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Molecular and phylogenetic analysis of *Mycoplasma synoviae* in Haryana

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Abstract
Mycoplasma synoviae is one of the pathogenic species causing sinusitis in chicken and turkey. *Mycoplasma synoviae* has also caused significant economic loses in commercial flocks of broilers. A total of 100 tissue samples (trachea, lungs and air sacs) were collected form 100 poultry flocks in different districts of Haryana. A total of 19/100 (19%) prevalence was found in Haryana by using 16S rRNA primers through Polymerase Chain Reaction (PCR). PCR is a simple and quick approach to screen field samples.

Keywords: *mycoplasma synoviae*, pcr, molecular, phylogenetic

Introduction
Mycoplasma synoviae is responsible for causing infectious sinusitis in chicken and turkey and is economically significant because of decreased growth rate, hatchability rate, egg production and arthritis [1] and air sacculitis are the major signs seen at the time of slaughtering of the carcass [2, 3]. The incubation in young birds is relatively short and respiratory tract of all birds are affected and the morbidity and mortality is different in arthritic form. The birds that are infected exhibit coryza and tracheal rales and swelling of infraorbital sinus have been seen in turkey [13]. In acute cases of arthritic form there is pale comb, marked depression, rapid loss of condition, retarded growth, swelling of joints and lameness. Breast blisters also occur. The disease may extend to chronic form and synovitis persists for entire life of flock. The primary breeder flocks have seen to be free from infection of *Mycoplasma synoviae*, but infection is seen in Commercial turkey and chicken breeding stock time to time and appears to be more widespread infection in layer birds. *Mycoplasma synoviae* has also caused significant economic loses in commercial flocks of broilers. In broilers, a decline of up to 20% to 30% is seen in weight gain, 10% to 20% reduction in food conversion efficiency, 10% to 20% of carcasses are condemned at the processing plant and a 5% to 10% mortality rate [4, 5]. The aim of the study is to detect the prevalence of *Mycoplasma synoviae* in different districts of Haryana.

Materials and methods
Sample collection
A total of 100 tissue samples were collected from birds affected with respiratory problems within different age groups and from different flock sizes from various districts of Haryana. The samples were collected from October 2018 to February 2019. The samples included lungs, trachea and air sacs from the dead birds. The samples were collected by certified veterinary practitioner in sterile containers without any preservative. The collected pooled samples were immediately taken to Department of Veterinary Public Health and Epidemiology, Hisar and stored at -20 °C [6].

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.

Amplification of 16 S rRNA of *Mycoplasma synoviae*
Polymerase Chain Reaction was carried out on 100 pooled tissue samples from various poultry flocks.

The amplification for 16S rRNA specific to *Mycoplasma synoviae* was as per the protocols described by OIE 2008 [7]. The primer selected for *M. synoviae* for 16S rRNA was; Forward 5'-GAGAAGCAAAATAGTGATATCA-3' and Reverse 5'-CAGTCGTCTCCGAAGTTAACAA- 3'. The amplified product size was 210 bp.

Amplification conditions with the primer were standardised. The method of OIE (2008) was followed with certain modifications for amplification of DNA of *Mycoplasma synoviae*. The initial denaturation was achieved at 95 °C for 10 min with Sapphire fast PCR- hot start master mix. It was further followed with 35 cycles of denaturation at 95 °C for 30 sec, annealing at 52 °C for 30 sec and extension at 72 °C for 30 sec. The final extension was achieved at 72 °C for 8 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 (1989) [8]. 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 and phylogenetic analysis

The PCR products of *M. synoviae* 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 synoviae* (16S 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 [9].

Results

19/100 (19%) samples were found positive when analysed by Polymerase Chain Reaction.

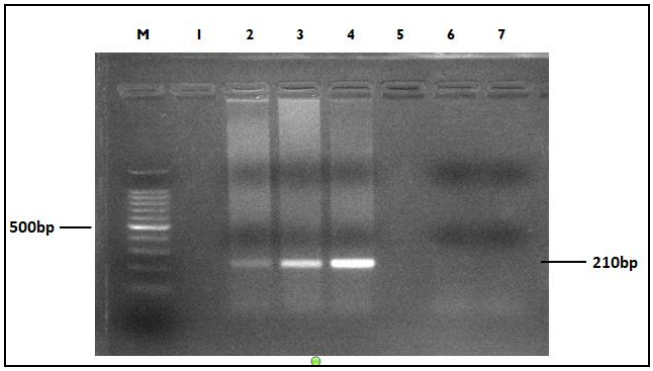

Fig 1: Agarose gel showing amplified products of 16S rRNA of *Mycoplasma synoviae* from poultry field samples. M- 100 bp ladder; 1- negative control; 2-4-samples.

Phylogenetic analysis

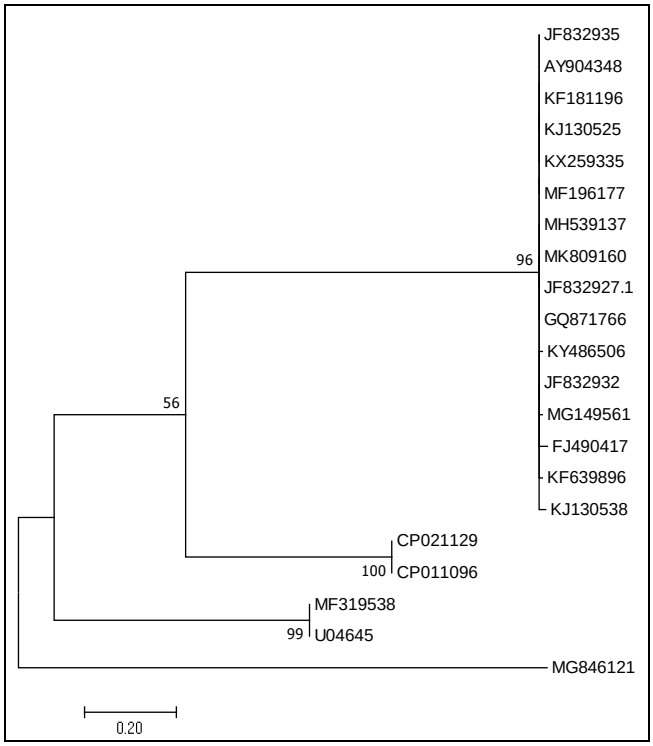

Fig 2: Phylogenetic tree based on partial nucleotide sequences of 16S rRNA of *M. synoviae*. Phylogenetic tree constructed by the maximum likelihood tree method using 1000 bootstrap replicates value in Mega7.0 software.

Table 1: Accession numbers of various sequences obtained for *Mycoplasma synoviae* 16S rRNA:

Accession number	Year	Country	Sample/Isolate
MK890160 (This study)	2018	India	Lungs, air sacs, trachea of birds with respiratory problems
JF832927	2011	Iran	Chicken
AY904348	2005	Tamil Nadu (India)	isolate="TN/427"
KF181196	2012	Egypt	Chicken Tracheal swab
KJ130525	2014	Lahore (Pakistan)	*Gallus gallus*
KX259335	2016	Gujarat	Poultry tracheal swab
MF196177	2017	South Africa	Chicken
MH539137	2018	South Africa	strain="B730-09-2"
JF832925	2011	Iran	Chicken
GQ871766	2009	Iran	Chicken tracheal swab
KY486506	2015	Haryana	Chicken trachea, lungs and air sacs
JF832932	2011	Iran	Chicken
MG846121	2017	Iraq	Chicken with respiratory sign
U04645	1993	Sweden	strain="WVU1853"
MF319538	2017	China	Poultry synovial fluid

CP011096	1957	West Virginia (USA)	*Gallus gallus*
CP021129	2017	Australia	Chicken
KJ130538	2014	Lahore (Pakistan)	*Gallus gallus* Trachea and joint swabs
KF639896	2013	Ludhiana (Punjab)	Chicken lung
FJ490417	2008	Andhra Pradesh (India)	Chicken choanal cleft swab
MG149561	2016	Saudi Arabia	Chicken

Phylogenetic analysis carried out by using the sequence of 16S rRNA of the present study with 20 other sequences (Table 1) and is depicted in Fig 2.

There was 19% prevalence of *Mycoplasma synoviae* in Haryana when detected through PCR.

The sequence of present study was in same clad with all the sequences reported from India and many sequences from different countries. The sequence was quite different from sequence from West Virginia (USA-CP021129), Australia-CP011096, China-MF319538, Sweden-U04645 and Iraq-MG846121**.** The results showed similarity with strains from India and was different from the strains obtained from other countries. The prevalence of *Mycoplasma synoviae* was quite high as compared to other infections.

Discussion

Mycoplasma synoviae was detected in various districts of Haryana [1, 10]. This study demonstrated the high prevalence of MS infection in Commercial poultry farms of different districts of Haryana. Various studies have shown similar results showing higher prevalence of *M. synoviae* [12]. Some studies have showed a prevalence of more than 50% [13]. Therefore, the high prevalence and wide distribution of MS infection warrants immediate attention and preventive strategies to minimize economic impact of MS infection [11]. The present study suggests that the PCR assays performed for MS and MS provide a simple, quick and precise tool to specifically detect these organisms from the field samples which are always found to be complicated by other pathogens. PCR is being more sensitive, specific and reliable test can be applied for the early diagnosis of avian mycoplasmosis in poultry flocks. The phylogenetic analysis showed similarity to different strains obtained from India [9]. The data had showed diverse distribution of *Mycoplasma synoviae* in various countries [14].

Conclusion

With majority of studies showing greater prevalence of *Mycoplasma gallisepticum* than *Mycoplasma synoviae*, we obtained 19% prevalence of *Mycoplasma synoviae*. This gives us a broader view to screen samples at poultry farms more frequently and adopting PCR as a real, simple and quick diagnostic method for diagnosing field samples.

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