Investigation on Seroprevalence of *Mycoplasma Gallisepticum* Infection in Commercial Layer Farms in Rajshahi District Using Rapid Serum Plate Agglutination Test

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ABSTRACT

Mycoplasma gallisepticum (MG) infection is very common in birds that cause respiratory infections in chickens, turkeys, and other avian species. It has brought about a considerable amount of financial losses to the poultry sector in Bangladesh. We conducted a study on the Seroprevalence of MG infection in two different geographical areas of Bangladesh under Rajshahi district, namely Paba and Bagmaraupazila. 800 sera samples were collected and tested with Rapid Serum Plate Agglutination Test (RSA) to identify the MG antibodies using commercial *Mycoplasma gallisepticum* antigen. The gross Seroprevalence of MG infection was 59.25% in the study area. The maximum rate (68.80%) of infection was found in the winter season, followed by the summer season (49.36%). The result further revealed that the condition was higher (69.01%) in larger-sized flocks than in small (53.63%). We noticed that younger birds having 10-20 weeks of age are more prone to be affected with avian mycoplasmosis with an infection rate of 72% compared to their adult counterpart of 71+ weeks with 52% morbidity. Our study revealed that *Mycoplasma gallisepticum* infection is prevalent in Paba and Bagmaraupazila in Rajshahi. The farms should take strict bio-security measures to mitigate this infection in the mentioned areas. Proper medications for the affected birds and timely prophylactic measures for the healthy ones could be practical and preventive strategies against avian mycoplasmosis. Amid limitations, we conducted our experiments, and thus further research is warranted to substantially assess and validates our observations.

Keywords: Seroprevalence, Commercial Poultry, *Mycoplasma Gallisepticum* Infection, Serum Agglutination Test.

Submitted : June 22, 2021

Published : July 14, 2021

ISSN: 2684-1827

DOI: 10.24018/ejfood.2021.3.4.331

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I. INTRODUCTION

Infectious diseases, especially bacterial and viral, generate major health problems for the poultry industry's proper growth and development. *Mycoplasma gallisepticum* is considered an essential deleterious microorganism in layer birds that causes financial losses [1], [2]. Significant losses in chickens can be caused by economically significant pathogen *Mycoplasma*, which causes feed efficiency, decreased growth, lower egg production as well as production of inferior quality carcasses. It's likely that the carcasses of slaughtered birds will be downgraded. This organism has been eradicated from commercial poultry breeding flocks in many countries with modern poultry operations. However, in many other poultry operations, it can still be a concern, such as multi age layer flocks, game bird raising facilities and backyard birds [3].

In terrestrial poultry, *Mycoplasma gallisepticum* is one of the most important agents which belongs to the family Mycoplasmataceae (class Mollicutes, order Mycoplasmatales). This organism comes in a variety of strains, which can have varying degrees of virulence and host preferences. The house finch lineage is a distinct that has become established in wild birds after diverging significantly from poultry strains. Some pathogenic strains of mycoplasmas in poultry include *M. synoviae*, *M. meleagridis* and *M. iowae*. Avian mycoplasmosis is the name given to the diseases they cause, but the clinical symptoms vary [4]. Pleuropneumonia like species emerged as other pathogenic and saprophytic isolates from veterinary and human sources accumulated. *Mycoplasma* (Greek: mykes, fungus plasma, moulded) refers to the filamentous (fungus-like) nature of certain species' cells, as well as the plasticity of the outer membrane, which results in pleomorphism [5]. Mycoplasmas, for example, are the tiniest prokaryotic species that can develop in a cell-free culture medium. Humans, animals, plants, insects, dirt, and sewage all contain them. The first mycoides spp. was isolated from pleuropneumonia-stricken cattle in 1898. Chickens, turkeys, and other game birds are all affected by *M. gallisepticum* such as ring necked pheasants (*Phasianuscolchicus*), chukar partridges (*Alectorisgraeca*), redlegged partridges (*Alectorisrufa*), grey partridges (*Perdixperdix*), bobwhite quail (*Colinusvirginianus*), Japanese quail (*Coturnixjaponica*) and peafowl (*Pavocristatus*). This organism has also been detected in ducks and geese, although it does not seem to be a significant pathogen in waterfowl. Pet or hobby birds are also affected, which are symptomatic yellow napped Amazon parrots (*Amazonaauropalliata*) and asymptomatic pigeons (*Columba livia*) [6].

Respiratory and ocular secretions, eggs and semen are the source of *M. gallisepticum*. *M. gallisepticum* which are in the egg and debris from broken eggs may be a source of the organism for the poultry and other birds. Egg borne transmission is more frequent in birds infected during laying than in birds infected before they mature. Long term asymptomatic carriage has been reported in poultry, some game birds and house finches. When birds are in a stressed condition, they develop clinical signs. *M. gallisepticum* can be spread by fomites and can survive for many days in the atmosphere. Some substrates, such as feathers and the contents of eggs, have been documented to have a higher rate

of survival. This organism was found to survive for a day or two on human skin and one day on bird feeders. Mycoplasmas can survive in the environment by forming biofilms. The amount of biofilm generated by different strains varies [7].

Illnesses which are induced by *M. gallisepticum* are possible to diagnose by isolating the organism from clinical samples or detecting nucleic acids by means of PCR, and flock serology. Loop mediated isothermal amplification assays have also been published. In mycoplasma free chicken embryos or chicken, MG can be retrieved; however, since the invention of PCR, this method has been seldom used. The colonal cleft, oropharynx, conjunctiva, infraorbital sinus, nasal cavity, esophagus, trachea, air sacs, and lungs are all typical sampling sites for culture and PCR. Swabs from the cloaca and phallus, as well as embryonated eggs, dead in shell embryos, and chicks or poults that have broken the shell but failed to hatch, can all contain organisms. Postmortem samples should be collected from recently dead animals or carcasses frozen soon after death. Frey's medium is the media through which MG can be isolated. It is not always easy to bounce back. To recognize cultured species, indirect immunofluorescence, immunoperoxidase staining, a growth inhibition procedure, metabolism inhibition, and PCR or other DNA methods are used. Biochemical tests can be helpful in the early stages of diagnosis. It may be difficult to tell the difference between *M. gallisepticum* and *M. imitans*, a species that is more commonly found in ducks and geese. If *M. imitans* is suspected, tests such as PCR restriction fragment length polymorphism (PCR-RFLP) or immunofluorescence using serial dilutions of antisera to the two organisms in parallel may be used to differentiate the two species.

The disease's microscopic lesions (multifocal parenchymal necrosis, meningitis, perivascular cuffing, and vasculitis) have been identified as consistent and distinct. Serology is particularly useful when it comes to screening in poultry flocks. Individual birds are less useful since non-specific reactions are normal in some studies. A rapid serum agglutination (RSA) test, ELISAs, and hemagglutination inhibition are examples of widely used assays. The hemagglutination inhibition test is more specific than the RSA test, which can produce false positives, but it is also strain specific and less sensitive. Other serological tests have been identified or used [8].

Thus, we examine the Seroprevalence of MG infection's current situation regarding age, season, and flock sizes in commercial layer chickens at two different areas in the western part of Bangladesh (Rajshahi district) using RSA.

II. METHODOLOGY

A. Location of the Study Area

This study was conducted in two different sites: The Laboratory of Quality feeds Limited and Paba and Baghmaraupazila of Rajshahi, Bangladesh. Geographically Rajshahi is situated within Barind Tract at 23m above sea level, where the divisional city centre stands upon the northern alluvial plains of the Padma River [9].

B. Sampling

800 blood samples were collected from the wing vein of unvaccinated and healthy chickens from January 2018 to May 2019. The sampling considered the age group, seasons (summer and winter), Flock size, and region.

C. Data Collection

Some information about qualitative variables (geographical area of flocks, seasons, commercial strain of layer type, present of maternal antibodies against MG, any vaccination against MG administered in their parent flocks) and quantitative variables (farm capacity, egg production rate, house number, flock age) were gathered from each farm to be calculated for correlation.

D. Preparation of Sera Samples

Blood samples were obtained aseptically from the wing vein of the selected birds using 5ml sterile disposable syringe and needles. The blood caused the syringe to coagulate and was kept for 1-2 hours at room temperature. After clotting, a clean straw colored serum was seen around the clotted clump, sera were separated, centrifuged and poured in sterile vials, labeled individually and stored at 4°C until used.

E. Mycoplasma Antigen

Standard *Mycoplasma gallicepticum* (MG RSA-CevaBiovac) antigen purchased from Biolab Company Ltd. Bangladesh was used for Rapid Slide Agglutination (RSA) test for the detection of *M. gallisepticum* antibodies in the sera samples.

F. Rapid Serum Plate Agglutination Test (RSA)

Rapid tests were carried out at 25°C and within 24 hours of collection of sera. The RSA test was performed by crystal violet stained commercial MG RSA-CevaBiovac antigen. For this test 0.02ml antigen and 0.2 ml serum was mixed thoroughly on a glass plate. In positive cases, agglutination was noticed within 2 minutes of gentle rocking. However, in negative cases, such a phenomenon was not evident. To calculate the prevalence agglutination strengths were scored from (0) to (+++) (Fig. 1). The sera's heat inactivation offset any false-positive reaction at 56°C for 30 minutes in a water bath.

Fig. 1. Agglutination reaction scored (0) to (+++) (a, b and c).

III. RESULTS

From each flock five chickens were randomly selected for blood collection. 2 ml of blood was collected aseptically from wing vein of each bird and then sera were separated and stored at -21°C until use for serum plate agglutination test to determine the Seroprevalence of MG infection. The Seroprevalence of mycoplasmosis was calculated by considering total number of samples screened for

mycoplasmosis and number of samples detected positive per formula.

$$\text{Seroprevalence of Mycoplasmosis (\%)} = \frac{\text{No. of positive cases}}{\text{No. of screened samples}} \times 100$$

A. *M. gallisepticum* Antibodies Detection by RSA

A total of 800 sera samples were collected during winter and summer season from different geographical areas of Rajshahi district tested by Rapid Serum Plate Agglutination (RSA) test with commercial MG antigen.

Fig. 2. Overall Prevalence of Mycoplasmosis.

The Seroprevalence of mycoplasmosis caused by MG was compared with respect to regions, seasons, age of birds and flock sizes and the test was performed in laboratory after collecting serum. Out of 800 sera samples tested 474 (59.25%) were found positive by RSA (Fig. 2).

B. Region Wise Seroprevalence of Mycoplasmosis

Seroprevalence of mycoplasmosis was observed in two different geographical areas of Rajshahi district. Out of 800 sera samples from two different geographical areas 407 sera samples were collected from the Bagmaraupazila and 393 samples from Pabaupazila. In Bagmara 239(58.72%) sera samples out of 407 were positive and in Paba upazila 235(59.79%) samples out of 393 were positive. Highest Seroprevalence of mycoplasmosis caused by MG was observed in Paba upazila about 59.79% (Table I, Fig. 3).

Fig. 3. Region wise Seroprevalence of Mycoplasmosis.

TABLE I: REGION WISE SEROPREVALENCE OF MYCOPLASMOSIS

Geographical Region	Total sample collected	MG positive	MG Negative
Bagmaraupazila	407	239 (58.72%)	168 (41.28%)
Paba upazila	393	235 (59.79%)	158 (40.21%)
Total	800	474 (59.25%)	326 (40.75 %)

C. Age wise Seroprevalence of Mycoplasmosis

800 sera samples of different ages of different layer birds were collected and grouped them to interpret age wise Seroprevalence of the disease. Total 125 serum samples were collected from birds between 10-20 weeks of age. And the Seroprevalence was 72% at the 10-20 weeks of age. Observed Seroprevalence in 21-30 weeks, 31-40 weeks, 41-50 weeks, 51-60 weeks, 61-70 weeks, and 71 weeks above was 68.14%, 60.48%, 48.25%, 61.85%, 51.85%, and 52.22% respectively (Table II, Fig. 4).

TABLE II: AGE WISE SEROPREVALENCE OF MG BY USING RSA TEST

Age of Bird (Weeks)	Total sample collected	MG Positive	MG Negative	Level of significance
10-20	125	90 (72%)	35 (28%)	0.95 NS
21-30	113	77 (68.14%)	36 (31.86%)	
31-40	124	75 (60.48%)	49 (39.51%)	
41-50	143	69 (48.25%)	74 (51.74%)	
51-60	97	60 (61.85%)	37 (38.14%)	
61-70	108	56 (51.85%)	52 (48.15%)	
71-Above	90	47 (52.22%)	43 (40.74%)	
Total	800	474(59.25%)	326(40.75%)	

NS= Nonsignificant at 5% probability.

** Significant at 5% ($p < 0.05$).

Fig. 4. Age wise Seroprevalence of MG by using RSA test.

D. Seasonal Variation in Seroprevalence of mycoplasmosis

A total number of 407 samples collected in winter and 280 were (69.01%) positive for MG antibodies. 393 samples were collected in summer season, out of which 194 (49.49%) were positive for MG antibodies (Table III, Fig. 5).

Fig. 5. Seasonal variation of mycoplasmosis.

TABLE III: SEASONAL VARIATION OF MYCOPLASMOSIS

Season	Total samples tested	MG Positive	MG Negative	Level of Significance
Winter	407	280 (68.80%)	127 (31.20%)	0.0000000116
Summer	393	194 (49.36%)	199 (50.64%)	**

E. Flock Size Wise Seroprevalence of MG

The highest infection rate was recorded (69.01%) in large scale flocks (3001-3500) in comparison (53.63%) to small size flocks having 500-1000 birds (Table IV, Fig. 6). Highest infection rate in large scale flocks probably due to the faulty management and bio-security.

TABLE IV: FLOCK SIZE WISE SEROPREVALENCE OF MG

Flock Size	Total Sample Collected	MG Positive	MG Negative	Level of significance
500-1000	138	74 (53.63%)	64 (46.37%)	0.0000088 **
1001-1500	132	71 (53.78%)	61 (46.21%)	
1501-2000	134	76 (56.71%)	58 (43.28%)	
2001-2500	139	88 (63.30%)	51 (36.69%)	
2501-3000	127	75 (59.00%)	52 (40.99%)	
3001-3500	130	90 (69.01%)	40 (30.99%)	
Total	800	474(59.25 %)	326 (40.75%)

Fig. 6. Flock size wise Seroprevalence of MG.

IV. DISCUSSION

Poultry is an emerging industry, and its progress is remarkable in Bangladesh. It has a bright prospect not only in Bangladesh but also in the whole world. Being a leading industry, it is contributing to the economic growth rate of Bangladesh. People of Bangladesh are getting inclined to this profession because of an unparalleled platform for a quick profit. So, it is now playing a major role in poverty alleviation. Despite this, it has some constraints, such as infectious diseases. Infectious diseases hampered poultry farming by its fatal outbreak. Avian mycoplasmosis is a kind of infectious disease which causes poor health condition and less in productivity, chronic respiratory disease, respiratory tract infection, and infectious sinusitis. It also causes considerable economic losses in chicken by reducing weight

gain, lower meat quality, and the significant drop in egg production in layer birds. Taking the tremendous economic impact of avian mycoplasmosis in layer chickens in various regions of the Rajshahi district into consideration in our project, we endeavored to determine and develop a quick and efficient diagnostic platform.

800 sera samples were collected from the Rajshahi district were subjected to RSA test. Out of 800 sera samples, 474 (59.25%) were found positive for MG. A case study on the Seroprevalence in Patuakhali district of Bangladesh was reported by 56.86% MG infection in commercial layer chicken [10]. It was reported that 58.90% of cases positive for MG infection in some model breeder poultry farms in the Feni district of Bangladesh [11]. There were also reported of 69%, 60.25%, 56.54%, 59.10%, 57.15%, 54.90% MG infection in layer chickens [12]-[15]. The parameter of age can influence mycoplasmosis. We have recorded age-wise seroprevalence, and the highest rate of MG infection, about 72%, was seen in 10-20 weeks of age group followed by 64.86%, 58.71%, 55.52%, 48.89%, 47.43%, and 45.36 in 21-30 weeks, 31-40 weeks, 41-50 weeks 51-60 weeks, 61-70 weeks and 71-above weeks of age respectively. A similar observation was reported by 72.72% at 18-25 weeks of age and lowest by 44% at above 66 weeks of age (Hossain et al., 2007). Equally increased vulnerability of younger birds to mycoplasmosis was demonstrated [10], [11].

During the winter season, seroprevalence of MG was recorded highest by 69.01% and lowest rate of infection as 49.49% during summer. It was noted that inclement weather such as cold and altered relative humidity could influence the seroprevalence. Our findings are relatively close, conforming to the earlier results [11]. The seroprevalence of MG was stated highest in the winter season [16]. Similar statements have been reported as 61.48% & 61.45% in winter come after summer 47.7% & 51.74%, respectively [17], [10]. Regarding flocking density, the maximum infection of MG was recorded in large-sized flocks having a bird's density of 3001-3500 (61.01%) compared to small ones (53.63%). These findings have similarities with other scientists from Bangladesh [17], [18].

The highest infection rate in larger flocks might be correlated with poor management and bio-security measures in addition to horizontal transmission of the organisms from one bird to the other. During the farm survey, compromised management regarding bio-security was evident. So, the prevalence of *Mycoplasma* is higher where the management system is inadequate. Thus, the occurrence of mycoplasmosis and the excellent management system is negatively correlated.

In earlier, it has also been reported that the MG infection was more prevalent in the flocks kept under poor management conditions. The other contributing factors of high prevalence in the Rajshahi district might include the construction of poultry farms in close vicinity and the opportunity to recycle the pathogens to persist longer in the area. The other factors that may contribute to MG infection include inadequate ventilation, contamination of litter, and no restriction on the movement of technical staff and visitors from one farm to other as basic bio-security measures.

V. CONCLUSION

The highest Seroprevalence of MG was found in Paba upazila 59.79%, and in Bagmaraupazila 58.72%. Regarding age group, 10-20 weeks layer chickens were mainly affected with MG. In the winter season, MG infection was noted highest and, the infection rate was 68.80%, and in the summer, it was 49.36%. According to flock size, large size flock was more affected than other age groups. Therefore, more attention should be paid to age, season, and flock size because mycoplasmosis causes significant economic losses due to reduced feed conversion rate, egg production, and growth rate in layer birds in Bangladesh. Layer chickens affected by MG infection should be isolated, give proper medication, and eventually culled. Strict bio-security should be maintained for proper management of poultry farms and the prevention of infectious diseases by means of prophylactic treatment and vaccination. A detailed country-wide investigation is warranted for designing a comprehensive MG offsetting strategy in Bangladesh.

ACKNOWLEDGMENT

The authors gratefully thank the Quality Feed Limited, Bangladesh for rendering the technical support in carrying out all the research experiments in its facilities.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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