Avian mycoplasmosis is responsible for heavy economic losses to the poultry industry in India. It results from a complex interaction of various factors like viral, bacterial and housing conditions. *Mycoplasma gallisepticum* (MG) belongs to class Mollicutes is small in size, lacks bacterial cell wall with minimum genetic information. It is the principle pathogenic agent causing Chronic Respiratory disease (CRD) in chickens and infectious sinusitis in Turkeys and has a worldwide distribution. The objective of this study was molecular detection of *Mycoplasma gallisepticum* in poultry with respiratory affections in Haryana (India). 100 tissue samples including trachea, lungs and air sacs were collected and pooled from 100 different poultry flocks in different districts of Haryana. The samples were screened by Polymerase chain reaction (PCR) for *Mycoplasma gallisepticum*. A prevalence of 16% (16/100) was seen for *Mycoplasma gallisepticum* by using 16S to 23 S rRNA primer. PCR can be used for a simple and quick way to identify *Mycoplasma gallisepticum* from field samples.
chicken and turkey and the harmful impacts were decreased with proper and synchronised management on the farms. Within intensive poultry farming, infection by CRD is found in association with avian influenza, Newcastle disease, colibacillosis and infectious bronchitis and further leads to more severe problems (Stipkovits et al., 2012). It is the principle pathogenic agent causing Chronic Respiratory disease (CRD) in chickens and infectious sinusitis in Turkeys and has a worldwide distribution (Li et al., 2010). 

*Mycoplasma gallisepticum* infections vary from asymptomatic to severe symptoms like reduced feed efficiency, reduced egg production and decreased growth rate (Sarkar et al., 2005). It is characterized by coughing, nasal discharge, rales and severe lesionson air sacs commonly air sacculitis (Tomar et al., 2017). Grossly, thickened air sacs with caseous or mucous exudate, catarrhal inflammation in bronchi, trachea and sinuses, fibrinous perihepatitis, interstitial pneumonia and adhesive pericarditis (Yamamoto, 1991; Charlton et al., 1996). There has been a horizontal and vertical transmission documentation (Kumaragurubaran et al., 2018).

**Material and Methods**

**Sample Collection**

A total of 100 samples were collected from seven different districts of Haryana. Bhiwani (n=2), Hisar (n=9), Jhajjar (n=19), Jind (n=20), Karnal (n=20), Panipat (n=23) and Sonepat (n=7). Sonepat The samples were collected from October 2018 to February 2019. Trachea, lungs and air sacs were collected from poultry flocks and were pooled, and together known as “sample”.

**DNA Extraction**

DNA was extracted from directly from tissues collected from various poultry farms using DNA extraction mini kit (QIAmp mini kit) as recommended by the manufacturer.

**Gene specific PCR**

PCR was carried out on 100 pooled tissue samples from various poultry flocks. 16 to 23 S rRNA PCR specific to *Mycoplasma gallisepticum* was per the protocols described by Raviv et al., 2007). The primer selected for *M. gallisepticum* for 16 to 23 S rRNA was; F-5’ GTAGGGCCGGT GATTGGAGTTA3’ and R-5’ CCCGTAGCATTTCGCA GGT T3’ and the size of the amplified product was 810bp. The positive control used for *Mycoplasma gallisepticum* was S-6 serotype antigen (Salsbury laboratories, U.K.).

The protocol of Raviv et al. (2007) was used with certain modifications. The initial denaturation was achieved at 94 °C for 10 min with Sapphire fast PCR- hot start master mix. It was further followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 62°C for 30 sec and extension at 72 °C for 45 sec. The final extension was achieved at 72°C for 10 min.

**Analysis of PCR product**

The amplified PCR products were analysed by agarose gel electrophoresis using 2% agarose containing 0.5 µg/ml ethidium bromide in tris-borate EDTA (TBE) buffer and visualized under UV trans illuminator, as per the method of Sambrooke et al., (1989).

The amplified DNA product was examined by comparison with standard DNA marker (100 bp DNA ladder, Takara dye plus). The image of gel was obtained using gel documentation system (Alpha Imager). PCR amplicons of the positive samples were purified as per the protocols recommended by MACHEREY-NAGEL NucleoSpin Gel and PCR Clean-UP.
Nucleotide Sequencing, Phylogenetic Analysis and Accession number

The PCR products of *M. gallisepticum* were purified and sent for sequencing at DNA sequencing facility of Department of Animal Biotechnology, College of Veterinary Sciences, LUVAS, Hisar. The nucleotide sequencing was done by Automated DNA sequencer, Applied Biosystem 3130 XL Genetic analyser in both directions. The sequences were trimmed and analysed in MeAlign program (Lasergene, DNASTAR). The sequences were aligned by ClustalW software. The nucleotide sequences obtained for *Mycoplasma gallisepticum* (16 to 23 S rRNA) by using Mega 7.0.26 (Molecular Evolutionary Genetic Analysis). They were aligned by ClustalW. The phylogenetic tree was made by maximum likelihood tree using 1000 bootstrap value. The sequences reported in this article have been deposited in the GenBank database under accession number MK922122.

**Fig.1** Agarose gel showing *Mycoplasma gallisepticum* from poultry field samples. M- 100 bp ladder; 1-8- samples; 9- negative control.

Results and Discussion

16/100 (16%) samples were found to be positive for *Mycoplasma gallisepticum* by Polymerase Chain Reaction.

*Mycoplasma gallisepticum* 16S-23S rRNA intergenic spacer region

Phylogenetic analysis carried out by using the sequence of the present study with 19 other sequences (Table 2) and is depicted in Figure 2.

The sequences were in the same clad along with other sequence reported from India (KX759108) and other countries Japan (AB098504), USA (KC247863, AY744942, JN935873). The sequences from other countries were present in different clads including Brazil (KT824823, KJ019166), Egypt (GU357708), Germany (KP710079), South Africa (MF196171) and USA (KJ468424, KJ468432). It was very divergent from South Africa (KY362223).
Fig. 2 Phylogenetic tree based on partial nucleotide sequences of 16S-23S rRNA IGSR gene of *Mycoplasma gallisepticum*. Phylogenetic tree constructed by the maximum likelihood tree method using 1000 bootstrap replicates value in Mega7.0 software.
Table.1 Accession number of various isolates obtained for *Mycoplasma gallisepticum*

| Accession number | Year | Country          | Strain/Host                      |
|------------------|------|------------------|----------------------------------|
| MK922122         | 2019 | Haryana, India   | Lungs, trachea, air sacs         |
| AB098504         | 2002 | Japan            | PG31                             |
| KC247863         | 2012 | USA              | USA/R/CK60                       |
| AY744942         | 2004 | USA              | ATCC19610                        |
| JN935873         | 2011 | Rockville, USA   | PG31CX95                         |
| KX759108         | 2016 | Haryana, India   | PT-89                            |
| MH571923         | 2018 | South Africa     | B2777-15A-8                      |
| KY362237         | 2016 | South Africa     | ZA_MGB1932_15                    |
| JQ770174         | 2012 | Australia        | 6-85                             |
| HQ143386         | 2010 | Jordan           | OR/83/CKB                        |
| HQ143380         | 2010 | Egypt            | EGY/67240/CK08                    |
| GQ406845         | 2009 | Egypt            | RabE2-09                         |
| KT824823         | 2015 | Brazil           | BRA/UFF/LSA020                   |
| GU357708         | 2008 | Egypt            | RabE4-08                         |
| KJ019166         | 2011 | Brazil           | 2011/UFMG1                      |
| KP710079         | 2011 | Germany          | 2038/11/CK                       |
| MF196171         | 2017 | South Africa     | B878-14-M2_157                   |
| KJ468424         | 2014 | USA              | LVT 9815                         |
| KJ468432         | 2014 | USA              | LVT 9815                         |
| KY362223         | 2016 | South Africa     | ZA_MG B642_08                    |

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How to cite this article:

Vaishali, Davinder Singh and Renu Gupta. 2020. Molecular and Phylogenetic Analysis of Mycoplasma gallisepticum in Haryana, India. Int. J. Curr. Microbiol. App. Sci. 9(01): 2518-2523. doi: https://doi.org/10.20546/ijcmas.2020.901.286