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RESEARCH ARTICLE

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.

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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

transferred to solid medium and plates were incubated at 37°C. The plates were observed daily for visible growth up to 21 days; afterwards the samples were regarded as negative for MS. The culture characteristics along with glucose fermentation and tetrazolium reduction (aerobic and anaerobic) were recorded for identification of MS isolates (Senterfit, 1983; Kleven, 1998).

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'-CAGTCGTCTCCGAAGTTAACAA-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 94ºC for 2min, followed by 35 cycles of denaturation at 94ºC for 30 sec, annealing at 58ºC for 30 sec., and extension at 72ºC for 60 seconds. This was followed by final extension at 72ºC (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/identification and only 42.35% samples showed visible MS colonies (Table 1). The typical growth of fried egg shaped colonies was observed within the span of 48-72 hours of incubation. The size of individual colonies was observed within the mean diameter of 1.2±0.02mm. Color of fresh colonies were varied from pale yellow to dull brown on Modified Frey's medium however, the growth of some colonies were changed to straw color after 1-week of storage under refrigeration temperature.

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.

Table 1: Distribution of samples positive for *Mycoplasma synoviae* cases screened through RSA test and identified through culture and PCR assay in broiler flocks.

Flock No.		Sera Samples tested		MS Positive Samples	
		Total Sera	MS RSA +	Culture	PCR
1		36	28	13	28
2		30	30	13	30
3		25	13	04	12
4		20	14	6	14
Total	No	111	85	36	84
	%	-	76.57	42.35	98.82

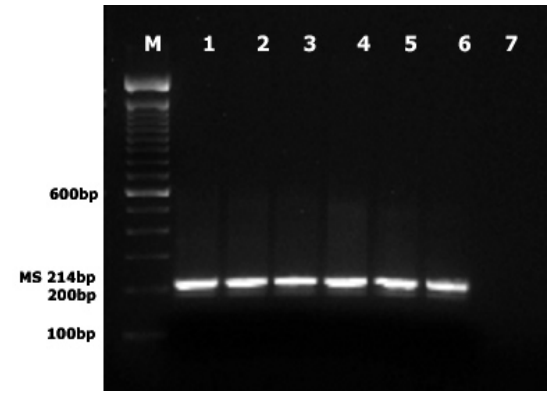

Fig. 1: PCR based amplification for detection of *M. synoviae* (*16srRNA* gene); Lane M=100bp DNA ladder (Invitrogen Life Technologies, USA). Lane 1= (trachea), Lane 2-3= (Synovial fluid). Lane 4-5= MS (indigenous culture). Lane 6= MS strain (WVU 1853). Lane 7= PCR buffer as (Negative control).

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" strains of *M. gallisepticum* and *M. synoviae* which are extremely difficult to recover from birds.

Recently a PCR assay for detection of variable heamagglutinin 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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