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Molecular Detection of *Mycoplasma gallisepticum* in Broiler Chicken Farms of Satara and Pune Districts of Maharashtra

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ABSTRACT

Mycoplasma gallisepticum is one of most important pathogen economically affecting poultry worldwide. In the present study, filed samples from broiler flocks showing respiratory distress were analyzed by specific 16S rRNA PCR. The PCR products were sequenced and analyzed *in silico* by bioinformatics tool. At the flock level, 04 out 11 (36.36%) flocks were found positive for presence *M. gallisepticum* infection. Further, 7.57 % samples from Bhor and 6.06 % samples from Wai tested positive for presence of *M. gallisepticum* with PCR. *In silico* sequencing analysis revealed that field *M. gallisepticum* sequence was 100% identical with *M. gallisepticum* PB1/06/Ind sequence whereas it was 99.5% identical with f-99, ts-11 and 6/85 *M. gallisepticum* vaccine strains.

HIGHLIGHTS

- *Mycoplasma gallisepticum* is the most virulent and economically important pathogen causing chronic respiratory disease (CRD) in chickens.
- *In silico* analysis of nucleotide sequence of field *Mycoplasma gallisepticum.*

Keywords: *Mycoplasma gallisepticum*, Broiler, Chicken, PCR

Mycoplasmas are small prokaryotes (300-800 nm in diameter) belonging to the class Mollicutes. They have a small genome and lack cell wall, as well as many biochemical pathways (Semashko *et al*., 2017). *Mycoplasma gallisepticum* (MG), most virulent and economically significant of the avian Mycoplasma, causes chronic respiratory disease (CRD) in chickens and infectious sinusitis in turkeys (Prajapati *et al*., 2018). CRD is a worldwide problem affecting in layer, broiler, and breeder poultry flocks and it causes heavy economic losses to poultry industry. Infection with MG may be in-apparent or result in varying degrees of respiratory distress. The MG infected birds exhibit respiratory symptoms that include sneezing, coughing, rales, swollen sinuses, suborbital swelling and exudation from the nostrils and eyes (Gondal *et al*., 2015). In MG infection, morbidity is high and mortality low in uncomplicated cases (Bahatti *et al*., 2013). Economic losses in CRD are due to egg and meat production, mortality, poor feed conversion ratio, carcass condemnation, costly monitoring programs and treatment cost (Kleven *et al*., 1984, Ferguson-Noel *et al*., 2020). Other pathogens which also cause similar respiratory symptoms in poultry include mild Newcastle disease and avian infectious bronchitis, *Avibacterium paragallinarum* and *Pasteurella multocida*. Thus, accurate and rapid diagnosis of MG is crucial to prevent the spread of infection. Diagnosis of MG can be made on

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the basis of characteristic gross and histological lesions, serology, isolation and identification of the organism (OIE, 2018; Prajapati *et al*., 2018; Ferguson-Noel *et al*., 2020). Detection of MG genetic material by polymerase chain reaction (PCR) has been increasingly used as a valuable alternative test for detection of MG as it has high specificity and sensitivity (García *et al*., 2005; Ferguson-Noel *et al*., 2020). This study aimed to detect the presence of MG in broiler flocks with respiratory signs in Satara and Pune districts of Maharashtra using molecular method.

MATERIALS AND METHODS

Tissue samples (trachea, lungs and airsacs) and choanal swabs for the present study were collected from 11 broiler chicken farms showing respiratory distress. The sample collection was done from different parts of Satara district and Pune district viz. Khandala, Phaltan, Wai, Bhor and Baramati during October, 2020 to November, 2020. Samples were collected in sterile containers under aseptic conditions. DNA extraction from tissue samples carried out by using Phenol chloroform method as described previously by Sambrook *et al*. (1989). The extracted DNA samples were stored in - 20° C until use. The DNA from field samples was screened with polymerase chain reaction (PCR) using MG specific primer pairs (MGF primer-GAG-CTA-ATC-TGT-AAA-GTT-GGT-C and MGR primer-GCT-TCC-TTG-CGG-TTA-GCA-AC) (OIE, 2018). The MG specific amplification PCR product of 185 bp was detected by loading the PCR products in 1.5% agarose gel containing ethidium bromide. The amplified products were subjected to nucleotide sequencing (geneOmbio Technologies Pvt. Ltd., India) and the obtained sequences were compared with other sequences from Gene Bank by BLAST analysis. The sequencing reports were analyzed using EditSeq, SeqMan and MegAlign of DNASTAR Lasergene (Version 7.0.0) software.

The PCR amplicons of 185 bp was obtained using specific primers (OIE, 2018). The PCR products were checked using 1.5 per cent agarose gel electrophoresis (Fig. 1). These findings are in agreement with the previous reports (OIE 2018; Poorna Chandhar *et al*., 2018; Rajkumar *et al*., 2018; Emam *et al*., 2020). Out of 11 flocks tested from six different places, two farms each from Bhor and Wai Tehsil were found positive with PCR whereas samples from Khandala, Phaltan and Baramati were negative. At the flock level, 04 out 11 (36.36%) flocks were found positive for presence MG infection from suspected farms. These results were in agreement with Tomar *et al.* (2017), wherein 27 % flocks were reported positive for MG infection while, in another study from Maharashtra conducted by Bagal *et al.* (2019), 10 % broiler flocks were found positive for MG.

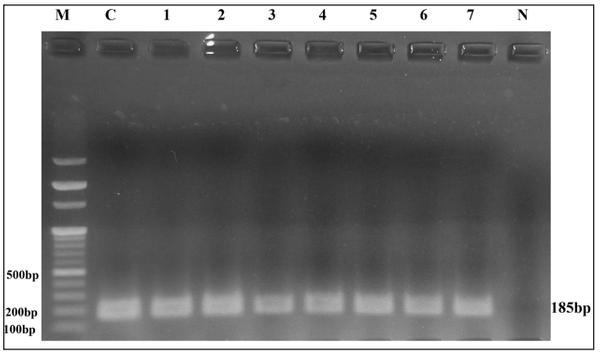

Fig. 1: 16S rRNA PCR amplification: M- DNA ladder, C- Positive control with DNA from standard 6/85 vaccine strain, Lane 1-7 field samples, N- negative control

In this study, a total of 19 Choanal swabs and 99 tissue samples were collected out of which all swabs were negative whereas, 07 tissue samples yielded positive result with 16S rRNA PCR. In the tissue samples tested, 05 (7.57%) birds from Bhor out of 66 and 02 (6.06 %) birds from Wai out of 33 tested positive for presence of MG with PCR which successfully yielded the desired PCR products of 185 bp. Thus, out of total 99 tissue samples tested by PCR, 07 (7.07%) were found positive for MG whereas, all the swab samples (19) were negative. These results were in correlation with results of simplex PCR performed by Ramdass *et al.* (2006), where in 3.46% prevalence of MG was reported in Tamil Nadu state of India. In concurrence with results of present study, a study from Maharashtra has reported MG in 10% of total tissue samples processed with PCR (Bagal, *et al*., 2019). Likewise, Rajkumar *et al.* (2018) have reported the prevalence of MG by PCR as 11.65% in Hyderabad. A recent study from Madhya Pradesh has reported an overall prevalence of MG as 13.33% in broilers by PCR. As compared to our findings a much lower rate of MG infection in broiler birds (1.47%) were reported by Bium *et al.* (2009). Different factors have been suggested for variation of MG prevalence and detection rates reported by different studies viz. rate of

Table 1: Results of *M. gallisepticum* 16S rRNA PCR in field samples

Sl. No.	Place	Samples					
		Farms	Birds	Type	Number	Positive	% Positive
1.	Khandala	02	08	Tissue	00	00	Nil
				Choanal Swab	08	00	Nil
2.	Bhor	03	66	Tissue	66	05	**7.57**
				Choanal Swab	00	00	Nil
3.	Phaltan	02	06	Tissue	00	00	Nil
				Choanal Swab	06	00	Nil
4.	Wai	02	33	Tissue	33	02	**6.06**
				Choanal Swab	00	00	Nil
5	Baramati	02	05	Tissue	00	00	Nil
				Choanal Swab	05	00	Nil
TOTAL		**11**	**118**	**Tissue**	**99**	**07**	**7.07**
				Choanal Swab	**19**	**00**	**Nil**

infection, sample collection time and stage of infection, sample size, sample type, detection techniques, biosafety and biosecurity in flocks (Rajkumar, *et al*., 2018; Ferguson-Noel, *et al*., 2020).

The results from sequencing were also in accordance with the fragment size observed (185bp). The sequencing reports were analyzed using DNASTAR Lasergene (Version 7.0.0) software. Relevant sequences for analysis were downloaded from NCBI. The nucleotide sequences were aligned by Clustal W slow-accurate method. The alignment results revealed that nucleotide sequence of field sample was exactly identical with PB1/06/Ind sequence (India) where as one substitution each was observed in f-99, ts-11 and 6/85 vaccine strains, respectively. Strains AS1UAF21 and A-5 had two substitutions each. The field MG was 100% identical with PB1/06/Ind sequence (Fig. 2). The field MG sequence was 99.5% identical with standard M. gallisepticum, f-99, ts-11 and 6/85 sequences. A percent identity of 98.9% and 98.4% was seen between field sample and strains AS1UAF21 and A-5, respectively (Fig. 2).

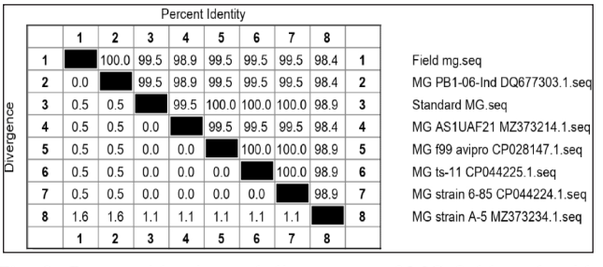

Fig. 2: Percent identity and divergence of MG field nucleotide sequence

SUMMARY

To sum up, findings of the present study revealed incidence of MG in Satara and Pune districts of Maharashtra. The MG infection was confirmed by sequencing and *in silico* analysis. These findings are important with respect to control and preventive measures against this economically important disease in poultry.

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