

Short Communication

Identification of avian *Mycoplasma* species in commercial broilers and layers with respiratory symptoms in Balochistan

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Among many avian mycoplasmas, only the *Mycoplasmas gallisepticum* (MG) and *Mycoplasmas synoviae* (MS) are responsible for causing respiratory disease in commercial poultry. This study reported for the very first time the serological occurrence of *M. gallisepticum* and *M. synoviae* in blood samples (n = 600) from sixty flocks (n = 42 broiler and n = 18 layer flocks) with respiratory symptoms in Quetta, Pishin and Kuchlak districts of Balochistan, Pakistan. Sera were tested for MG and MS antibody responses by serum plate agglutination (SPA) test and by enzyme-linked immunosorbent assay (ELISA) Synbiotics kit. It was found that *M. gallisepticum* antibodies in broiler flocks detected by SPA and by ELISA tests were 10.47 and 19.76%, whereas *M. synoviae* were 7.86 and 11.19%, respectively. In layer flocks the MG and MS antibodies detected by SPA and ELISA were 19.44, 31.66 and 8.8, 25%, respectively. The overall antibodies of MG and MS in both broiler and layer flocks tested by SPA and ELISA was found to be 13.16, 23.33 and 8.16, 15.33%, respectively. In broiler and layer flocks the presence of antibodies against both *M. gallisepticum* and *M. synoviae* were found in tested flocks with respiratory symptoms. Further studies on prevalence and diagnosis of both the *M. gallisepticum* and *M. synoviae* in causing respiratory disease in commercial broilers and layers in Balochistan are required.

Key words: Mycoplasma, broilers, serum plate agglutination, enzyme-linked immunosorbent assay.

INTRODUCTION

Over 200 species of *Mycoplasma* have been found in ruminants, non ruminants, insects, plants and humans. *Mycoplasma* is soft skin bacteria that lack cell wall and hard to grow *in vitro* due to fastidious and cholesterol dependent growth requirements (Razin, 2005). Among many avian mycoplasmas isolated from commercial poultry worldwide both *M. gallisepticum* (MG) and *M. synoviae* (MS) are responsible for causing chronic respiratory disease (CRD) and sinusitis in layer and broiler flocks. Signs of infectious sinusitis caused by MS

in chicken are pale combs, lameness and swollen joints. Swollen joints contain viscous and grey exudates (Kleven and Bradbury, 2008). Respiratory signs in (CRD) caused by MG are sneezing, coughing, difficulty in breathing (Land man et al., 2008). The MG infection is diagnosed by detection of its DNA and by detection of specific humoral antibodies (Liu et al., 2001). Presence of MG and MS can be confirmed by conducting serological identification through serum plate agglutination SPA and ELISA (Kleven and Bradbury, 2008). Herrero et al. (2011) reported presence of both *M. gallisepticum* and *M. synoviae* in a single case of turkey.

Serological identification is useful for an early diagnosis of CRD caused by MG to plan an antibiotic therapy to restrict the disease. A positive serological test along with

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Table 1. Serological tests of serum samples for detection of antibodies against *M. gallisepticum* (MG) and *M. synoviae* (MS).

Type of flock	Serum sample (n)	SPA test ¹ MG (%)	ELISA test ² MG (%)	SPA test ¹ MS (%)	ELISA test ² MS (%)
Broiler	420	44 (10.47)	83 (19.76)	33 (7.85)	47 (11.19)
Layer	180	35 (19.44)	57 (31.66)	16 (8.8)	45 (25)
Overall	600	79 (13.16)	140 (23.33)	49 (8.16)	92 (15.33)

¹, Serum plate agglutination test; ², Enzyme linked immunosorbent assay.

history and signs typical of the CRD, make assumption of initial diagnosis. Later on the isolation and identification of the organism should be done (Ley, 2003). The MG suspected flocks are identified by using SPA test and further confirmed by ELISA (Kleven and Bradbury, 2008).

MATERIALS AND METHODS

A total of sixty flocks (n = 42 broiler and n = 18 layer flocks), were visited. Blood samples (n = 600) representing ten samples from each of the suspected flocks were tested for the presence of MG and MS antibodies. Serum from blood samples (n = 600) was separated for MG and MS serological monitoring. All of these flocks were not vaccinated against any *Mycoplasma* disease. The samples were brought to the Centre for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan for serological studies.

SPA test

The serum plate agglutination (SPA) test was performed by mixing an equal amount of 0.03 ml MG or MS antigen and fresh serum on a glass slide. Results were read within 2 min.

Elisa test

Two separate commercial ELISA kits (Synbiotics Corporation, ProFlock, USA) to detect specific antibodies against MG and MS were used according to the manufacturer's instructions. Optical density values were set at 405 nm wave length on IDEX (BDSL Immunoskan, England) micro plate reader. All SP values ≥ 0.6 were considered positive and optical density values ≤ 0.6 were negative for the presence of *M. gallisepticum* and *M. synoviae* antibodies.

RESULTS

The *M. gallisepticum* antibodies detected by SPA test and ELISA in broiler serum samples (n = 420) from forty two flocks was 44/420 (10.47%) and 83/420 (19.76%) respectively. MG antibodies detected by SPA and ELISA in layer serum samples (n = 180) from eighteen flocks was 35/180 (19.44%) and 57/180 (31.66%), respectively. The *M. synoviae* antibodies detected by SPA and ELISA in broiler serum samples was 33/420 (7.86%) and 47/420 (11.19%), respectively. The *M. synoviae* antibodies detected by SPA and ELISA in layer serum samples was 16/180 (8.8%) and 45/180 (25%), respectively (Table 1). The overall occurrence of MG in both broiler and layer flocks detected by SPA and ELISA

tests were 79/600 (13.16%) and 140/600 (23.33%), respectively. The overall presence of MS in both broiler and layer flocks detected by SPA and ELISA were found as 49/600 (8.16%) and 92/600 (15.33%).

DISCUSSION

In this study, the occurrence of *M. gallisepticum* and *M. synoviae* from suspected broilers and layers showing symptoms of respiratory disease was carried out in three districts of Quetta, Kuchlak and Pishin province of Balochistan. The occurrence of MS and MG in blood samples of commercial broiler and layer flocks is shocking. Serological tests were found helpful for monitoring the respiratory disease caused by MG and MS in suspected flocks and for choosing the line of treatment in chickens with respiratory symptoms. In this study, ELISA detected more MG infected samples than SPA test (Table 1). An overall on occurrence of *M. gallisepticum* in both broiler and layer from blood samples by SPA test and by ELISA were 13.16 and 23.33%, respectively, are in agreement with the studies of Atif and Najeeb (2007). The higher occurrence of MG in layer flocks matches with the findings of Barua et al. (2006). The SPA test detects antibody IgM in the serum within a week of infection. However, the SPA test also gives cross reactions positive due to some viral infections in MG positive SPA tests (Cziffra et al., 1993). It is essential to monitor poultry flocks displaying culture negative and serological findings positive to enable best possible antibiotic management and infection control. During brief respiratory symptoms, the infection is at an early stage and there is no MG colonization that may get false ELISA results.

At present no prophylactic measure to control the respiratory symptoms caused by MG or MS are in use in Balochistan. Probably, no serological monitoring of flocks and MG isolation techniques, are used in any commercial broiler and layer flocks. It is found that the occurrence of *M. gallisepticum* and *M. synoviae* infection is present in commercial poultry flocks in Balochistan. Further research is required to evaluate the impact of *M. gallisepticum* and *M. synoviae* on the commercial poultry production system. Probably, the losses to the

commercial poultry flocks can be reduced through serological monitoring, rapid diagnosis, planning an effective antibiotic treatment and implementation of strict biosecurity measures.

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