



## Research article

# Seroprevalence of *Mycoplasma gallisepticum* (MG) infection in poultry and impact of biosecurity practices

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## Abstract

*Mycoplasma gallisepticum* (MG) infection is considered as one of the significant problems for the poultry industry in Bangladesh. Therefore, this study was to determine the seroprevalence of MG in poultry (Layer, Broiler, and Sonali) and the impact of biosecurity strategies. The study was carried out in the Mymensingh and Gazipur districts of Bangladesh from January to September 2021. A total of 338 serum samples were considered for the Serum Plate Agglutination (SPA) test. Findings indicated 56.80% overall seroprevalence, and comparatively, Mymensingh had a greater seroprevalence (59.02%) than Gazipur (54.19%). The highest seroprevalence (63.87%) was observed in Layers, whereas 56.55% and 45.95% were in Broilers and Sonali, respectively. Considering the seasonal variation, winter seasons had a considerably ( $p < 0.05$ ) higher chance of seroprevalence than summer, indicated by 83.33% and 68.35% in Layer and Broiler, respectively. Flock size has a meaningful ( $p < 0.01$ ) impact on MG seroprevalence established by the higher seroprevalence (79.41%) in Layer birds of flock sizes  $> 2000$  to  $< 2500$ . In logistic regression, MG seroprevalence was significantly ( $p < 0.01$ ) less like to occur where the farm had concrete floor (OR=0.446; 95% CI: 0.268-0.745), distance  $> 1000$  meters from nearby farms (OR=0.485; 95% CI: 0.289-0.812), used disinfectant regularly (OR= 0.362; 95% CI: 0.214-0.614), controlled the rodents (OR=0.374; 95% CI: 0.222-0.63), controlled the visitors (OR=0.553; 95% CI: 0.331-0.926), had well ventilation (OR=0.300; 95% CI: 0.178-0.503) and cleaned the waterer-feeder regularly (OR=0.518; 95% CI: 0.307-0.876). In short, reducing the sources of contamination and transmission by ensuring strict biosecurity measures, could be the strategy for controlling MG seroprevalence.

**Keywords:** Biosecurity, Mycoplasmosis, Prevention, Serum Plate Agglutination, Small-scale

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## INTRODUCTION

In the past few decades, Bangladesh's poultry industry has grown from a small backyard business to a highly advanced commercial enterprise (Akhter et al., 2018). Mainly, these industries furnish the dietary protein demands because it is a quicker and less expensive way to get animal protein (Islam et al., 2014). It is also observed that the farmer's initial financial commitment is to the poultry industry (Sobuj et al., 2021). The four types of poultry are white-feathered Broiler chickens (like Hybro-PN, Hubbard classic, Ross, and Cobb 500) (Dolberg, 2008), Sonali poultry (a crossbreed between a Fayoumi female and a Red Island Red male) (FAO, 2015), Layer chickens, and deshi (local chickens raised in backyards) are mainly reared poultry industry in the country. The first three poultry types are grown on commercial farms.

Despite Bangladesh's rapid expansion of poultry farms, several infectious diseases afflict this lucrative subsector (Sarkar et al., 2005; Haque et al., 2015). The most common and hazardous microbial illnesses in poultry are Mycoplasmosis and chronic respiratory diseases (Heleili et al., 2011). The threat to Bangladesh's poultry industry is the most pervasive, persistent, and financially damaging (Arefin et al., 2011; Ali et al., 2015). The financial loss carried on by lower egg production in Layer flocks, decreased hatchability in breeder flocks, a poor feed conversion ratio in Broilers, poor grade Broiler meat and condemnations (Carpenter et al., 1982; Kabir et al., 2021). Mymensingh and Gazipur districts are one of the poultry-based parts of Bangladesh where the poultry economy plays a significant role.

The microbes of the genus *Mycoplasma* cause mycoplasmosis under the family of Mycoplasmataceae (Freundt, 1955). These microbes are considered unusual bacteria (Dingfelder et al., 1991; Kempf et al., 1997). *Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae* (MS), *Mycoplasma meleagridis* (MM), and *Mycoplasma iowae* (MI) are four of the 25 species of *Mycoplasma* that have been identified in poultry (Bradbury, 2001; Evans et al., 2005). MG is most significantly responsible for Mycoplasmosis (Ley, 2008). MG can live for a few days to months, depending on the environment. They could survive on cotton, rubber, hair, and feathers for one to four days. In poultry embryos, the allantoic fluid and yolk can hold on to MG, letting it spread vertically and horizontally (Clark, 2019). However, total eradication through testing and slaughter is the most efficient way to combat Mycoplasmosis (Ley, 2008). Nevertheless, this is expensive in reality, and the emergence of multiage complexes in the commercial Layer industry makes this method impractical (Evans and Hafez, 1992; Levisohn and Kleven, 2000). On the other hand, biosecurity practices with improved management techniques, treatment, and immunization could be the crucial approaches to prevent Mycoplasmosis in flocks.

Biosecurity is preventing or limiting the exposure of animal or human populations to pathogenic germs. In contemporary poultry farming, biosecurity approaches hold off the diseases-producing microorganisms from the birds. Firstly, obtaining fertile eggs and chicks from *Mycoplasma*-free sources is the initial step in preserving biosecurity on the farm premises. Next, the birds must be raised with the appropriate hygiene and disinfectants (Hong et al., 2004). Risk factors for MG infection include inadequate ventilation, litter

pollution, unrestricted mobility of employees and guests, and other biosecurity precautions (Feizi et al., 2013). Minimizing the risk factors for Effective disease control depends on regular evaluations of farm biosecurity status (Meher et al., 2020). However, there needs to be more information on the seroprevalence of Mycoplasmosis in considering the farm biosecurity status in Bangladesh's poultry industrial area (Mymensingh and Gazipur). Numerous researches on *Mycoplasma gallisepticum* seroprevalence have been conducted in Bangladesh. Most of these studies emphasized on better biosecurity practices for controlling *Mycoplasma gallisepticum* infection in poultry (Barua et al., 1970; Sikder et al., 2005; Hossain et al., 2007; Hossain et al., 2010; Islam et al., 2014; Sobuj et al., 2021; Kabir et al., 2021; Raquib et al., 2022). However, considering the biosecurity parameters to evaluate the impact of biosecurity practices on MG controlling remains unknown. Particularly, the strict biosecurity practices may have significant impact on reducing the MG seroprevalence. Hence, this study aimed to ascertain the seroprevalence of *Mycoplasma gallisepticum* in commonly raised poultry species (Layer, Broiler, and Sonali) in small-scale poultry farms in Mymensingh and Gazipur districts of Bangladesh. Moreover, this study also sought to assess the connection between the seroprevalence of *Mycoplasma gallisepticum* and the biosecurity status of these farms.

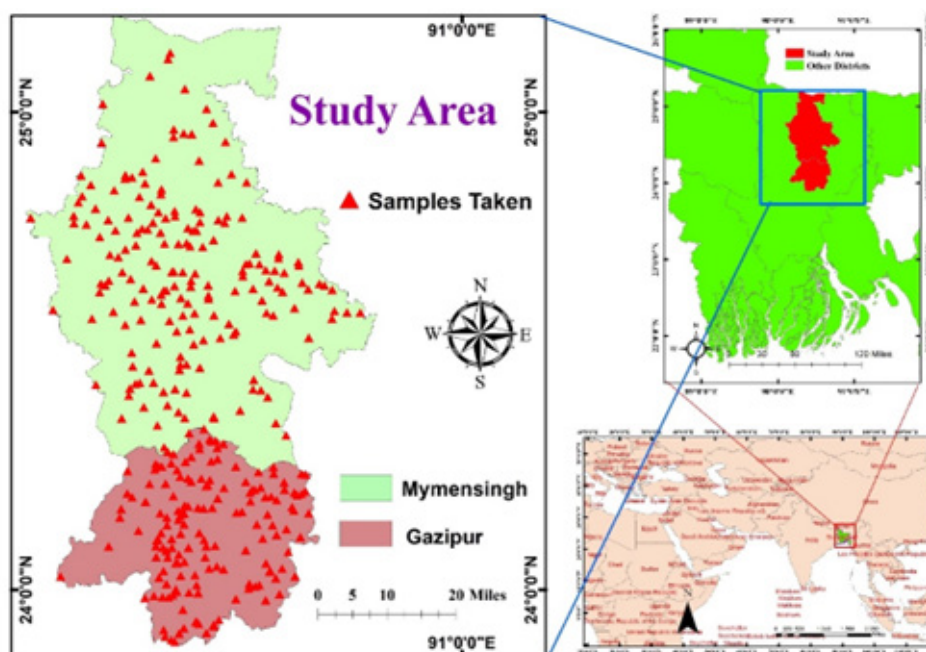
## MATERIALS AND METHODS

### Ethical approval

This study followed the research ethics of 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)/2020/04. Moreover, the verbal consent was also obtained from the poultry farmers.

### Study area and period

In order to carry out the study's objective, samples were collected from Mymensingh and Gazipur, two districts in Bangladesh that are situated between latitudes 24.30°N and 24.88°N and longitudes 90°164E and 90.73°E (Figure 1). ArcGIS-ArcMap (ESRI, USA) version 10.8 was used to demonstrate the study area. From January to September of 2021, the study was conducted.



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

### Sample Size Estimation

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

$$n = \frac{Z^2 P(1 - P)}{d^2}; n = \frac{(1.96)^2 \times 0.36(1 - 0.36)}{(0.05)^2}; n = 354.04 \cong 354$$

Where,

$n$  = sample size,

$Z = 1.96$  (95% confidence level),

$P$  = expected prevalence or proportion (in proportion of one; 36.25%,  $P = 0.36$ ), and

$d$  = precision (in proportion of one; whereas  $P=0.36$ , therefore  $d = 0.05$ ).

The authors of the previous study (Sobuj et al., 2021) discovered a 36.13% overall prevalence of *Mycoplasma gallisepticum* in southern region of Bangladesh. Therefore, 36.13 percent was assumed to be the expected prevalence. 354 was the approximate number of samples. A total of 355 blood samples were gathered, and after separation of serum from the blood only 338 samples were considered to test for seroprevalence.

### Sample collection

Without using anticoagulants, blood samples were taken from the wing veins of the selected birds under aseptic conditions. The study's entire flock of birds was chosen from small poultry farms with less than 2500 chickens. For the serum plate agglutination (SPA) test to determine the seroprevalence of *Mycoplasma gallisepticum*, a total of 338 blood samples were obtained. The serum was separated from the blood after blood was drawn using a sterile syringe and needle, and stored in syringes in a cold box in a standing position

for six hours. After being separated, serum samples were transferred to 1.5 ml microcentrifuge tubes until the serum plate agglutination (SPA) test was run. All of the blood samples were drawn from unvaccinated birds as their sources. The farmer was simultaneously asked about the chicken operation to gather the information. The findings from a past study were used to design the questionnaire to gather information on biosecurity procedures.

### ***Mycoplasma* spp. antigen**

Using the crystal violet stained *Mycoplasma gallisepticum* commercial antigen (Lillidale Diagnostic®, England), antibodies resulting from infections caused by both the standard and variant strains of *Mycoplasma gallisepticum* were found in sera samples. The manufacturer's (Lillidale Diagnostics-England) instructions for the SPA test were followed.

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

The steps specified by [Sikder et al., \(2005\)](#) were followed for performing the SPA test. Briefly, 0.02 ml of antigen and 0.02 ml of bird's serum were pipetted in equal volumes and placed side by side on a glass plate. Then, a toothpick was used to blend the antigen and serum samples carefully. The glass panel was illuminated from below to avoid unwanted heat from the light source while viewing the reaction. Within 2 minutes of combining the serum and antigen in the case of a positive response, identifiable clumps appeared. Along the edge of the liquid, clumps typically started to appear and condense. Absence of an agglutination reaction was the confirmation of a negative response. Precautions were taken to avoid false-positive results caused by natural granulation of the antigen.

### **Level of Infection**

The strength of the clumps was used to determine the degree of infection ([Figure 2](#)). The clumps generally started at the edges of the mixture and concentrated there. The strength of the agglutination reaction was determined using the procedures described by [Hossain et al., \(2007\)](#). In summary,

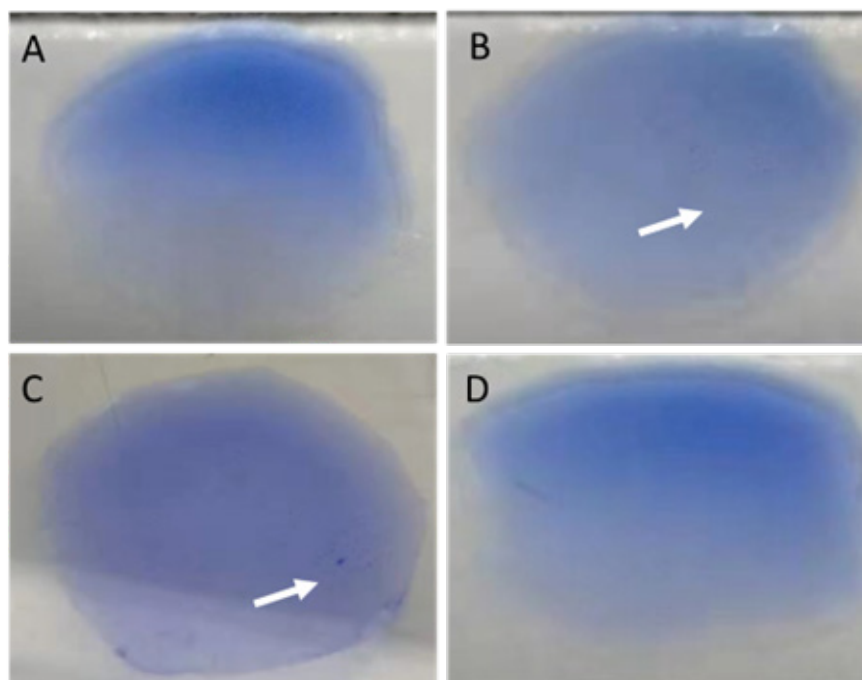
No infection (–) = No clumps with no background clearing.

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

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

Heavy infection (+++) = Large to huge clumps (mainly in the periphery) with complete background clearing.





**Figure 2** 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

### Gross Pathological Lesions

Dead birds were collected randomly from the poultry farms, where blood samples were taken for the SPA test. Organs from dead birds were dissected systemically, and the changes in the organs were noted. Changes in the specific internal organs are considered to confirm cases of clinically suspected *Mycoplasma gallisepticum* infection (Kumari et al., 2013). Each case used a different sanitized set of tools for the post-mortem examination. The same methodologies that Hossain et al., (2017) used here were applied.

### Statistical analysis

Before being imported into "Statistical Package for Social Sciences (SPSS)" version 25.0 for statistical analysis, all data were arranged in Microsoft Excel (Microsoft, 2019). Pearson's Chi-square test calculated the correlation between the categorical explanatory variable and the result. The p-value of continuity correction was considered when more than 20% of the cells in a 2×2 contingency table had an expected count of less than 5. However, the p-value of Fisher's exact tests was considered when the table comprised more than 2×2 contingencies. Additionally, a single sample Chi-square test was conducted if the table contains 1×3 contingencies. The correlations between conventional biosecurity practices employed on chicken farms and cases of *Mycoplasma gallisepticum* seropositivity were examined using a regression model. The binary logistic regression analysis was performed using the enter techniques. The appropriate assumptions were challenged before achieving all of the statistical tests. A p-value of  $\leq 0.05$  was regarded as significant.

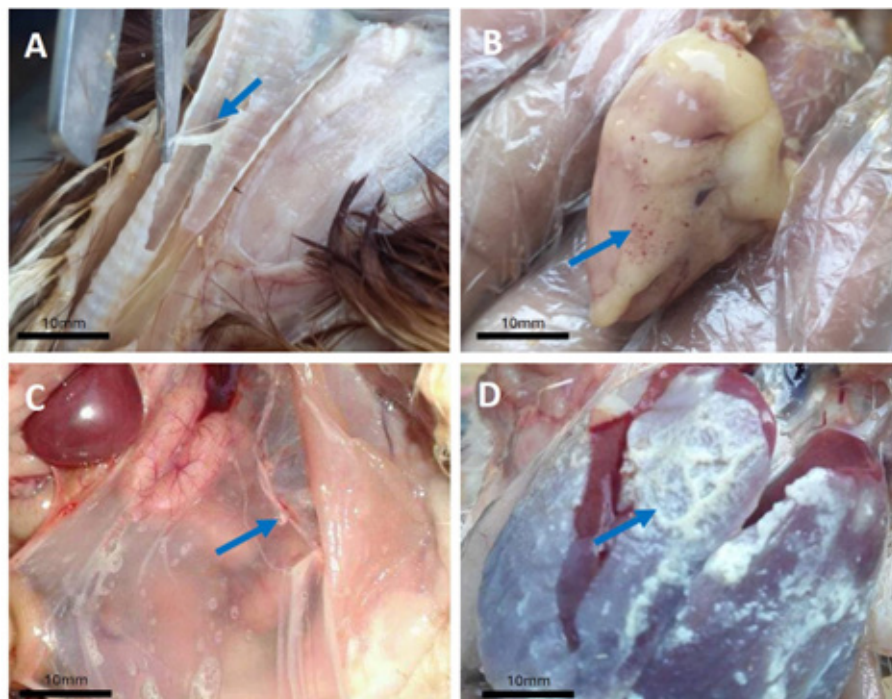
## RESULTS

A total of 338 samples were tested, and 192 were found to be positive. The overall *Mycoplasma gallisepticum* seroprevalence was 56.80%. Between the two study areas (Gazipur and Mymensingh), the higher seroprevalence of *Mycoplasma gallisepticum* was 59.02% in Mymensingh district. Among the different types of poultry, higher seroprevalence was observed in Layer birds of 63.87% (Table 1). Clinical manifestations of Mycoplasmosis included tracheal rale, nasal discharge, coughing, facial oedema, lacrimation, and decreased feed intake. Additionally, Cloudy air sac, pericarditis, perihepatitis, congested, and mucoid trachea were all found during post-mortem examination (Figure 3).

**Table 1** Prevalence of *Mycoplasma gallisepticum* infection in different poultry farms of Gazipur and Mymensingh district of Bangladesh.

Categories		No. of Sample Tested	Positive Cases (Prevalence)					
Variable	Level		Overall	P value	Farm Species			P value
			n (%)		Layer (%)	Broiler (%)	Sonali (%)	
Area	Gazipur	155	84 (54.19)	0.380	34(61.82)	36(53.73)	14(42.42)	0.219
	Mymensingh	183	108 (59.02)		42(65.63)	46(58.97)	20(48.78)	0.245
Total		338	192 (56.80)		76(63.87)	82(56.55)	34(45.95)	0.049

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



**Figure 3** The gross pathological lesions show that the (A) mucous in the trachea, (B) pericarditis, (C) Cloudy air sac and inflammation, and (D) Perihepatitis.

## Prevalence of *Mycoplasma gallisepticum* infection in commercial Layer birds

The seroprevalence of *Mycoplasma gallisepticum* was significantly ( $p<0.05$ ) higher in the winter season (83.33%) than summer season 39.6% in the study area in Layer birds (Table 2). The Layer birds of the farms having a flock size of >2000 to <2500 birds had comparatively ( $p<0.01$ ) higher prevalence than the other flock sizes. Among the different levels of flock size, the significantly ( $p<0.01$ ) higher percentages (66.67%) were moderately infected, which was in the >1500 to  $\leq$ 2000 flock size group.

Though the birds of <10 weeks of age showed the highest (80.00%) prevalence among the different levels of age variable, there were no significant ( $p>0.05$ ) variations. Between the two districts of the study area, the result showed a higher (65.63%) prevalence in Mymensingh district than in Gazipur (61.82%) without any significant ( $p>0.05$ ) variations. In considering the level of infection in Layer birds, the significant ( $p<0.05$ ) proportions (48.68%) of *Mycoplasma gallisepticum* seropositive were moderately infected.

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

Categories			Positive Case			Level of Infection			
Variable	Level	No. of Sample Tested	(N)	Prevalence (%)	P value	Low	Moderate	High	P value
Season	Winter	66	55	83.33	0.000	17(30.91)	27(49.09)	11(20)	0.680
	Summer	53	21	39.62		5(23.81)	10(47.62)	6(28.57)	
Flock Size	$\leq 500$	26	10	38.46	0.006	0(0)	8(80)	2(20)	0.006
	>500 to $\leq 1000$	20	10	50.00		2(20)	6(60)	2(20)	
	>1000 to $\leq 1500$	14	11	78.57		8(72.73)	2(18.18)	1(9.09)	
	>1500 to $\leq 2000$	25	18	72.00		2(11.11)	12(66.67)	4(22.22)	
	>2000 to <2500	34	27	79.41		10(37.04)	9(33.33)	8(29.63)	
Age (Weeks) of the Birds	<10	30	24	80.00	0.353	10(41.67)	10(41.67)	4(16.67)	0.637
	10-19	25	16	64.00		2(12.5)	9(56.25)	5(31.25)	
	20-29	18	10	55.56		2(20)	7(70)	1(10)	
	30-39	16	8	50.00		3(37.5)	3(37.5)	2(25)	
	40-49	19	11	57.89		4(36.36)	4(36.36)	3(27.27)	
	Above 50	11	7	63.64		1(14.29)	4(57.14)	2(28.57)	
Area	Gazipur	55	34	61.82	0.811	9(26.47)	16(47.06)	9(26.47)	0.731
	Mymensingh	64	42	65.63		13(30.95)	21(50)	8(19.05)	
Total		119	76	63.87		22(28.95)	37(48.68)	17(22.37)	0.014

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



## Prevalence of *Mycoplasma gallisepticum* infection in commercial Broiler birds

The seroprevalence of *Mycoplasma gallisepticum* in Broiler birds according to different categorical variables is shown in Table 3. In this case, the seroprevalence was significantly ( $p<0.01$ ) higher (68.35%) in winter in comparison to summer (42.42%) seasons. The Broiler birds of >1500 to  $\leq 2000$  flock size showed insignificantly ( $p>0.05$ ) greater (75%) seroprevalence than other flock size farms. Higher seroprevalence was 65.63%, observed in the birds of <5 days old, but had no significant ( $p>0.05$ ) variation with the other age groups. The Broiler birds of the Mymensingh district showed a higher prevalence of 58.97% than the Broiler of the Gazipur district. In considering the level of infection in Broiler birds, the significant ( $p<0.05$ ) proportions (46.34%) of *Mycoplasma gallisepticum* seropositive were moderately infected.

**Table 3** Prevalence of *Mycoplasma gallisepticum* infection in commercial Broiler birds in contrast to different parameters.

Categories		No. of Sample Tested	Positive Case			Level of Infection			P value
Variable	Level		(N)	Prevalence (%)	P value	Low	Moderate	High	
Season	Winter	79	54	68.35	0.001	17(31.48)	23(42.59)	14(25.93)	0.639
	Summer	66	28	42.42		7(25)	15(53.57)	6(21.43)	
Flock Size	$\leq 500$	23	12	52.17	0.205	4(33.33)	6(50)	2(16.67)	0.200
	>500 to $\leq 1000$	28	14	50.00		3(21.43)	10(71.43)	1(7.14)	
	>1000 to $\leq 1500$	21	10	47.62		3(30)	3(30)	4(40)	
	>1500 to $\leq 2000$	32	24	75.00		4(16.67)	12(50)	8(33.33)	
	>2000 to <2500	41	22	53.66		10(45.45)	7(31.82)	5(22.73)	
Age (Days) of the Birds	<15	64	42	65.63	0.144	9(21.43)	21(50)	12(28.57)	0.475
	15-30	40	19	47.50		7(36.84)	7(36.84)	5(26.32)	
	31- 45 and above	41	21	51.22		8(38.1)	10(47.62)	3(14.29)	
Area	Gazipur	67	36	53.73	0.641	9(25)	17(47.22)	10(27.78)	0.700
	Mymensingh	78	46	58.97		15(32.61)	21(45.65)	10(21.74)	
Total		145	82	56.55		24(29.27)	38(46.34)	20(24.39)	0.039

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

## Prevalence of *Mycoplasma gallisepticum* infection in commercial Sonali birds

The *Mycoplasma gallisepticum* infection (seroprevalence) in Sonali birds concerning different categorical variables and levels are indicated in Table 4. Surprisingly, there was no significant variation in seroprevalence among the different levels of other categorical variables, even though the level of infection was insignificantly ( $p>0.05$ ) different among the seropositive Sonali chickens. However, the summer season (47.2%), birds of >1500 to  $\leq 2000$  flock size (56.25%), age of 61 to  $\geq 90$  days (50%) and Sonali birds of Mymensingh district (48.78%) showed higher seroprevalence.

**Table 4** Prevalence of *Mycoplasma gallisepticum* infection in commercial Sonali 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
Season	Winter	38	17	44.74	0.830	4(23.53)	7(41.18)	6(35.29)	0.904
	Summer	36	17	47.22		5(29.41)	7(41.18)	5(29.41)	
Flock Size	≤500	14	7	50.00	0.516	2(28.57)	3(42.86)	2(28.57)	0.977
	>500 to ≤1000	9	2	22.22		1(50)	1(50)	0(0)	
	>1000 to ≤1500	13	5	38.46		2(40)	2(40)	1(20)	
	>1500 to ≤2000	16	9	56.25		2(22.22)	3(33.33)	4(44.44)	
	>2000 to <2500	22	11	50.00		2(18.18)	5(45.45)	4(36.36)	
Age (Days) of the Birds	<30	22	9	40.91	0.815	3(33.33)	4(44.44)	2(22.22)	0.491
	31-60	24	11	45.83		3(27.27)	6(54.55)	2(18.18)	
	61- 90 and above	28	14	50.00		3(21.43)	4(28.57)	7(50)	
Area	Gazipur	33	14	42.42	0.585	4(28.57)	6(42.86)	4(28.57)	0.923
	Mymensingh	41	20	48.78		5(25)	8(40)	7(35)	
Total		74	34	45.95		9(26.47)	14(41.18)	11(32.35)	0.72

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

### Role of biosecurity practices on the prevalence of *Mycoplasma gallisepticum* infection

The association of *Mycoplasma gallisepticum* seroprevalence with different biosecurity practices in poultry farms is analysed in Table 5. The poultry house floor made with soil was significantly ( $p<0.01$ ) more seroprevalent (66.47%) than the poultry house having concrete floor (47.02%). Among the positive samples, a significantly higher rate (46.9%) was observed in Layer birds raised in soil floor houses. The birds of the farm that had a distance of  $\leq 1000$  meters showed higher seroprevalence (65.87%) than the distance of  $>1000$  meters (47.95%). Surprisingly, a significant ( $p<0.01$ ) proportion (52.44%) of seropositive Layer birds' farms were situated at a distance of  $>1000$  meters. The regular use of disinfectant significantly ( $p<0.01$ ) reduced the seroprevalence (44.16%) of *Mycoplasma gallisepticum* than the irregular use (67.39%). Though the poultry farms had the control on the access of wild birds showed significantly ( $p<0.01$ ) higher seroprevalence (62.91%), the control on rodents and visitors showed a significantly ( $p<0.05$ ) lower proportion of seroprevalence which was 43.95% and 46.6% respectively. The sufficient ventilation of the poultry farm and regular cleaning of the feeder and water also played significant ( $p<0.01$ ) roles in reducing the seroprevalence, indicated by the lower proportion of seroprevalence (43.60% and 43.88%, respectively). The other biosecurity practices like mixing birds' species in a shed, foot bath in front of the farm, separate clothes for farm handlers and proper disposal of dead birds had no significant ( $p>0.05$ ) influence on *Mycoplasma gallisepticum* seroprevalence.

**Table 5** Prevalence of *Mycoplasma gallisepticum* infection in commercial birds with respect to biosecurity parameters.

Categories		N	Positive Case						
Variable	Level		Overall			Farm Species			
			(n)	Prevalence (%)	P value	Layer	Broiler	Sonali	P value
Floor of poultry house	Concrete	168	79	47.02	0.000	23(29.11)	34(43.04)	22(27.85)	0.003
	Soil	170	113	66.47		53(46.9)	48(42.48)	12(10.62)	
Distance (meter) between the farms	>1000	171	82	47.95	0.001	43(52.44)	33(40.24)	6(7.32)	0.001
	≤1000	167	110	65.87		33(30)	49(44.55)	28(25.45)	
Mixed birds' species in a shed	Yes	181	107	59.12	0.417	39(36.45)	51(47.66)	17(15.89)	0.298
	No	157	85	54.14		37(43.53)	31(36.47)	17(20)	
Foot bath in front of farm gate	Yes	156	86	55.13	0.641	32(37.21)	40(46.51)	14(16.28)	0.631
	No	182	106	58.24		44(41.51)	42(39.62)	20(18.87)	
Use of disinfectants	Regularly	154	68	44.16	0.000	31(45.59)	25(36.76)	12(17.65)	0.384
	Irregularly	184	124	67.39		45(36.29)	57(45.97)	22(17.74)	
Separate cloth for farm handler	Yes	131	69	52.67	0.268	26(37.68)	30(43.48)	13(18.84)	0.940
	No	207	123	59.42		50(40.65)	52(42.28)	21(17.07)	
Proper disposal of dead birds	Yes	151	89	58.94	0.547	37(41.57)	38(42.7)	14(15.73)	0.766
	No	187	103	55.08		39(37.86)	44(42.72)	20(19.42)	
Control of wild bird	Yes	151	95	62.91	0.042	39(41.05)	41(43.16)	15(15.79)	0.778
	No	187	97	51.87		37(38.14)	41(42.27)	19(19.59)	
Control of rodents	Yes	157	69	43.95	0.000	21(30.43)	31(44.93)	17(24.64)	0.23
	No	181	123	67.96		55(44.72)	51(41.46)	17(13.82)	
Well ventilation	Yes	172	75	43.60	0.000	28(37.33)	36(48)	11(14.67)	0.448
	No	166	117	70.48		48(41.03)	46(39.32)	23(19.66)	
Cleaning of waterer and feeder	Regularly	139	61	43.88	0.000	23(37.7)	25(40.98)	13(21.31)	0.446
	Irregularly	199	131	65.83		53(40.46)	57(43.51)	21(16.03)	
Control of visitors	Yes	147	68	46.26	0.001	24(35.29)	29(42.65)	15(22.06)	0.671
	No	191	124	64.92		52(41.94)	53(42.74)	19(15.32)	
Total		338	192	56.80		76(39.58)	82(42.71)	34(17.71)	0.049

### Logistic regression analysis for *Mycoplasma gallisepticum* prevalence in poultry

As is presented in Table 6, the binary logistic analysis clarifies the consequence of biosecurity practices (common farm biosecurity) in poultry farms on *Mycoplasma gallisepticum* seroprevalence. The result revealed that more than a few variables of familiar biosecurity practices on the probability affected *Mycoplasma gallisepticum* seroprevalence. The logistic model contained twelve independent variables (Floor of the poultry house, Distance (meter) between farms, Mixed birds' species in a shed, Foot bath in front of the farm gate, Use of disinfectants, Separate cloth for farm handler, Proper disposal of dead birds, Control of wild bird, Control of rodents, Well ventilation, Cleaning of waterer and feeder and Control of visitors). The regression model was statistically significant ( $p < .001$ ), indicating that the model can differentiate between *Mycoplasma gallisepticum* seropositive and seronegative samples in considering the biosecurity practices in poultry farms. Overall, this model could clarify between 26.3% (Cox and Snell R square) and 35.3% (Nagelkerke R squared) of the variance in seroprevalence of *Mycoplasma gallisepticum* status. The model was also appropriately organized in 73.4% of instances. Hence, the

goodness of fit for this model was determined by the p-value of 0.295 ( $p>0.05$ ) in the “Hosmer and Lemeshow” test, which indicates that the final model is fit. The results of logistic regression suggest that the farm had a concrete floor (OR=0.446; 95% CI: 0.268-0.745) and distance >1000 meters from nearby farms (OR=0.485; 95% CI: 0.289-0.812) were significantly ( $p<0.01$ ) less like to *Mycoplasma gallisepticum* seroprevalence. Moreover, the significantly ( $p<0.01$ ) less tendency to *Mycoplasma gallisepticum* seroprevalence was also found in the farms that used of disinfectant regularly (OR=0.362; 95% CI: 0.214-0.614), controlled the rodents (OR=0.374; 95% CI: 0.222-0.63), controlled the visitors (OR=0.553; 95% CI: 0.331-0.926), had good ventilation (OR=0.300; 95% CI: 0.178-0.503) and cleaned the waterer and feeder regularly (OR=0.518; 95% CI: 0.307-0.876). Surprisingly, the farm that controlled the access of wild birds had a significantly ( $p<0.05$ ) higher tendency to seroprevalence, indicated by the odd ratio of 1.717 ( $p=0.041$ ; 95% CI: 1.022-2.885). The other biosecurity variables like having a foot bath (OR=0.685; 95% CI: 0.404-1.155) and separate cloth for farm handler (OR=0.866; 95% CI: 0.51-1.471) had insignificant ( $p>0.05$ ) propensity to seroprevalence. On the other hand, the farms that had mixed bird species in the same shed (OR=1.138; 95% CI: 0.681-1.902) and had no proper disposal system for dead birds (OR=1.232; 95% CI: 0.732-2.073) had insignificantly ( $p>0.05$ ) higher tendency to seroprevalence.

**Table 6** Logistic regression analysis of common biosecurity practices in poultry farm associated with *Mycoplasma gallisepticum* prevalence.

Categories		Wald	Odd Ratio	P value	95% C.I. for O. R.	
Variable	Level				Lower	Upper
Floor of poultry house	Concrete	9.546	0.446	0.002	0.268	0.745
	Soil					
Distance (meter) between Farm	>1000	7.56	0.485	0.006	0.289	0.812
	≤1000					
Mixed birds' species in a shed	Yes	0.244	1.138	0.622	0.681	1.902
	No					
Foot Bath in front of Farm	Yes	2.018	0.685	0.155	0.406	1.155
	No					
Use of Disinfectants	Regularly	14.19	0.362	0	0.214	0.614
	Irregularly					
Separate Cloth for Farm Handler	Yes	0.284	0.866	0.594	0.51	1.471
	No					
Proper Disposal of Dead Birds	Yes	0.618	1.232	0.432	0.732	2.073
	No					
Control of wild bird	Yes	4.167	1.717	0.041	1.022	2.885
	No					
Control of rodents	Yes	13.651	0.374	0	0.222	0.63
	No					
Well ventilation	Yes	20.76	0.3	0	0.178	0.503
	No					
Cleaning of waterer and feeder	Regularly	6.027	0.518	0.014	0.307	0.876
	Irregularly					
Control of Visitors	Yes	5.081	0.553	0.024	0.331	0.926
	No					

$R^2= 0.263$  (Cox & Snell R Square), 0.353 (Nagelkerke R Square)

Hosmer and Lemeshow test p value: 0.295; Significant at 1% ( $P<0.01$ ); Significant at 5% ( $P<0.05$ ); C.I.= Confidence Interval; O.R. = Odd Ratio.

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## DISCUSSION

The present study revealed the seroprevalence of *Mycoplasma gallisepticum* in three commonly reared types of chicken (Layer, Broiler, and Sonali) in the Mymensingh and Gazipur districts of Bangladesh. In Layer birds, the observed seroprevalence of *Mycoplasma gallisepticum* was 63.87%, which is a bit higher and comparable to the findings of previous research conducted by the SPA test in different parts of Bangladesh. Like as, Sikder et al., (2005) observed 56.86% seroprevalence in the Patuakhali district, Hossain et al., (2007) found 55.13% seroprevalence in the Rajshahi district, Haque et al., (2015) reported 27% in Kotwali thana of Chittagong district and Sobuj et al., (2021) reported 36.13% in southern region of Bangladesh. Though these observations were lower than our findings, a higher seroprevalence of 73% was observed in the Kishoreganj district of Bangladesh (Raquib et al., 2022). In another research (Ali et al., 2017), 64.48% seroprevalence was determined in northern Bangladesh by the iELISA test, which is very close to our observed seroprevalence. In the case of Broiler-type poultry, the observed seroprevalence was 56.55%. In another study, the *Mycoplasma gallisepticum* seroprevalence was 49.50% in the Chittagong district of Bangladesh (Barua et al., 1970). Other than Bangladesh, the SPA test recorded 42.22% seroprevalence of *Mycoplasma gallisepticum* in Broilers of rural areas in Iran (Feizi et al., 2013). These findings have variations to our conclusions in Broiler birds. In the case of Sonali birds, the current observation was 45.95% by the SPA test. In the other studies on Sonali birds, Ali et al., (2017) reported 60.64% seroprevalence by SPA test in the northern region of Bangladesh. In considering the clinical and postmortem findings, Talukdar et al., (2017) reported 12.79% *Mycoplasma gallisepticum* prevalence. These observations have remarkable differences from our results. This might be due to differences in the detection methods for *Mycoplasma gallisepticum* prevalence and regional specificity. The discrepancies between the present and earlier findings can be attributed to changes in the sample size, selection of samples, methodologies employed in serological diagnosis, geographic regions, climatic factors, and operation of commercial poultry farms (Sobuj et al., 2021). However, the result showed that the significantly ( $p < 0.05$ ) higher seroprevalence was in winter for Layer and Broiler birds. These findings are in line with the other research, Sikder et al., (2005), Hossain et al., (2007), Islam et al., (2014), Raquib et al., (2021), Sobuj et al., (2021) found the highest seroprevalence in winter that was 61.49%, 80%, 60.42%, 45.54%, and 61.45% respectively. However, current findings indicate that Sonali birds show insignificantly ( $p > 0.05$ ) higher seroprevalence in summer seasons. This seasonal variation in seroprevalence of *Mycoplasma gallisepticum* might be due to the sudden change in temperature and cold stress on the birds, depress the natural resistance of birds, leading to an increased susceptibility to infections (Haque et al., 2015). The seroprevalence could be affected by unfavorable weather conditions, such as cold and changing relative humidity.

The findings of this current study show that the flock size is a significant factor that influences the spread of *Mycoplasma gallisepticum* infection in Layer birds other than Broiler and Sonali birds. Broiler and Sonali chickens are mainly reared for shorter periods than Layer chickens, and this could be the



reason for the significant relation between flock size density and *Mycoplasma gallisepticum* seroprevalence. Comparatively, the highest seroprevalence was observed in larger flocks than in smaller ones. Specifically, the highest seroprevalence was recorded at 79.41% in >2000 to <2500 flock size Layer farms, where the poultry farms with flock size <2500 birds were only included for this research. Similarly, the other authors, [Hossain et al., \(2007\)](#), [Mukhtar et al., \(2012\)](#), [Ali et al., \(2017\)](#), [Raquib et al., \(2022\)](#) reported that the highest seroprevalence of *Mycoplasma gallisepticum* in large size flocks of the commercial Layer farm. Moreover, [Hossain et al., \(2010\)](#) observed that seroprevalence was increased in Layer birds with the increased flock size density, which agrees with the findings of this present research. Increased seroprevalence of *Mycoplasma gallisepticum* in large-scale flocks might be due to the faultiness of management and biosecurity measures as well as horizontal transmission of the organisms from one bird to the other ([Chandiramani et al., 1996](#)). However, [Sobuj et al., \(2021\)](#) reported the highest *Mycoplasma gallisepticum* seroprevalence in medium-sized flocks, followed by small and large-sized flocks. Poor management practice and improper biosecurity in different-sized flocks play a significant role in the fluctuation of *Mycoplasma gallisepticum* seroprevalence of Layer chickens ([Raquib et al., 2022](#)). Although age is a significant factor influencing the incidence of Mycoplasmosis ([Whitford et al., 1994](#)), age had no significant impact on another study, which could be due to regional specificity. In Layer birds, seroprevalence of *Mycoplasma gallisepticum* declined with the increase of age. Remarkably, the highest seroprevalence was 80% in younger birds (<10 weeks) compared to older ones. A similar report was demonstrated in Layer birds by other research; for instance, [Hossain et al., \(2007\)](#) found 72.72% at 18-25 weeks of age, [Kabir et al., \(2021\)](#) reported about 72% in 10-20 weeks of age, [Islam et al., \(2014\)](#) found highest seroprevalence (54.84%) in pullets. Surprisingly, the seroprevalence of *Mycoplasma gallisepticum* gradually decreased with the increase of age in the case of Sonali birds. Some researchers also reported on an upward tendency of *Mycoplasma gallisepticum* seroprevalence with age ([Islam et al., 2015](#); [Raquib et al., 2022](#)). Younger chickens may have higher seroprevalence due to vertical transmission of the organisms from the parent flock or lower immunity levels, and it is possible to predict that the illness cannot be controlled from commercial flocks until and unless parent flocks are clear of Mycoplasmosis. All the changes in organs after postmortem inspection were in line with the findings of [Talukdar et al., \(2017\)](#). For assessing the relation of biosecurity practice with *Mycoplasma gallisepticum* seroprevalence, a total of twelve parameters were considered. The birds raised in a house with a concrete floor tended less tendency (OR=0.446) to *Mycoplasma gallisepticum* seroprevalence; this indicates that the concrete floor in poultry farms improves the working conditions as well as the most accessible sanitary control and disinfection of poultry sheds. A similar report also found that the concrete floor farm had less prevalence of Newcastle diseases ([Meher et al., 2020](#)). The poultry farm situated less distant (<1000 meters) had a higher chance of seroprevalence because *Mycoplasma gallisepticum* led to a carrier state and can be transmitted both horizontally and vertically ([McMartin, 1968](#); [Vardaman et al., 1973](#)).

Horizontal spread can occur via direct contact and indirect contact through aerosol transmission or by introduction of contaminated materials or persons ([Feberwee et al., 2022](#)). Most of the above reasons for the probability

of *Mycoplasma gallisepticum* spread are higher in nearby farms. Additional risk factors for introducing and transmitting *Mycoplasma gallisepticum* include multi-age sheds and inadequate hygiene management. However, the farms that used disinfectant regularly were much less likely (OR=0.362) to be infected by *Mycoplasma gallisepticum*. Because most of the *Mycoplasma* spp. are sensitive to disinfectant, especially to 0.5% sodium hypochlorite or 2% chlorhexidine (Justice-Allen et al., 2010). In the early study, Stallknecht et al., (1982) reported that wild birds may have served as a mechanical carrier of *Mycoplasma gallisepticum* but did not appear to be engaged in its transmission or maintenance. In another study, Michiels et al., (2016) claimed that wild birds were positive (1.7%) to *Mycoplasma gallisepticum*. This research agrees with our findings that control of wild birds is less likely (OR=0.041) to be seropositive. Although rodents are also infected (24.5%) by hemotrophic *Mycoplasma* (Alabí et al., 2020), our study found a lower tendency of *Mycoplasma gallisepticum* seroprevalence in the farms that had control on rodents, probably due to disruption of mechanical transmission of *Mycoplasma gallisepticum*. Ventilation enhances poultry's respiration system, which could be the reason for the lower tendency of *Mycoplasma gallisepticum* seroprevalence in our study. Regularly cleaning of waterer and feeder and control of visitor access had less propensity to seroprevalence. Other studies have shown that mycoplasma species can survive on different materials like straw, rubber, nose, hair, dust, feed, water, and egg debris and can be regarded as a source of indirect transmission (Abolnik and Gouws, 2014). The visitors' feeder, waterer, nose, hair, nails, and clothing may be a source of indirect transmission.

Nevertheless, survival time is low on most materials (1-4 days) except for egg material, which can survive for several months (Abolnik and Gouws, 2014). Survival potential outside the host is also evidenced by the biofilm formation by MG, which provides survival advantage and increased resistance to disinfectants (Wang et al., 2017). Notably, some other factors like as poor ventilation, litter contamination, and a lack of restrictions on the movement of technical staff, visitors, and other individuals, as well as poor biosecurity practices, are more frequent parameters for the occurrences of MG infection (Hoshyar et al., 2019).

## CONCLUSIONS

*Mycoplasma gallisepticum* is prevalent in the Layer's, Broiler, and Sonali birds both in the Mymensingh and Gazipur districts of Bangladesh. The overall seroprevalence was high in Mymensingh in comparison to Gazipur district. The Layer birds are more prone to seroprevalence among three types of poultry. Mostly, *Mycoplasma gallisepticum* infection is prevalent in winter seasons, larger flocks, and younger Layer and Broiler birds, but it is vice versa in Sonali birds. Most of the biosecurity parameters have significant associations with *Mycoplasma gallisepticum* seroprevalence. Results indicate that strict farm hygienic practices and improved biosecurity measures are significantly linked to decreasing *Mycoplasma gallisepticum* seroprevalence. Therefore, the control of *Mycoplasma gallisepticum* seroprevalence depends on limiting the sources of contamination and transmission by implementing biosecurity measures. Other efforts need to be made towards educating poultry farmers regarding

vaccination programs and adopting appropriate prophylactic or therapeutic measures for adequate control of MG infection in Layer birds. However, further study could include the serotyping and molecular identification of *Mycoplasma gallisepticum* from poultry farms having different levels of biosecurity practices, along with the calculation of losses caused by *Mycoplasma gallisepticum* every year.

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## AUTHOR CONTRIBUTIONS

**MMM** involved in conception and design of the experiments, questionnaire development, implementation of research, statistical analysis and manuscript writing.

**MA** contributed to revise the manuscript.

**AAB and ABH** collected the questionnaire data and experimentation. All authors read and approved the manuscript and also contributed it critically for important intellectual content.

## CONFLICT OF INTEREST

There is no conflict of interest among the authors.

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