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Seroprevalence of *Mycoplasma gallisepticum* infection in layer chickens of Bangladesh

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Abstract

Mycoplasma gallisepticum causes major health hazards in poultry birds in Bangladesh which results in huge economic losses every year. This study was carried out to estimate and analyze the prevalence of *Mycoplasma gallisepticum* (MG) infection in commercial layer chickens at Kishoreganj district of Bangladesh during the period from November 2018 to October 2019. A total of 505 serum samples from 94 commercial layer farms of Kishoreganj Sadar Upazila and Pakundia Upazila of Kishoreganj district were collected. Serum plate agglutination (SPA) was performed to detect the antibody against MG. Prevalence was found 73% in the Kishoreganj district by SPA test. MG was significantly ($P<0.01$) more prevalent in Pakundia Upazila 82% than Kishoreganj Sadar Upazila 61.11%. In case of season, winter season had significantly higher ($X^2=30.94$, $p=0.000$) prevalence of MG infection. In relation to age, seroprevalence of MG infection was highest 78% in birds of 65 weeks' age and lowest 71% in 6-25 weeks' age birds. Any significant ($P>0.05$) association was not found between flock size and seroprevalence of MG. Seroprevalence was highest in flock containing above 2600 birds. MG infection is prevalent in the chicken population of Kishoreganj district, Bangladesh. Measures should be taken for successful prevention and control of this disease in Bangladesh.

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Introduction

Poultry industry has made a remarkable progress in livestock sector of Bangladesh in last decades (1,2). Despite the rapid growth of the industry, one of the major constraints of poultry farming in Bangladesh is the outbreaks of different infectious diseases (3). Among the major health hazards caused by different microbial agents being faced by poultry birds, mycoplasmosis is of great importance which brings serious monetary damage for poultry industry (4-6). Mycoplasmosis is a chronic respiratory disease of poultry (7) caused by *Mycoplasma gallisepticum* and *Mycoplasma synovia* (8,9) and less commonly by *Mycoplasma meleagridis* and *Mycoplasma iowae* (10). This pathogen can be transmitted through a direct contract from infected birds to healthy birds (horizontally) and newly hatched birds can get this pathogen by transovarian (vertically) transmission (11-14). Affected birds can show signs of nasal discharge, tracheal rales, ocular discharge, and coughing (15-17) or they can be asymptomatic showing no clinical signs which results in undetected MG infection in the farm causing latent infection (18). Mycoplasmosis weakens the immune system of infected birds that facilitates infection with other pathogens (19). Birds of all ages can be affected by

mycoplasmosis but young birds are more susceptible to it (9,15). Morphological, cultural, biochemical and serological properties of mycoplasma can be studied for the diagnosis of mycoplasmosis. Among the serological tests, serum plate agglutination (SPA) is mostly used in field conditions for the rapid diagnosis of mycoplasmosis because it is easy to perform and cost-effective. The test and slaughter program can be an effective measure for the total eradication of mycoplasma (20,21).

Control of MG infection in poultry farms can be achieved by fetching birds from MG infection free sources and employing vaccination program (5,22,23). Mycoplasmosis has been a huge burden for the poultry industry of Bangladesh for many years because it costing Bangladesh a huge economic loss by alleviating feed conversion ratio, weight gain and by diminishing egg production and egg hatchability in layers. Among 64 districts of Bangladesh Kishoreganj is one the most densely populated poultry region.

For the lack of enough data about mycoplasmosis, it has been very difficult to take proper control measures against it. So this serological study of *Mycoplasma gallisepticum* (MG) infection was done to know the actual status of MG infection in layer chicken at the Kishoreganj district of Bangladesh.

Materials and methods

Ethical approval

The birds were handled according to the rules and regulations of the Animal Care and Use Committees (ACUC) of Directorate of Livestock, Ministry of Fisheries and Livestock, Bangladesh.

Study area and population

The study was conducted on 94 commercial layer farms in two Upazilas (KishoreganjSadar and Pakundia) of Kishoreganj District during the period of November 2018 to October 2019 at Vetlab Kishoreganj.

The entire samples were brought in the Vetlab Kishoreganj by the farmers in study period for routine diagnosis of diseases in their farms.

Blood collection and serum preparation

Blood samples 2 ml were collected aseptically from the wing vein of unvaccinated live birds using sterile syringes 5 ml and kept in room temperature for 2 hours for clot formation and serum was collected by decanting. The serum was poured into a labeled screw capped vial and stored at -20ºC until use (24).

Serum plate agglutination (SPA) test

This test was done at room temperature 25ºC with LilliTest MG antigen (Lillidale Diagnostics-England) to detect MG antibodies in serum samples. The SPA test was performed according to the manufacturer's (Lillidale Diagnostics-England) instructions. For this test 0.03 ml of MG antigen was placed on a clean dry glass plate and immediately an equal volume of serum sample was placed next to the antigen. Then antigen and serum samples were mixed using a glass rod. In case of MG positive samples, colored agglutination formed within 2 minutes and no agglutination was the indication of MG negative samples. Agglutination was assigned score from + to +++. The sera samples having agglutination score ++ or greater were recorded as positive and used for calculation of prevalence.

Statistical analysis

The location of each study areas were geographically visualized in maps by using ArcGIS (Geographic information system) desktop 10.7. In case of data entry, management and creating of graphs Microsoft Excel® 2013 was used. All the serological data were analyzed by using SPSS 25 for windows (SPSS, Inc., Chicago, IL). Pearson's Chi-square test was used to calculate the significant differences among the variables. A p-value of less than 0.05 was thought as statistically significant.

Results

Overall prevalence of MG infection

This study shows that 505 sera samples were obtained from 94 commercial layer farms of Kishoreganj district and from those samples 368 cases were found to be positive with *Mycoplasma gallisepticum* (MG) infection after subjected to SPA test (Table 1). So, overall seroprevalence of MG infection was 73.10% (95% Cl, 69.23-77.10) in the study area.

Table 1: Seroprevalence of MG infection in selected farms

Area	No. tested	+ve cases	Prevalence
Kishoreganj	505	368	73 (69.23-77.10)

Seroprevalence of MG infection based on the study area

Table 2 represents the seroprevalence of MG infection in layer chickens on two Upazilas of Kishoreganj district. In this study seroprevalence of MG infection was found to be significantly higher (X^2=26.41, p=0.000) in Pakundia Upazila 82%.

Season-wise seroprevalence of MG infection

In our study, there was a highly significant (P<0.001) relationship between MG infection and season (Table 3). In the winter season 80% seroprevalence of MG infection was higher than the summer season 56.41%.

Seroprevalence of MG infection by age

Age wise number of samples collected from Pakundia and Kishoreganj upazilla.

According to the present investigation, the seroprevalence of MG infection was recorded 71% in 6-45 weeks, 69% in 46-65 weeks and 75% in above 65 weeks of age birds (Table 4). Seroprevalence of MG infection was not significantly (X^2=4.13, p=0.25) higher in the above 65 weeks layer chicken in relation to the young group of layer chickens.

Seroprevalence of MG infection in relation to flock size

Flock wise number of samples collected from Pakundia and Kishoreganj upazilla. The seroprevalence of MG infection was 71% within 600-1600 bird's flocks, 74% within 1600-2600 bird's flocks and 75% above 2600 bird's flocks. Flock size was not significantly (p>0.05) differ with MG infection in layer chickens (Table 5).

Table 2: Seroprevalence of MG infection based on the study areas

Location	No. flocks	No. tested	+ve cases	Sero prevalence (%)	X^2	P-value
Kishoreganj	42	216	132	61	26.4	0.000
Pakundia	52	289	236	82		

Table 3: Seroprevalence of MG according to season

Season	No. flocks	No. tested	+ve cases	Seroprevalence (%)	X^2	P-value
Winter (Nov-Mar)	62	349	280	80	30.94	0.000
Summer (Apr-Oct)	32	156	88	56		

Table 4: Seroprevalence of MG infection in different ages

Age (Weeks)	No. flocks	No. tested	+ve cases	Seroprevalence (%)	X^2	P-value
6-25	11	68	48	71	4.13	0.25
26-45	30	148	105	71		
46-65	22	118	81	69		
Above 65	31	171	134	78		

Table 5: Seroprevalence of MG infection in relation to flock size

Flock size	No. flocks	No. tested	+ve cases	Seroprevalence (%)	X^2	P-value
600-1600	54	224	159	71	0.74	0.69
1601-2600	30	217	161	74		
Above 2600	10	64	48	75		

Discussion

A total of 505 sera samples were collected from 12 commercial layer farms at laying period and were subjected to SPA test. Out of these, 368 samples were found positive for MG infection by SPA test. The overall prevalence of MG infection was 73%. The overall seroprevalence of this study was support the previous report of Jalil *et al.* (21) who found 67.4% seroprevalence in layer farms of Khulna district. The prevalence of this study was higher than that of Islam *et al.* (25) and Sikder *et al.* (26) and Ali *et al.* (27) and Sarker *et al.* (3) who reported 55.83, 56.9, 56.13 and 58.9% seroprevalence of MG infection in Bhola district, Patuakhali district, Bogra, and Feni district of Bangladesh. Lower prevalence of MG infection was also found in India (28), Pakistan (29) and Saudi Arabia (30) with 53.40, 44.9, and 46.11% respectively. This variation might be due to differences in management practice, nature of poultry farming, higher density of farms in the area, environmental, geographical location, and higher incidence of MG infection and existence of MG infection for a higher duration in the study area.

Seroprevalence in Kishoreganj Sadar Upazila was 61% which agrees with the findings of Sikder *et al.* (26) who recorded 58.73% and 63.28% seroprevalence at two unions of Patuakhali district of Bangladesh. Findings in Kishoreganj Sadar Upazila also are in close agreement with the findings of Hussain *et al.* (31) and Chrysostome *et al.* (32) but findings in Pakundia Upazila is higher than those earlier findings. Pakundia Upazila is a pastoral region in the Kishoreganj district where almost every house has a poultry farm but their knowledge about poultry farm is very limited which might be a cause for the higher prevalence of MG infection in this area.

The present study showed highly significant (P<0.001) relationship between MG infection and season. In the winter season 80% seroprevalence of MG infection was higher than the summer season 56.41%. Similar findings were recorded

by (21) who reported 75.59% seroprevalence of MG infection in the winter season and 66.31% in the summer season. Similarly, Islam *et al.* (25) also found a higher seroprevalence of MG infection in the winter season 60.42% compared to the summer season 51.25%.

Within the different age groups seroprevalence was highest in above 65 weeks old layers. Higher prevalence of MG infection in older layers was also recorded by Islam *et al.* (2) and Ayim *et al.* (33) in Bangladesh and Ghana. However, findings of this study do not agree with the findings of Hossain *et al.* (10) and Islam *et al.* (25) who recorded a higher prevalence of MG infection in young layer birds. Improper bio-security, poor ventilation and intensive nature of commercial poultry farms might be the cause of high infection of MG in older birds (34).

In the study, it was found that the prevalence of MG infection was highest 75% in above 2600 flock size layer farms and lowest 71% in 600-1600 flock size layer farms. There was a slight increase of seroprevalence with the increase flock density which agrees with the findings of Hossain *et al.* (10) and Ayim *et al.* (33) who found a consistent increase of seroprevalence with the increase of flock size. Poor management practice and improper biosecurity in different size flocks play a significant role in the fluctuation of seroprevalence of MG infection in different flock size of layer chickens.

Conclusion

From the present investigation, it is evident that *Mycoplasma gallisepticum* infection is highly prevalent in the layer's birds of the Kishoreganj district. The finding represents prevalence is high in winter season, irrespective of different ages; different flock sizes and it may be due to lack of farmer's knowledge about biosecurity and management practice about poultry farm. And it is suggested that training about the management of poultry farms should frequently be arranged to educate the farmers. Further studies on the countrywide prevalence of MG should be done to know the current status and losses caused by MG in Bangladesh.

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Conflict of interest

The authors declare that there is no conflict of interest.

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الانتشار المصلي للإصابة بالمَفْطورَةُ المُنْتِنَةُ للدَّجاج في الدجاج البياض في بنغلاديش

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الخلاصة

تسبب المَفْطورَةُ المُنْتِنَةُ للدَّجاج مشاكل صحية كبيرة في الدواجن في بنغلاديش والتي تسبب خسائر اقتصادية كبيرة في كل سنة. تم إجراء هذه الدراسة لتقدير وتحليل انتشار الإصابة بالمَفْطورَةُ المُنْتِنَةُ للدَّجاج في قطعان الدجاج البياض في مقاطعة كيشوريجانج في بنغلاديش خلال الفترة المتمددة من تشرين الثاني ٢٠١٨ ولغاية تشرين الأول ٢٠١٩. تم جمع ٥٠٥ عينة مصل دم من ٩٤ قطيع دجاج بياض من سردار ابازيل وبكونداي ابازيل من مقاطعة كيشوريجانج. استخدم طبق تلازن مصل الدم لغرض الكشف عن الأجسام المضادة ضد المَفْطورَةُ المُنْتِنَةُ للدَّجاج. كانت نسبة الانتشار في مقاطعة كيشوريجانج ٧٣% باستخدام طبق تلازم مصل الدم. حيث كانت المَفْطورَةُ المُنْتِنَةُ للدَّجاج اعلى معنويا في منطقة بكونداي ابازيل ٨٢% من النسبة المسجلة في سردار ابازيل والتي كانت بنسبة ٦١,١١%. أما فيما يخص الموسم فان فصل الشتاء كان اعلى في نسبة الإصابة المَفْطورَةُ المُنْتِنَةُ للدَّجاج من باقي فصول السنة (س^٢= ٣٠,٩٤، أ= ٠,٠٠٠). أما فيما يتعلق بالعمر فان الانتشار المصلي للمَفْطورَةُ المُنْتِنَةُ للدَّجاج كانت عالية بنسبة ٧٨% في الطيور بعمر ٦٥ أسبوعاً، والأقل معنوياً كانت بنسبة ٧١% في الأسبوعين ٦ و ٢٥ من عمر الطيور. لم يتم ملاحظة أية ترابط معنوي بين حجم القطيع ونسبة انتشار المَفْطورَةُ المُنْتِنَةُ للدَّجاج، وكانت نسبة الانتشار المصلي اعلى في القطعات اللي ضمت أعداد من الطيور أكثر من ٢٦٠٠ طائر. إن انتشار المَفْطورَةُ المُنْتِنَةُ للدَّجاج في قطعان الدجاج عالية في مقاطعة كيشوريجانج، في بنغلاديش، إذ يجب تطبيق إجراءات الوقاية وبشكل فعال لغرض منع والسيطرة على هذا المرض في بنغلاديش.

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