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RESEARCH ARTICLE

Seroprevalance of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in Commercial Broilers and Backyard Poultry in Five Districts of Khyber Pakhtunkhwa-Pakistan

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ABSTRACT

Serological survey for *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) was conducted in serum samples of broilers and backyard poultry collected from five major districts of Khyber Pakhtunkhwa. The serum samples were examined by serum plate agglutination (SPA) test using stained MG & MS antigens separately. Out of total (n=648) broiler serum samples MG and MS prevalence was recorded as 35.03 and 16.67%, respectively. The highest prevalence of MG was recorded in Dera Ismail Khan 43.33% (65/150) followed by Tank 40% (60/150), Peshawar 36.67% (33/90), Abbottabad 31.46% (45/143) and Mansehra 20.86% (24/115) while in case of MS the highest prevalence was reported in Peshawar 23.33% (21/90) followed by Dera Ismail Khan 20% (30/150), Mansehra 18.26% (21/115), Tank 12.67% (19/150) and Abbottabad 11.88% (17/143). In open shed the prevalence of MG & MS was comparatively high (40.51 & 17.94%) than in closed shed (26.74 & 14.72%), respectively. Moreover, prevalence of MG and MS was higher during winter season (38.18 & 18.48%) than summer (31.76 & 14.77%). Combined MG/MS prevalence percentage was recorded as Peshawar (17.78%), Dera Ismail Khan (14%), Tank (12%), Mansehra (10.43%) and Abbottabad (7.69%) and it was found slightly higher (12.72%) in winter than (11.32%) in summer. Similarly in open shed reared birds, MG/MS prevalence was high (14.10%) than in closed shed (8.91%). One hundred (n=100) backyard poultry samples collected from Tank and Dera Ismail Khan (50 from each district) showed high MG (80%) & MS (50%) prevalence in Dera Ismail khan than in Tank with MG (56%) and MS (44%) prevalence. Overall results indicated a high prevalence of MG and MS in backyard poultry which may be a source of infection for commercial broiler especially for open shed reared birds.

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INTRODUCTION

In Pakistan poultry industry is the second largest industry with 11.8% annual growth rate (Anonymous, 2014-2015). Numerous infectious diseases are responsible for economic losses to this industry (Abbas *et al.*, 2017a, 2017b, 2017c; Hussain *et al.*, 2017; Idris *et al.*, 2017) but avian mycoplasmosis is one of the major causes due to Chronic Respiratory Disease (CRD) caused by MG and synovitis and subclinical respiratory infection caused by MS (Ehtisham-ul-haq *et al*., 2011, Catania *et al.*, 2016). These mycoplasma infections result in weight loss, poor meat production, high FCR, low egg output, more embryonic mortalities and bad carcass condition (Kleven and Noel, 2008). MG and MS are communicated horizontally as well as vertically which is the basic reason of their subclinical persistence in environment and a possible future threat for any outbreak (Bradbury and Jordan, 2003). Concurrent infection with other pathogens such as infectious bronchitis virus (Catania *et al.*, 2016) and avian influenza virus H9 subtype (Roussan *et al.*, 2015) can increase the severity of the disease. The seasonal effect on MG and MS prevalence percentage was also reported in this study and it was higher in winter and lower in summer as reported earlier by Sarkar *et al.* (2005). Overcrowding and poor biosafety conditions are

possible reasons for high rates of avian mycoplasmosis (Kleven and Noel, 2008). Northern areas of Khyber Pakhtunkhwa (KP) are rich in poultry population specially Mansehra, Swabia, Charsadda and Abbottabad. In these areas poor families are socio-economically dependent on household poultry. As backyard poultry eggs and meat are very much popular among the people due to good taste so their demand is increasing every day. Backyard poultry meat and eggs are more costly than broiler, but these birds are neither routinely monitored serologically nor vaccinated. These birds are also devoid of balanced diet (Xavier *et al.*, 2011). Therefore, revisiting the control measures, improving biosecurity standards, launching modern surveillance techniques and effective vaccination are the basic approaches for the control and prevention of avian mycoplasmosis.

MG and MS prevalence should be measured for effective vaccination. To overcome avian mycoplasmosis MG and MS should be detected serologically as well as antigenically. For serological screening SPA test, enzyme linked immunosorbent assay (ELISA) and haemagglutination inhibition test (HI) are directed and for antigen detection culture techniques and polymerase chain reaction (PCR) are performed (Yasmin *et al*., 2014). The SPA test identifies IgM, so it can detect antibodies earlier than HI or ELISA which detects IgG. SPA test is easy to perform, rapid, sensitive and does not require any special equipment (Wanasawaeng *et al.*, 2015; Ali *et al*., 2017). Therefore, SPA test can be used for the detection of MG and MS as field trials. In Pakistan seroprevalance of avian mycoplasmosis was conducted using SPA test, ELISA and HI by Rasool *et al.* (2017).

This study was carried out for the serological prevalence of MG and MS in poultry birds in KP Province for better future planning in controlling avian mycoplasmosis as no such effort had been made in past.

MATERIALS AND METHODS

Collection of serum sample: A total of 648 serum samples were collected from 40 commercial broiler farms randomly selected in different areas of five districts namely Tank (n=150), Dera Ismail Khan (n=150), Peshawar (n=90), Mansehra (n=115) and Abbottabad (n=143) of KP province, Pakistan during December 2016-June 2017. The sample size was calculated at 20% prevalence, 95% confidence interval, with a desired absolute precision of 5% (Thrusfield, 1997).

One hundred (100) backyard poultry blood samples were collected from Dera Ismail Khan and Tank (50 from each). The blood samples were collected aseptically from the brachial vein of 6-8 weeks old healthy birds. These birds were not vaccinated against MG & MS. Then each blood sample was transferred into a sterilized plastic bottle by using 5ml sterile disposable syringe and kept undisturbed at room temperature till clotting (Sarkar *et al.*, 2005). After clotting sera were separated, centrifuged and poured into Eppendorf tubes, labelled individually and stored at 4°C.

Serum plate agglutination (SPA) test: The SPA test was performed using stained MG antigen (SPAFAS, MG Plate Antigen CHARLES RIVER laboratories INC Wilmington USA 01887) and MS stained antigen was procured from the Institute of Microbiology, University of Agriculture, Faisalabad. A quantity of 25µl of antigen and 25µl of chicken sera were placed side by side with the help of an automatic dispenser on a glass slide, assorted and moved with a small tooth pick followed by gentle swaying at room temperature (Alan *et al*., 1988). After 2 minutes results were read. Granules formation indicated positive result which was seen during rocking, but in negative case no such granules formed. The flocks with more than 10% positive reactions were considered positive serologically as suggested by Kleven and Bradbury (2008). Using known MG and MS antiserum control positive SPA test was also conducted. These known antisera were obtained from the Institute of Microbiology, University of Agriculture Faisalabad.

RESULTS

Total 648 serum samples were collected randomly from 40 commercial broiler farms located in five districts. The names of these districts along with the number of samples collected from each district are given in the Table 1. In Tank, Dera Ismail Khan and Peshawar the blood samples were collected from broilers reared in open sheds while in Mansehra and Abbottabad the samples were taken from broilers kept in closed sheds.

Table 1: Results of SPA test for serological survey against MG & MS in broiler chicken conducted at five districts of KP.

Antigen	DIK n=150	TANK n=150	PESH n= 90	ABBOT n=143	MANSE n=115	Total n=648
MG+%	43.33	40.00	36.67	31.46	20.86	35.03
MS+%	20.00	12.67	23.33	11.88	18.26	16.67

DIK=Dera Ismail Khan, PESH=Peshawar ABBOT=Abbottabad, MANSE =Mansehra

SPA test: Out of 648 broiler serum samples the prevalence percentage of MG and MS were 35.03 and 16.67% respectively. The highest prevalence percentage of MG was recorded in Dera Ismail Khan 43.33% followed by Tank 40%, Peshawar 36.67%, Abbottabad 31.46% and Mansehra 20.86%. While in case of MS the highest prevalence was reported in Peshawar 23.33% followed by Dera Ismail Khan 20%, Mansehra 18.26%, Tank 12.67% and Abbottabad 11.88% as elaborated in Table 1. MG and MS prevalence recorded during winter season was high (38.18% & 18.48%) and during summer it was low (31.76 & 14.77%) as shown in Table 2. In samples taken from open shed kept birds, the prevalence of MG and MS was comparatively high (40.51 & 17.94%) than in closed shed (26.74 & 14.72%) respectively as indicated in Table 3. MG/MS combined prevalence was highest in Peshawar (17.78%) followed by Dera Ismail Khan (14%), Tank (12%), Mansehra (10.43%) and Abbottabad (7.69%). Difference in the concurrent prevalence of MG/MS was found slightly high 12.72% in winter than in summer 11.32%. In house reared poultry MG and MS prevalence was (80%) and (50%) in Dera Ismail khan while in Tank the MG and MS prevalence was 56 and 44% respectively as given in Table 4.

Table 2: SPA test results showing season wise prevalence of MG and MS in broiler poultry in five districts of KP

Season	Antigen	DIK	TANK	PESH	ABBOT	MANSE	Total
Winter	MG+%	48.00	42.67	37.78	36.00	23.33	38.18
(n=330)	MS+%	20.00	16.00	24.44	10.67	25.00	18.48
Summer	MG+%	38.67	37.33	35.56	26.47	18.20	31.76
(n=318)	MS+%	20.00	9.33	22.22	13.23	10.90	14.77

Table 3: SPA test results elaborating comparative prevalence of MG and MS in broiler chickens reared in open and closed shed systems in five districts of KP

Antigen	Open shed system (n=390)				Closed shed system (n=258)		
	TANK	DIK	PESH	Total	ABOT	MANSE	Total
MG+%	40	43.33	36.67	40.51	31.46	20.86	26.74
MS+%	12.67	20.00	23.33	17.94	11.88	18.26	14.72

Backyard poultry samples (n=100) were collected from Dera Ismail Khan and Tank (50 samples from each district) to perform SPA against MG & MS. Results showed higher MG & MS prevalence (80%) & (50%) in Dera Ismail khan than in Tank (56%) and (44%) respectively as shown below.

Table 4: SPA results against MG&MS in Backyard poultry from Dera Ismail Khan and Tank

Antigen	Dera Ismail Khan (n=50)	Tank (n=50)
MG+ %	80	56
MS+%	50	44

DISCUSSION

Poultry is the second largest industry with marvelous annual growth rate of 11.8% in Pakistan (Anonymous, 2014-2015). In present study, SPA test was performed for the detection of antibodies against MG and MS using stained antigen in 648 broiler samples taken from five districts of KP.

SPA test indicated the highest seroprevalence against MG in Dera Ismail Khan (43.33%) and lowest in Mansehra (20.86%). Samples collected from Peshawar showed the highest prevalence (23.33%) against MS and the lowest in Abbottabad (11.88%) as already reported by Tomar *et al.* (2017) who recorded 38.77% seroprevalence for both MG and MS in broiler birds of 6-8 weeks old. Similarly Srinivassan *et al.* (2014) indicated 50% seroprevalence of MG and MS in Tamil Nadu. Table 2 indicated the higher prevalence of MG and MS in winter 38.18 and 18.48% than in summer 31.76 and 14.77% respectively. Similarly high seroprevalence of avian mycoplasma in winter was also reported earlier by Heleili *et al.* (2012) and Mukhtar *et al.* (2012) due to low temperature and relative high humidity in winter season.

Moreover, high seroprevalence of MG (40.51%) and MS (17.94%) in open shed reared birds and low in closed shed 26.74 & 14.72%, respectively was recorded in present study, which may probably be due to heavy exposure of birds through contaminated environment and weak biosecurity measures prevailing around the open sheds. In closed shed there is more restriction on the movement of workers, officials as well as wild, fancy and domestic birds as reported by Michiels *et al.* (2016) in Belgium. Also de Wit *et al.* (2004) and Haesendonck *et al*. (2014) recorded a very high prevalence of both MG and MS with 73.2 and 96.4% of flocks, respectively suggesting that this group of birds might act as a potential reservoir for *Mycoplasma*. Interaction between MG and domestic chickens has been assisted through systems in which these birds breathing together with other domestic poultry species and wild birds (Luttrell *et al*., 1991). It is recognized that the core bases of infection in the transmission chain of MG are sick birds including both reservoirs and carriers (Nascimento *et al.*, 2005).

During this study the combined serological study of MG & MS was also conducted. Out of (n=330) samples collected during winter season only 42 samples (12.72%) showed positive reaction to both antigens while 36 samples (11.32%) were positive out of 318 samples collected in summer. Out of 390 samples from open shed system, 55 samples were positive (14.10%) as compared to closed shed with 8.91% (n=23) out of 258 samples. In Haryana (India), Tomar *et al.* (2017) reported low prevalence (2.04%) of both MG & MS in individual samples taken from adult birds and more prevalence in day old chicks (4.22%).

Differences in the prevalence percentage in different areas are possibly due to loopholes in the management and rearing systems. SPA results showed high prevalence of both MG & MS antigens in the backyard poultry sera, which indicates the possible source of infection for commercial broiler (Xavier *et al.*, 2011). Mean prevalence rates of avian mycoplasmosis in house-reared poultry in Argentina provided by Xavier *et al.* (2011) in three periods between 2003-2007 were 32.8, 55.1 and 76.2%, respectively, while Sílvio *et al.* (2015) reported wide range (21.7 to 100%) of prevalence variation. Shadmanesh and Mokhtari (2013) also detected very high prevalence of MG (85%) by SPA in native hens of Eghlid, Iran which is comparable to the results of our study. Junior *et al.* (2017) also detected very high percentages of anti-MG and anti-MS antibodies through ELISA in non-vaccinated backyard chicken in different villages of Mozambique. Due to free flying birds and rodents at the open shed of poultry farms the chances of mycoplasmosis occurrence become high and persisted for long time (Haesendonck *et al*., 2014; Michiels *et al*., 2016).

The congested backyard poultry was found comparatively more in Dera Ismail Khan and Tank as compared to other three districts so we preferably collected samples from Dera Ismail Khan and Tank only.

Over all presence of MG and MS in broilers may be due to vertical transmission of these agents from breeder and further transmission may be minimized through strict biosecurity measures or promoting control shed in the areas. Moreover, MG and MS may be routinely performed during the early age of chicks so that proper culling of the infected chicks may be practiced at the farms (Michiels *et al*., 2016; Tomar *et al*., 2017)

Conclusions: It is concluded that time to time monitoring of MG and MS prevalence may be carried out in commercial and backyard chicken for screening purposes and to combat with avian mycoplasmosis.

Authors contribution: SU Rahman designed the study. AU Rehman performed experiment and analyzed the serum samples under the supervision of AH Shah at Department of Biological Sciences, Gomal University, Dera Ismail Khan in collaboration with the Institute of Microbiology, University of Agricultural, Faisalabad. All authors interpreted the data and approved the final version.

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