



Pathology and Molecular Diagnosis of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* Infections in Broiler Chickens from Western Maharashtra, India

Uday Bagal, Vitthal Dhaygude*, Bhupesh Kamdi, Chandrashekhar Mote,
Mrunalini Pawade and Shruti Bhosale

Department of Veterinary Pathology, KNPCVS, Shirwal (Satara), Maharashtra, INDIA

*Corresponding author: V Dhaygude; E-mail: drvitthalp@gmail.com

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ABSTRACT

Mycoplasma gallisepticum and *Mycoplasma synoviae* are worldwide in distribution including India and causes heavy economic losses to the poultry industry. The present study confirms the incidence of *M. gallisepticum* and *M. synoviae* infections by gross and microscopic pathology and PCR in broiler chickens. Incidence of pathogenic *Mycoplasma* spp. in broiler chickens with respiratory lesions was found to be 26.66%. Out of total 60 flocks, 6 flocks (10%) were found positive for *M. gallisepticum* and 12 (20%) were positive for *M. synoviae*. The incidence of *M. synoviae* was found to be higher than *M. gallisepticum*. Catarrhal tracheitis, focal pneumonic consolidation of lungs and airsacculitis were consistent lesions in *M. gallisepticum* and *M. synoviae* positive broiler chickens. However, synovitis/arthritis was not observed in any of the flock positive for *M. synoviae*. Incidence of *E. coli* amongst broiler chickens with respiratory lesions was found to be 40% by PCR targeting 16S rRNA gene and was higher than *Mycoplasma* spp. Mixed infection of *M. gallisepticum* and *E. coli* was detected in 3(9.09%) flocks, *M. synoviae* and *E. coli* in 4 (12.13%), *M. gallisepticum* and *M. synoviae* in 1(3.03%) and *M. gallisepticum*, *M. synoviae* and *E. coli* combined infection was detected in 1 (3.03%) flock. Mixed infection of *M. gallisepticum* and *E. coli*, *M. synoviae* and *E. coli* and *M. gallisepticum*, *M. synoviae* and *E. coli* revealed moderate to severe lesions of chronic complicated respiratory disease.

Keywords: *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *E. coli*, Incidence, PCR

Indian poultry industry represents a major success story. In 1960s, it was largely a backyard venture and now it is transformed in to a commercial business with an average annual turnover of ₹ 30,000 crores. It ranks 3rd in egg production and 9th in broiler meat production in the world having annual growth rate of 8-10%. The broiler birds grow very fast and they are continuously under stress of over production and are more prone to the diseases of respiratory and circulatory systems. Amongst different causes of respiratory disease complex, *Mycoplasmosis* is one of the most prevalent and important. *Mycoplasma* spp. causes sub clinical or mild disease in variety of birds including chickens and turkeys. It is also present in multiple disease complexes with other pathogenic agents including *Escherichia coli*, *Avibacterium paragallinarum*, *Ornithobacterium rhinotracheale*, Newcastle disease

virus and Infectious bronchitis virus (Naylor *et al.*, 1992; Kleven, 1998). Low Pathogenic Avian Influenza virus is also involved in the respiratory disease complex. When it is present along with other pathogens as complex, it causes high morbidity and mortality particularly in birds under managerial and other stress conditions. The losses caused by *Mycoplasma* infections are due to increase in embryonic and early chick mortality which may be up to 10 to 20%. It is also responsible for poor feed conversion ratio; drop in egg production (up to 10-20%) together with a high medication cost for the treatment (Ley, 2003).

Pathogenic *M. gallisepticum* causes Chronic Respiratory

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Disease (CRD) in poultry leading to heavy economic losses due to decreased egg and meat production, high mortality, poor feed conversion ratio and costly treatment. It mainly causes disease in young chicken and signs attributed to *Mycoplasma* infections include tracheal rales, coughing, sneezing and airsacculitis. Adult birds rarely show signs and the losses occur only in the form of decrease in egg production and poor egg quality. *M. synoviae* is also associated with causation of CRD. Most commonly, *M. synoviae* infections are usually sub-clinical. Sometimes *M. synoviae* infections become systemic and affect the synovial membrane of joints and tendon causing acute and chronic infectious synovitis. Both *M. gallisepticum* and *M. synoviae* are transmitted laterally via direct contact with infected carrier birds and fomites (Stanley *et al.*, 2001) and vertically through infected eggs (OIE, 2008). Although vaccination against *M. gallisepticum* is practiced at many farms in the country, various prevalence studies in the recent indicated higher prevalence of *M. synoviae vis-à-vis M. gallisepticum* in the poultry. Similarly, information regarding prevalence of mycoplasmosis in particular to *M. synoviae* and *M. gallisepticum* is very scanty from the western part of the Maharashtra. Hence, the present study reports incidence of both pathogens from broiler flocks of western part of Maharashtra.

MATERIALS AND METHODS

Samples

Samples for the present study were collected from broiler chicken farms located in different parts of western Maharashtra viz. Khandala, Wai, Katraj, Nasrapur during October, 2018 to June, 2019 by personal visits. Pooled tissue samples from trachea, lungs and airsacs from dead birds with respiratory lesions were collected from 60 commercial broiler farms in sterile container taking utmost care to maintain aseptic conditions without contamination. The tissue samples taken in sterile tubes were transported on ice and processed within 24-48 hrs of collection for extraction of nucleic acid (DNA). Tissue samples from trachea, lungs, air sac, liver, spleen and heart etc. were collected for histopathological examination in 10% neutral buffered formalin and preserved for further processing.

Pathological studies

The birds which are died due to respiratory symptoms

were subjected to necropsy examination as per standard procedure. The gross lesions were noted and tissue samples like trachea, lungs, liver, airsacs, spleen and heart were collected in 10% neutral buffer formalin for histopathological examination. The trimmed tissues were processed by routine embedding method using automatic tissue processor (Leica, Germany) and sections of 4-6 micron thickness were cut with automatic microtome machine (Mediate, Germany). The tissue Sections were stained with standard Hematoxylin and Eosin method (Luna, 1968).

Molecular Diagnosis

DNA Extraction

The DNA from tissue samples was extracted by using Phenol chloroform method as described by Sambrook *et al.* (1989) with slight modification. The extracted DNA sample was stored in - 20° C until use.

Polymerase Chain Reaction

Primers

DNA samples were screened for *M. gallisepticum* targeting 16S rRNA gene, The forward primer sequence MG-F CTGCAGCACCGAAGTATTCGCTC and reverse primer sequence MG- CCAAGGCGA TGACGTGT GT AGTT to yield 590 bp PCR product as described by Lierz *et al.* (2008) were used. For *M. synoviae* MS-F (forward primer) GAGAAGCAAAATAGTGATATCA and MS-R (reverse primer) CAGTCGTCTCCGAAGTTAACAA were used to amplify 16S rRNA gene (OIE, 2008).

The 16SrRNA gene fragment of *E. coli* was detected by using, ECO-F CAGTCGTCTCCG AAGTTAACAA as s forward primer and ECO- R CTCTACGCATTTACCGCTAC as a reverse primer (Tonu *et al.*, 2011). The amplified DNA was checked for presence of 590 bp, 207 bp and 704 bp product specific for MG, MS and *E. coli* by electrophoresis in 1.5% agarose gel, 100 bp ladder was used for marker and ethidium bromide as a tracing dye under U. V eliminator.

RESULTS AND DISCUSSION

A total of 60 pooled samples of tracheal swabs and tissues viz. trachea, lungs and airsacs from 60 flocks were

collected. Out of total 60 flocks, 6 flocks (10%) were found to be positive for *M. gallisepticum* (Fig. 1) and 12 (20%) positive for *M. synoviae* (Fig. 2).

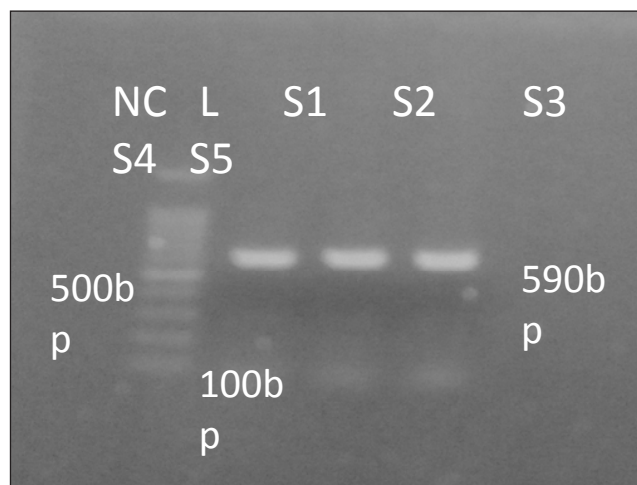


Fig. 1: Ethidium bromide stained agarose gel electrophoresis (1.5%) of *M. gallisepticum* PCR amplicon in TBE (0.5%) showing band of 590 bp. (Lane L= 100 bp ladder, NC- Negative control, S1- S3 samples).

Samples from total 16 (26.66%) broiler flocks affected with respiratory lesions were detected positive for pathogenic *Mycoplasma* spp. (MG/MS) by PCR. In this study, the incidence of *M. synoviae* was found to be higher than *M. gallisepticum*. Rajkumar *et al.* (2018) in similar kind of study recorded 25.98% and 9.45% incidence of *M. synoviae* and *M. gallisepticum* respectively from dead birds, which is in accordance with findings of the present study. On similar lines, Buim *et al.* (2009) in one of their studies reported 33% and 9.09% samples of tracheal swabs and piped embryos positive for MG and MS respectively from 33 farms (layers, breeders, broilers or hatchery) located in the Brazilian states with respiratory problems or drops in egg production. On the contrary, Tomer *et al.* (2017) in similar kind of study conducted in Haryana state of India in broiler chickens recorded high prevalence of MG over MS. They reported 27% and 2.1% samples positive for *M. gallisepticum* and *M. synoviae* respectively by PCR. Ramadass *et al.* (2016) also reported only 6.44% samples from broiler chicken positive for *M. synoviae* in different parts of Tamil Nadu.

Detection of *E. coli* was also attempted from all 60 pooled swab and tissue samples by PCR targeting 16S rRNA

gene. The expected amplicon of 704 bp size was detected in 24 (40.00%) samples (Fig. 3). Incidence of *E. coli* was found higher than *Mycoplasma* spp. in broiler chicken with respiratory lesions. Out of 17 flocks positive for only *E. coli* infection, 7(41.17%) flocks showed typical airsacculitis. Gowthman *et al.* (2013) reported *E. coli* as a major invading pathogen in respiratory disease complex. Similar findings were also reported by Saeb *et al.* (2001) wherein they identified *E. coli* in 88.2% cases of airsacculitis in broiler chickens in Jordan and their study suggests that airsacculitis can also be major lesion in *E. coli* infection. Minharro *et al.* (2001) also detected *E. coli* in 80.64% samples from airsac lesions of broilers in Brazil. Finding of the present study and reports by earlier suggests *E. coli* as a major pathogen in respiratory disease and airsacculitis in chicken.

Mixed infections were detected in samples from total 8 flocks out of total 33 positive flocks. Mixed infection of *M. gallisepticum* and *E. coli* was detected in 3(9.09%) flocks, *M. synoviae* and *E. coli* in 4 (12.13), *M. gallisepticum* and *M. synoviae* in 1(3.03%) and *M. gallisepticum*, *M. synoviae* and *E. coli* combined infection was detected in 1 (3.03%) flock (Table 1).

Table 1: Combined result of MG, MS and *E. coli* from 33 positive flock samples based on PCR detection

Name of the Microorganism	No. of Positive flocks	Percent cases in positive flocks	Percent cases in all 60 flocks
MG alone	01	3.03%	1.67%
MS alone	06	18.18 %	10.00%
<i>E. coli</i> alone	17	51.51 %	28.33%
MG + <i>E. coli</i>	03	9.09%	5.0%
MS + <i>E. coli</i>	04	12.13%	6.66%
MG + MS	01	3.03%	1.67%
MG + MS + <i>E. coli</i>	01	3.03%	1.67%

Minharro *et al.* (2001) also detected *E. coli*; *E. coli* and *M. synoviae*; *E. coli* and *M. gallisepticum* as well as *E. coli*, *M. gallisepticum* and *M. synoviae* in 80.64%, 16.13%, 12.90% and 9.68% samples respectively from airsac lesions of broilers in Brazil. Syuhada *et al.* (2013) detected *E. coli* infection in 17% cases of CCRD in broiler chicken in Johor state in Malaysia. Similarly, Sivaseelan and Balasubramanian (2013) in one of their studies concluded

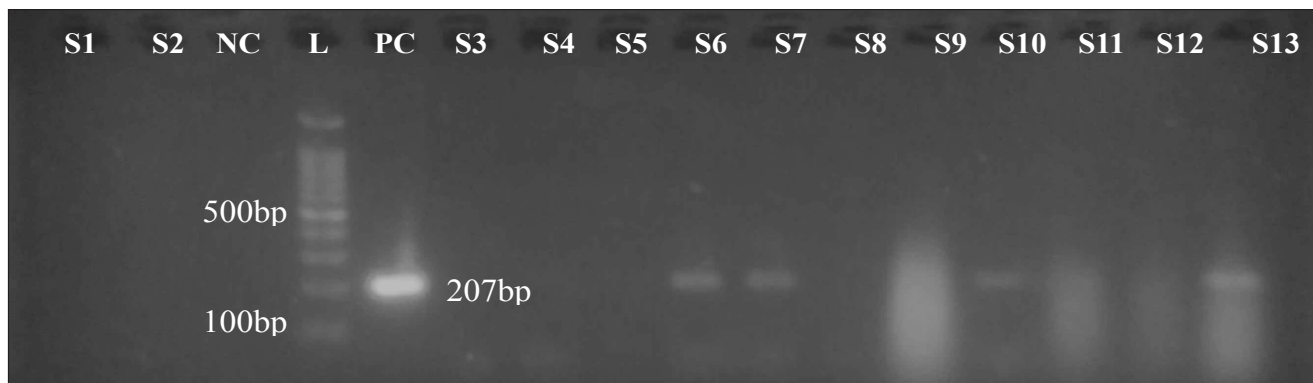


Fig. 2.: Ethidium bromide stained agarose gel electrophoresis (1.5%) of *M. synoviae* PCR amplicon in TBE (0.5%) showing band of 207 bp. (Lane L= 100 bp ladder, Pc- positive control, NC- Negative control, S1- S13 samples)

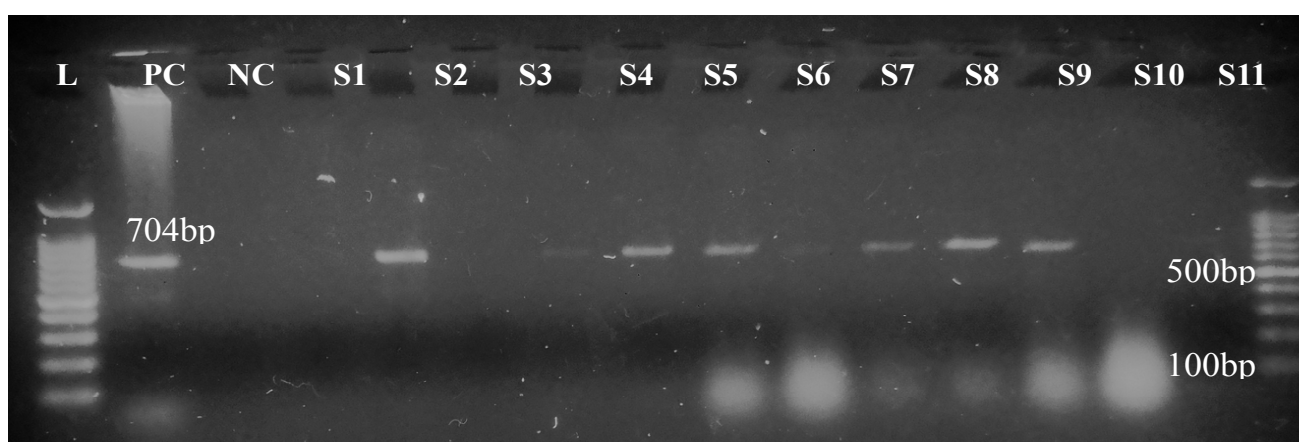


Fig. 3: Ethidium bromide stained agarose gel electrophoresis (1.5%) of *E. coli* PCR amplicon in TBE (0.5%) showing band of 704 bp. (Lane L= 100 bp ladder, PC- positive control, NC- Negative control, S1- S11 samples)

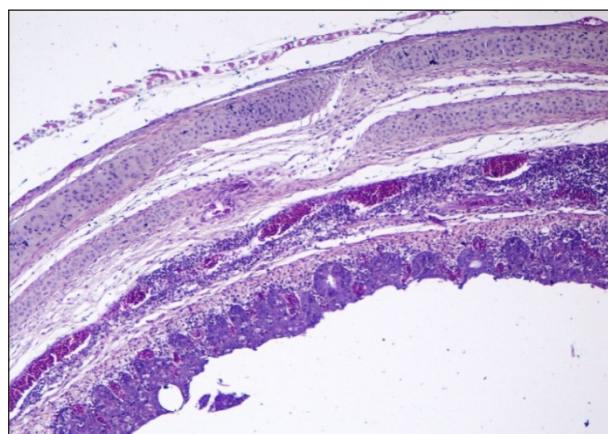


Fig. 4: Photomicrograph of trachea showing marked congestion, moderate hyperplasia of surface epithelium, infiltration of inflammatory cells with loss of cilia in *M. gallisepticum* affected birds (400 x H&E)

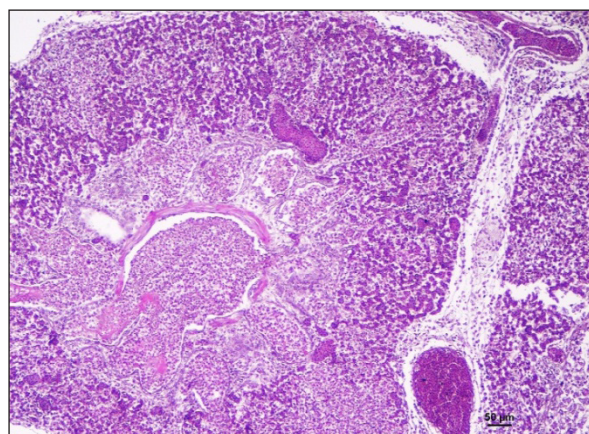


Fig. 5: Photomicrograph of lung showing multifocal areas of necrosis with infiltration of inflammatory cells and exudate in parabronchial lumen (H&E)

that, mortality due to CRD associated with *E. coli* was found to be 15% while due to CRD alone was only 8%. The result of present study indicates *E. coli* as one of the most common complications along with *M. gallisepticum* and *M. synoviae* infections.

Necropsy examination of most of the affected birds either by MG or by MS showed congestion in tracheal mucosa along with presence of catarrhal exudate. Lungs revealed varying lesions of mild to moderate congestion and focal to multifocal areas of consolidation. The airsacs were thickened and cloudy with presence of lumps of cheesy exudate suggestive of airsacculitis. All these lesions observed in the present study were similar to the lesions described by Ley, (2003) and Gabriel *et al.* (2005) in MG and MS affected chickens respectively. It is because of main portal of entry of pathogen through respiratory route where it adheres, colonies and produces lesions. Birds having mixed infections with one or more pathogens had respiratory lesions with more severity. However, synovitis/arthritis was not noted in any of the flock positive for *M. synoviae*. Similarly, Kleven, (2003) described purely respiratory involvement in MS affected chickens when concomitantly infected with avian paramyxoviruses.

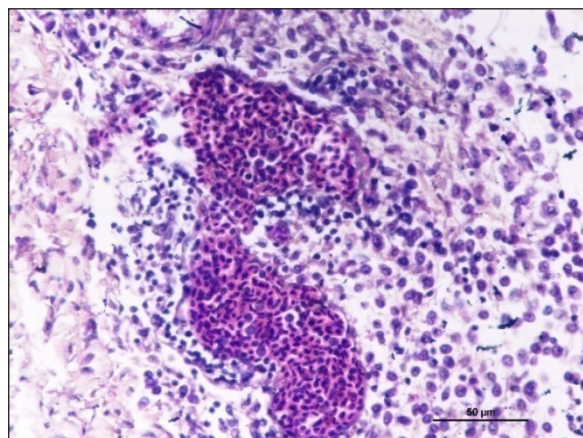


Fig. 6: Photomicrograph of airsac showing severe mononuclear cell infiltration along with few PMN and congestion (H&E)

Microscopic observation of trachea in MG positive birds showed thickening of tracheal mucosa due to hyperplasia of goblet cells and mild infiltration of inflammatory cells. Loss of ciliated epithelium in the mucosa was also noted (Fig. 4). Lungs showed mild congestion in the parabronchial wall and focal areas of necrosis along with

infiltration of degenerated inflammatory cells and epithelial cells in parabronchial lumen (Fig. 5). Atrial lumen also showed infiltration of degenerated inflammatory cells. There was thickening of airsacs along with pinkish exudate and infiltration of inflammatory cells (Fig. 6). Birds affected with MS revealed mild respiratory lesion like tracheal congestion with infiltration of inflammatory (predominantly mononuclear) cells in the submucosa, airsacculitis and pulmonary congestion. Birds with mixed infection had more severe lesions. All the *E. coli* infected birds revealed airsacculitis as a consistent lesions. All gross and microscopic lesions noted in MG/MS and *E. coli* infection in the present study were in accordance with the findings of Ley, (2003), Kleven, (2003) and Thilagavathy *et al.* (2016).

CONCLUSION

The present investigation revealed 10 and 20 % incidence of MG and MS respectively in broiler flocks showing respiratory lesions from the western part of Maharashtra. Incidence of *E. coli* (40 %) was found to be higher vis-à-vis MG and MS. Mixed infection of one or more selected pathogens was detected in 8 flocks out of total 33 positive flocks and produced severe lesions. As *Mycoplasmosis* is an economically important disease, present study warrants to create awareness amongst farmers and to implement strategies to prevent *Mycoplasmosis* and other respiratory infections.

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