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# Detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* Antigens by Immunohistochemical Method in Pneumonic Broiler Chicken Lungs

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**Abstract:** The purpose of this study was to detect *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) antigens by immunohistochemical method in pneumonic broiler chicken lungs. The material of the study was composed of paraffin blocks of lungs taken from 275 pneumonic chickens. The cross-sections which were taken to paraffin blocks were stained with Avdin Biotin Complex (ABC) technique. MG antigens in 7, MS antigens in 11 samples were determined out of 275 pneumonic chicken lungs. It was found that *E. coli* and *Proteous* sp. in 6 cases were identified in 11 positive MS cases and *E. coli* were identified in 3 cases of 7 MG positive samples. No bacterial agent grew in 9 of the MG and MS positive cases. Compared to the results of histopathological findings where MS and MG antigens were detected, it was found that all of the cases had penuomonia that were in catarrhal or catarrhal hemorrhagic nature. As a result, in this study made retrospectively, the researchers suggest that MS and MG are the causative agent of the pneumonia, primarily or mix in the 18 chickens of 275 pneumonic broiler chicken lungs by immunohistochemical method.

**Key words:** Immunohistochemistry, broiler chicken, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, pneumonia, lungs, agent, Turkey

## INTRODUCTION

Diseases of the respiratory tract have an important role in poultry. In survey studies which were carried out on diseased or dead chickens it is reported that among the causes of the diseases, respiratory system diseases generally ranked first (Kusama *et al*., 1985; Rathore *et al*., 1985; Reece *et al*., 1986). These diseases may emerge as the reflection of a primary or a multisystemic disease (Glisson, 1998). In the aetiology of respiratory system diseases, bacteria, virus, parasite, fungi, nutrition and environmental factors are effective (Brugere-Picoux, 1984). In the aetiology of respiratory system diseases bacterial agents have an important role.

Avian mycoplasmosis is caused by the members of Mycoplasmataceae family (Kleven, 2003). More than a dozen species of mycoplasma cause respiratory system of chickens or turkey. *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) are considered as the most pathogen strains (Buim *et al*., 2009). MG is the aetiological agent of chronic respiratory diseases in chickens and infectious sinusitis in turkeys resulting in reduced feed consumption and egg production and significant downgrading of carcasses at slaughter (Yoder, 1984). *Mycoplasma synoviae* may cause either respiratory disease or synovitis (Kleven, 1998).

MG and MS infections of avian species are transmitted horizontally by direct contact with infected chickens and turkeys or vertically by egg transmission therefore, they are significant for multiplier breeder flocks. Many researchers stated that clinical, macroscopic and microscopic findings in the naturally or experimentally MG and MS infections of avian species (Kempf *et al*., 1993; Radi *et al*., 2000; Ley, 2003) are not sufficient to diagnose the disease and could be mistaken with other diseases such as *Ornithobacterium rhinotracheale* infection, Fowl cholera, *Escherichia coli* infection, Newcastle disease observed in respiratory system (Kempf *et al*., 1993; Radi *et al*., 2000; Ley, 2003; Sentes-Cu *et al*., 2005). Immunohistochemical (IHC) methods are successfully used to specifically diagnose *Mycoplasma* species in many animals including avian species (Hill, 1978; Radi *et al*., 2000; Ley, 2003; Yilmaz *et al*., 2011). The purpose of this study is retrospectively to detect the antigens of MG and MS by immunohistochemical technique in pneumonic broiler chicken lungs in the region where broiler production is common.

## MATERIALS AND METHODS

The material of the current study was composed of paraffin blocks of lungs taken from 275 pneumonic broiler chickens. These blocks were kept in paraffin about

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3.5 years. Pathological and microbiological findings of these samples were published previously (Timurkaan *et al*., 2008). The cross-sections which were taken to paraffin blocks after routine processes were stained with Avidin Biotin Complex technique (Bourne, 1983) according to the procedure suggested by the manufacturer. Tissue sections were deparaffinised, rehydrated and incubated in 3% $H_2O_2$ methanol solution in order to block endogenous peroxidase activity. Subsequently, tissues were washed 3 times with Phosphate Buffer Saline (PBS) for 5 min. For the purpose of antigen retrieval, tissues were kept in citrate buffer solution at pH 6.0 in a microwave oven for 20 min (distilled water was added every 5 min). Thereafter, tissues were left cooling down at room temperature and washed in PBS. In order to prevent non-specific staining, tissues were subjected to ultra V block solution (Lab vision) for 10 min. The MG, MS and non-immune rabbit sera diluted with PBS to 1:800 were added and after incubation at 4°C overnight in a humidified chamber and washing again 3 times with PBS for 5 min, tissue samples were treated with biotinylated goat anti-rabbit antibody (Lab vision) for 10 min. After washing in PBS, tissues were incubated with streptavidin peroxidase (Lab vision) for 10 min and an additional three washes in PBS, sections were allowed to react with 3-Amino-9-Ethylcarbazole (AEC) chromogene (Dako corporation) for 5 min. Subsequently, sections were counterstained with Mayer haematoxylin and covered with the mounting medium. Rabbit polyclonal anti-*Mycoplasma gallisepticum* and rabbit anti-*Mycoplasma synoviae* antibodies (specific hyper-immune sera) were provided by Dr. Nicholas (Weybridge Lab.). MG and MS positive chicken tissues, previously known in culture were used as positive controls while negative controls were obtained by omitting polyclonal primary antibodies.

## RESULTS AND DISCUSSION

Gross, histopathological and microbiological findings seen in this study have been presented previously (Timurkaan *et al*., 2008). Briefly, in the previous study in which cultivations were made only on bloody agar in 244 of 275 samples, bacterial agents were isolated and identified and in 31 samples, no agent was isolated or identified. The researches found that among the agents which were produced in lungs with lesion of broilers, *E. coli* ranked first and *Proteus* sp., *Staph.* sp. and *Staph.* sp.+*E. coli* followed it. In the previous study, according to histopathological evaluation, exudative pneumonia, especially catarrhal and catarrhal-hemorrhagic pneumonia was seen at the higher ratio.

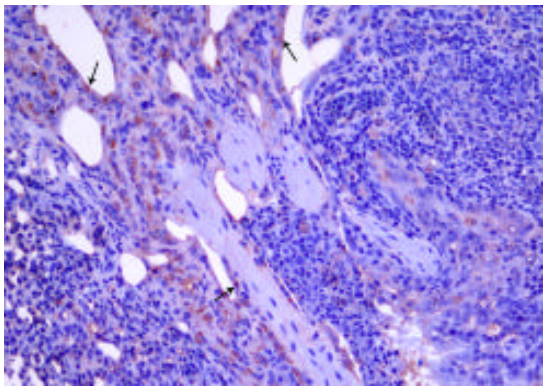

Fig. 1: MS positive staining in the epithelial cells of parabronchus (arrows) in a chicken lung. ABC method, Mayer haematoxylin counterstain, X200

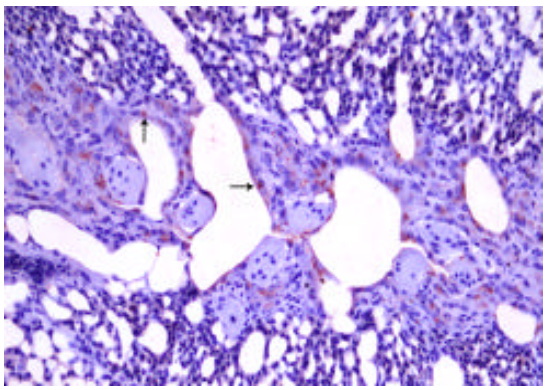

Fig. 2: MG positive staining in the epithelial cells of parabronchus (arrows) in a chicken lung. ABC method, Mayer haematoxylin counterstain, X200

MS positive staining was observed in 11 cases and MG positive staining in 7 cases among the 275 pneumonic lung chickens by ABC method. In the two cases, strong MG positive reaction was associated with weak MS positive reaction. Nevertheless although, no cross-reaction was observed between MG and MS hyper-immune sera, the double positive samples were considered as positive only for MG. A non-specific background staining easily distinguished from positive staining was observed especially in connective tissue. MS and MG antigens were in similar locations in the lungs. Positive MG and MS labelling was observed in epithelial cells of parabronchus (Fig. 1 and 2) and the intrapulmonary primary bronchus (Fig. 3 and 4). In addition, the immune staining was also determined on the cilia of the intrapulmonary primary bronchus epithelial cells and in inflammatory cells and partially in the exudate obstructing the air ways.

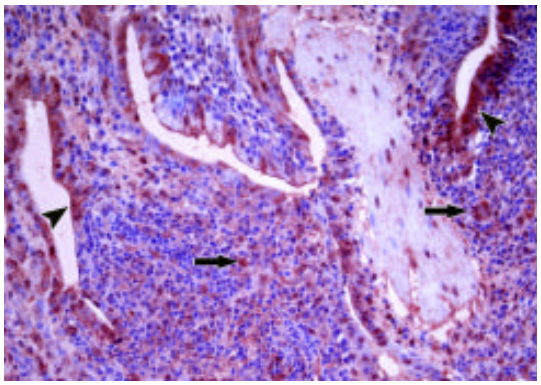

Fig. 3: MS positive staining in the epithelial cells of the intrapulmonary primary bronchus (arrowheads) and in the cytoplasm of inflammatory cells (arrows) in a chicken lung. ABC method, Mayer haematoxylin counterstain, X200

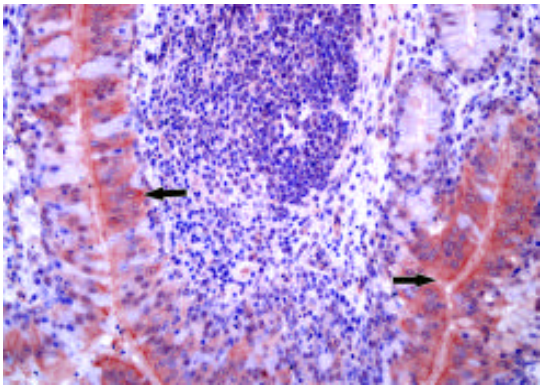

Fig. 4: MG positive staining in the epithelial cells of parabronchus (arrows) in a chicken lung. ABC method, Mayer haematoxylin counterstain, X200

Compared to the results of the previous study in which microbiological data of these cases were published, it was found that *E. coli* in four cases and *Proteous* sp. in two cases were identified in 11 positive MS cases and *E. coli* were identified in three cases of 7 MG positive samples. On the other hand, no bacterial agent that can grow in blood agar grew in 5 of MS and in 4 of MG cases. Compared to the results of histopathological findings where MS and MG antigens were detected, it was found that all of the cases had pneumonia that were in catarrhal or catarrhal hemorrhagic nature.

There are many studies carried out to determine mycoplasma agents in mammals using IHC methods however, the number of studies on avian mycoplasma is quite limited (Nunoya *et al*., 1997; Radi *et al*., 2000; Yilmaz *et al*., 2011). In a study, made comparison of diagnostic methods in MG and MS infections, the respiratory system organs and synovial membranes of seropositive broiler chickens were investigated by culture, PCR and IHC and the researchers revealed that IHC was relatively more sensitive than the other methods (Yilmaz *et al*., 2011). In the present study, a strong MS positive labelling was observed in 11 cases and MG positive labelling in 7 cases among the 275 pneumonic lung chickens. This finding demonstrated that MS was more common in broiler chickens investigated in the study compared to MG similar to the previous study (Yilmaz *et al*., 2011). In addition, recent studies carried out in broiler and breeding flocks reported that MS infection was serologically more common in Turkey.

*Mycoplasma gallisepticum* is a primary cause of chronic respiratory disease. *Mycoplasma synoviae* may cause either respiratory disease or synovitis (Kleven, 1998). These two agents can cause a mix infection with other bacterial and viral agents and the disease can exhibit a more severe course (Bradbury, 1984). In the current study, detection of *E. coli* and *Proteus* sp. in 6 of 11 MS positive cases and detection of *E. coli* in 3 of 7 MG positive cases indicate the presence of a mix infection in these cases. No bacterial agent grew in 9 of the MG and MS positive cases when cultured in blood agar. Researcher in the previous study (Timurkaan *et al*., 2008) cultured the samples in blood agar only therefore, detection of any viral agent was not considered. In the light of the findings of the current study, it can be considered that the pneumonia in these cases could be associated primarily with MS or MG or combination of viral aetiology.

In the present study, two cases simultaneous exhibited strong MG positive labelling and weak MS labelling in the lungs. Although, Opitz *et al*. (1983) stated a possibility for cross reaction between MS and MG antibodies in a study performed with ELISA and though, similarly, Bradley *et al*. (1988) reported common antigenic determinants for MG and MS, the results of the present study preferentially evoked a mixed infection in the two cases than occurrence of cross reactions. In addition, the polyclonal rabbit MG and MS antibodies used in the study were reported to cause non-specific staining whereas monoclonal antibodies in the diagnosis of mycoplasma infections are considered to be more specific according to Radi *et al*. (2000).

## CONCLUSION

As a result, in this study made retrospectively MS positive labelling was observed in 11 cases and MG positive labelling in seven cases among the 275 pneumonic lung chickens. The researchers suggest that MS and MG are the causative agent of the pneumonia, primarily or mix, in the 18 chickens by ABC method.

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