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Article

Macroscopic and microscopic characterization of mycoplasmosis in commercial chickens in Barishal, Bangladesh

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Abstract: Avian mycoplasmosis in commercial chicken is a serious problem around the world, and responsible for high economic losses and decreased poultry productivity. This study aimed to characterize the mycoplasma infection macroscopically and microscopically in commercially important poultry like broiler and layer in Barishal, Bangladesh. Diagnosis of mycoplasmosis was made on the basis of history, clinical signs, necropsy findings and histopathology of the suspected organs. The study was conducted on a total no. of 460 birds (broiler n=237; layer n=223) of 20 different farms of three different upazillas of Barishal district in Bangladesh. The overall prevalence of micoplasmosis was 26.52%. Layer chicken (30.04%) was more susceptible to mycoplasmosis than broiler chicken (23.20%). The highest prevalence of mycoplasmosis was observed in 3-6 weeks-aged chicken in both broiler (54.55%) and layer (35.82%) chicken. A significant difference ($p<0.05$) was observed in mycoplasmosis prevalence rate among the seasons. The highest prevalence rate of mycoplasmosis was in winter season followed by rainy and seasons. Major necropsy findings of mycoplasmosis include catarrhal hemorrhage and mucus exudation on tracheal mucosa and severe congestion, consolidation and reddish gelatinous mass over the lung surface. Mycoplasmosis caused severe tracheitis, bronchitis, air sacculitis, and pneumonia in chickens. Mycoplasma affected different organs in the chicken and hinder the production through morbidity and mortality. Present study will provide the baseline data for future in-depth research on mycoplasmosis to draw an effective policy for controlling and eradicating mycoplasmosis from commercial poultry.

Keywords: prevalence; gross and microscopic pathology; mycoplasmosis; broiler; layer; Barishal district

1. Introduction

Poultry industry is one of the rapid growing sectors in the world. Bangladesh has a huge population where over 80% people live in rural areas and depends on agriculture. Poultry contributes about 22-27% of total animal protein supply in the country (Hamid *et al.*, 2017). Traditionally, poultry rearing is one of the most important sources of income for rural women of Bangladesh. In the last decades, the progress of poultry production is

being seriously hampered by diverse factors; where infectious diseases encountered 30% mortality in chickens annually in Bangladesh (Noor *et al.,* 2005). Diseases in birds significantly affect the productivity and health status and many of them also have public health importance (Badruzzaman *et al.,* 2015). Avian mycoplasmosis is one of the major problems encountered for poultry industry of all over the world. Mycoplasmosis of chickens caused by four commonly recognized pathogens namely *Mycoplasma gallisepticum, Mycoplasma synoviae, Mycoplasma meleagridis and Mycoplasma iowae.* In poultry the infection is spread vertically through infected eggs and horizontally by close contact. The clinical signs of *Mycoplasma gallisepticum* affected bird are characterized by major respiratory signs (sneezing, coughing, nasal discharge, breathing difficulty and closed eyelid) including mild sinusitis, tracheitis and air sacculitis (Faiz *et al.,* 2001). Moreover mycoplasma gallisepticum infection in poultry can vary from subclinical to obvious respiratory signs. Initial respiratory signs of mycoplasmosis showed excess mucous exudates followed by catarrhal and caseous exudates, which may form amorphous masses in the air sacs (Katinka *et al.,* 2019). Postmortem examination reveals mucopurulent exudates in nasal cavities, trachea, and bronchi, fibrinopurulent pericarditis and peri hepatitis. Serological (ELISA) and molecular diagnostic protocol (PCR) have been recognized and practiced for sensitive and confirmatory diagnosis but isolation and identification is the gold standard for the diagnosis of Mycoplasmosis (Khatoon *et al.,* 2018). However, during field practice necropsy has been considering the major techniques for diagnosis of poultry disease in Bangladesh. Poultry diseases occur singly or in combination with other pathogens (Rahman *et al.,* 2017). The prevalence of diseases in a particular area depends on several factors like geographical condition, management practices by the farmer, bio-security and immunization status of the farm and quality of the chicks (Badruzzaman *et al.,* 2015). Histopathological assessment of local outbreak of infectious diseases in poultry is important to mitigate economic losses in farming system (Khatoon *et al.,* 2018). Barishal is located at the southern part of Bangladesh. Poultry industry is not so developed in this region compared to other poultry populated zones of Bangladesh like Kishoregonj, Narsindi, Gazipur, Comilla (Badruzzaman *et al.,* 2015). However, poultry industries have been developing at Barisal region. Previous studies on the prevalence and pathology of avian mycoplasmosis were conducted at different parts of Bangladesh (Rahman *et al.,* 2003; Belal *et al.,* 2015; Rahman *et al.,* 2016; Hassan *et al.,* 2016; Jalil *et al.,* 2012; Hossain *et al.,* 2007; Rahman *et al.,* 2008). A couple studies have been conducted at Bola (Islam *et al.,* 2014) and Patuakhali district (Sikder *et al.,* 2005). However, there are very limited reports on avian mycoplasmosis at Barishal region. Therefore, current study has been designed to investigate the prevalence and histopathology of mycoplasmosis in broilers and layers at Barishal district in Bangladesh.

2. Materials and Methods

2.1. Experimental design and layout

A total no. of 460 birds (broiler n=237; layer n=223) were randomly selected from different farms of three different upazillas (Barishal Sadar, Babugonj, Banaripara) of Barishal district in Bangladesh during the period from January 2018 to December 2018. The duration of the study period was divided into three sessions; summer (March-June); rainy (July–October), and winter (November-February) seasons. Randomly selected birds were stratified into four age groups (0-3 wks; >3-6 wks; >6-12 wks; >12 wks) to observe the age-wise prevalence. Tissues from suspected or infected samples were collected for further macroscopic and microscopic observations.

2.2. Field diagnosis

Field diagnosis of mycoplasmosis was made on the basis of history, farms owner's complain, presenting clinical signs including nasal discharge, coughing, and tracheal rales, depression, conjunctivitis, swollen eye, eyelid edema and other more general signs were included such as in appetence, depression, weight loss and a drop in egg production and post mortem examination of dead birds. Necropsy of dead birds was performed systemically and post mortem changes were recorded during necropsy.

2.3. Post mortem and macroscopic examination

Most of the vital organs are affected during mycoplasmosis and the highest morbidity and mortality occurred in the presence of concurrent infections and environmental stress (Haque *et al.,* 2015). To get a better understanding of mycoplasmosis we did postmortem examination of dead birds noted macroscopic lesions from suspected samples like pneumonic and congested lung tissues, catarrhal trachea, enlarged mottled spleen and congested enlarged liver with sub capsular hemorrhage. The sample further processed for histopathological study.

2.4. Histopathological examination

Hematoxylin and eosin (H & E) staining was conducted according to routine protocols (Hassan *et al.,* 2014). Briefly, after deparaffinization and rehydration, 5 μm sections were stained with hematoxylin solution for 5 min followed by 5 dips in 1% acid ethanol (1% HCl in 70% ethanol) and then rinsed in distilled water. Then the sections were stained with eosin solution for 3 min and followed by dehydration with graded alcohol and clearing in xylene. The mounted slides were then examined and photographed using microscope (Samsung WB150F, South Korea).

2.5. Statistical analysis

All data were summarized with the help of MS Word, MS Excel and observations were made on mathematical parameters (quantitative analysis), like- number of birds and prevalence of the infection. Statistical analyses were performed with Microsoft Excel and statistical software available online (https://astatsa.com/OneWay_Anova_with_TukeyHSD/). Differences in prevalence rate between the layer and broiler chicken, and among the seasons were evaluated by one-way analysis of variance (ANOVA), followed by Turkey HSD post-hoc analysis (where possible). *p* values less than 0.05 were considered significant.

3. Results and Discussion

3.1. Overall prevalence of mycoplasmosis in commercial poultry

In present study a total 460 birds (broiler and layer) were examined and 122 cases were diagnosed as Mycoplasmosis. The overall prevalence of micoplasmosis in selected area was calculated as 26.52% that was in agreement with a previous study (Hassan *et al.,* 2014) where it was recorded as 24.78%. In Pakistan an overall prevalence of Mycoplasmosis was detected 29.88% and 20. 67% using ELISA and SPA test respectively (Haider *et al.,* 2019). But the prevalence of mycoplasnosis in present study showed a bit higher than the previously published research (Rahman *et al.,* 2016; Haque *et al.,* 2015; Abdullah *et al.,* 2019; Uddin *et al.,* 2010; Talha *et al.,* 2001) and lower than other some studies (Hossain *et al.,* 2007; Islam *et al.,* 2014; Sikder *et al.,* 2005; Barua *et al.,* 2006; Hossain *et al.,* 2010). This variation might be due to diagnostic procedure, geographical location sample size. In this study, diagnosis was done based on history, clinical signs, and gross and microscopic feature of representative organs.

3.2. Susceptibility to mycoplasma infection between layer and broiler chicken

The prevalence of mycoplasmosis in broiler and layer chicken was estimated in the selected areas. Current study revealed a significant difference ($p<0.05$) in mycoplasmosis prevalence rate between broiler and layer chicken. The higher prevalence of mycoplasmosis was recorded in layer (30.04%) than broiler (23.20%) chicken (Table 1). Previous studies reported the prevalence of mycoplasmosis in broiler 10.89% (Hassan *et al.,* 2016) or 12.56% (Ahmed *et al.,* 2009) at Gazipur district in Bangladesh that was lower than the present findings. However, a study was carried out in Mymensingh that reported 50% mycoplasmosis in broiler chicken (Rahman *et al*., 2003). Previous studies showed lower rate 14.7% (Hassan *et al.,* 2016) and higher rate 67.4% (Jalil *et al.,* 2012), 58.9% (Hossain *et al.,* 2007), 46.88% (Sikder *et al.,* 2005), 55.13% (Sarker *et al.,* 2005) of myciplasmosis than the present study in layer chicken at different parts in Bangladesh. This variation may be due to uneven sample size, different geographical locations, variation in diagnostic procedure and samples, differences in farm management and farm bio-security.

Table 1. Incidence of mycoplasmosis in broiler and layer chickens.

Types of Birds	No. of Birds Examined	No. of Infected Birds	Prevalence (%)
Broiler	237	55	23.20^{a}
Layer	223	67	30.04^{b}

Note: Values with different superscripts in a column indicate significant differences between broiler and layer chickens ($p<0.05$).

3.3. Effect of age in occurrence of mycoplasmosis in broiler and layer chicken

Age-wise study is an important consideration to boost the management practice against mycoplasmosis. Present study noted the highest prevalence of mycoplasma infection at age group >3-6 weeks in the broiler (54.55%) and layer (35.82%). On the other hand broiler chickens more than 6 weeks (3.64%) and layer more than 12 weeks (11.94%) of age were less susceptible (Table 2). This study was in consistent with the previous report (Jalil *et al.,*2012; Haque *et al.,* 2015) that had been conducted in Chittagong and Khulna districts of Bangladesh.

However, some reports (Badruzzaman *et al.*, 2015; Rahman *et al.*, 2016; Islam *et al.*, 2003) on mycoplasmosis in Sylhet district showed higher prevalence of this in commercial chicken. The variation of the prevalence data may be due to different risk factors that aggravate the occurrence of diseases like- breed, strain of birds, load of pathogens, epidemiological location, availability of reservoirs, and carrier hosts play crucial roles.

Table 2. Incidence of mycoplasmosis in poultry on the basis of age.

Types	No. of birds infected	Age group (weeks)	No. of infected birds at different age	Prevalence (%)
Broiler	55	0-3	23	41.81
		>3-6	30	54.55
		>6-12	2	3.64
		>12	-	-
Layer	67	0-3	21	31.34
		>3-6	24	35.82
		>6-12	13	19.40
		>12	8	11.94

3.4. Seasional influence of mycoplasmosis in broiler and layer

The overall seasonal prevalence of mycoplasmosis in commercial chickens (broiler and layer) was 21.95%, 25.15%, and 31.18% in summer, rainy and winter season respectively (Table 3). Seasons have great impact on the prevalence of this disease, as described by a number of researchers (Badruzzaman *et al.*, 2015; Rahman *et al.*, 2016; Sikder *et al.*, 2005). Poultry are particularly vulnerable to climate change as they can tolerate a very narrow range of thermal conditions (Rahman *et al.*, 2016). The highest sero-prevalence of mycoplasma was recorded in winter (61.45%) and lower in summer (51.74%) (Sikder *et al.*, 2005). This alteration might be due to variation in geographical location, thermal profile of the study areas, different in management practices. However, a couple of researchers reported higher prevalence mycoplasmosis in summer followed by winter and rainy seasons (Badruzzaman *et al.*, 2015; Rahman *et al.*, 2016). In the present study, we found a significant difference ($p<0.05$) in the mycoplasmosis prevalence rate among the seasons. The highest prevalence rate of mycoplasmosis we found in winter season followed by rainy and seasons.

Table 3. Effect of season in the prevalence of mycoplasmosis in commercial poultry.

Season (n)	Broiler (n=237)		Layer (n=223)		Total no. of infected birds	Prevalence (%)
	Examined (n)	Infected No. (%)	Examined(n)	Infected No (%)		
Summer (n=123)	64	12 (18.75)	59	15 (25.42)	27	21.95^{a}
Rainy (n=167)	82	19 (23.17)	85	23 (27.05)	42	25.15^{b}
Winter (n=170)	91	24 (26.37)	79	29 (36.71)	53	31.18^{c}

Note: Values with different superscripts in a column indicate significant differences among the seasons ($p<0.05$).

3.5. Gross pathologic lesions

Affected birds having clear signs of mycoplasmosis were euthanized, and necropsy revealed significant macroscopic lesions in different visceral organs. Suspected organs like trachea, lungs, liver, spleen and heart from mycoplasma infected broiler and layer chicken were collected and observed macroscopically. Tracheal mucosa showed catarrhal hemorrhagic congestion in broiler at 3 weeks of age (Figure 1a) and fibrinous exudate (arrow) in the tracheal lumen of layer at 6 weeks of age (Figure 1b). Lungs showed severe congestion, consolidation and yellowish gelatinous mass over surface (arrow) in broiler at 3 weeks of age (Figure 1c and d). These observations were in consistent with previous reports (Haque *et al.*, 2015; Pazani *et al.*, 2008; Stipkovits *et al.*, 2012). Above mentioned pathognomonic post-mortem lesions were also in agreement with the previous reports (Hassan *et al.*, 2016; Ahmed *et al.*, 2009; Talukder *et al.*, 2017; Islam *et al.*, 2014).

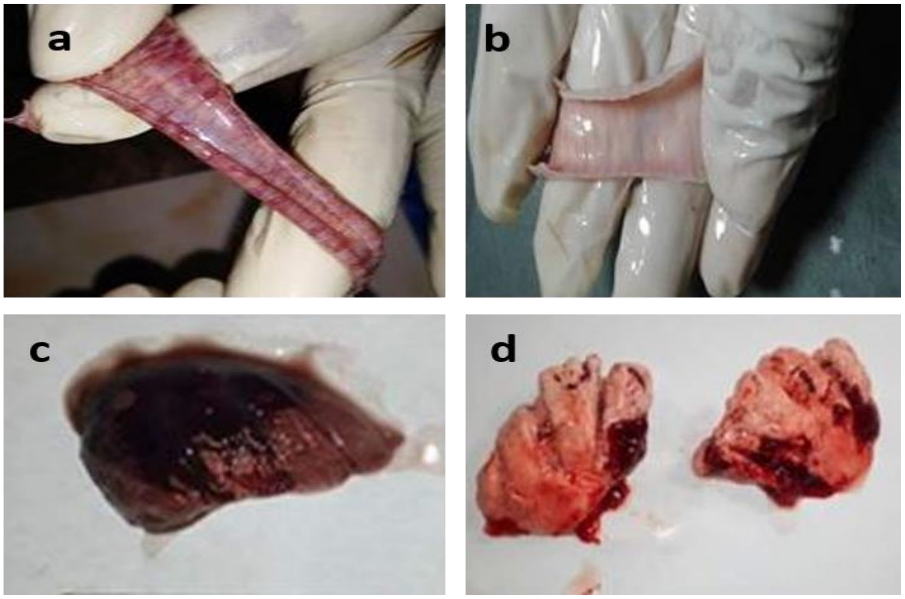

Figure 1. Micoplasmosis causes marked lesions in trachea and lung of affected birds. Catarrhal hemorrhage (a) and mucus exudation (b) on tracheal mucosa. Severe congestion, consolidation (c) and reddish gelatinous mass (d) of affected lungs.

Liver showed severe hemorrhage and congestion with fragile consistency in broiler at 3 weeks of age (Figure 2a and b). Typical swollen and hemorrhagic liver was marked in layer at 6 weeks age (Figure 2c). Enlarged mottled spleen showed remarkable congestion in 3 weeks old broiler chicken (Figure 2d). Observation of heart in affected broiler chicken of 4 weeks age showed fibrinopurulent pericarditis characterized by fibrinous exudation in pericardial sac (Figure 2e). On the other hand, profuse hemorrhagic deposits was found in pericardial sac of 12 weeks aged layer chickens (Figure 2f) those were in agreement with previous study (Nasir *et al.*, 2018).

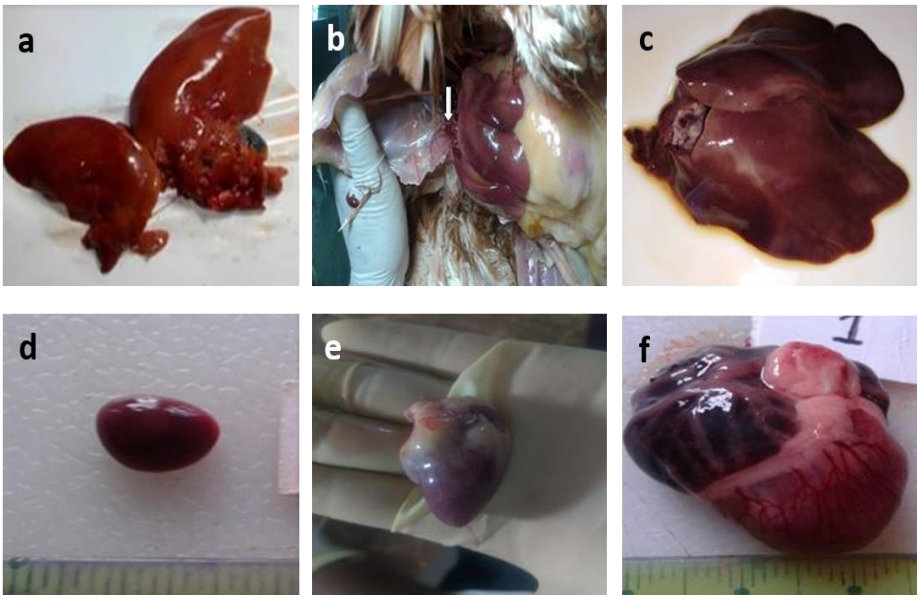

Figure 2. Mycoplasma affected liver, spleen and heart of birds. Congested, fragile liver in broiler at 3 weeks of age (a, b), hemorrhagic liver of layer chicken (c), enlarged molted spleen (d), fibrinopurulent pericarditis (e) and hemorrhagic deposits in pericardial sac (f).

3.6. Microscopic pathology of mycoplasma infected birds

Histopathologic section of trachea showed hemorrhagic sub mucosa and thickened tracheal mucosa. Profuse congestions in lung alveoli were found (Figure 3a, b, and c). These findings were consistent with previous report (Rottem 2003; Pazani *et al.*, 2008). Infiltration of inflammatory cells along with necrotic tissues in the liver

parenchyma recorded in infected birds (Figure 3d). Hemorrhage in the splenic parenchyma and fibrous deposition were marked in myocardium (Figure 3e and f). Wu *et al.* (2019) and Stipkovits *et al.* (2012) reported the aggressive inflammatory response in liver parenchyma those are in agreement with the present study.

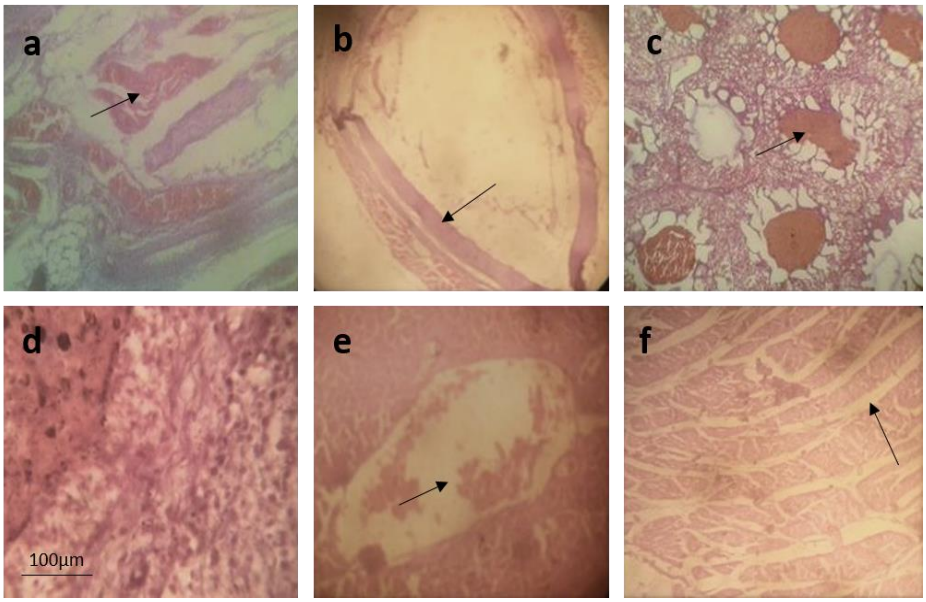

Figure 3. Histopathologic micrographs (hematoxylin and eosin staining, 10x) of different organs of mycoplasmosis affected poultry. Histopathologic findings (arrow) showed hemorrhage in tracheal submucosa (a) and thickened tracheal mucosa (b) in affected poultry, accumulated blood in the alveoli of suspected lungs (c), infiltration of inflammatory cells along with necrotic tissue in liver parenchyma (d), hemorrhage in splenic tissue (e), thick fibrous layer inside the myocardium (f).

4. Conclusions

Layer chicken was more susceptible to mycoplasmosis than broiler chicken. The highest prevalence of mycoplasmosis was observed in 3-6 weeks-aged chicken (in both broiler and layer) and in winter season. Mycoplasmosis caused severe tracheitis, bronchitis, air sacculitis, and pneumonia in chickens. It also has detrimental effects on the body's essential organs, such as the liver, spleen, and heart. As a result of poor weight gain, decreased egg output, and death, mycoplasmosis in commercial chickens can result in severe economic losses. Given the widespread prevalence of mycoplasma infections in chicken, a surveillance of this infection in Barishal and nearby districts in Bangladesh is required.

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Conflict of interest

None to declare.

Authors' contribution

Sujala Bepary – sample collection and did the experiment, Md. Mostafijur Rahman – manuscript writing, Md. Saidur Rahman – Histological support and manuscript writing, Shib Shankar Saha and Md. Shah Alam – Monitoring of the work and guidance, Md. Royhan Gofur – data analysis and manuscript writing, Mst. Ismat Ara Begum – manuscript writing, Khondoker Jahengir Alam – deigned the study and supervised the experiment. All authors have read and approved the final manuscript.

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