



RESEARCH ARTICLE

Sero-Prevalence and Associated Risk Factors of *Mycoplasma Gallisepticum*, *Mycoplasma Synoviae* and *Salmonella Pullorum / Gallinarium* in Poultry

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ABSTRACT

In the present study, a total of 1667 sera samples were collected from breeder, broiler and layer birds to determine sero-prevalence of *Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae* (MS), *Salmonella pullorum* and *Salmonella gallinarium* (SPG) infections. Serum Plate Agglutination (SPA) test was used to determine the presence of antibodies against commercial MG, MS and SPG antigens. The overall sero-prevalence of MG, MS and SPG in case of broilers was 7.14, 10 and 5.35%, in the layer birds was 44.9, 42.6 and 51.32% and in broiler breeders was 59.6, 50.13 and 44.1% respectively. The study shows that sero-prevalence of MG and MS was higher in breeder as compared to that of layers and broilers. However, for SPG the prevalence was found highest in layers. The sero-prevalence of MG was higher in female birds while of MS and SPG higher in males. The highest prevalence for all pathogens was found in the period from October to December.

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INTRODUCTION

Infectious diseases have been a continuous threat in poultry production systems (Naqvi *et al.*, 2017; Mahmood *et al.*, 2018; Rehman *et al.*, 2018; Abbas *et al.*, 2017, 2019a, 2019b; Zhang *et al.*, 2020). Likewise, Mycoplasmosis and Salmonellosis are diseases of paramount importance in every type of chicken. Two main pathogens that cause mycoplasmosis in poultry birds are *Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae* (MS). Chronic respiratory disease (CRD) in chickens is caused by MG (Shah, 2018). Young birds are more susceptible to MG although birds of all age are prone to this pathogen (Seifi and Shirzad, 2012). MG is transmitted vertically by trans-ovarian method or horizontally by direct or indirect contact (Shah, 2018). Respiratory sounds, nasal and ocular discharge, coughing, conjunctivitis and drop in egg production are major clinical signs of MG infection (Shah, 2018).

MS causes upper respiratory tract infection leading to damage to air sacs and bed for other respiratory pathogens (Kleven, 2003; Seifi and Shirzad, 2012). MS infection

causes chronic or acute synovitis if infection becomes systemic. Along with respiratory signs, birds infected by MS show joint abnormalities and egg shell and production losses. Layer flocks infected with MS show sharp decrease in egg production. MS may be transmitted both by horizontal or vertical means and may persist in flock as sub clinical form (Heleili *et al.*, 2012). MS may be detected on basis of clinical signs, epidemiological findings and serology followed by culture and isolation (Luciano *et al.*, 2011). Fowl typhoid and pullorum disease are two most important bacterial diseases of chickens caused by *Salmonella gallinarium* and *Salmonella pullorum* (Ansari *et al.*, 2017). Pullorum disease and fowl typhoid has become a wide spread problem due to expansion in poultry farming. Both these diseases show similar clinical signs and are of serious concern in all types of young and adult chickens. These diseases are vertically transmitted through egg to embryos. *Salmonella pullorum* and *Salmonella gallinarium* are frequently diagnosed by culturing and serological tests. Infected flocks are detected by serum agglutination test and used to find prevalence of infection in the flock (Sarkar *et al.*, 2005).

Keeping in view of the economic importance of MG, MS and SPG, this study is designed to determine sero-prevalence of all four pathogens in different types of chickens.

MATERIALS AND METHODS

A total of 1667 serum samples were taken randomly from broiler (n=280), layer (n=657) and broiler breeder (n=730) from the poultry farms located in the different regions of Rawalpindi during 2016-2017. Blood (1.5-2.0 mL) was collected from wing vein aseptically in sterile disposable plastic syringes and kept at room temperature for 1-2 hours. Sera samples were separated and stored in sterile vials at 4°C until further usage.

MG, MS and SPG antigen: Standard MG antigen (code SL 212, inactivated coloured antigen), MS antigen (code SL 222, inactivated coloured antigen) and SPG antigen (code SL 242, inactivated coloured antigen) from Soleil diagnostics France were purchased for detecting antibodies in sera by serum plate agglutination test (SPA).

Serum plate agglutination (SPA) test: The SPA test was performed by placing side by side 20 µl of serum and 20 µl of antigen on glass slide with help of micropipette. Antigen and serum were mixed properly with help of stirrer and results were recorded within two minutes. Samples were considered positive where agglutination or granule formation occurred, otherwise samples were marked negative. Positive samples were graded (+) to (+++++) according to extent of agglutination. The test was performed in similar way for detection of MG, MS and SPG antibodies in the serum samples. Known positive and negative control sera were used for validating evaluation of SPA test.

Statistical analysis: Chi-square test was performed to test the significance of the prevalence results.

RESULTS AND DISCUSSION

Overall sero-prevalence of MG, MS and SPG: In broiler breeders the overall Sero-prevalence of MG and MS was found the highest as compared to layers and broilers. For SPG highest sero-prevalence was found in layer birds. Sero-prevalence of MG, MS and SPG in layer birds was found to be 44.9, 42.60 and 51.32% respectively (Table 1).

According to the present study sero-prevalence of MG in case of broiler breeder was 59.6% while in broilers it was 7.14%. The overall sero-prevalence of MG in chicken on average was 45.96%. Earlier studies conducted show that over all sero-prevalence of MG in chicken on average was 48.8% by Junior *et al.* (2017). The incidence of MG by SPA was found to be 44.9% whereas by Islam *et al.* (2014) it is less than 53.61%. The sero-prevalence of MG in breeder flock is 58.9% by Sarkar *et al.* (2005) which is also in line with the results of present study. The incidence of MG in broiler breeder was shown to be 14.2% in 2002, 21.4% in 2003, 10.3% in 2004, 3.9% in 2005 and 2.5% in 2017 (Seifi and Shirzad, 2012; Alavinia *et al.*, 2017). The study showed that there was variation in the incidence of MG in the different years. The variation in sero-prevalence findings of MG in chicken in different

studies may be due to difference in management practices, treatment regime and bio-security measures (Junior *et al.*, 2017).

The overall sero-prevalence of MS in all types of chickens was found to be 40.43%. The sero-prevalence of MS in chickens was found to be 66.33% by Heleili *et al.* (2012) which is higher than results of present study. The incidence of the MS was highest in the broiler breeder (50.14%) and lowest in broiler (10%), while in case of layer incidence of MS was found to be 42.60%. In another study by Michiels *et al.* (2016) the MS was found very low in broilers (12.9%). The low prevalence of MS in broilers is may be due to short life span of broilers and extensive antibiotic treatment (Michiels *et al.*, 2016).

The overall sero-prevalence of SPG was found to be 41.8% in all types of chickens. The sero-prevalence of SPG in case of broiler was very low i.e. 5.36% as compared to that of the El-Sharkawy *et al.* (2017). A total of 45% of the sampled 1-week-old broiler flocks and 38% of the 5-week-old broiler flocks tested positive for SPG (El-Sharkawy *et al.*, 2017). The sero-prevalence of SPG in broiler was two to four times less as compared to layers. This difference might be due to longer life of layers and breach in the managerial conditions during their longer life span. The incidence of SPG in layers was reported to be 63.5% (Rahman *et al.*, 2013). This finding is discordant with the present study in which incidence of SPG in layers is 51.32%. These reports show different incidence than current study and it may be due to difference in geographical variation and difference in management conditions.

Sero-prevalence of MG, MS and SPG and effect of age: Sero-prevalence of the pathogens was compared in the different groups based on age in the case of layers and broiler breeders (Table 2). The sero-prevalence of all the three pathogens was more in case of birds which were older than 20 weeks. The highest prevalence of MG was 73.94% in broiler breeders, whereas, in layers it was 33.15% at the age of 20 weeks. The prevalence of MS was highest in layers (54.09%) as compared to broiler breeders (48.04%) at the age 21 weeks above. The highest prevalence of SPG was found in broiler breeders (54.52%) at the age 21 weeks and above (Table 2). Mukhtar *et al.* (2012) showed the highest sero-prevalence of MG in case of pullets than adults and laying birds. Similar results are also reported by Hossain *et al.* (2010) and Sarkar *et al.* (2005). In model breeder poultry farms the sero-prevalence of MG was found to be 71% at 18 week of age and 50% at 49 week of age (Sarkar *et al.*, 2005). It has also been seen that that prevalence of MG in the breeder was decreased with increase in age. According to Sarkar *et al.* (2005) it was recorded 73% in the 20 week old birds and 60% in the 42 week old birds. Higher prevalence in the younger birds might be due to the vertical transmission of the disease.

Sero-prevalence of MG, MS and SPG and effect of gender: Sero-prevalence of MG, MS and SPG pathogens was compared in different groups based on sex of birds in case of broiler breeder. Gender wise prevalence revealed higher in females; MG (52%) and SPG (24%) was in case of female birds while MG (46%) and SPG (15%) in case of male birds.

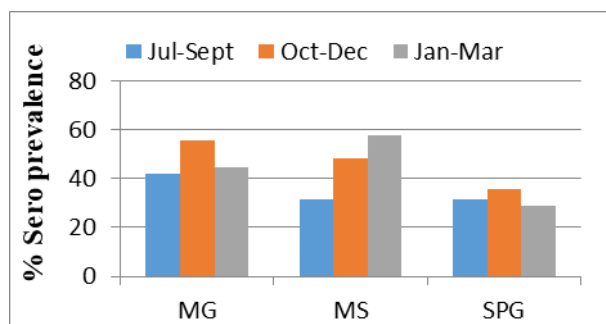


Fig. 1: Sero-prevalence of MG, MS and SPG in layers and effect of season.

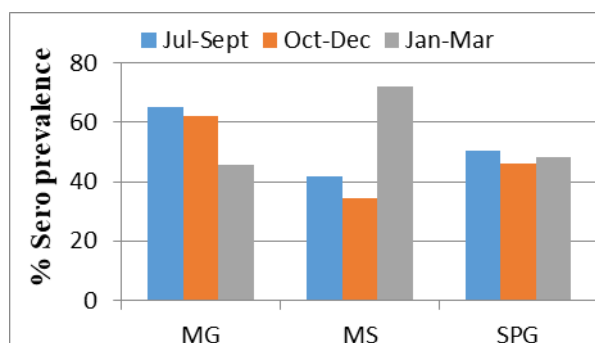


Fig. 2: Sero-prevalence of MG, MS and SPG in broiler breeder and effect of season.

Table 1: Percentage sero-prevalence of MG, MS and SPG in broiler, layer and broiler breeder

Type of chicken	MG (%)	MS (%)	SPG (%)
Broiler	7.14	10	5.36
Layer	44.9	42.60	51.32
Broiler breeder	59.6	50.14	44.11
Overall prevalence	45.96	40.43	41.80

Table 2: Sero-prevalence (%) of MG, MS and SPG in different age groups

	Layer		Broiler breeder	
	0-20 week	21 week and above	0-20 week	21 week and above
MG	33.15	50.92	73.94	51.15
MS	23.53	52.09	42.10	48.04
SPG	23.22	40.07	41.61	54.52

Sero-prevalence of MG, MS and SPG and effect of season: Effect of season on sero-prevalence of MG, MS and SPG was determined in different months of the year. Prevalence was checked during rainy season (July to September), winter season (October to December) and spring season (January to March). The sero-prevalence of MG and SPG in layer birds was highest during winter season and of MS was highest during spring season (Fig. 1). It has been observed that during winter season there was more incidences of diseases which might be due to more pre disposition of birds to these pathogens when the temperature is low. In the present study the MS was found highest during January to March (57.64%) (Fig. 1-2). Different studies reported similar findings (Heleili *et al.*, 2012; Mukhtar *et al.*, 2012; Islam *et al.*, 2014). The incidence of MG in layers was 61.8% in winter and 47.74% in the summer in Faisalabad district (Mukhtar *et al.*, 2012). The prevalence of MG infection was more in the winter (70%) as compared to summer (60%) (Heleili *et al.*, 2012). According to Islam *et al.* (2014) in layer birds the sero-prevalence was found to be more in winter season (60%) as compared to summer season (51%). The

higher sero-prevalence in winter might be due to sudden changes in temperature and cold stress.

Salmonella pullorum and *Salmonella gallinarium* both contains O antigen 9 and 12 and may also have O antigen 1. There is variation in O antigen 12₁, 12₂ and 12₃ in case of *Salmonella pullorum*. There is more concentration of 12₂ than 12₃ in variant forms while reverse is true for accurate form. In the case of *Salmonella gallinarium* there is no such variation. In the diagnostic test polyvalent antigen should be used because of this variation. Same antigen is used for detection of *Salmonella pullorum* and *Salmonella gallinarium* but the results for *Salmonella gallinarium* may be poor (Sarkar *et al.*, 2005; Junior *et al.*, 2017). Most common test used for the diagnosis of Mycoplasmas and Salmonella is SPA. Sensitivity and specificity of SPA test was compared with culture, PCR technique and ELISA. SPA test occasionally gives a very high prevalence which may be due to false positive results because of cross reactivity among serotypes, administration of killed vaccines, contaminated serum samples and age of flock (Luciano *et al.*, 2011; El-Sharkawy *et al.*, 2017). For proper diagnosis and control, programs based on sero-conversion may be inadequate, so sero-monitoring should be combined with culture and molecular techniques (Luciano *et al.*, 2011).

Conclusions: The study has shown that sero-prevalence of MG and MS was higher in breeder than layers and broilers and for SPG prevalence was highest in layers. A very high sero-prevalence of MG, MS and SPG requires strict biosecurity measures for control of these pathogens in poultry.

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Authors contribution: All the authors contributed equally in research and preparation of manuscript.

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