Involvement of *Mycoplasma synoviae* in Respiratory Distress Cases of Broilers


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**ABSTRACT**

*Mycoplasma synoviae* (MS) is an important pathogen of poultry worldwide, causing respiratory tract infection and infectious synovitis in chickens and turkeys. The study was designed to detect *M. synoviae* through serology, culture isolation and polymerase chain reaction (PCR) assay to document the involvement of MS infection in respiratory distress cases of broiler birds. The validated PCR assay amplifying the conserved gene region of 16SrRNA gene was applied for the detection of *M. synoviae* from culture as well as in clinical samples. The results indicated that 04 out of total 17 commercial broiler flocks showing respiratory distress signs were found positive with *M. synoviae* infection indicating 76.57% sero-positivity as, determined with rapid serum agglutination (RSA) test. Out of 85 clinical specimens (collected from sero-positive birds); *M. synoviae* culture isolation was successfully attained in 36 (42.35%) samples. Whereas, PCR test has detected 84 (98.82%) positive cases. The prevalence of MS in broiler birds was observed maximum as measured through PCR. It is suggested that the true prevalence of MS may best be reflected by combining RSA and PCR test findings.

INTRODUCTION

*Mycoplasma synoviae* (MS) is an economically important pathogen of poultry worldwide, causing respiratory infection and infectious synovitis in chickens and turkeys (Kleven, 1997); it may result in a sub clinical infection (Bencina et al., 2001; Anonymous, 2008). Provisional diagnosis and monitoring of *M. synoviae* infection is usually made using serological assays, while definitive diagnosis is made by isolation and identification of the organism. Cross reactivity between antigens is common problem in conducting serological testing of *M. gallisepticum* and *M. synoviae* (Ben-Abdelmoumen and Roy, 1995). Where, culture isolation is generally expensive and time consuming (requiring 1–2 weeks to complete). Polymerase chain reaction (PCR) assays have been employed for identification of suspected cultures and for rapid detection of *M. synoviae* directly from clinical samples (Lauerman et al., 1993; Garcia et al., 1995; Wang et al., 1997; Ahmed et al., 2009). The 16SrRNA gene based PCR method (Lauerman, 1998) is commonly used for confirmation of *M. synoviae* infection in chicken’s world over. Keeping in view the expected prevalence of *M. synoviae* in commercial broiler birds, the present study was designed to provide serological, cultural and molecular based evidence for the involvement of MS directly from respiratory distress cases.

MATERIALS AND METHODS

Serology

A total of 142 commercial broiler flocks located in and around district Faisalabad, Pakistan were contacted and 17 flocks were reported with respiratory distress signs. Sample size for sera collection was calculated on the basis of 10% infection rate with 99% confidence interval (Cannon and Roe, 1982). Rapid serum agglutination (RSA) test was performed (Anonymous, 2008).

Culture isolation and identification

The specimens of either, tracheal and nasal swab were collected from RSA positive cases whereas, synovial fluid was aspirated from swollen hock joints of the birds (Zain and Bradbury, 1996; Salisch et al., 1999). Specimens were processed on Modified Frey's medium (Kleven, 1998), incorporating nicotinamide adenine dinucleotide (NAD) as growth requirement for the primary isolation of MS. The positive growth on broth (with turbidity and colour change in medium) was...
DNA extraction and PCR based identification

The mycoplasma cultures were lysed by adding sodium dodecyl sulphate (SDS) at a final concentration of 1% and the DNA was extracted and purified using phenol-chloroform method (Wang et al., 1997). Similarly, clinical swab samples collected from RSA positive birds were also processed directly for DNA extraction. For positive control, the reference strain of *M. synoviae* (WVU 1853) was processed simultaneously with the test samples. PCR buffer was used as negative control.

The PCR was performed with modified primers already described for MS-16SrRNA conserved gene region (Lauerman, 1998; Moscoso et al., 2004) were MS-F 5’-GAGAAGCAAAATAGTGATATCA-3’ and MS-R 5’-CAGTCTGTCTCCGAATTTAACAA-3’. The PCR reaction was operated in thermal-cycler (Peqlab, Germany) using master mix (Platinum® PCR SuperMix, Invitrogen, USA) incorporating 20pm/µl of each forward and reverse primer at Molecular Diagnostic Laboratory, Department of Microbiology, University of Agriculture, Faisalabad. The amplification was carried out with an initial denaturation at 94°C for 2min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec., and extension at 72°C for 60 seconds. This was followed by final extension at 72°C (1 cycle) for 5 min. PCR amplicons were separated on 2% agarose gel prepared with 1X Tris-Actate EDTA buffer and ethidium bromide (0.2µg/ml) at 90V. The 100bp DNA ladder (Invitrogen Life Technologies, USA) was used and gels were visualized under ultraviolet light.

**RESULTS**

The flock wise prevalence of respiratory distress cases were recorded as 11.97%. Rapid serum agglutination (RSA) test showed the sero-positivity of *M. synoviae* (MS) in 23.5% flocks out of 17 commercial broiler flocks having respiratory distress signs. Whereas, 85 out of 111 sera samples revealed 76.57% sero-positivity as detected through RSA test. All the sero-positive cases of birds were further re-confirmed through culture isolation and only 42.35% samples were visualized under ultraviolet light.

The polymerase chain reaction (PCR) test had detected 98.82% positive results from sero-positive cases of specimens as detailed in (Table 1). The MS primers used in this study has successfully amplified 214 bp amplicon product, not only in the culture but also from the direct clinical specimens (Fig. 1). Overall prevalence of MS was observed as 32.43% through culture isolation, and 75.67% with PCR. The above results indicated that the PCR test may be a useful tool to describe the exact carrier status of *M. synoviae* involved in the respiratory infection of broiler birds.

**Fig. 1:** PCR based amplification for detection of *M. synoviae* (16srRNA gene); Lane M=100bp DNA ladder (Invitrogen Life Technologies, USA). Lane 1= (trachea), Lane 2-3= (Synovial fluid). Lane 4-5= MS (indigenous culture). Lane 6= MS strain (WVU 1853). Lane 7= PCR buffer as (Negative control).

**DISCUSSION**

In this study, PCR method was applied to document the involvement of *M. synoviae* infection in respiratory distress cases of chickens and the results obtained were compared with rapid serum agglutination (RSA) test and culture isolation. This molecular detection method also successfully eliminated the need of biochemical tests and specific antiserum for the detection of *M. synoviae* colonies.

In this study, non-specificity (false positive) result was observed with RSA test because of well known cross reactivity reported for *M. gallisepticum* (MG) and *M. synoviae* (MS) antigens as described by Kleven *et al.* (2000). In present study MS culture identification was based on culture characteristics and biochemical reactions. It is known that, biochemical reactions may assist in identification, but are not considered specific for MG or MS and necessitate purification of the culture by cloning (Anonymous, 2008). So, specific cultures identification are generally made using immunofluorescence procedures applied to suspect laboratory isolates (Anonymous, 2008). In present study, the possible reason for lower culture identification as compared to PCR assay might be due to none incorporation of fluorescein-conjugated antiserum for direct or indirect identification of MS.
colonies; bacterial overgrowth of non-pathogenic mycoplasma species (Salisch et al., 1999); low numbers of cells on the swabs (Zhao and Yamamoto, 1993), and samples collected from birds treated with antibiotics (Moalic et al., 1997). These findings have also been supported from the observation of Bradbury (1998) that, the diagnosis using isolation is slow and laborious with reports of “atypical” observation of Bradbury (1998) that, the diagnosis using isolation is slow and laborious with reports of “atypical” strains of M. gallisepticum and M. synoviae which are extremely difficult to recover from birds.

Recently a PCR assay for detection of variable haemagglutinin gene (vlhA gene) of M. synoviae (Hong et al., 2004; Harada et al., 2009) proved useful tool in detection and typing of different MS strains. Comparing vlhA-PCR with 16SrRNA-PCR (Hong et al. 2004) also projected that in very early stage of infection the 16SrRNA procedure was more sensitive than the vlhA method.

In conclusion, PCR based detection of M. synoviae infection was useful because of the time and difficulties associated in obtaining pure cultures and identification. Also, the true prevalence of MS may best be reflected by combining PCR results with RSA test findings.

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REFERENCES


