Haryana Vet. (June, 2022 **61(1),** 110-112

Research Article

ISOLATION AND IDENTIFICATION OF *MYCOPLASMA* spp. FROM COMMON RESPIRATORY INFECTIONS OF POULTRY IN ANDHRA PRADESH

NAGENDRA REDDY THOPIREDDY[1]*, SREEDEVI BOLLINI[2], VINOD KUMAR NAGARAM[3], SRILATHA C.H.[4] and MADHAVA RAOT[5]

[1]Assistant Professor, [2]Professor and University Head, [3]Associate professor, Department of Veterinary Microbiology, [4]Professor and University Head, Department of Veterinary Pathology, [5]Professor and University Head, Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati-517502

Received: 18.08.2021; Accepted: 04.01.2022

ABSTRACT

Pooled samples of 19 poultry farms that includes tracheal swabs (228), cloacal swabs (228), nasal swabs (228), trachea from dead birds (152), lungs from dead birds (152) and oviduct from dead birds (76) were collected and labelled separately as per farm and tested for the presence of *Mycoplasma* by culturing in Pleuro Pneumonia Like Organisms (PPLO) agar media and targeting the 16 *SrRNA* gene by PCR. The study found that 17 farms were positive for genus *Mycoplasma*, with percent positivity of 89.47% by PCR. Among the 17 positive farms, 60, 96 and 120 cloacal, nasal swabs and tracheal swabs were positive with positivity of 26.31, 42.10 and 52.63%, respectively. A total of 64 lung tissues (42.10%), 72 tracheal tissues (47.36%) and 8 (10.52%) oviduct samples were positive for genus *Mycoplasma*. Histopathological examination was conducted for *Mycoplasma* infected lung specimens. This study found that the confirmatory diagnosis of mixed respiratory infections in poultry is accurate when histopathology and molecular detection methods like PCR are used.

Keywords: Histopathology, *Mycoplasma*, PCR, Poultry, PPLO agar

How to cite: Thopireddy, N.R., Bollini, S., Nagaram, V.K., Srilatha, C.H. and Raot, M. (2022). Isolation and identification of *Mycoplasma* spp. from common respiratory infections of poultry in Andhra Pradesh. *Haryana Vet.* **61(1)**: 110-112.

Avian mycoplasmosis is caused by several pathogenic mycoplasmas. *Mycoplasma* pathogens spread rapidly both vertically and horizontally via the eggs and contaminated fomites. Because of similarities in exhibition of clinical signs and lesions in mixed respiratory infections, it is often difficult to diagnose the specific disease condition. At present there is a need for accurate diagnosis of respiratory pathogens from mixed infections and is highly essential to reduce the significant economic losses to the poultry industry. Accurate diagnosis is required for implementation of control strategies, hence present work was under taken to isolate and identify the common respiratory *Mycoplasma* pathogen from poultry.

MATERIALS AND METHODS

Samples

Samples were collected from farms with history of vaccination against IB and RD from different poultry pockets of Andhra Pradesh. From a total of 19 suspected farms, samples were collected from birds that were showing respiratory symptoms and the samples were pooled farm wise and organ wise separately. From each poultry farm few live birds, few dead birds and pooled samples of 12 cloacal swabs, 12 tracheal swabs, 8 tracheal tissues, 8 lung tissues and 4 oviducts were collected. The total pooled samples of all the poultry farms includes tracheal swabs (228), cloacal swabs (228), nasal swabs (228), trachea from dead birds (152), lungs from dead birds (152) and oviduct from dead birds (76) were labelled separately as per farm.

Isolation of Avian *Mycoplasmas*:

The samples after collection were immediately inoculated into sterile Eppendorf tubes containing sterile PPLO broth. Eppendorf tubes were incubated anaerobically at 37 ºC with 90 percent relative humidity in BOD incubator, until the phenol red indicator changed from red to yellow after 4 days of incubation. The cultures were tested for the presence of Mycoplasma by genus specific PCR (Table 1) and simultaneously plated on PPLO agar medium and incubated anaerobically in candle jar to avoid drying of plates, at 37 ºC with 90 per cent relative humidity for 8 days in BOD incubator. The colonies were detected by microscopic examination at low power magnification for "Fried egg" appearance (Sumitha *et al.*, 2015). The DNA extraction was carried out from the suspected samples by Trizol method. PCR was standardized by using the positive DNA from MG Vaccine. It was found that an annealing temperature of 56 ºC for 30 seconds was optimum for amplification of 16*SrRNA* gene yielding 280 bp PCR product (Fig. 2).

*Corresponding author: tnreddy32@gmail.com

Molecular detection of genus *Mycoplasma*

Table 1

Primers used for detection of genus Mycoplasma (Cetinkaya *et al.*, 2009)

Primers	Primer Name	Nucleotide Sequence	Amplicon Size
16*SrRNA* gene	GPO3F	5'-TGGGGAGCAAACAG GATTAGATACC-3'	280 bp
	MGSO	5'-TGCACCATCTGTCAC TCTGTTAACCTC-3'	

Histopathology

A transverse section of tissue approximately 0.5 cm in thickness was taken from trachea, lungs and oviduct of birds showing respiratory and associated organ lesions. Tissues were fixed in 10 per cent formalin and trimmed to a thickness of about 3 mm. Tissues were dehydrated, cleared and embedded in paraffin in a routine manual processing. Tissues were cut at 3 to 5 mm thickness, mounted on glass slides, stained with haematoxylin and eosin and were places with DPX for histopathological examinations. The stained slides were read under X100 magnification and histopathological changes were recorded.

RESULTS AND DISCUSSION

Indian poultry industry is one of the fastest growing segments of the agricultural sector. The organized poultry sector is contributing to 70 per cent output and the remaining 30 per cent comes from unorganized sector (Kiilholma, 2007). Among the poultry diseases, the respiratory infections due to *Mycoplasma*, infections bronchitis virus (IB) and infectious laryngotracheitis virus (ILT) are very common and causes heavy economic losses to the poultry industry.

The affected birds showed respiratory rales, dyspnoea, sneezing, mucoid nasal discharges, swelling of lower eyelids, oedema of facial sub-cutis, growth retardation and lameness. Similar clinical manifestations were reported by Ley (2003). Based on these clinical signs, the flocks were suspected for respiratory infections like *Mycoplasma*, ILT and IB. Some of the birds in the affected farms also exhibited lameness due to joint infections and facial oedema suggestive of *Mycoplasma* infections.

All the suspected samples were initially tested for the presence of *Mycoplasma* by targeting the 16*SrRNA* gene. Amplification of 16*SrRNA* gene was also used by Cetinkaya *et al.* (2009) and Srinath (2019) for the detection of genus *Mycoplasma*.

Out of the 19 farms screened, 17 farms were found to be positive, with percent positivity of 89.47% (Table 2). Several other researchers also reported similar findings. Positivity of 91.6% was reported by Sumitha *et al.* (2015), 100% by Gharaibeh *et al.* (2011), 72.7% by Buim et al. (2009) in suspected poultry respiratory infections. Among the 17 positive farms, 60, 96 and 120 cloacal, nasal swabs and tracheal swabs were positive with positivity of 26.31, 42.10 and 52.63%, respectively. A total of 64 lung tissues (42.10%), 72 tracheal tissues (47.36%) and 8 (10.52%) oviduct samples were positive for genus *mycoplasma*. In similar studies conducted by other scientists, Gharaibeh *et al.* (2011) reported 100% positivity from tracheal swabs, cloacal swabs (Dhondt *et al.*, 2007), tracheal tissues (Gharaibeh and Roussan, 2008) and oviduct samples (Sumitha *et al.*, 2015). *Mycoplasma* infections were recorded in all age groups of birds and during all the seasons. These findings coincided with the findings of Feberwee *et al.* (2009) and Sumitha *et al.* (2015).

In the present study an attempt was made for isolation of *Mycoplasma* by using selective media, PPLO broth supplemented with horse serum, yeast extract, thallus acetate and penicillin. Sumitha *et al.* (2015) used 10% pig serum as supplement for PPLO broth. All the positive samples changed the colour of the PPLO broth from pink to yellow in 4 days after inocubation.

Preliminary identification of *Mycoplasma* was carried out based on postmortem lesions and histopathological studies. PM lesions noticed include pneumonia in lungs, inflammation of oviduct, joint swellings and similar lesions were reported by Sumitha *et al.* (2015). Further, histopathological examination of lung revealed pneumonic changes in addition to thickening of interstitial septa and infiltration of lymphocytes (Fig. 4). Similar histopathological changes were reported in lungs in *Mycoplasma* infections by Gaunson *et al.* (2000). Histopathology of trachea revealed degenerated epithelial cells with infiltration of mononuclear cells in the sub mucosa and lamina propria with mucus in the lumen of trachea under X100 magnification (Fig. 3). Similar histopathological changes of trachea were reported by Bagal *et al.* (2019).

Table 2

Pooled samples results for genus *Mycoplasma*

	No. of farms		No. of cloacal swabs		No. of Tracheal swabs		No. of Nasal swabs		No. of Tracheal tissues		No. of Lung tissues		No. of Oviduct samples	
	Tested	positives	tested	positives	tested	positives	tested	positives	tested	positives	tested	positives	tested	Positives
Genus *Mycoplasma*	19	17	228	60	228	120	228	96	152	72	152	64	76	08

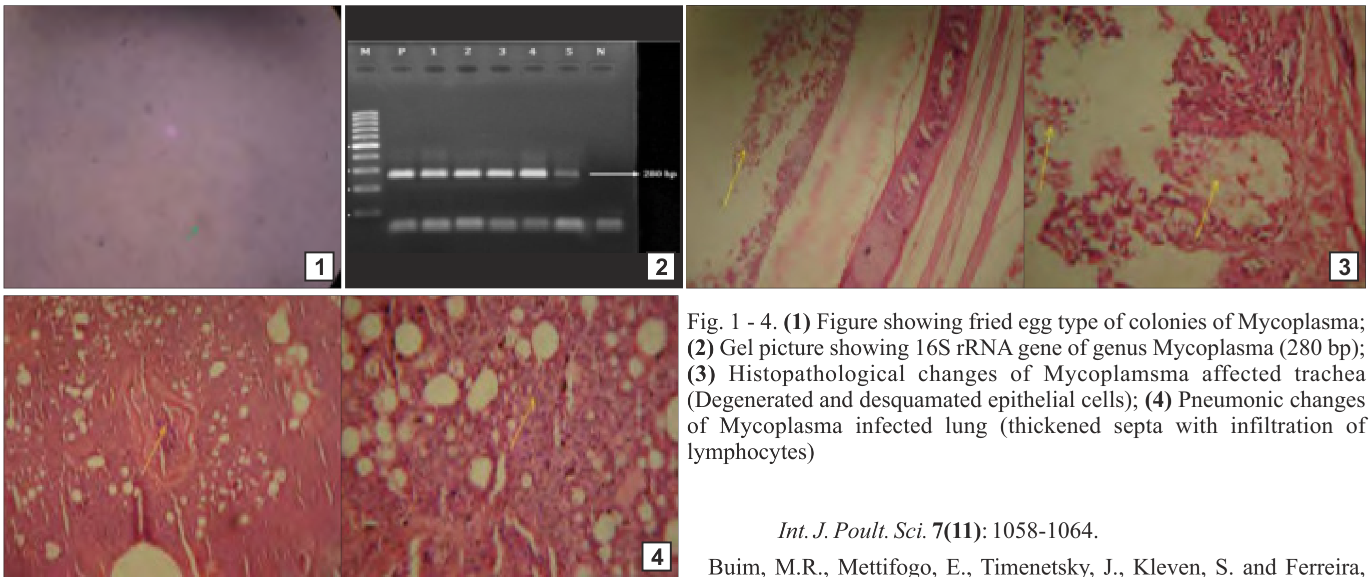

Fig. 1 - 4. **(1)** Figure showing fried egg type of colonies of Mycoplasma; **(2)** Gel picture showing 16S rRNA gene of genus Mycoplasma (280 bp); **(3)** Histopathological changes of Mycoplamsma affected trachea (Degenerated and desquamated epithelial cells); **(4)** Pneumonic changes of Mycoplasma infected lung (thickened septa with infiltration of lymphocytes)

Six samples showed raised, round, micro colonics with fried egg appearance (Fig. 1) of the colony, which was noticed after 8 days of incubation under anaerobic conditions. These findings are in accordance with Pourbakhsh (2014). There was no observable growth in other samples which could be due to medication of birds with antimycocidal drugs and lower concentration of the organisms. These findings are in accordance with the findings of Behbahan *et al.* (2008).

CONCLUSION

Mycoplasma infections are more common in poultry. Economic losses due to *Mycoplasma* are devastating. Early detection and timely deployment of treatment in infected flock is more important to reduce the morbidity and mortality of *Mycoplasma* infections. In field level, post-mortem of infected poultry birds is done to know the etiology of infections. Isolation and identification of *Mycoplasma* infections takes more time. The alternate methods with feasible, accurate and rapid diagnostic techniques available are molecular methods like PCR and histopathology. These methods can further reduce the time taken for diagnosis and could be used as confirmatory diagnostic tools. In this present study, histopathology and PCR are conducted along with cultural isolation and identification of *Mycoplasma* spp. This study concluded that PCR and histopathology are promising tools of rapid and accurate diagnosis of *Mycoplasma* infections.

REFERENCES

Bagal, U., Dhaygude, V., Kamdi, B., Mote, C., Pawade, M. and Bhosale, S. (2019). Pathology and Molecular Diagnosis of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* Infections in Broiler Chickens from Western Maharashtra, India. *J. Anim. Res.* **9(6)**: 897-902.

Behbahan, N.G.G., Asasi, K., Afsharifar, A.R. and Pourbakhsh, S.A. (2008). Susceptibilities of *Mycoplasma gallispeticum* and *Mycoplasma synoviae* isolates to antimicrobial agents *in vitro*. *Int. J. Poult. Sci.* **7(11)**: 1058-1064.

Buim, M.R., Mettifogo, E., Timenetsky, J., Kleven, S. and Ferreira, A.J.P. (2009). Epidemiological survey on *Mycoplasma gallisepticum* and *M. synoviae* by multiplex PCR in commercial poultry. *Pesq. Vet. Bras.* **29(7)**: 552-556.

Cetinkaya, B., Kalin, R.E.C.E.P., Karachan, M., Atil, E., Manso-Silvan, L. and Thiaucourt, F. (2009). Detection of contagious caprine pleuropneumonia in East Turkey. Revue scientifique et Technique-Office International des Epizooties, **28(3)**: 1037-1044.

Dhondt, K.V., Dhondt, A.A. and Ley, D.H. (2007). Effects of route of inoculation on *Mycoplasma gallisepticum* infection in captive house finches. *Avian Pathol.* **36(6)**: 475-479.

Feberwee, A., De Wit, J.J. and Landman, W.J. (2009). Induction of eggshell apex abnormalities by Mycoplasma synoviae: field and experimental studies. *Avian Pathol.* **38(1)**: 77-85.

Gaunson, J.E., Philip, C.J., Whithear, K.G. and Browning, G.F. (2000). Lymphocytic infiltration in the chicken trachea in response to *Mycoplasma gallisepticum* infection. *Microbiol.* **146(5)**: 1223-1229.

Gharaibeh, S. and Al Roussan, D. (2008). The use of molecular techniques in isolation and characterization of *Mycoplasma gallisepticum* from commercial chickens in Jordan. *Int. J. Poult. Sci.* **7(1)**: 28-35.

Gharaibeh, S., Laibinis, V., Wooten, R., Stabler, L. and Ferguson-Noel, N. (2011). Molecular characterization of *Mycoplasma gallisepticum* isolates from Jordan. *Avian Dis.* **55(2)**: 212-216.

Kiilholma, J. (2007). Food-safety concerns in the poultry sector of developing countries. Food and Agricultural Organisation of the United Nations, 1-20.

Ley, D.H. (2003). *Mycoplasma gallisepticum* infection In: Diseases of Poultry, Saif Y.M. (Edt.), (11th Edn.), Iowa State Pres, USA, 722-744.

Pourbakhsh, S.A. (2014). Molecular characterization of *Mycoplasma synoviae* isolates from commercial chickens in Iran. *Archives of Razi Institute*, **69(1)**: 1-14.

Srinath, M.G.R. (2019). Studies on *Mycoplasma ovipneumoniae* infection and its associated bacterial pathogens in pneumonic sheep. (MVSc. Thesis, SVVU, Tirupati).

Sumitha, P., Moorthy, M., Sukumar, K., Mani, K. and Eswaran, M.A. (2015). Prevalence of *Ornithobacterium rhinotracheale*, *Mycoplasma gallisepticum* and *Mycoplasma synoviae* antibodies in Layers. *Indian Vet. J.* **92(8)**: 78-79.