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Molecular Prevalence and Seroprevalence of *Mycoplasma gallisepticum* and *M. synoviae* in Indian Poultry Flocks

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ABSTRACT

Mycoplasma gallisepticum and *M. synoviae* are the two important pathogens affecting poultry worldwide and responsible for huge economic losses to the poultry industry. Here, we studied the prevalence of *M. gallisepticum* and *M. synoviae* in poultry flocks from different geographical regions in India. Prevalence of *M. synoviae* and *M. gallisepticum* as 33.0% and 11.65% was observed in samples from live birds of different states and 25.98% and 9.45% of samples were positive for *M. synoviae* and *M. gallisepticum* in post mortem examined birds of a single farm. ELISA results showed seroprevalence of 52.1 and 32.6%, respectively of *M. synoviae* and *M. gallisepticum* in a total of 635 samples. The PCR and ELISA results revealed an overall higher prevalence of *M. synoviae* than *M. gallisepticum* in live birds from different states and in dead birds with Chronic Respiratory Disease and the results shows the urgent need for adoption of better control measures against *M. synoviae* including vaccination.

Keywords: *Mycoplasma gallisepticum*, *M. synoviae*, prevalence, seroprevalence

There are numerous species of Mycoplasmas capable of infecting birds, of which *M. gallisepticum* and *M. synoviae* are the most important species affecting poultry. Both these pathogens have a worldwide distribution. *M. gallisepticum* causes Chronic Respiratory Disease (CRD) in chicken characterized by respiratory rales, coughing, nasal discharge, conjunctivitis, sinusitis and air sac lesions. Infection most frequently occurs as a subclinical infection of the upper respiratory tract and the birds remain infected life long (Ley, 2008). *M. synoviae* frequently causes subclinical upper respiratory tract infection and air sacculitis.

Systemic infection of this pathogen results in infectious synovitis characterized by inflammation of the synovial membranes of joints and tendon sheaths. Carrier birds and vertical transmission play an important role in maintaining these organisms in the poultry farms. Both these pathogens have significant economic impact on poultry production in terms of high morbidity, reduced feed and egg production efficiency and losses due to carcass condemnation and cost of treatment.

Mortality due to respiratory diseases is very common in poultry flocks. In India, in poultry farms where multiple aged populations is maintained and complete disinfection of premises is not possible, there is always a persistence of *Mycoplasma* pathogens. Vaccination with live attenuated strains MG ts-11 or MG 6/85 is being used by some farmers for control of *M. gallisepticum* infection where as it is not practiced in case of *M. synoviae.*

Routine postmortem examination at an organized poultry farm at Hyderabad has shown occurrence of Chronic Respiratory Disease characterized by lesions in the air sac and trachea *viz*., mild to severe air sacculitis and congestion and thickening of tracheal mucosa. Understanding the epidemiology of both these pathogens is essential for adopting proper control measures against these pathogenic

Mycoplasmas. Hence in the present study, the prevalence of both these pathogens is studied in poultry flocks from different states of India by Polymerase Chain Reaction (PCR) and Enzyme Linked Immuno Sorbent Assay (ELISA).

MATERIALS AND METHODS

Ethical approval

Ethical approval was not required as no live birds were used for experimentation in this study. However, adequate measures were taken to minimize pain or discomfort while sample collection from live birds in accordance with the International Animal Ethics Committee.

Samples

Tracheal, choanal and synovial swab samples were collected during post mortem examination at an organized poultry farm showing lesions characteristic of CRD or swollen joints (508 numbers). Choanal swabs (309 numbers) were also collected from apparently healthy birds or birds showing respiratory signs from 26 poultry flocks of 7 Indian states (Telangana, Karnataka, Tamilnadu, Gujarat, Himachal Pradesh, West Bengal and Odisha). Samples were collected during the period from March 2013 to January 2014. Except for one flock (Layer, 74 week, Bangalore, vaccinated with MG ts-11), none of the flocks were vaccinated for *M. gallisepticum* or *M. synoviae*. Swab samples were placed in 2 ml screw cap tubes containing Frey's medium (Ley *et al.,* 2008). A total of 635 blood samples were collected from 40 poultry flocks from 5 states which were not vaccinated and serum was separated and stored for carrying out seroprevalence study.

Isolation and PCR screening

Immediately after collection samples were transported to lab on ice. Swab samples were cultured in Frey's medium as described earlier (Ley, 2008). The broth culture was incubated till mild colour change from red to orange or till 5th day. DNA isolation was carried out from 2 ml of broth culture by phenol chloroform isoamyl alcohol method. DNA concentration was estimated using Genova nano spectrophotometer and approximately 10 ng DNA was used for PCR test. DNA samples were screened by PCR for *M. gallisepticum* using MG-14F and MG-13R primers (OIE, 2008) and for *M. synoviae* using primer pair MSlink-F and MScons-R (Jeffery *et al.,* 2007). The DNA isolated from commercial attenuated MG 6/85 vaccine and *M. synoviae* isolate MSG510 available at Avian Health Laboratory, Directorate of Poultry Research, Hyderabad, Telangana were used as positive controls for *M. gallisepticum* and *M. synoviae*, respectively.

Sero-prevalance

Serum samples collected from different poultry farms were screened for presence of antibodies against *M. gallisepticum* and *M. synoviae* by ELISA test using commercially available kit (IDEXX Laboratories, Inc., Maine, USA). The assays were performed and analysed following manufacturer's instructions. The absorbance was measured at the wavelength of 650 nm using µQuant Microplate reader (Biotech Instruments Inc.). From the absorbance, sample to positive (S/P) ratio and titer were calculated for each sample. Serum samples, with S/P ratio greater than 0.5 or titer greater than 1,076 were considered as positive.

RESULTS AND DISCUSSION

PCR amplification of 16S rRNA gene of *M. gallisepticum* using MG-14F MG-13R primer pairs yielded an amplicon of 185 bp. PCR amplification of *vlhA* gene using MSlinkF and MSconsR primer pairs produced a product which varied in size among different isolates between 300 to 400 b. Prevalence study showed that among the samples (309) from live birds of 26 flocks from different states, 33.0% and 11.65% were positive for *M. synoviae* and *M. gallisepticum,* respectively, in PCR. Out of 26 flocks tested, 50% and 27% had prevalence of *M. synoviae* and *M. gallisepticum*, respectively and 11.5% of the flocks had prevalence of both the pathogens. The study shows that only few flocks of older birds of age >50 weeks had very high prevalence of *M. synoviae* (70-100%) where as in case of *M. gallisepticum* no such higher prevalence was observed in older flocks >50 weeks. *M. gallisepticum* had a maximum prevalence of 40% in 39 and 40 week old birds. Flock details along with PCR results are presented in the Table 1. These results show a high prevalence of

both Mycoplasma species in the screened poultry farms. Similar to present results, a high prevalence of *M. synoviae* (49.1%) was reported in commercial broiler breeders in Tamil Nadu (Senthilnathan *et al.,* 2015).

Table 1: Incidence of *M. gallisepticum* and *M. synoviae* based on isolation and identification by PCR

Flock No.	Location	Age in weeks	No of samples	Percent prevalence	
				M. *gallisepticum*	*M. synoviae*
1	Hyderabad	63	85	28.24	48.24
2	Hyderabad	45	8	0	75
6	Hyderabad	50	10	0	70
7	Hyderabad	50	10	0	10
8	Hyderabad	36	10	0	0
9	Kolkata	12	5	0	0
10	Kolkata	50	5	0	100
11	Kolkata	72	5	0	20
12	Kolkata	83	5	0	100
13	Kolkata	12	5	0	60
14	Palampur	Mixed	12	0	25
15	Palampur	64	12	0	33.3
16	Palampur	48	12	0	58.3
17	Anand	40	5	0	0
18	Anand	40	5	0	0
19	Anand	45	5	20	0
20	Anand	45	5	40	0
21	Anand	39	5	40	0
22	Anand	45	5	0	0
23	Anand	45	5	20	0
24	Anand	35	5	0	0
25	Anand	35	5	0	0
26	Anand	45	5	0	0
27	Bangalore	74	40	12.5	40
43	Namakkal	74	11	9.1	27.3
44	Bhubneswar	42	24	0	0
Total			**309**	**11.65%**	**33.0%**

A high PCR prevalence of *M. gallisepticum* (27%) and very low prevalence of *M. synoviae* (2.1%) were reported in commercial broilers in Haryana (Tomar *et al.,* 2017). A comparatively lower prevalence of *M. synoviae* (6.4%) and *M. gallisepticum* (3.4%) were reported by *Ramadass et al,* (2006) in poultry flocks of Tamil Nadu, India. The incidence of 11.5% of Chronic Respiratory Disease (CRD) caused by *M. gallisepticum* cases observed in total mortality (Susitha *et al.,* 2017) also indicates the high prevalence of this pathogen in Indian poultry farms.

Mycoplasma gallisepticum and *M. synoviae* are the most relevant *Mycoplasma* species in poultry industry due to its clinical and economic importance. Occurrence of CRD with characteristic lesions in trachea and air sac is very common in poultry farms in India and presence of *M. gallisepticum* and *M. synoviae* has been reported in poultry farms of different Indian states (Jignesh Kumar, 2010; Saritha *et al.,* 2010 and Saha *et al.,* 2011). In case of postmortem samples collected from CRD affected birds, 25.98% and 9.45% were positive for *M. synoviae* and *M. gallisepticum*, respectively. From this farm a total of 13,350 carcasses were examined during a period from March, 2013 to February, 2014 and the incidence of CRD was found to be 11.5% by post mortem examination (Susitha *et al.,* 2017). Detection of both these pathogens from carcasses with CRD lesions shows that infection with this pathogen is one of the important causes of mortality in the particular farm.

ELISA results showed that the seroprevalence of *M. synoviae* and *M. gallisepticum* in 635 samples analyzed was 52.1 and 32.6%, respectively. Out of the 40 flocks tested, 36 flocks (90%) were positive for *M. synoviae* of which 14 flocks had very high seroprevalence ie. 80-100% of the samples tested positive. A total of 21 flocks (52.5%) were positive for *M. gallisepticum* and of which, 8 flocks had 80-100% samples positive. Out of the 40 flocks tested, 18 flocks (45%) were positive for both *M. synoviae* and *M. gallisepticum*. Very high seroprevalence (80-100% samples positive) of *M. synoviae* and *M. gallisepticum* were generally seen in older flocks (above 50 weeks) as compared to younger flocks (<25 weeks). However, such high seroprevalence was also observed in few young flocks (<25 weeks). Most of the older flocks showed high prevalence of either one or both the *Mycoplasma* species except for one flock of 52 weeks old which had zero prevalence for *M. gallisepticum* and a low (10.71%) seroprevalence of *M. synoviae.* Another 5 commercial broiler flocks aged 40-48 days tested were found negative for *M. gallisepticum* and 2 flocks had low prevalence (16.67%) of *M. synoviae.* Flock details along

with seroprevalence results are given in Table 2. A lower seroprevalence of *M. gallisepticum* (22.44%) and *M. synoviae* (18.36%) in 6-8 week old broiler chicken and prevalences of *M. gallisepticum* (28.87%) and *M. synoviae* (10.56%) in day old broiler chicks was reported in Haryana (Tomar, 2017a) Seroprevalence in day old chicks might be due to maternal antibodies which indirectly indicates that parent flocks had seroprevalence of both these pathogens.

Table 2: Seroprevalence of *M. gallisepticum* and *M. synoviae*

Flock No.	Location	Age in weeks	Number of Samples	Percent Seroprevalence	
				M. gallisepticum	***M. synoviae***
1	DPR, Hyd	72	84	85.71	100.00
3	DPR, Hyd	83	21	19.05	100.00
4	DPR, Hyd	12	11	0.00	45.45
5	DPR, Hyd	9	10	0.00	40.00
6	Hyderabad	50	15	0.00	80.00
7	Hyderabad	50	15	0.00	93.33
8	Hyderabad	36	14	0.00	0.00
9	Kolkatha	12	10	0.00	80.00
10	Kolkatha	50	10	10.00	100.00
11	Kolkatha	72	10	10.00	100.00
12	Kolkatha	83W	10	20.00	100.00
13	Kolkatha	12W	10	30.00	100.00
14	Palampur	Mixed age	24	0.00	45.83
15	Palampur	64W	12	0.00	100.00
16	Palampur	48W	12	8.33	91.67
17	Anand	40W	12	0.00	25.00
18	Anand	40W	12	0.00	25.00
19	Anand	45W	11	100.00	9.09
20	Anand	45W	11	81.82	27.27
21	Anand	39W	11	90.91	63.64
22	Anand	45W	11	100.00	72.73
23	Anand	45W	11	90.91	27.27
24	Anand	35W	11	9.09	63.64
25	Anand	35W	11	0.00	9.09
26	Anand	45W	11	0.00	9.09
27	Anand	25W	11	0.00	27.27
28	Anand	25W	11	0.00	36.36
29	Anand	25W	11	45.45	90.91
30	Anand	25W	11	36.36	72.73
31	Anand	25W	4	0.00	100.00
32	Anand	25W	4	0.00	25.00
33	Karimnagar	38W	24	50.00	100.00
34	Bangalore	41 days	18	0.00	0.00
35	Bangalore	48days	18	-0.00	16.67
36	Bangalore	40 days	18	0.00	0.00
37	Bangalore	48 days	18	0.00	0.00
38	Bangalore	42 days	18	0.00	16.67
39	Bangalore	36W	30	83.33	23.33
40	Bangalore	52W	28	0.00	10.71
41	Bangalore	52W	31	80.65	6.45
			635	32.60	52.13

W-Week

A higher seroprevalence of 60%-100% for *M. synoviae* and *M. gallisepticum* has been reported in other countries also (Gole *et al.,* 2012; Xavier *et al.,* 2011; Feberwee *et al.,* 2008). Present study also showed a high seroprevalence (80-100%) in few young flocks of 25 weeks or less age maintained in farms where multiple aged birds were kept and complete disinfection of cages and premises were not possible. High seroprevalence in younger flocks may be due to vertical transmission or due to the continuous persistence of these pathogens in farm premises and horizontal transmission. Landman (2014) has reviewed a higher global prevalence of *M. synoviae* than *M. gallisepticum.* The result of the present study also shows an overall higher prevalence of *M. synoviae* than *M. gallisepticum* in the flocks screened.

CONCLUSION

To sum, it up it may be stated that infection with *M. gallisepticum and M. synoviae* is still an important problem in Indian poultry flocks and the present study also shows an overall higher prevalence of *M. synoviae* than *M. gallisepticum* in the Indian poultry flocks. As the infection with both of these pathogens adversely affect the egg production, hatchability, weight gain and feed efficiency of poultry flocks and these pathogens have the ability to interact with other respiratory pathogens to cause severe infections and mortality, the results of the present study shows the urgent need for application of various control measures. As in general vaccination is not practiced in India against *M. synoviae,* and there is a higher prevalence of *M. synoviae* than *M. gallisepticum* in poultry flocks from various states and also in dead birds having lesions

of CRD, the present study recommends on urgent need for adoption of preventive measures against *M. synoviae* including vaccination.

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CONFLICT OF INTEREST

There is no conflict of interest among authors and there is no financial or personal relationship between authors and other organisations which that might inappropriately influence or bias their work.

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