity practices in preventing the seroprevalence of *Salmonella spp.* in poultry farm has not been studied before. Moreover, update information on the seroprevalence of *Salmonella spp.* in different types of poultry (Layer, Broiler and Sonali) is essential to design a prevention and control strategies. In fact, the hygienic property of the poultry products depends on the management and health status of poultry and predominantly on the conception and the biosecurity grade of the poultry houses. Hence, this study was aimed to estimate the seroprevalence of *Salmonella spp.* infection in commonly farming types of poultry (Layer, Broiler and Sonali) along with biosecurity practices that are associated with this infection in Mymensingh and Gazipur district of Bangladesh.

MATERIALS AND METHODS

Study area and period

This study was designed to collect the samples from two districts namely Mymensingh and Gazipur of Bangladesh, lie between the latitudes of 24.30°N to 24.88°N and longitudes of 90°16′4″E to 90.73°E (Figure 1). The software ArcGIS-ArcMap version 10.8 (ESRI, USA) was applied to show the study area. The study was conducted during the period from January to September, 2021.

Sample Size Estimation

The sample size in the study area was determined by the formula of Daniel (1999).

$$\mathbf{n} = \frac{\boldsymbol{Z}^2\boldsymbol{P}(1-\boldsymbol{P})}{\boldsymbol{d}^2}; \mathbf{n} = \frac{(1.96)^2 \times 0.31(1-0.31)}{(0.05)^2};$$

$$\mathbf{n} = 328.69 \cong 329$$

Where,

n = sample size,

Z = 1.96 (95% confidence level),

P = expected prevalence or proportion (in proportion of one; 31.25%, P = 0.31), and

d = precision (in proportion of one; whereas P=0.31, therefore d = 0.05).

In the previous study, the authors (Mridha et al., 2020) found the 31.25% overall prevalence of *Salmonella spp.* in

Gazipur, Tangail, and Dhaka districts of Bangladesh. So, the expected prevalence was considered as 31.25%. The estimated sample size was 329. A total of 330 samples was collected and finally, 314 samples were considered to test for determining the seroprevalence.

Sample collection

The blood samples were collected aseptically from wing vein of the selected birds without any anticoagulant. All the selected birds were from small-scale poultry farm, and the farms having less than 2500 birds were considered as small-scale farming. A total of 314 samples were for detection of seroprevalence of *Salmonella spp.* infection. After collection of blood using sterilized syringe and needle, the syringes having the blood sample were placed in a standing position in a cool box and kept for 6 hours to separate the serum. After separation of serum, the serum samples were transferred to 1.5 ml micro centrifuge tubes and stored in refrigerator until perform the Serum Plate Agglutination (SPA) test.

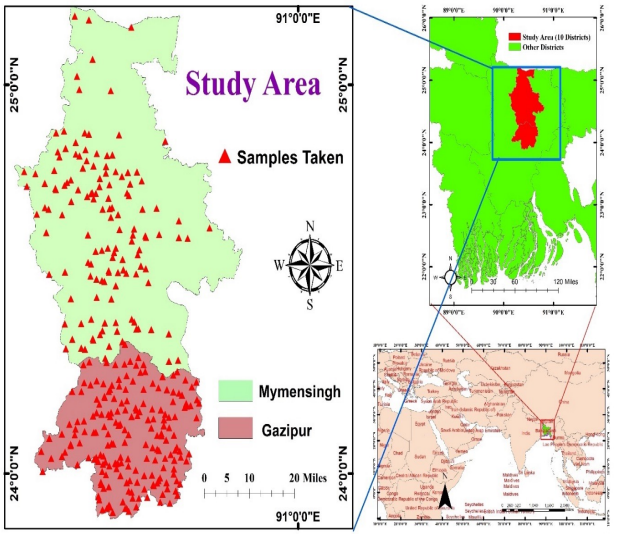

Figure 1. Spatial location of the study area (Mymensingh and Gazipur District) in Bangladesh.

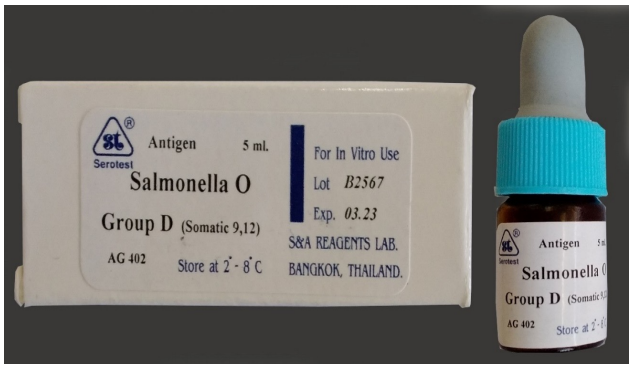

Figure 2. *Salmonella spp.* antigen for serum plate agglutination (SPA) test to detect seroprevalence.

All the blood samples were collected from non-vaccinated birds. At the same time, data on poultry farm were collected from the farmer. The questionnaire to collect the data on biosecurity practices was prepared according to the previous study (Meher et al., 2020).

Salmonella spp. antigen

Salmonella spp. antigen (Serotest® SP, S& A Reagent Lab, Thailand) was used for rapid serum plate agglutination (SPA) test to detect antibodies due to infections caused by both standard and variant strains of *Salmonella pullorum* and *Salmonella gallinarum* in the sera samples. The antigen was killed and colored *Salmonella O* group D (Somatic 9, 12) (Figure 2).

Detection of *Salmonella spp.* infection by serum plate agglutination (SPA) test

The SPA test was performed according to the methods described by Sikde et al. (2005). Briefly, equal amount of antigen and serum (0.02 ml of antigen and 0.02 ml of bird's serum) were positioned on a glass plate side by side with micropipettes. Afterward, antigen and serum sample were mixed methodically by mixing with a small tooth pick. To observe the reaction, the glass plate was brightened from below for avoiding unnecessary heat from the light source. In case of positive reaction, the definite clumps were formed within 2 minutes just after mixing the serum and antigen. The clumps usually began to appear and became condensed at the periphery of the mixture. The absence of agglutination reaction was characterized as negative reaction. Precaution was taken to avoid the false positive result due to natural granulation of the antigen.

Level of Infection

The level of infection was determined by the strength of the clamps (Figure 3). In general, the clumps begun and concentrated from the periphery of the mixture. The strength of the agglutination reaction was measured according to the methods followed by Hossain et al. (2007). In brief,

No infection (–) = No clumps with no background clearing.

Low infection (+) = Small clumps with no background clearing.

Medium infection (++) = Medium sized clumps with almost complete background clearing.

Heavy infection (+++) = Large to very large clumps (mostly in the periphery) with complete background clearing.

Gross Pathological Lesions

Some of dead birds were randomly selected from the poultry farms where the blood samples were taken for SPA test. After a systemic dissection of the organs of dead

birds, the changes in the organs were recorded and the variations were compared with the results of Kumari et al. (2013) to confirm suspect clinically the *Salmonella spp.* infection cases. The sterilized distinct set of apparatuses were used for each case to complete the post-mortem examination and the methods applied which followed by the authors Hossain et al. (2017).

Statistical analysis

All the data were arranged in Microsoft excel (Microsoft corp. 2019) and then transferred to "Statistical Package for Social Sciences (SPSS)" version 25.0 to perform the statistical test. The association between the categorical explanatory variable with outcome were estimated by Pearson's Chi-square. On the other hand, when more than 20% of cells of 2×2 contingency table had expected count less than 5, the P value of continuity correction was considered but when the table more than the 2×2 contingency then P value of Fisher exact tests was accounted. Moreover, if the table is 1×3, the one sample Chi-square test was performed. A regression model was used to determine the significant associations of *Salmonella spp.* seropositive case with the common biosecurity practices of poultry farm. The Binary logistic regression analysis was performed using the enter methods. Before performing all the statistical test, the assumptions were checked found suitable. The p value ≤ 0.05 was considered as significant result.

Results

This study revealed that the seroprevalence of *Salmonella spp.* infection was 47.77% in the study area (Table 1). Among the two districts, the seroprevalence of *Salmonella spp.* infection was higher in Mymensingh (51.59%) than Gazipur (45.21%), though there was no significant (p>0.05) difference. Among the 150 positive cases, highest proportion (51.33%) was detected in broiler species, where in layer and sonali was 32.67% and 16% respectively.

In case of layer birds, seroprevalence was significantly (p<0.05) higher in summer seasons (56.45%) than the winter seasons (Table 2). In winter season the moderate level of infection was significantly (p<0.05) higher (64.29%). The Salmonella spp. seroprevalence of the farms that flock size was >2000 to <2500 birds had significantly (p<0.05) higher (71.43%) seroprevalence. Although, the 30-39 weeks old birds were more seroprevalent (70.00%) but had no any significant (p>0.05) differences with the other ages. similarly, the seroprevalence was higher (56.76%) in Mymensingh without any significant (p>0.05) difference with Gazipur district for the layer birds. However, in Gazipur the significant amount of layer birds (57.14%) was moderately infected.

The table 3 shows the seroprevalence in broiler birds, where the seroprevalence was significantly (p<0.05) higher in summer season (60.64%) and 15 to 30 days old birds (77.05%). The flocks having the birds of >1500 to ≤2000 was higher (59.09%) seroprevalent. The moderate level of infection was significantly (p<0.05) higher (61.11%) in the birds of the flock size about >2000 to <2500. Among the infected broiler birds, highest proportion (44.16%) had the moderate level of infection.

The seroprevalence of Salmonella spp. Infection in sonali chickens is presented in table 4. The result shows the significantly (p<0.05) higher seroprevalence in summer seasons of 51.22%. Moreover, the higher seroprevalence was observed in the sonali birds of flock size ≤500 (54.55%), 61 to ≥90 days of age (52.17%) and in Mymensingh district (40.91%). Among the infected sonali birds, highest proportion (41.67%) had the low level of infection.

The Figure 4 shows the gross pathological lesions which indicated that the *Salmonella spp.* infection. The pathological lesions including the black color enlarged liver with congestion and necrotic foci, splenomegaly, pericarditis, misshapen and congested ova had been found in all dead birds of the farms that had the SPA test positive samples.

In Table 5, considering the biosecurity parameters, the significant (p<0.05) number of infected birds were in the farms used surface water (67.74%), used disinfectant irregularly (59.78%), had the density of >10 birds/meter2 (55.70%), cleaned water and feeder irregularly (55.56%), and had no any visitors' restrictions (59.35%). However, among the different types of poultry (Layer, Broiler and Sonali), there was no any significant differences in term of different categorical levels of biosecurity parameters that commonly practiced.

Table 1. Prevalence of Salmonella spp. infection in different poultry farms of Gazipur and Mymensingh district of Bangladesh.

Categories		No. of Sample Tested	Positive Case (Prevalence)					
			Overall		Farm Species			
Variable	Level		n (%)	P value	Layer (%)	Broiler (%)	Sonali (%)	P valueOSC
Area	Gazipur	188	85 (45.21)	0.321	28(32.94)	42(49.41)	15(17.65)	0.323
	Mymensingh	126	65 (51.59)		21(32.31)	35(53.85)	9(13.85)	0.494
	Total	314	150 (47.77)		49(32.67)	77(51.33)	24(16)	0.429

"OSC" = p value of one sample Chi-square test. Significant at 1% (P<0.01); Significant at 5% (P<0.05).

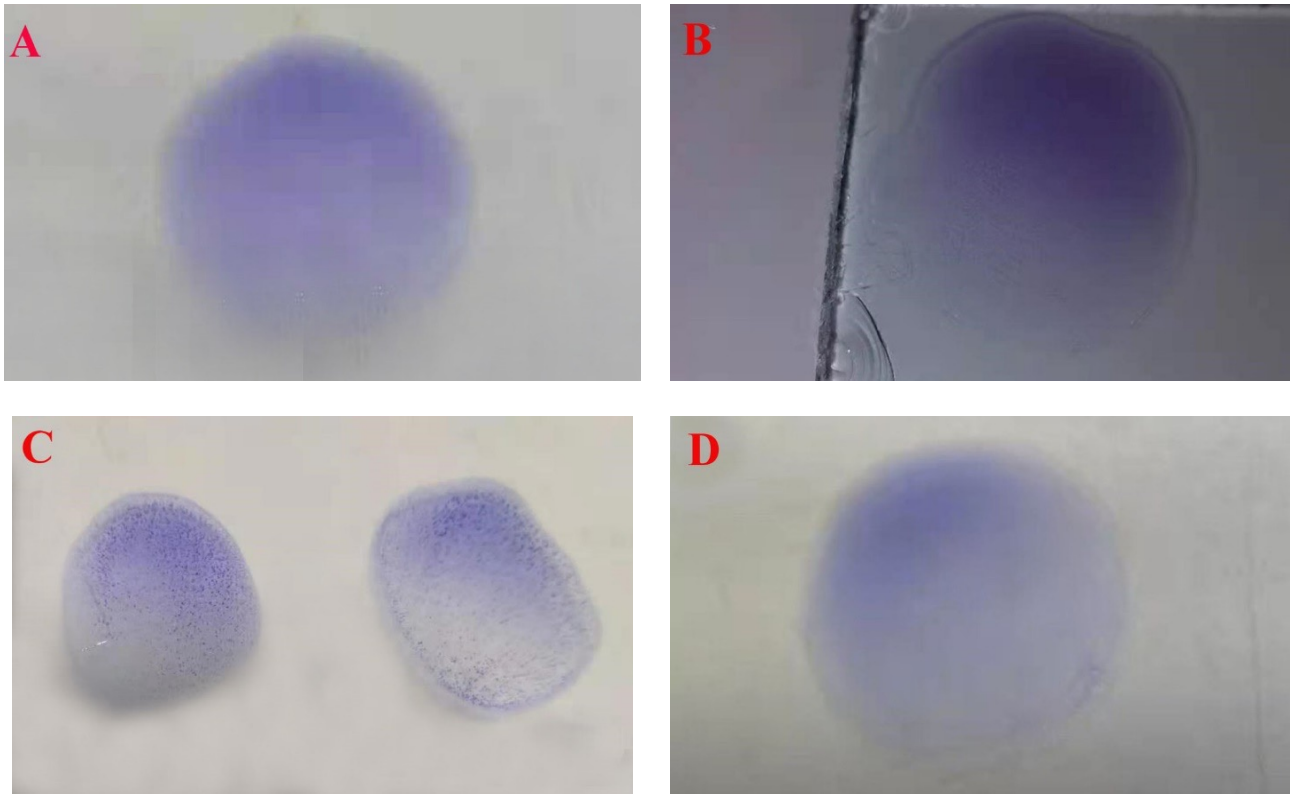

Figure 3. The strength of the agglutination reaction showed (A) Low level of infection, (B) Moderate level of infection, (C) High level of infection and (D) No infection

Table 2. Prevalence of *Salmonella spp.* infection in commercial Layer birds with respect to different parameters

Categories		No. of Sample Tested	Positive Case			Level of Infection			
Variable	Level		(N)	Prevalence (%)	P value	Low	Moderate	High	P value [osc]
Season	Winter	39	14	35.90	0.044	2(14.29)	9(64.29)	3(21.43)	0.046
	Summer	62	35	56.45		9(25.71)	15(42.86)	11(31.43)	0.449
Flock Size	≤500	15	5	33.33	0.015	1(20)	4(80)	0(0)	0.074
	>500 to ≤1000	22	6	27.27		2(33.33)	1(16.67)	3(50)	0.607
	>1000 to ≤1500	19	11	57.89		4(36.36)	5(45.45)	2(18.18)	0.529
	>1500 to ≤2000	17	7	41.18		1(14.29)	4(57.14)	2(28.57)	0.368
	>2000 to <2500	28	20	71.43		3(15)	10(50)	7(35)	0.157
Age (Weeks) of the Birds	<10	13	6	46.15	0.160	1(16.67)	3(50)	2(33.33)	0.607
	10-19	15	8	53.33		2(25)	6(75)	0(0)	0.030
	20-29	17	10	58.82		1(10)	4(40)	5(50)	0.273
	30-39	10	7	70.00		3(42.86)	2(28.57)	2(28.57)	0.867
	40-49	26	7	26.92		1(14.29)	5(71.43)	1(14.29)	0.102
	Above 50	20	11	55.00		3(27.27)	4(36.36)	4(36.36)	0.913
Area	Gazipur	64	28	43.75	0.208	4(14.29)	16(57.14)	8(28.57)	0.018
	Mymensingh	37	21	56.76		7(33.33)	8(38.1)	6(28.57)	0.867
	Total	101	49	48.51		11(22.45)	24(48.98)	14(28.57)	0.059

"OSC"= p value of one sample Chi-square test. Significant at 1% (P<0.01); Significant at 5% (P<0.05)

Table 3. Prevalence of Salmonella spp. infection in commercial Broiler birds with respect to different parameters.

Categories		No. of Sample Tested	Positive Case			Level of Infection			
Variable	Level		(N)	Prevalence (%)	P value	Low	Moderate	High	P Value[OSC]
Season	Winter	56	20	35.71	0.003	5(25)	8(40)	7(35)	0.705
	Summer	94	57	60.64		13(22.81)	26(45.61)	18(31.58)	0.104
Flock Size	≤500	23	12	52.17	0.699	2(16.67)	6(50)	4(33.33)	0.368
	>500 to ≤1000	36	20	55.56		5(25)	7(35)	8(40)	0.705
	>1000 to ≤1500	34	14	41.18		4(28.57)	5(35.71)	5(35.71)	0.931
	>1500 to ≤2000	22	13	59.09		2(15.38)	5(38.46)	6(46.15)	0.368
	>2000 to <2500	35	18	51.43		5(27.78)	11(61.11)	2(11.11)	0.030
Age (Days) of the Birds	<15	49	16	32.65	0.001	4(25)	7(43.75)	5(31.25)	0.646
	15-30	61	47	77.05		10(21.28)	20(42.55)	17(36.17)	0.186
	31- 45 and above	40	14	35.00		4(28.57)	7(50)	3(21.43)	0.395
Area	Gazipur	83	42	50.60	0.842	5(11.9)	22(52.38)	15(35.71)	0.005
	Mymensingh	67	35	52.24		13(37.14)	12(34.29)	10(28.57)	0.819
	Total	150	77	51.33		18(23.38)	34(44.16)	25(32.47)	0.082

"OSC" = p value of one sample Chi-square test. Significant at 1% (P<0.01); Significant at 5% (P<0.05)

Table 4. Prevalence of Salmonella spp. infection in commercial Sonali birds in respect to different parameters.

Categories		No. of Sample Tested	Positive Case			Level of Infection			
Variable	Level		(N)	Prevalence (%)	P value	Low	Moderate	High	P Value[OSC]
Season	Winter	22	3	13.64	0.003	1(33.33)	1(33.33)	1(33.33)	1.000
	Summer	41	21	51.22		9(42.86)	7(33.33)	5(23.81)	0.565
Flock Size	≤500	11	6	54.55	0.305	2(33.33)	2(33.33)	2(33.33)	1.000
	>500 to ≤1000	10	5	50.00		2(40)	3(60)	0(0)	0.247
	>1000 to ≤1500	13	2	15.38		0(0)	0(0)	2(100)	0.135
	>1500 to ≤2000	12	5	41.67		2(40)	2(40)	1(20)	0.819
	>2000 to <2500	17	6	35.29		4(66.67)	1(16.67)	1(16.67)	0.223
Age (Days) of the Birds	<30	27	9	33.33	0.179	4(44.44)	2(22.22)	3(33.33)	0.717
	31-60	13	3	23.08		1(33.33)	1(33.33)	1(33.33)	1.000
	61- 90 and above	23	12	52.17		5(41.67)	5(41.67)	2(16.67)	0.472
Area	Gazipur	41	15	36.59	0.736	8(53.33)	7(46.67)	0(0)	0.022
	Mymensingh	22	9	40.91		2(22.22)	1(11.11)	6(66.67)	0.097
	Total	63	24	38.10		10(41.67)	8(33.33)	6(25)	0.607

"OSC" = p value of one sample Chi-square test. Significant at 1% (P<0.01); Significant at 5% (P<0.05)

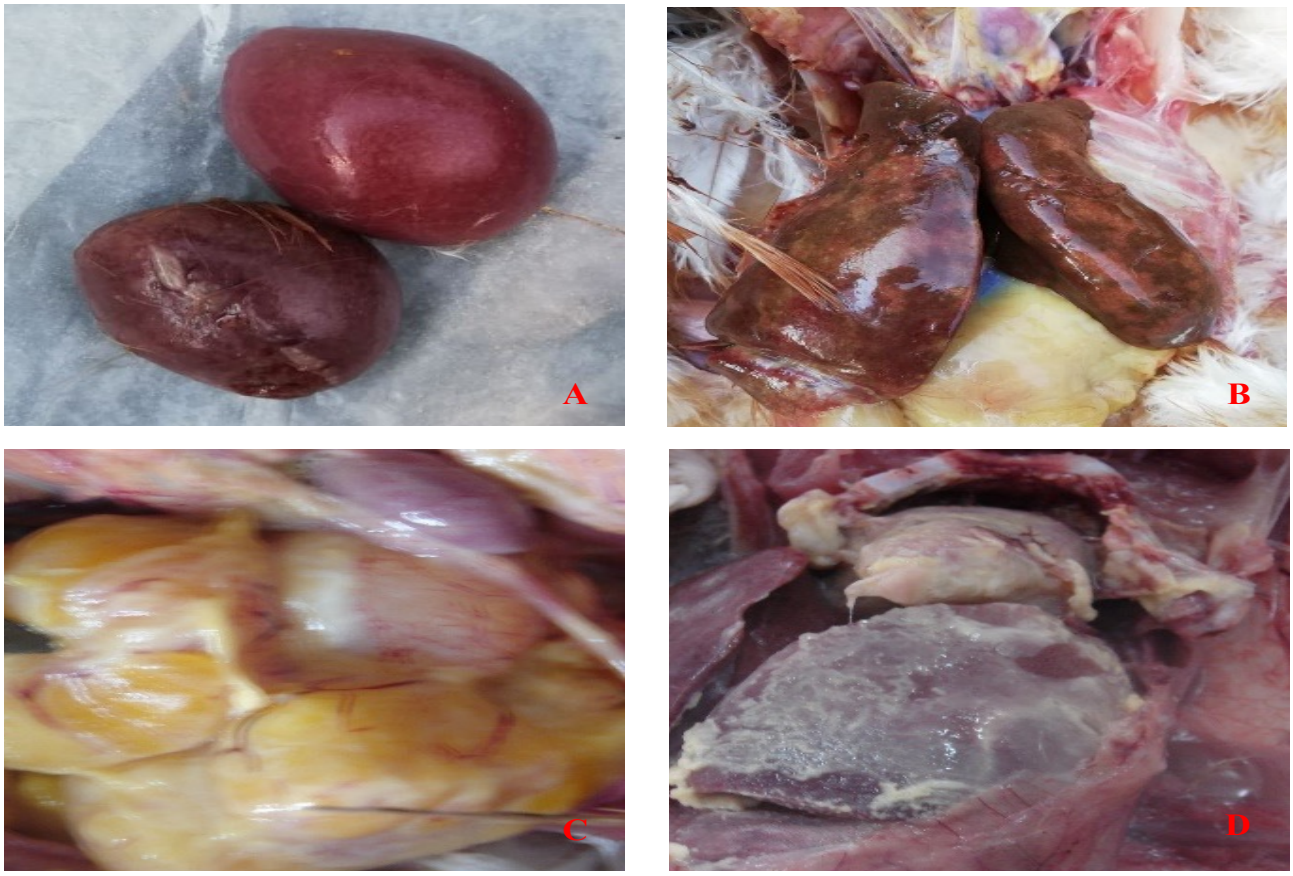

Figure 4. The gross pathological leshions. (A) Spleen larger than normal size with blackish discoloration. (B) Enlaged congested liver with bronze discoloration. (C) Congested and misshapened egg follicles. (D) Pericarditis conjugation with Escherichia coli infection

Table 5. Prevalence of *Salmonella spp.* infection in commercial birds in respect to biosecurity parameters.

Categories		N	Positive Case						
			Overall			Farm Species			
Variable	Level		(n)	Prevalence (%)	P value	Layer	Broiler	Sonali	P value
Source of water in farm	Underground	159	45	28.30	0.00	13(28.89)	21(46.67)	11(24.44)	0.197
	Surface	155	105	67.74		36(34.29)	56(53.33)	13(12.38)	
Use of disinfectants	Regularly	130	40	30.77	0.001	16(40)	17(42.5)	7(17.5)	0.406
	Irregularly	184	110	59.78		33(30)	60(54.55)	17(15.45)	
Density of birds (birds/meter2)	8 to 10	156	62	39.74	0.007	23(37.1)	34(54.84)	5(8.06)	0.061
	>10	158	88	55.70		26(29.55)	43(48.86)	19(21.59)	
Disposal of dead birds	Burned/buried	148	65	43.92	0.239	18(27.69)	32(49.23)	15(23.08)	0.111
	Thrown away	166	85	51.20		31(36.47)	45(52.94)	9(10.59)	
Well ventilation	Yes	140	61	43.57	0.221	19(31.15)	33(54.1)	9(14.75)	0.876
	No	174	89	51.15		30(33.71)	44(49.44)	15(16.85)	
Cleaning of waterer and feeder	Regularly	152	60	39.47	0.006	19(31.67)	30(50)	11(18.33)	0.831
	Irregularly	162	90	55.56		30(33.33)	47(52.22)	13(14.44)	
Visitors restricted	Yes	159	58	36.48	0.001	22(37.93)	28(48.28)	8(13.79)	0.554
	No	155	92	59.35		27(29.35)	49(53.26)	16(17.39)	
Total		314	150	47.77		49(32.67)	77(51.33)	24(16)	0.429

Significant at 1% (P<0.01); Significant at 5% (P<0.05)

Table 6. Logistic regression analysis of common biosecurity practices in poultry farm associated to *Salmonella spp.* seroprevalence.

Categories		Wald	Odd Ratio	P value	95% C.I. for O. R.	
Variable	**Level**				**Lower**	**Upper**
Source of water in farm	Underground	37.663	0.182	0.000	0.106	0.314
	Surface			Reference		
Use of disinfectants	Regularly	18.983	0.296	0.000	0.171	0.511
	Irregularly			Reference		
Density of birds (birds/meter2)	8 to 10	12.121	0.379	0.000	0.219	0.654
	>10			Reference		
Disposal of dead birds	Burned/ buried	2.625	0.641	0.105	0.375	1.098
	Thrown away			Reference		
Well ventilation	Yes	0.628	0.805	0.428	0.47	1.378
	No			Reference		
Cleaning of waterer and feeder	Regularly	6.266	0.503	0.012	0.294	0.862
	Irregularly			Reference		
Visitors restricted	Yes	12.747	0.375	0.000	0.219	0.643
	No			Reference		
Constant		47.818	17.446	0.000		
R^2= 0.284 (Cox & Snell R Square) , 0.379 (Nagelkerke R Square)						
Hosmer and Lemeshow test p value: 0.400; Significant at 1% (P<0.01); Significant at 5% (P<0.05); C.I.= Confidence Interval; O.R. = Odd Ratio.						

The Table 6 shows the binary logistic analysis to determine the effect of common biosecurity practices in poultry farm on the likelihood that the farm had the seroprevalence of *Salmonella spp.* The result revealed that the several variables of common biosecurity practices on the probability had the influence on *Salmonella spp.* seroprevalence. The logistic model contained seven independent variables (Source of water, Use of disinfectants, Density of birds, Disposal of dead birds, Ventilation, Cleaning of waterer and feeder, and Visitors restricted). The regression model was statistically significant, $\chi 2$ (7, N = 314) = 105.02, p < .001, representing that the model was able to distinguish between *Salmonella spp.* seropositive and seronegative farm in terms of biosecurity practices. This model as a whole, could clarify between 28.4% (Cox and Snell R square) and 37.9% (Nagelkerke R squared) of the variance in *Salmonella spp.* seroprevalence status. The model was also correctly classified 73.9% of cases. Hence, the goodness of fit for this model was determined by the Hosmer and Lemeshow test, in which the p value of 0.400 (p>0.05) indicates that final model is fit. The seroprevalence of *Salmonella spp.* had a lower (OR=0.182; 95% CI: 0.106-0.314) tendency (p<0.001) in the farm used underground water compared with the farm used surface water. The other categorical level of biosecurity practices, regular use of disinfectant, density of 8-10 birds/meter2, disposal of dead birds by buried or burned, Ventilation, Cleaning of waterer and feeder regularly, and restriction of visitor access ensued the odd ratio (OR) of 0.182 (p=0.00, 95% CI=0.106-0.314), 0.296 (p=0.00, 95% CI=0.171-0.511), 0.379 (p=0.00, 95% CI=0.219-0.654), 0.641 (p=0.105, 95% CI=0.375-1.098), 0.805 (p=0.428, 95% CI=0.47-1.378), 0.503 (p=0.012, 95% CI=0.294-0.862), and 0.375 (p=0.00, 95% CI=0.219-0.643) respectively. All these values of odd ratio indicate that the less likelihood to *Salmonella spp. seroprevalence*.

DISCUSSION

The present study revealed that the seroprevalence of *Salmonella spp*. in Mymensingh was higher than the Gazipur district in Bangladesh. The overall seroprevalence is very close to the findings of another research (Sabuj et al., 2019) where report was 42% seroprevalence in layer birds in Cox's Bazar district of Bangladesh. But the overall seroprevalence of this study was higher than the findings of Hossain et al. (2010), Sikder et al. (2005), Barua et al. (2012) where they reported that 14.1% in Rajshahi and surrounding districts, 23.46% in Patuakhali district, 18% in Chittagong district of Bangladesh respectively. On the other hand, the authors Jalil & Islam, (2012) reported 65.9% seroprevalence in chicken in Khulna district of Bangladesh. Among the two study districts, in Mymensingh the seroprevalence was higher than the Gazipur district. This might be due to variation in the farming strategies and number of farms in these two different study areas.

In the study area, mainly three types of poultry (Layer, Broiler and Sonali) are commercially reared. Among them, the higher seroprevalence was observed in broiler. Similarly, Naurin et al. (2013) also observed higher prevalence in broiler than the indigenous chicken. The highest seroprevalence of *Salmonella spp.* in broiler might be due to overcrowding along with inadequate hygienic measures in the farms. In addition, the higher seroprevalence in broiler indicated that broilers could be an important

reservoir of *Salmonella spp.* Naurin et al. (2013). Among the variation of seroprevalence of *Salmonella spp* in different types of poultry, the author Naurin et al. (2013) found significantly higher prevalence about 70% in broiler than layer and sonali birds. This result is in line with our findings where the seroprevalence was higher in broiler.

There was a significant impact of seasonal variation on the seroprevalence of *Salmonella spp.* infection in all types of poultry (layer, broiler and sonali). In accordance with the present findings, Jalil & Islam (2012) also reported the seroprevalence of *Salmonella spp.* infection was significantly higher in summer (82%) than winter (50%) seasons. Similarly, a study conducted by Naurin et al. (2013) reported that the significantly higher prevalence of *Salmonella spp.* in poultry in the Mymensingh area during summer as compared to rainy season. The increasing bacterial growth in summer season along with the hot humid weather that could decrease the immunity of birds against infection, ultimately increase the *Salmonella spp. seroprevalence* (Hossain et al., 2010). Moreover, the warm temperature may be a major factor to provide the suitable environment for the growth and proliferation of *Salmonella spp.* (Guthrie, 1992). Similarly, a study conducted in Nepal recorded that the highest prevalence of *Salmonella spp.* during summer in chicken raw meat (Maharjan et al., 2006). Though the seroprevalence in broiler and sonali birds did not significantly influenced by the flock size, but layer birds had significant differences. Among the categorized flock size, the higher flock size led to higher seroprevalence. Similarly, the author Hossain et al., (2010) and Jalil & Islam (2012) reported the higher seroprevalence of 17.8% and 81.4% in the farm of flock size 4501 to 5000 and 5000 to above respectively.

In different study, the seroprevalence of *Salmonella spp.* in different ages layer birds was variable. The authors, Jalil & Islam (2012), Hossain et al. (2010) and Sabuj et al. (2019) found the highest seroprevalence of *Salmonella spp.* infection was 76.6% at 56 weeks of age, 27.2% at ≥64 weeks of age and 68% at >55 weeks of age respectively. These findings contradicted our result. However, our study was in line with the authors Sikder et al. (2005) who reported the highest seroprevalence was 30.8% at 39 weeks of age. In different study, there was variation of seroprevalence according to age which might be due to dissimilarities in study area and management practices. In case of broiler the *Salmonella spp.* infection was higher at the age of 15 to 30 days. This observation is supported by the Djeffal et al. (2018) who reported that the farms having the broiler at the age of 15-30 days were more infected than the birds at the age of 45-60 days.

On the other hand, the gross pathological changes of the organs of dead birds from SPA test positive farms indicated the salmonellosis which supported the findings of Kumari et al. (2013).

In this study seven common practices for farm biosecurity were considered as parameters to determine the association with *Salmonella spp.* seroprevalence. The *Salmonella spp.* seroprevalence had significant association with all the parameters except the parameters of dead bird's disposal system and ventilation system. The samples taken from the farms using surface water were significantly ($p<0.01$) more seroprevalent to *Salmonella spp.* than the farm using the ground water. Because the surface waters including those waters used for irrigation and refreshment or as a drinking water which are the potential source for *Salmonella spp.* contamination (Levantesi et al., 2012). However, when the surface water is treated mainly by the organic acids, it may reduce the number of *Salmonella spp.* (Argüello et al., 2013). The use of disinfectant, especially when the regularly is also a significant biosecurity practice to minimize the *Salmonella spp.* seroprevalence (OR=0.296). Irregular and insufficient cleaning and disinfection may lead to persistence of *Salmonella spp.* in poultry houses (Trampel et al., 2014). The density of birds (birds/meter^2) also had the significant ($p<0.01$) role, where the farms had 8 to 10 birds/meter^2 had lower prevalence (39.74%) and less likelihood (OR=0.379) to seroprevalence than the farms had >10 birds/meter^2. Though this observation contraindicates the finding of Djeffal et al. (2018) who reported that, <10 birds/meter^2 is a risk factor (OR=2.25) without any significant association. But in another study, Elgroud et al. (2009) noted that there was significant($p<0.01$) effect (OR=7.7). Moreover, our findings are in agreement with another literature relating that poultry house having the high density of birds, is a risk factor favoring the infection by *Salmonella spp.* (Heyndrickx et al., 2002). The irregular cleaning of feeder and waterer in the poultry farm contributed to significantly ($p<0.01$) higher seroprevalence and the regular cleaning practices (OR=0.503) farms were less like to seroprevalence. This finding is supported by another research mentioning that the poultry can be infected by the horizontal transmission of *Salmonella spp.* during the rearing period through feeding drinking water, and contaminated equipment (Tabo et al., 2013). Another important biosecurity practices, the restricted access of visitors in poultry farms that had less tendency (OR=0.375) to *Salmonella spp.* seroprevalence than the farms having free access. Though these findings differ from the study conducted by Hamilton & Dornan (1992), they found that culture of *Salmonella spp.* from floor litter or drinking water has no any significant association with restrictions on visitors. But the restriction of visitors is one of the important steps for poultry biosecurity to minimize the contamination.

Limitations

The present study has some limitations. First, only the

sample were subjected to test rapid serum plate agglutination (SPA). Second, only eight farm practices were considered for biosecurity parameters. Lastly, presence of data collector in the working situation may had some influence on responses to a questionnaire.

CONCLUSIONS

The seroprevalence of *Salmonella spp.* in different types of poultry was detected both in Mymensingh and Gazipur district. The seroprevalence was comparatively higher in Mymensingh and in broiler farm. Summer is the more prevalent season than winter for salmonellosis in poultry. Strict farm hygienic practice and biosecurity measures are significantly linked to decrease the *Salmonella* infection. Therefore, the control of *Salmonella spp.* seroprevalence depends on limiting the sources of contam¬ination and transmission by implementing the biosecurity measures. However, further study could be the serotyping and molecular identification of salmonellosis in poultry maintained in different grade of biosecurity measures.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

There is no conflict of interest among the authors

Author contribution

MMM involved in conception and design of the experiments, questionnaire development, implementation of research, statistical analysis and manuscript writing. MAS contributed to revise the manuscript. ALB collected the questionnaire data and experimentation. All authors read and approved the manuscript and also contributed it critically for important intellectual content.

Ethical approval

The ethics of this study was in accordance to research ethics followed by the Department of Microbiology and Public Health, Faculty of Veterinary Medicine and Animal Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University. The ethical consent number was BSMRAU/FVMAS/MPH/20(Ethical Approval)/2020/03. Moreover, the verbal consent was also obtained from the poultry farmer.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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