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Short Communication

Detection of *Mycoplasma gallisepticum* from field samples of Poultry using conventional PCR

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ABSTRACT

The present study reports the use of polymerase chain reaction (PCR) for detection of *Mycoplasma gallisepticum* (MG) infection in poultry using tissues directly. A total of 51 samples (trachea and airsacs) were collected from clinically suspected birds originating from district Hisar, India. PCR was carried out using specie specific primers for MG and identified 18 samples (35.3%). It is concluded that tissues may be used for rapid screening and detection of MG in poultry.

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Mycoplasmosis is one of the major respiratory problems in poultry. Avian Mycoplasmosis, caused mainly by *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS), can cause considerable economic losses in chicken due to chronic respiratory disease, reduced weight gain and meat quality and increased feed conversion ration and mortality in broilers, tremendous drop in egg production in layers and increase in embryonic mortality in breeders (Kleven and Noel, 2008; Ley, 2008). Out of four major pathogenic *Mycoplamas*, *Mycoplasma galliseptcium* has been described as the most important species affecting poultry, others include *Mycoplasma synoviae* (chicken and turkey pathogen), *Mycoplasma synoviae* (turkey pathogen), and *Mycoplasma meleagridis* (turkey pathogen) (Mohammed Et al., 1987; Thomas and Sharp, 1990; Ley, 2008). The annual economic impact of MG infections in the United States has been estimated between $118 and $150 million for the layer industry alone in 1994 (Patterson, 1994). In the recent past, the MG outbreaks have caused significant losses to the poultry industry (Ley, 2008).

The flock screening for the pathogen is done by serological assays and the confirmation can be done by cultivation of pathogen or PCR detection (Ramadass Et al., 2006). Isolation of *Mycoplasma* is very difficult and cumbersome to perform. Diagnosis of *Mycoplasma* infections by serological procedures is sometimes hampered by interspecies cross–reactions and nonspecific reactions (Hagan Et al., 2004). Therefore, molecular methods for diagnosing the disease may be more effective in comparison to cultural and serological methods. These include a validated PCR assay for MG, MS, and other avian *Mycoplasmas* based on unique sequences contained in the 16S rRNA gene and nucleic acid probes (Khan and Kleven 1993; Kempf, 1998; Lauerman, 1998; McAuliffe Et al., 2005). The technique has been proven to be very specific and sensitive method even for amplifying low amounts of nucleic acid to a level that cannot be easily detected by other methods.

Therefore, present study was aimed to test feasibility of using tissues directly PCR based identification of *Mycoplasma* in poultry flocks without any need of enrichment or culture.

Tissues (trachea and airsacs) from poultry flocks (one representative samples was taken from each affected flock) showing typical signs of chronic respiratory disease *viz* rales, coughing, nasal discharge, sinusitis, brought for diseases investigation were collected at Disease Investigation Lab., College of Veterinary Sciences, LLR UVAS, Hisar. Broiler flocks around Hisar region of Haryana were included with flock size ranging from 5000 to 10,000 birds. Necropsy of the dead birds revealed pathologic findings such as air sacculitis, tracheitis, and pneumonia. Tissue samples (trachea and airsacs) were collected from such birds in buffered glycerol (50%) and stored at –20^{O}C till further use.

Tissues were triturated directly without any enrichment in pestle and mortar. Genomic DNA was extracted using DNeasy Blood & Tissue Kit® (Qiagen) following manufacturer's instructions. The total DNA was measured at 260 nm optical density as per method described by Sambrook and Russel (2001). The extracted DNA was kept at –20^{O}C till further use.

PCR was carried out by using species–specific primer pair for MG (Kiss *et al.*, 1997) (Table 1). PCR was carried out in 0.5 mL PCR tubes in a final reaction of 25 µL volume. Each reaction mixture contained 2X PCR Master Mix (12.5µL) (TopTaq Master Mix ®, Qiagen), 10 pmol/µL each of MG–F and MG–R primers and 100 ng of the DNA template. The reaction mixtures were adjusted to the final volume by adding Nuclease free water (NFW) and subjected to PCR amplification in MyCycler (Biorad, USA).

The PCR amplified products were separated by agarose gel electrophoresis (2% agarose) in 1X Tris–borate–EDTA buffer (Tris–base10.8g 89 mM, Boric acid 5.5g 2mM, EDTA 4 ml of 0.5 M EDTA pH 8.0). Gel was stained with ethidium bromide (@ 0.5 µg/mL of gel).

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Table 1: Primers and PCR conditions applied for the detection of MG

	Primer	SEQUENCE (5'–3')	PCR Conditions (°C/s)			Reference
			Denaturation	Annealing	Elongation	
MG	MG_F	AACACCAOAGGCGAAGGCGAG	94 ^{O}C/ 60 sec	58 ^{O}C/ 45 sec	72 ^{O}C / 60 sec	Kiss *et al.* 1997
	MG_R	ACGGATTTGCAACTGTTTOTATTGG				

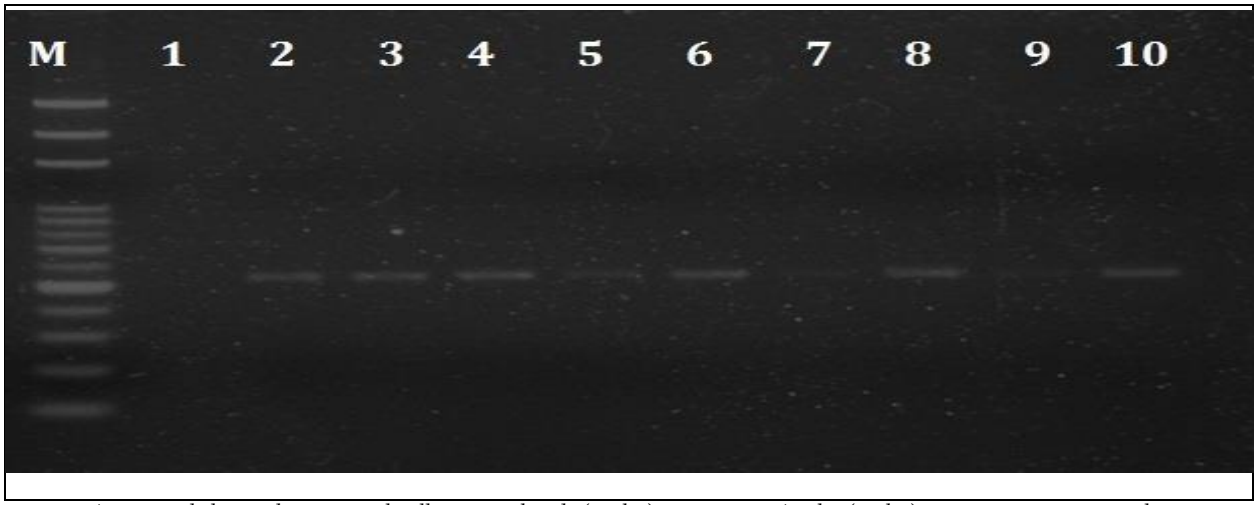

Figure 1: Agarose gel electrophoresis results illustrating bands (530bp); Lane M: DNA ruler (100bp); Lane–1: negative control; Lane 2–9: Positive samples

Aliquots of 6 µL were applied to the gel. Standard molecular size marker, Gene Ruler 100 bp DNA ladder (Fermentas, USA) was included in gel. Product separation was done at constant voltage of 75V for 1 hour. DNA fragments were observed by ultraviolet transilluminator and photographed in a gel documentation system (Alpha Imager, Germany).

The species specific primers used in the study successfully amplified the portion (713–1243 bp) of 16s RNA gene of MG (530 bp) (Figure 1). Out of 51 samples tested, 18 samples (35. 3%) were found PCR positive for the *Mycoplasma gallisepticum.* This study showed that MG can be detected from field samples directly without need of culturing this fastidious organism as reported by McAuliffe Et al. (2005). Ramadass Et al., (2006) had documented that tracheal samples gave more isolations than from lung tissues; however the present study reports the use of airsacs along with tracheal tissues for extraction of DNA.

Thus this species specific PCR may be applied for rapid screening and diagnosis of the MG in naturally infected birds before the culture results are obtained as reported by various workers (Lauerman Et al., 1993; Ramadass Et al., 2006).

Mycoplasmas are among those fastidious organisms which are very hard to culture, as they have unique medium requirements. It requires 4–5 days for growth and initial cultures commonly contain other contaminants also (McAuliffe Et al., 2005; Kleven, 2008, Bagheri Et al., 2011). Present study illustrates the combined advantage of simple, cheap and rapid molecular test of PCR, having high sensitivity and specificity, to diagnose MG infection as compared to traditional culture of the organism.

Culture testing can be costly and time–consuming, and can also be inconclusive (Ewing Et al 1998), besides this, culture of Mycoplasma is laborious and can take 3–4 weeks, and even then, the result can be negative or be hampered by mixed infections (Bradbury Et al., 1982; Zain Et al., 1995). As for culture and PCR tests, despite the comparable sensitivity, PCR test lead to rapid results and relatively low costs compared with culture (Lauerman Et al., 1993; Feberwee Et al., 2005; Sakhaei Et al., 2009; Pourbakhsh Et al., 2010). For such reasons, use of rapid and sensitive molecular detection methods, like PCR, can be advantageous (Hyman Et al., 1989; Fernandez Et al., 1993; Kempf Et al., 1993).

In the present study, PCR was used to demonstrate the involvement of *Mycoplasma gallisepticum* infection in broiler flocks in Haryana, suspected for respiratory disease involvement. Results indicated that *M. gallisepticum* infection is prevalent in poultry flocks of Haryana as indicated by PCR testing of clinical tissues (trachea along with airsacs). Thus, PCR could be a rapid, effective, sensitive and inexpensive method as compared to the standard cultural technique which is cumbersome, time consuming and laborious, hence PCR directly from tissues can be an alternative and useful method for screening compared to traditional culture for the detection of MG in poultry at various diagnostic laboratories.

In conclusion, PCR application on tissues like trachea and air sacs seems to give encouraging results for detection of *Mycoplasma gallisepticum* infection in poultry flocks suffering from respiratory disease. Further studies are needed to test the comparative efficacy, sensitivity and specificity of such PCR separately on different tissues, which will allow rapid and specific diagnosis of this infection which would subsequently help in its control.

Conflict of interest:

The authors encountered no conflict of interest during the study.

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