



## Pathomorphological and Immunohistochemical Changes in Lungs of Poultry Affected with Chronic Respiratory Disease in Ayodhya District of Eastern Uttar Pradesh

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### Abstract

*The present study was conducted to describe the gross and microscopic lesions in lungs due to chronic respiratory disease of chickens in Ayodhya district of Eastern Uttar Pradesh with special reference to immunohistochemical diagnosis. The naturally dead birds from 10 different private farms in Ayodhya district of Eastern Uttar Pradesh were collected for 6 months (October, 2018 to March, 2019). After post-mortem examination, the grossly suspected lungs were kept for further histopathological and immunohistochemical study. Macroscopic lesions in lungs showed yellowish to creamy thick coverings. On removal of superficial coverings, lungs revealed dark red colour appearance and showed congestion and haemorrhages. Microscopically, lung showed oedema in the lumen, congestion of capillaries and lymphocytic infiltration in the interstitial tissue. MG antigen was evident as brown colored staining of parabronchiolar epithelial cells, primary bronchiolar epithelial cells besides inflammatory cells in immunohistochemical studies.*

**Keywords:** Chronic Respiratory Disease, Immunohistochemistry, Lung, Pathomorphology, Poultry

## Introduction

Poultry rearing in India has taken a quantum jump during the last four decades emerging from a backyard practice to a full-fledged industry which is indicated by its growth rate in both table eggs and meat production (Blake *et al.*, 2014). Due to selective breeding policies for higher production, intensive rearing and too many vaccinations, the birds are facing all sorts of stress beyond the limit of their physiological tolerance which are making them more vulnerable to diseases. Among reported diseases of poultry, Chronic respiratory disease is reported from several parts of the world in both poultry layer and broiler stocks which is characterized by coughing, sneezing, respiratory rales, difficulty in breathing (through partially opened beak), frothy ocular exudates and nasal discharge, conjunctivitis, decrease in feed consumption (poor weight gain) and egg production, increased mortality, poor hatchability and increased embryo mortality (Steinlage *et al.*, 2003; Okwara, 2016 and Islam *et al.*, 2011). All age group birds are susceptible to *Mycoplasma gallisepticum* but young birds are considered to be affected severely as compared to older birds (McMullin *et al.*, 2004). Infected birds remain carriers for extended periods of time. There is marked interaction between respiratory viruses, *Escherichia coli*, and *Mycoplasma gallisepticum* in the pathogenesis and severity of chronic respiratory disease (Ley, 2018 and Sarika *et al.*, 2019).

Different members of Mycoplasmataceae family are the causative agents of mycoplasmosis in poultry (Kleven, 2003). Mycoplasmas are cell wall less pleomorphic bacteria and lacks the ability of Gram staining, therefore the characterization of mycoplasmas based on morphological characteristics is non-effective. They belong to the Class Mollicutes (mollis-soft and cutes-skin) and order Mycoplasmatales. Different Mycoplasmas strains are characterized by differences in infectivity, tissue tropism and pathogenicity (Razin *et al.*, 1998). *Mycoplasma gallisepticum* and *Mycoplasma synoviae* are considered to be the most pathogenic strains in poultry causing chronic respiratory diseases in chickens (Buim *et al.*, 2009 and Bradely *et al.*, 1998).

Mycoplasmas are considered as smallest free-living microbes capable of self-replication characterized by worldwide occurrence, lack of conventional bacterial cell wall but are bounded by a plasma membrane (Kleven, 1997). These characteristics account for the fried egg type of colonial morphology exhibited by mycoplasmas, their complete resistance to antibiotics that affect cell wall synthesis and their complex nutritional requirements (Kleven, 1998). It has slow growth cycle because of absence of cell cycle and depend upon host for many nutrients for survival (Bradely *et al.*, 1998). The isolation of mycoplasmas is very time taking process due to its fastidious nature but molecular techniques helps in early diagnosis (Yoder, 1991; Radi *et al.*, 2000). Histopathology has been considered a good technique for MG infection diagnosis. Immunohistochemistry has been proved as an excellent tool for effective detection of mycoplasma antigens in many animals including avian species and sensitivity and specificity of IHC made it superior to all prevailing methods like RT-PCR and in-situ hybridization in diagnostic pathology and research (Nunoya *et al.*, 1997; Kempf, 1998; Radi *et al.*, 2000; Yilmaz *et al.*, 2011). Though extensive work on mycoplasmosis in birds has already been done in different states, but it was not covered in Ayodhya district of Eastern Uttar Pradesh. So, the objectives of the present study were to observe the pathological changes related to causal agents and to diagnose the cases of chronic respiratory disease by immunohistochemistry.

## Material and Methods

For the present study, dead birds from ten different private farms of Ayodhya district were collected on the basis of stratified random sampling. Two private farms from each tehsil of Ayodhya district were selected for the above purpose. The birds were of different age group and of both sexes. The pathological investigation was carried out at all the 10 different randomly selected farms. These farms were visited during study period for 6 months (October 2018 to March 2019) to screen the cases of chronic respiratory disease on the basis of clinical signs and symptoms like respiratory rales, open mouth breathing, ocular and nasal discharge, marked depression and weakness, decreased feed intake, feed conversion, egg production and hatchability.

In addition, sometimes some sick birds were kept under careful observation with feed and water ad libitum till death to record the detail clinical signs along with other abnormalities and all of them were necropsied soon after death. The naturally dead birds from the above different private farms were collected during study period and brought to the Veterinary Pathology Laboratory, ANDUAT, Kumarganj, Ayodhya for the postmortem examination. The post-mortem examination was conducted thoroughly and carefully for any gross lesions in the lungs. Post mortem examination was also conducted at the farms. The lungs were collected in 10% neutral buffered formalin for further histopathological and immunohistochemical studies. The representative tissue pieces from lungs were fixed in 10%

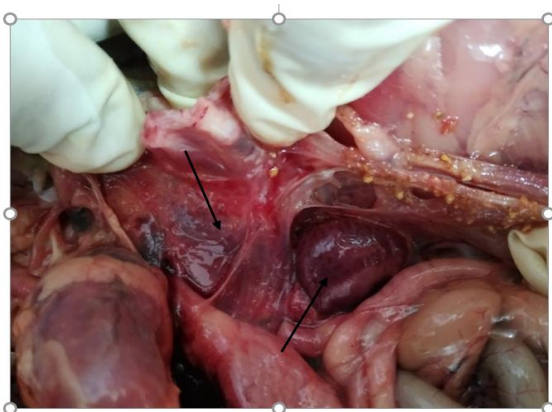
formal saline and were processed for paraffin block and sectioning into 3-5 mm thickness by microtome machine and were stained with haematoxylin and eosin (H&E) stain for histopathological examination (Luna, 1968). Immunohistochemical study was carried out as per the procedure of Brar *et al.* (2017) using primary antibody, chicken polyclonal to *Mycoplasma gallisepticum* (Abcam, UK). Immunohistochemistry (IHC) is selective identification of antigens in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues.

## Results and Discussion

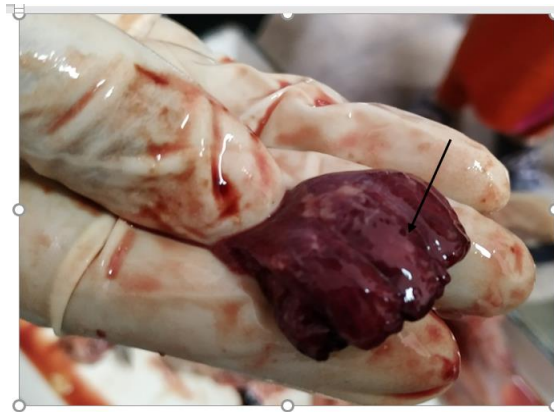
The diagnosis of the disease was based on the clinical signs viz. open mouth breathing, respiratory rales, ocular and nasal discharge, and reduced feed intake and characteristic gross lesions, microscopic tissue alterations and immunohistochemical studies.

### Gross Lesions

Grossly, pneumonic changes were found in the lungs that included haemorrhages and consolidation (Fig. 1) and frothy exudates came out from cut surface. Yellowish to creamy thick coverings were present on lungs. On removal of superficial coverings dark red to brown consolidated lungs were evident (Fig. 2) and lung showed congestion, light colour foci and haemorrhages. Accumulation of caseous material in the bronchi and pneumonic areas in the lung were observed. Islam *et al.* (2011), Vitula *et al.* (2011), Subtain *et al.* (2016), Brar *et al.* (2017) and Karthik *et al.* (2018) also described the similar gross lesions.

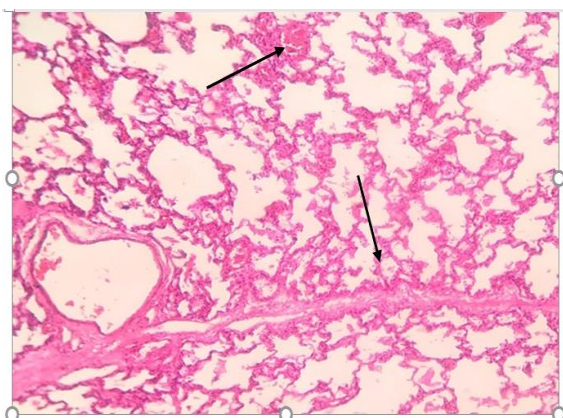


**Figure 1:** Pneumonic changes (consolidated and haemorrhagic) in the lung of affected bird

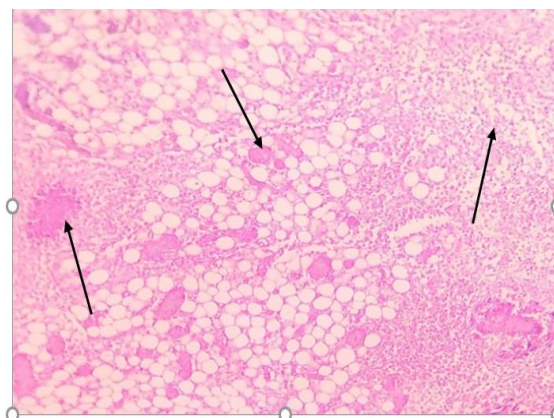


**Figure 2:** Red to brown colour consolidated and haemorrhagic lung of affected bird

### Microscopic Lesions

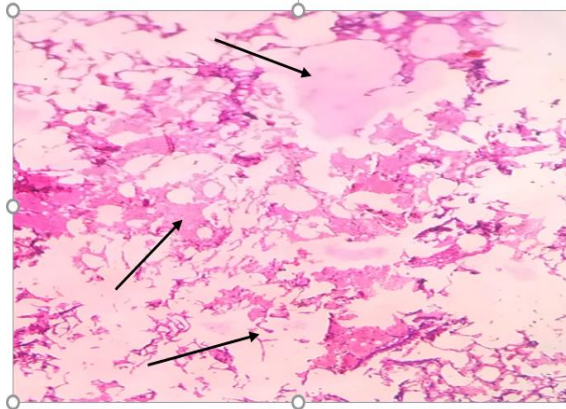


**Figure 3:** Congestion and lymphocytic infiltration in interstitial area along with denuded epithelial cell (interstitial pneumonia) (H&E X 200)

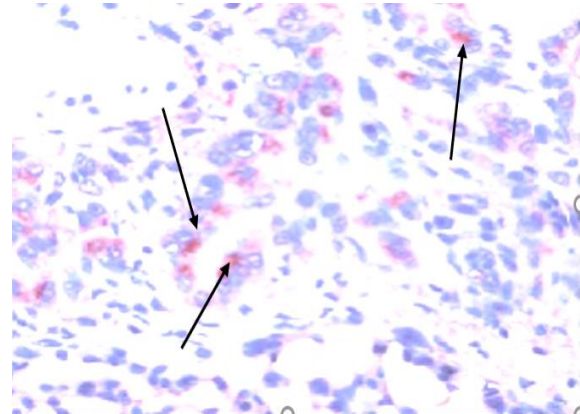


**Figure 4:** Congestion, haemorrhages and lymphocytic infiltration in interstitial area of lung (interstitial pneumonia) H&E X 100

Microscopically, lung section revealed congestion and haemorrhages, lymphomononuclear cell infiltrates in interstitial area (Fig. 3) besides necrotic and denuded epithelial cells (Fig. 5), which are suggestive of interstitial pneumonia. Lung showed oedema in the lumen of the alveoli, congestion of capillaries and lymphocytic infiltration in the interstitial tissue (Fig. 4 & 5). Degeneration, necrosis or hyperplasia were seen in the epithelium of the secondary and tertiary bronchi. The histopathological observations of lung sections corroborated the earlier reports of Islam *et al.* (2011), Vitula *et al.* (2011), Thilagavathi *et al.* (2016), Brar *et al.* (2017), Karthik *et al.* (2018) and Ley (2018).



**Figure 5:** Oedema in the lumen of the alveoli, congestion, denuded epithelial cells and lymphocytic infiltration in the interstitium of lung (interstitial pneumonia) H&E X 200



**Figure 6:** *Mycoplasma gallisepticum* as positive brownish red colour (antigen-antibody reaction) in primary bronchial epithelial cells of the lungs (IHC X 200)

### Immunohistochemical Studies

In the present study, the immunohistochemical studies performed on the suspected cases of chronic respiratory disease as per the gross and histopathological findings. *M. gallisepticum* antigen was evident as intense brown colored staining of parabronchiolar epithelial cells, primary bronchiolar epithelial cells (Fig. 6) besides inflammatory cells especially macrophages. MG antigen was also present in the extracellular surface of ciliated brush border and/or in the top of epithelium of bronchi. Similar findings were observed in other studies of Nunoya *et al.* (1995), Yilmaz *et al.* (2011), Gharaibeh and Hailat (2011), Yilmaz and Timurkaan (2011) and Brar *et al.* (2017) where *M. gallisepticum* antigen were detected in epithelial cells of parabronchus and the intrapulmonary primary bronchus as well as cilia of intrapulmonary primary bronchus epithelial cells in pneumonic broiler chicken lungs. IHC helps in understanding the distribution and localization of antigen antibody complexes in different parts of lung tissue.

### Conclusion

The present investigation throws light on the pathological changes of chronic respiratory disease-causing mortality in chickens in Ayodhya district of Eastern Uttar Pradesh. These findings indicate that chronic respiratory disease is a major disease in the poultry farms causing morbidity and mortality in Ayodhya district of Eastern Uttar Pradesh but morbidity and mortality varies from farm to farm depending upon the management practices and other superimposed infections. Tentatively diagnosed chronic respiratory disease on the basis of clinical signs and gross lesions can be confirmed by histopathological and immunohistochemical study and infection can be checked by proper treatment if diagnosed early. So, bird morality can be reduced or prevented and more income can be generated by the poultry farmers by regular preventive treatment using antimicrobial agents along with improved sanitation, hygiene and better husbandry practices.

### Conflict of Interests

There is no conflict of interest.

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