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Article

Seroprevalence and pathology of *Mycoplasma gallisepticum* infection in layer birds

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Abstract: Avian mycoplasmosis, caused by *Mycoplasma gallisepticum* (MG), imposes a significant threat to the poultry industry in Bangladesh, leading to considerable economic losses. This study aimed to determine the seroprevalence of MG and characterize the associated pathology in MG-infected layer birds from different geographical areas within the Nilphamari district. Our hypothesis suggested that the seroprevalence of MG infection in layer birds may vary based on geographical location within the Nilphamari district of Bangladesh, with higher prevalence expected in areas with colder temperatures, such as during the winter season, and in densely populated poultry farming regions. For the serum plate agglutination test, 400 random blood samples were collected, while tissue samples from suspected layer birds with respiratory distress were obtained from 40 commercial layer farms for histopathological analysis. The serum plate agglutination test revealed an overall seroprevalence of MG of 64.25%, with the highest prevalence occurring during the winter season (70%), particularly among layer birds aged 20-40 weeks. Moreover, the seroprevalence of MG was significantly higher in Kishoregonj upazila (68%) compared to Nilphamari sadar (62%). Gross examination of MG-infected layer birds unveiled characteristic features such as tracheal hemorrhages and mucous accumulation, pulmonary edema and hemorrhages, and air sac cloudiness. Histopathological observations included tracheal mucosal sloughing and increased goblet cell numbers, as well as lung edema, hemorrhages, and tissue destruction, alongside significant reactive cell infiltration and exudation in the air sacs. By shedding light on the current MG infection status in layer chickens within the Nilphamari district, this study provides valuable insights to aid poultry industry stakeholders in formulating preventive strategies and enhancing the overall health and welfare of poultry flocks.

Keywords: avian mycoplasmosis; seroprevalence; histopathological analysis; respiratory distress; commercial layer farms

1. Introduction

Over the past few decades, Bangladesh's poultry industry has undergone remarkable expansion and advancement, evolving from a modest backyard endeavor into a flourishing business sector (Rahman *et al.*, 2015, 2017). It has emerged as a vital component of the country's agricultural landscape, contributing substantially to economic growth and generating employment opportunities, particularly in rural areas (Nugroho *et al.*, 2021). Livestock and poultry farming, including the poultry sector, play a crucial role in meeting the demand for animal protein and addressing poverty alleviation in Bangladesh (Moller *et al.*, 2023). Poultry farming provides an affordable and efficient means of producing animal protein, accounting for a considerable portion of the whole meat requirement in the country (Akhter *et al.*, 2018).

However, the poultry industry faces challenges, including the risk of infections that can result in substantial economic losses. *Mycoplasma gallisepticum* (MG), a chronic respiratory disease that affects poultry, particularly layer birds, is the cause of avian mycoplasmosis (Ayim-Akonor *et al.*, 2018; Shiferaw *et al.*, 2022). MG infection leads to decreased egg production, carcass condemnation, and low mortality rates among layer birds. It is considered a major problem in the poultry industry worldwide, resulting in significant direct and indirect losses, such as reduced hatchability, poor-quality chicks, decreased growth rates, and increased costs associated with disease control and eradication programs (Basak *et al.*, 2021). MG infection is prevalent globally, and its impact on the poultry industry necessitates a comprehensive understanding of its epidemiology, clinical signs, and pathological manifestations. The disease spreads through both vertical transmission via infected eggs and horizontal transmission through close contact (Armour and Ferguson-Noel, 2015). Clinical signs of MG infection in birds include respiratory symptoms such as conjunctivitis, sneezing, coughing, nasal discharge, breathing difficulties, and swollen infraorbital sinuses (Bharathi *et al.*, 2018; Zhang *et al.*, 2020). Moreover, some infected birds may not exhibit clinical signs, complicating detection and control efforts. Nevertheless, comprehensive scientific research on the seroprevalence and pathology of avian mycoplasmosis, attributed to MG, remains limited in Bangladesh. Although studies in the southern region (Sobuj *et al.*, 2021), Bhola district (Islam *et al.*, 2014), Kishoregonj district (Raquib *et al.*, 2021), and Rajshahi district (Kabir *et al.*, 2021) have contributed valuable insights, there is still a notable absence of detailed information regarding avian mycoplasmosis in the northern region, particularly in the Nilphamari district of Bangladesh.

We hypothesized that the seroprevalence of MG infection in layer birds may vary based on geographical location within the Nilphamari district of Bangladesh, with higher prevalence expected in areas with colder temperatures, such as during the winter season, and in densely populated poultry farming regions. Understanding the prevalence and characterization of MG infection in this region is crucial for formulating effective prevention and control strategies. Diagnosis of MG infection often involves serological tests, with the serum plate agglutination (SPA) test being a rapid and valuable tool for detection. Furthermore, accurate pathological examination is essential for definitive diagnosis and the implementation of appropriate treatment and disease control measures. The current study aimed to determine the seroprevalence of MG infection in layer birds in Nilphamari district, Bangladesh, and to characterize the disease based on clinical signs and pathological observations.

2. Materials and Methods

2.1. Ethical approval and informed consent

This study did not require ethical permission from the university. However, verbal consent was obtained from the farm owner during the sample collection and necropsy procedures.

2.2. Experimental areas

Samples were collected from the livestock office and veterinary hospital of Nilphamari Sadar and Kishoregonj Upazila and the surrounding villages of these two upazilas of Bangladesh (Figure 1).

2.3. Experimental birds

Birds displaying clinical symptoms were physically examined at various locations around the Nilphamari Sadar and Kishoregonj upazilas. Those birds brought to the Upazila livestock office and veterinary hospital for diagnosis and treatment were classified as experimental subjects.

2.4. Collection of samples

For the serum plate agglutination test, 400 random blood samples were collected. Tissue samples (lungs, trachea, and air sacs) from layer birds with respiratory distress were also collected for histopathological studies. These samples came from 40 commercial layer farms in the experimental areas during the experiment period.

2.5. Blood collection and preparation

For live birds, a volume of 1-2 ml of blood was obtained from the wing vein using a new disposable plastic syringe (3ml) without any anticoagulant. The blood was then left to clot for 1 hour in the syringe at room temperature. A syringe containing blood was stored in a refrigerator at 4°C for 4-5 hours. The blood was then centrifuged at 2,500 g rpm for 5 minutes in order to obtain a clarified serum. The serum was thereafter collected in a sterile eppendorf tube and stored at -20° C until it was ready to be processed for the serological investigation.

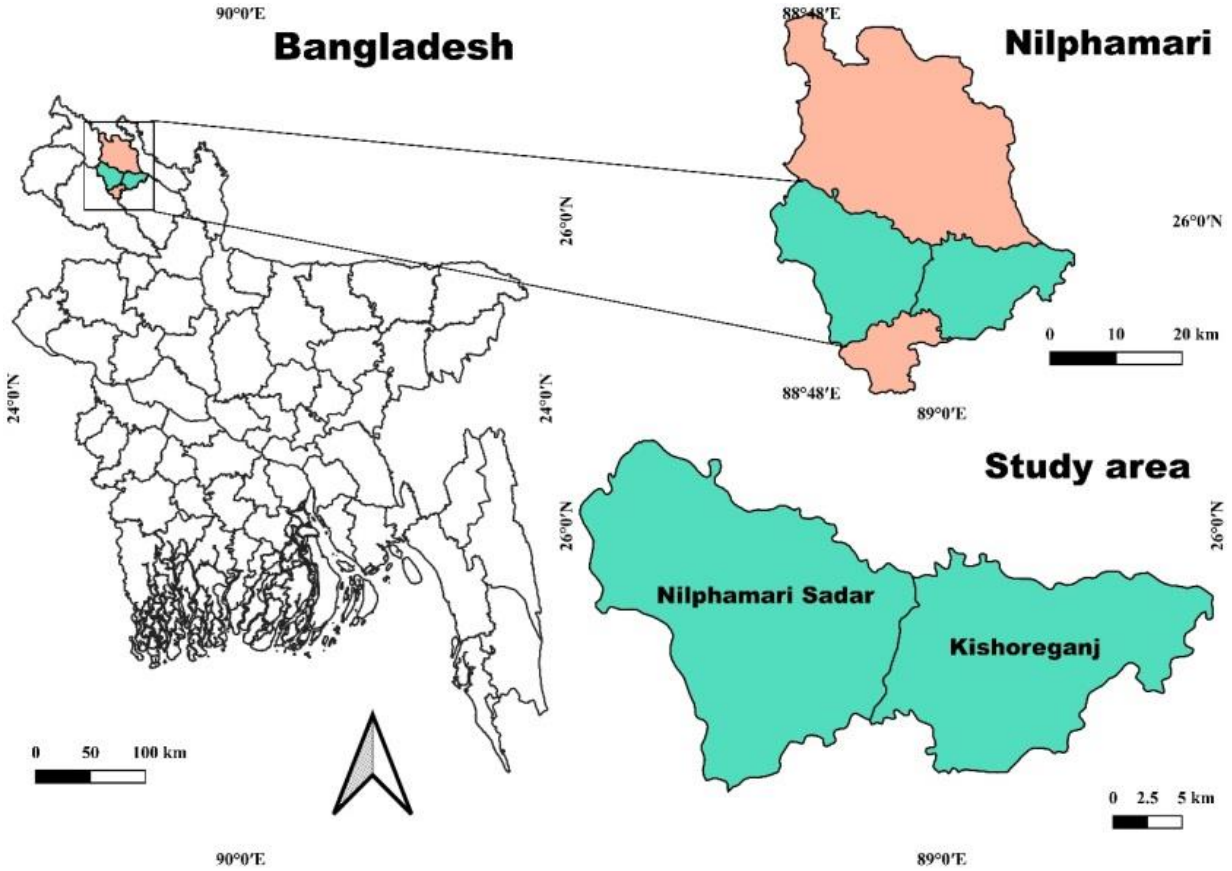

Figure 1. The study was conducted in the areas of Nilphamari Sadar and Kishoregonj upazilas within the Nilphamari district.

2.6. Serum plate agglutination (SPA) test

The SPA test followed the procedure outlined by Sobuj *et al.* (2021), conducted at 25° C within 24 hours of sera collection. Utilizing crystal violet stained with Nobilis MG commercial antigen (Intervet, Netherlands), the test was executed according to manufacturer's guidelines. Briefly, 0.03 ml of antigen and serum were pipetted onto a glass plate, agitated with a toothpick, and illuminated from below to observe the reaction. Within two minutes, distinct clumps indicated a positive reaction, while the absence of agglutination denoted a negative result. Precautions were taken to prevent misinterpretation due to natural granulation in the antigen.

2.7. Clinical examination

The presented clinical manifestations of the MG infections in layer birds were recorded during the physical visits, following submission to the hospital and the farmer's complaints in relation to the affection were also emphasized.

2.8. Pathological examination

The gross morbid lesions of the diseases were systematically examined, noted and categorized. The tissue samples (lungs, trachea, and air sacs) from suspected layer birds with respiratory distress were collected for diagnosis and treatment for further histopathological study. The tissues were preserved in a 10% formalin solution, then prepared by embedding in paraffin, cutting into sections, and stained with hematoxylin and eosin for histopathological analysis (Abdanaser *et al.*, 2019).

2.9. Statistical analysis

The statistical analysis involved utilizing SPSS version 25 (SPSS 25, 2016). The seroprevalence of mycoplasmosis was determined by dividing the number of positive cases by the total number of samples examined for mycoplasmosis, multiplying the result by 100 to obtain a percentage. Subsequently, a Chi-square test was conducted for further analysis of the data. The map of the study site was generated using QGIS software (QGIS Development Team, 2019).

3. Results

3.1. Seroprevalence of MG in layer birds

Out of 400 serum samples, 250 were taken from Nilphamari Sadar and 150 were taken from Kishoregonj, Nilphamari. Clumping or agglutination formed after the mixing of commercial antigen and bird serum were considered as seropositive (Figure 2). A total of 155 (62.00%) were found positive in Nilphamari sadar, and 102 (78%) were found positive in Kishoregonj for antibodies (Table 1). The highest prevalence of mycoplasmosis was found in the Kishoregonj region of Nilphamari, with 102 cases representing 78%.

Table 1. Seroprevalence of MG infections based on study areas.

Name of upazila	No. of farms	No. of sera tested	SPA (+) positive	SPA (-) negative	Prevalence rate (%)
Kishoregonj	15	150	102	48	68%
Nilphamari sadar	25	250	155	95	62%
Total	40	400	257	143	64%

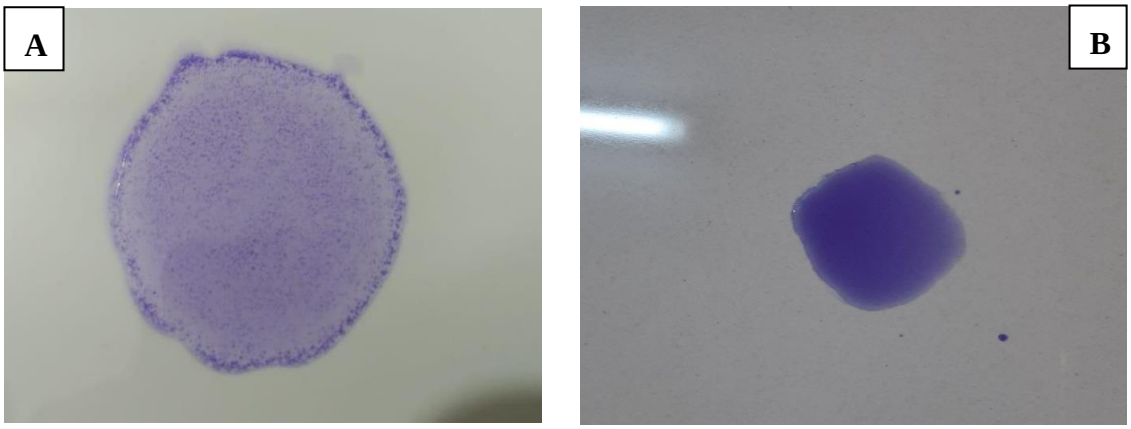

Figure 2. Seroprevalence of MG by serum plate agglutination test, (A) Agglutination/ clumping were formed slowly (B) No agglutination/clumping.

3.2. Epidemiological study of MG infections in layer birds

The prevalence of MG in layer chickens in the research region was 64.25% out of 400 birds. There was a great seasonal variation in the occurrences of MG infections, which were significantly higher in the winter season (70%) than the summer season (57.2%). The infection was highest in the winter season due to cold shock and stress (Table 2).

Table 2. Season wise *Mycoplasma gallisepticum* infections in layer birds.

Season	No. of farms	No. of sera tested	SPA (+) positive	SPA (-) negative	Prevalence rate (%)	*P*-value
Winter	22	220	154	66	70%	0.008*
Summer	18	180	103	77	57.2%	

*=Significant at 1% level of probability.

MG occurrence in layer chickens was observed across various age groups. The highest prevalence was recorded in the 20-40 week age group, with 71%, followed by the >40 week age group at 60% and the <20 week age group at 55% (Table 3).

Table 3. Age wise *Mycoplasma gallisepticum* infections in layer birds.

Age of layer birds (weeks)	No. of farms	No. of sera tested	SPA (+) positive	SPA (-) negative	Prevalence rate (%)	*P*-value
<20	11	100	55	45	55%	
20-40	16	200	142	58	71%	0.014**
>40	13	100	60	40	60%	

**=Significant at 5% level of probability.

3.3. Clinical manifestations

Layer birds affected with MG infections exhibited signs ranging from moderate (sub-acute) to severe (acute). The distinction between acute and sub-acute levels of the disease was based on factors such as the degree of severity, presence of complications or concurrent infections, extent of lesions, disease stage, and overall health conditions of the birds. Various levels of respiratory distress were observed, including slight to marked rales, difficulty in breathing, coughing, and sneezing. Additionally, swelling of the eyelids and sinuses, nasal discharge, conjunctivitis with frothiness of the eyes, reduced feed efficiency, weight gain, egg and meat production, as well as dullness and depression with ruffled feathers, were recorded in both forms of the disease (Figure 3A and B).

Figure 3. Characteristics clinical signs of MG infection in layer, (A) Swelling of the eyelids and closed eyelids (B) dull and depressed.

3.4. Gross lesions of MG infection in layer birds

Postmortem findings showed dark red color appearance, edema, congestion and haemorrhage in the lungs (Figure 4A, B).

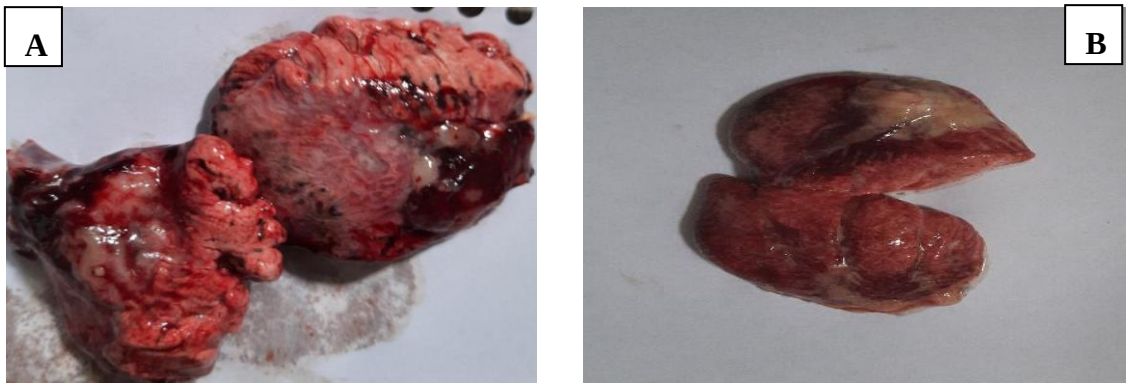

Figure 4. Gross lesions in the lungs affected with MG, (A) Edema, congestion, and haemorrhages in lungs (B) Dark red color appearance in lungs.

Catarrhal exudates and haemorrhage were found in the trachea as well as the presence of mucous in the trachea (Figure 5A, B). Air saculitis was observed in the air sacs (Figure 5C), these become thickened and covered with caseous exudates, and cloudiness was seen.

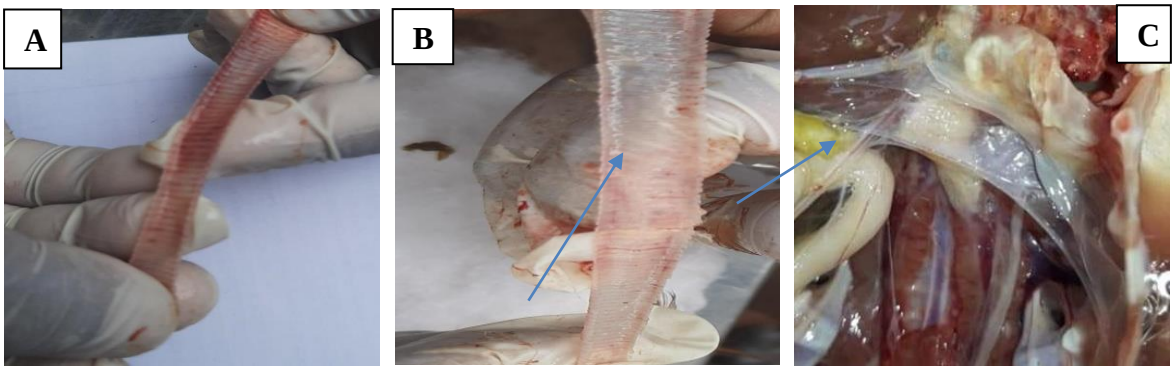

Figure 5. Gross lesions in the trachea and air sacs affected with MG. (A) Haemorrhages present in trachea (B) Mucous present in trachea (C) Cloudiness in air sacs.

3.5. Histopathological lesions

Microscopic examination of the lungs showed edema, hemorrhage, and tissue destruction (Figure 6A), accompanied by significant reactive cell infiltration and exudation in the air sacs (Figure 6B), with additional observations of hemorrhages and tissue destruction in the lungs (Figure 6C).

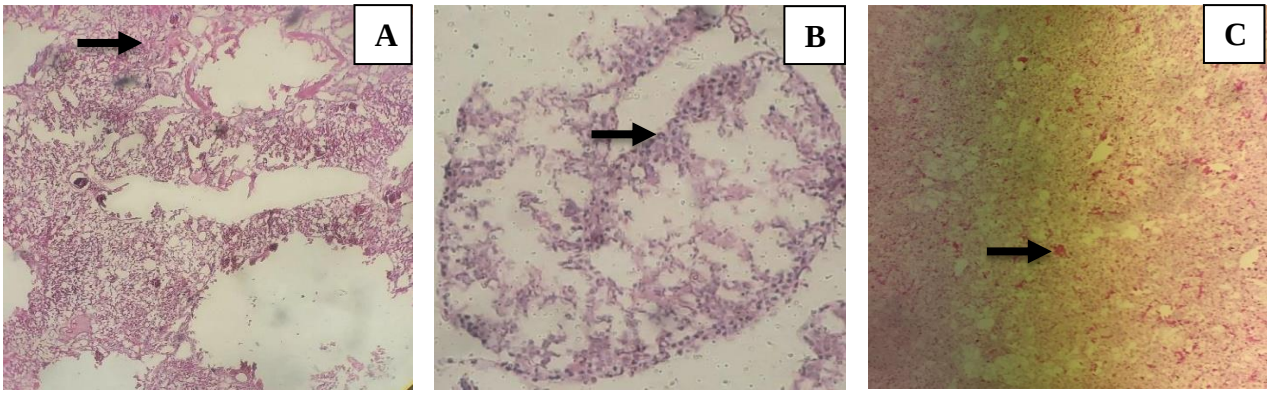

Figure 6. Microscopic lesions in the lungs and air sacs affected with MG, (A) Edema and haemorrhages in the lungs and tissue destruction (arrow) [10x]; (B) Huge lymphocytic infiltration and exudation in air sacs (arrow) [10x]; (C) Haemorrhages in the lungs and tissue destruction (arrow) [10x].

Sloughing off the mucosa from the trachea, and haemorrhage of variable degree and an increase number of goblet cells in the trachea were also noted (Figure 7).

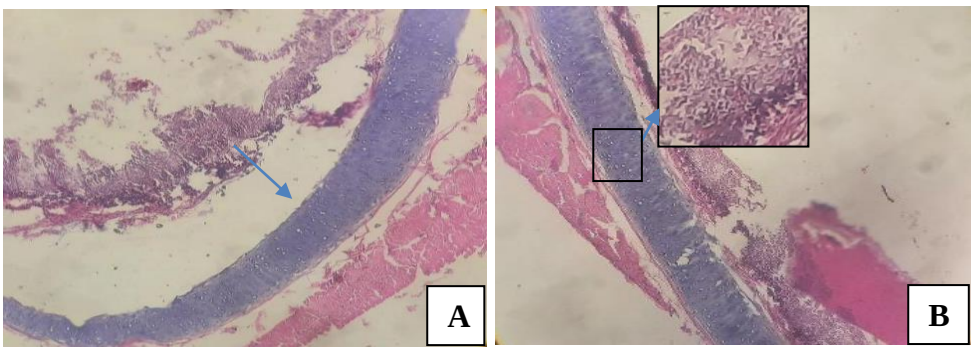

Figure 7. Microscopic lesions in the trachea affected with MG. (A) Haemorrhages in trachea and sloughing off the mucosa from trachea [10x], (B) Increase number of goblet cell (inset) [40x].

4. Discussion

Avian mycoplasmosis is an important poultry disease responsible for economic losses for the poultry industry. Despite many studies conducted on avian mycoplasmosis, control and prevention of mycoplasmosis in poultry

are still not visible in Bangladesh (Islam *et al.*, 2014; Raquib *et al.*, 2021). The current study was carried out to determine serological prevalence of avian mycoplasmosis caused by MG and its pathological effects on poultry. A total of 68.00% prevalence rate was found in Kishoregonj upazila and 62% prevalence was found in Nilphamari sadar. While almost similar seroprevalence in layer chicken was noted by Raquib *et al.* (2021) in Kishorgonj district, while other study recorded much lower seroprevalence of avian mycoplasmosis in Bogra districts in Bangladesh (Ali *et al.*, 2015). The variation in prevalence might be due to geographical locations, biosecurity and the management of commercial layer farms (Kim *et al.*, 2021; Mirzaie *et al.*, 2023). Based on the seasonal prevalence, the largest proportion of MG was seen during the winter season (70%) and the summer season (57.2%). The studies of Islam *et al.* (2014) reported a higher occurrence of MG in the winter season, with percentages of 58.33%, compared to 48.89% in the summer season. These findings are consistent with the results of the present study. The age group with the highest prevalence was 20-40 weeks in the present study, with a significantly higher rate of 71%. This was followed by the age group over 40 weeks, which had a prevalence rate of 60%, and the age group under 20 weeks, which had a prevalence rate of 55%. In a study conducted by Islam *et al.* (2014), it was discovered that the prevalence of MG was highest in pullets (60.63%), followed by adults (55.63%) and elderly hens (51.25%). According to Hossain *et al.* (2007), there was a much higher occurrence of MG in young individuals (72.72%) compared to adults (44.0%). This finding suggests a strong inclination towards MG in the younger population. The disparity in the occurrence of MG in various age groups of layer chickens could be attributed to the influence of low stocking density and management practices, which may account for the reduced incidence of MG in older birds (Islam *et al.*, 2014).

Layer birds afflicted with MG exhibited symptoms including lethargy, despondency, unkempt feathers, coughing, sneezing, nasal discharge, and respiratory distress accompanied by wet rales (Marouf *et al.*, 2022). Birds affected by complex diseases involving other organisms often display clinical signs that mirror those of single infections but are notably exacerbated, resulting in elevated mortality and morbidity rates. Birds with complex diseases involving other organisms typically show clinical signs that are similar but more severe, leading to increased mortality and morbidity rates (Montesdeoca *et al.*, 2017; Yehia *et al.*, 2023). The primary clinical observations were respiratory symptoms and deteriorating circumstances, which align with the findings reported by Yehia *et al.* (2023). The eyelids exhibited edema along with the presence of ocular discharge and drainage from the nares, which aligns with the observations made (Muhammad *et al.*, 2017)

The present study noted a strong red color and displayed signs of congestion and hemorrhaging in the lungs. The nasal passages contained catarrhal exudates, which were sticky and frothy. These exudates were firmly attached to the tracheal wall. The trachea exhibited signs of congestion and hemorrhaging. There was serious involvement of the trachea, lungs, air sacs, heart, and liver (Abdanaser *et al.*, 2019). Air sacculitis, characterized by thickening and the presence of caseous exudates, was seen in the air sacs, with noted cloudiness (Sivaseelan *et al.*, 2015; Abdanaser *et al.*, 2019). In the present study, most consistent histopathological alterations were observed in the trachea and lungs. Lungs showed edema, hemorrhages and tissue destruction. Huge reactive cell infiltration and exudation in air sacs. Sloughing off of the mucosa from the trachea and hemorrhage of variable degree, along with an increase in the number of goblet cells, were also noted, coinciding with the findings of previous study (Carnaccini *et al.*, 2022).

5. Conclusions

The current study unveiled a notable prevalence of MG in layer birds within the Nilphamari district of Bangladesh. Layer birds aged between twenty to forty weeks exhibited the highest rates of MG infection. Moreover, a heightened incidence of mycoplasmosis was observed during the winter season compared to other seasons. Gross and histopathological examinations revealed significant lesions, particularly in the trachea, air sacs, and lungs. In light of these findings, it is imperative to enforce robust efforts and stringent biosecurity measures to effectively curb the spread of this infection. Additionally, there is a pressing need for nationwide investigations to comprehensively assess the prevalence of MG across Bangladesh and understand the current status of the disease.

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Data availability

The data of the study is available from the corresponding author on request.

Conflict of interest
None to declare.

Authors' contribution
Shahina Akter Shova: investigation, data curation and writing original draft; Md. Haydar Ali: data analysis, writing and editing manuscript; Mahfuza Akther: performed histopathology, writing and editing manuscript; Md. Mominul Islam: Conceived and conceptualized, data curation and writing and editing original draft manuscript. All authors have read and approved the final manuscript.

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