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Short Communication

Seroprevalence of *Mycoplasma gallisepticum* in Commercial Poultry Birds Showing Respiratory Clinical Signs in Chakwal District, Pakistan

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SU, AM and TP designed the study. SH performed the experimental work. SU supervised the work. AA, SP, ZI, JI, SM and AK helped in sample collection, provided technical supports and participated in drafting the manuscript.

Key words
***Mycoplasma gallisepticum*, Chronic respiratory disease, iELISA test, RPA test, Poultry flocks, Chakwal.**

ABSTRACT

Bacterial diseases are a huge concern for poultry farmers and cause huge economic loss to poultry industry every year. Various pathogens can initiate respiratory diseases in poultry, including mycoplasmosis caused by *Mycoplasma gallisepticum* (MG). The distribution pattern of MG in layer flocks is not known in Chakwal. Keeping this in mind, current study was designed to know about distribution of MG. The present study was conducted on 25 commercial layer flocks from different regions of district Chakwal, Pakistan. A total of 358 blood samples were collected from different regions of district Chakwal and subjected to serological tests including enzyme linked immusorbant assay (ELISA) and rapid plate agglutination assay (RPA). The overall seroprevalence of MG detected through iELISA and RPA was 29.88 % and 20.67% respectively. Moreover, age-wise study revealed high prevalence of MG in 24-31 weeks old layers (44.17%) as compared to 55-63 weeks old layer flock (14.49%). The study of seasonal effect revealed highest MG prevalence in December (44%) while lowest in October (20%). Furthermore, higher prevalence was recorded in layer flocks with large number (4000-5000) as 33.33% than flocks with small number of birds (1000-2000) as 24.28%. In addition, it was also found that iELISA test is more sensitive and specific for detection of MG antibodies in serum samples as compared to RPA/SPA test. This evidence emphasizes the need of more systemic approaches for the investigation ofMG distribution and prevalence in otherparts of Pakistan in order to design effective control strategies.

Mycoplasmas are important pathogens infecting the respiratory tract of chickens. *Mycoplasma gallisepticum* is a bacterium belonging to the class Mollicutes and the family Mycoplasmataceae. There are a number of Mycoplasmas namely *Mycoplasma gallisepticum* (MG), *M. synoviae* (MS), *M. meleagrides* (MM) and *M. iowae* (MI) which cause mycoplasmosis in birds. Avian mycoplasmosis is worldwide in occurrence and causes huge economic losses to poultry industry in the form of loss of production and mortality. Transmission may be transovarian or lateral via respiratory aerosols and direct contact. MG causes infectious sinusitis in turkeys and chronic respiratory disease (CRD) in chicken with nasal discharge, sneezing, conjunctivitis and coughing as main clinical signs (Ley, 2008). MG not only disturbs the growth and viability but can also lead to co-infections and thus exacerbating clinical signs and lesions (Umar *et al*., 2017). MG is a cell wall less bacteria thus resists to all range of antibiotic drugs which affect synthesis of cell wall (OIE, 2008). Proper vaccination and biosecurity measures can be viable options to control MG infections at poultry farms (Kleven, 2008).

Serological assays such as Enzyme Linked İmmusorbant Assay (ELISA) and Rapid Plate Agglutination assay (RPA) are still considered valuable to detect MG in poultry flocks (OIE, 2008). In the past, regional studies on poultry disease surveillance and clinical surveys have been conducted to better understand the disease distribution pattern in different regions of Pakistan (Alam *et al*., 2012; Siddique *et al*., 2012; Rehman *et al*., 2013). Similarly, some researchers have reported on seroprevalence of MG in different regions of Pakistan (Gondal *et al*., 2015; Abbas et al., 2018). No data are available regarding seroprevalence of MG in commercial poultry in Chakwal,

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Pakistan. To the best of our knowledge this is the first attempt on seroprevalence of MG in chicken in Chakwal.

Materials and methods

This research study plan was approved by Institutional research and animal ethics committee Virtual University of Pakistan Lahore. The study was conducted on 25 randomly selected non vaccinated commercial open sheds of layers reporting severe respiratory problems with the coordination of District Livestock Department Chakwal (Poultry Wing) from July 2017 to February 2018 in Tehsil Chakwal, Tehsil Talagang, and Tehsil Kallar Kahar in Chakwal district, Punjab Pakistan. This study was conducted on selected 25 layer farms with approximately 85500 birds having hyline and leghorn breed. During this study 10 blood samples were collected from flock size of 1000-2000 and 15 blood samples were collected from flock size range from 2001-5000, and 20 blood samples from flock size with above 5001. A total of 358 blood samples were collected aseptically from wing vein or brachial vein of individual birds with 3 ml sterilized disposable plastic syringe without anticoagulant. The blood samples were transferred to Eppendorf tubes and placed at 4°C for 3 to 4 h, centrifuged (2,500 rpm for 5 to 7 min) to harvest the serum, whcih was preserved at -20°C until further processing.

The presence of MG antibodies in collected samples was detected by a commercial kit for MG RPA test (BİOVAC, France, Catalogue No. AS9) following the instructions of the OIE Manual (OIE, 2008) and by a 96 well MG coated indirect ELİSA plate (Pro FLOK USA; Catalogue No. 210332) as described previously (Ali et al., 2015). All data obtained for RPA and ELİSA assays were analyzed with Microsoft Office Excel 2007. Chi-square test was applied to find statistical significance for MG infection. A *P* value <0.05 was considered significant for statistical analysis.

Results

RPA test is commonly for screening of young flocks for MG infections. Our findings revealed that out of 358 serum sample, 74 (20.67%) were found positive through RPA test (Table I) while 107/358 (29.88%) samples were detected positive through iELISA indicating that ELİSA is more sensitive for detection of specific antibody (IgG) against MG (Table I) and hence age, month and density wise seroprevalence described in present study is exclusively based of ELİSA findings.

Layer chicken having age in between 24-31 week old showed highest seroprevalence of MG through iELISA (41.17%, 28/70), while the layers with age 32-39 weeks, 40-47 weeks, 48-55 week and 56-63 week showed a seroprevalence of 36.98% (27/75), 30.26%(26/74), 26.38% (16/69) and 14.49% (11/70), respectively. The statistical analysis revealed a significant correlation between age and seroprevalence of MG as described in Table II ($P<0.05$).

Table I.- Overall seroprevalence of MG through SPA and iELISA tests.

Farm ID	Flock size	Age (week)	Serum samples collected	iELISA positive samples (%)	SPA test positive samples (%)
1	1100	24	10	6 (60)	4 (40)
2	1400	56	10	0 (0)	1 (10)
3	1800	46	10	4 (40)	3 (30)
4	1500	48	10	2 (20)	3 (30)
5	1500	50	10	2 (20)	1 (10)
6	1500	52	10	2 (20)	1 (10)
7	2000	56	10	1 (10)	0 (0)
8	2200	26	15	3 (20)	1 (6.66)
9	2500	36	15	4 (26.66)	2 (13.33)
10	2500	32	15	5 (33.33)	4 (26.66)
11	2600	40	15	4 (26.66)	3 (15)
12	3000	36	15	5 (33.33)	5 (33.33)
13	3500	42	14	4 (26.66)	4 (26.66)
14	3500	54	15	5(33.33)	4 (26.66)
15	3800	39	15	6 (40)	5 (33.33)
16	4000	28	15	5 (33.33)	3 (15)
17	4200	58	15	2 (13.33)	0 (0)
18	4400	47	15	4 (26.66)	4 (26.66)
19	4500	28	15	6 (40)	4 (26.66)
20	4500	55	14	5 (35.71)	3 (21.42)
21	5000	31	15	8 (53.33)	1 (6.66)
22	5500	34	20	7 (35)	6 (30)
23	6000	44	20	9 (45)	6 (30)
24	6000	63	20	4(20)	3 (15)
25	7000	61	20	4 (20)	3 (15)
Total	85500	24-63	358	107 (29.88)	74 (20.67)

Table II.- Seroprevalence of MG among various age groups of birds.

Age group (weeks)	Flocks of birds	Total sera tested	Positive sera sample	Prevalence % (iELISA Test)	Chi-square value
24-31	5	70	28	41.17	13.827
32-39	5	75	27	36.98	P value=
40-47	5	74	26	30.26	0.008
48-55	5	69	16	26.38	Sig.
56-63	5	70	11	14.49	(P<0.05)

Monthly based seroprevalence of MG in layer flocks was conducted from July 2017 to February 2018 and revealed a lowest seroprevalence (20%) in the month of October whereas a higher seroprevalence was observed during the months of July, August, September, November, December, January and February which was 24.38, 25.49%, 25.64%, 39.13%, 44%, 27% and 32% respectively. However, seroprevalence for the month of March, April, May and June could not be determined due to lack of samples (Table III). Furthermore, no statistical significant difference was seen between months and MG prevalence (P>0.05).

Table III.- Seroprevalence of MG antibodies detected month wise.

Month	Total sera tested	Positive sera samples	Prevalence % (iELISA Test)	Chi-square value
July 2017	41	10	24.39	10.150
August	51	13	25.49	P value=0.180
September	39	10	25.64	Sig. (P>0.05)
October	40	08	20.00	
November	46	18	39.13	
December	50	22	44.00	
January 2018	48	13	27.00	
February	43	14	32.00	

Seroprevalence in different flock sizes were also investigated. A seroprevalence of 24.28%, 28.37%, 31.25%, 33.33% and 30% was observed for flock sizes of 1000-2000 birds, 2001-3000 birds, 3001-4000 birds, 4001-5000 birds and 5001-above birds, respectively. It was noted that highest prevalence 33.33% was observed in 4000-5000 while the lowest seroprevalence (24.28%) in 1000-2000 flock size (Table IV). No significant relationship was noticed between flock size and MG infection during statistical analysis of data (P>0.05).

Table IV.- Seroprevalence of MG antibodies detected with respect to flock density.

Flock size	No. of flocks	Total serum tested	Positive sera samples	Prevalence % (iELISA Test)	Chi-square value
1000-2000	7	70	17	24.28	1.559
2001-3000	5	74	21	28.37	P value= 0.816
3001-4000	4	64	20	31.25	Sig.
4001-5000	5	75	25	33.33	(P>0.05)
5001->	4	80	24	30.00	

Discussion

In present study, overall seroprevalence of MG detected through iELISA and RPA was 29.88% and 20.67%, respectively. Previous studies from Pakistan revealed similar findings through iELISA and SPA in commercial poultry (Atique *et al*., 2017; Mukhtar *et al*., 2012; Ahmed *et al*., 2008). MG prevalence detected through iELISA and SPA was 35 and 21% respectively in Quetta district of Baluchistan (Atique *et al*., 2017) and 49.38 % in district Faisalabad, (Mukhtar *et al*., 2012). İn another study, seroprevalence of 49.74% and 27.2% using PCR and culture techniques was reported in Pakistani poultry (Gondal *et al*., 2015). Recent studies reported MG prevelance in poultry and pheasantry birds of Northern Pakistan as 46.56% and 27.2 % respectively (Abbas et al, 2018). Previous surveys from China, Ghana, France, Italy, Egypt and Jordan reported varying prevalence of 43.07,84.5% 84%, 31%, 60% and 73.5% of MG by indirect ELISA, respectively in commercial layers (Kempf *et al*., 1997; Osman *et al*., 2009; Hong, 2018; Mesaa *et al*., 2017; Ayim-Akonor *et al*., 2018). Another survey in commercial layer chickens of Poland revealed 65.2% sero-positivity of MG antibodies (Alina *et al*., 2000). In Bangladesh, 45.1%, 32% and 64.47% sero-prevalence of MG was reported in layer chickens (Hossain *et al*., 2007; İslam *et al*., 2015; Ali *et al*., 2015). These above findings are concurrence with the present study and our results are very close in accordance with another finding obtained in India with a positivity rate of 54.4% (Reddy, 2014). Similarly, Baksi *et al*. (2016) conducted the serological study about prevalence of MG and detected 32.06% positive broiler breeders in different seven states of India.

Age wise analysis revealed highest MG prevalence as 41.17% in 24-31 weeks old birds followed by 36.98%, 30.26%, 26.38% and 14.49% in 32-39 weeks, 40-47 weeks, 48-55 weeks and 56-63 weeks older layers respectively (P<0.05). It was noticed that with the increase in age the seroprevalence of MG decreased. Similarly, Ali *et al*. (2015) reported highest MG prevalence as 66.35% in 38-43 old birds and lowest as 53.26% in age group of 56-61 in layer chicken. Similar kinds of reports were obtained in Pakistan, İndia, İran and Bangladesh (Ahmad *et al*., 2008; Hossain *et al*., 2007; Baksi *et al*., 2016; Mukhtar *et al*., 2012). The best possible reason for the decrease in seroprevalence with age could be due to immunity development againt MG with the passage of time.

According to the present study, seasons have some effect on the prevalence of MG (p>0.05). The prevalence of MG was higher in December (44%) than other months of the years. Baksi *et al*. (2016) reported high MG prevalence in winter season as 58.10% as compare to summer 7.43%. Similarly Ali *et al*. (2015) reported a prevalence of 70.13%

and 63.64% in December and July respectively in poultry in Bangladesh. This seasonal variation in prevalence might be due to the sudden change in temperature and cold stress on the birds. Similarly, our findings agrees with the findings of several previously published findings from different countries (Hossain *et al*., 2007; Mukhtar *et al*., 2012; Baksi *et al* 2016; Heleili *et al*., 2012).

Stocking density can impact the occurance of diseases. During present study, the maximum infection of MG was recorded in large size flocks having a bird density of 4000-5000 as compared to small ones. Similar findings were reported by Ali *et al*. (2015) from poultry. Hossain *et al*. (2007) also revealed highest MG infection (51.4 %) in large flocks as compare to smaller ones (41.3 %). Poor biosecurity and mangement could enhance the transmission of the MG via horizontal and vertical transmission.

Conclusion

Our findings revealed a higher seroprevalence of MG in commercial layer flocks in district Chakwal through iELISA and RPA. İmmuninization of poultry with effective vaccines is required for the effective control of the MG. In addition, it was also found that iELISA test is more sensitive and specific for the detection of MG antibodies in serum samples as compared to RPA test.

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Statement of conflict of interest

The authors declare no conflict of interest.

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