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Epidemiological studies on *Mycoplasma synoviae* infection in poultry in parts of Haryana

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Abstract
Avian mycoplasmosis is one of the most economically important poultry disease with *Mycoplasma gallisepticum* and *Mycoplasma synoviae* being most pathogenic species with worldwide distribution. One hundred Tissue samples comprising of trachea, lungs and air sacs were collected during post mortem of poultry birds and were pooled. For studying different epidemiological factors, a questionnaire was designed for collection information comprising of total birds on farm, number of birds affected, number of birds died, ventilation facilities, number of farms in 1 km² area, agro climatic zone, flock size, distance from nearby road, location of farm etc. The results was statistically analysed by STATA and interpreted. however statistical analysis of the data concluded that the statistical association between these factors and occurrence of *Mycoplasma synoviae* infection could not be established, which may be due to small sample size as required. For establishment of association of these epidemiological factors, further studies with increased sample size with larger area of study and for a longer period is needed.

Keywords: *Mycoplasma synoviae*, Age, Ventilation, Flock size, statistics

Introduction
Avian mycoplasmosis is one of the most economically important poultry disease. *Mycoplasma gallisepticum* and *M. synoviae* are the two most important pathogenic species with worldwide distribution and causing heavy economic losses in poultry industry [3,4]. *Mycoplasma synoviae* (MS) usually causes respiratory disease and acute to chronic infectious synovitis in chickens and turkeys [1, 2]. MS causes upper respiratory tract infection [4]. The infection can also be vertically transmitted because of contamination of eggs. MS causes infection of upper respiratory tract and damages air sacs and serve as bed for other pathogens [5]. Keeping in view of dense poultry population in Haryana state and economic importance of *M. synoviae* infection in poultry, present study was conducted to study effects of different epidemiological factors in *M. synoviae* infection.

Materials and Methods
A total of 100 samples were collected from seven different districts of Haryana *viz.* Bhiwani (n=2), Hisar (n=9), Jhajjar (n=19), Jind (n=20), Karnal (n=20), Panipat (n=23) and Sonepat (n=7). Tissue samples comprising of trachea, lungs and air sacs were collected during post mortem of poultry birds and were pooled, and together known as "sample". For studying different epidemiological factors, a questionnaire was designed for collection information comprising of total birds on farm, number of birds affected, number of birds died, ventilation facilities, number of farms in 1 km² area, agro climatic zone, flock size, distance from nearby road, location of farm etc. On farm location along with geo co-ordinates of farm were analysed by using android application "ODK collect". The location was mapped through QGIS software. The statistical analysis was carried out by STATA for analysis and interpretation of data thus collected.

Results
Data thus collected was recorded, analysed and interpreted to study effect of different factors for *M. synoviae* infection in poultry. Detailed results are presented in Table 1 as below:

Table 1: Association of different parameters with detection of *Mycoplasma synoviae* in poultry with respiratory problems in Haryana

Variable	Category (n)	Positive for *Mycoplasma synoviae* (%)	95% CI	Wald χ^2	P
PCR results					
	100	19 (19.00)	11.84-28.07		
Age of flock				1.89	0.170
	0-2 wk (4)	0	0.00-60.23		
	>2-6 wk (66)	16 (24.24)	14.54-36.36		
	>6-10 wk (17)	2 (11.76)	1.46-36.44		
	>10 wk (13)	1 (7.69)	0.19-36.03		
District				1.28	0.257
	Bhiwani (2)	1 (50.00)	1.26-98.74		
	Hisar (9)	1 (11.11)	0.28-48.25		
	Jhajjar (19)	7 (36.84)	16.29-61.64		
	Jind (20)	1 (5.00)	0.13-24.87		
	Karnal (20)	5 (25.00)	8.66-49.10		
	Panipat (23)	4 (13.04)	2.78-33.59		
	Sonepat (7)	0	0.00-40.96		
Zone				1.10	0.295
	Eastern (70)	11 (15.71)	8.11-26.38		
	Western (30)	8 (26.67)	12.28-45.89		
Flock size				2.27	0.132
	Small-≤10,000 (54)	7 (15.71)	8.11-26.38		
	Medium->10,000-30000 (40)	10 (25.00)	12.69-41.20		
	Large->30,000 (6)	2 (33.33)	4.33-77.72		
Duration of respiratory problems				1.98	0.159
	0-7 days (77)	17 (22.08)	13.42-32.98		
	8-21 days (7)	1 (14.29)	0.36-57.87		
	>21 days (16)	1 (6.25)	0.16-30.23		
Use of antibiotics during last 1 month				1.57	0.210
	Yes (78)	17 (21.79)	13.24-32.59		
	No (22)	2 (9.09)	1.12-29.16		
Location of farm				0.01	0.923
	Rural (83)	16 (19.28)	11.44-29.41		
	Semi-urban (14)	2 (14.29)	1.78-42.81		
	Urban (3)	1 (33.33)	0.84-90.57		
No. of farms in 1 Km^2 area				0.79	0.375
	1-5 (89)	18 (20.22)	12.45-30.07		
	6-10 (10)	1 (10.00)	0.25-44.50		
	>10 (1)	0	0.00-97.5		
Ventilation facilities				0.32	0.570
	Natural (51)	11 (21.57)	11.29-35.32		
	Only Fan/Cooler (8)	1 (12.5)	0.32-52.65		
	Only Exhaust (8)	2 (25.00)	3.18-65.09		
	Both Fan/Cooler + Exhaust (33)	5 (15.15)	5.11-31.90		
Distance from nearby road				-	-
	0-1 Km (89)	19 (21.35)	13.37-31.31		
	>1-3 Km (11)	0	0.00-28.49		

Age of flock: The maximum number of samples positive for *M. synoviae* were observed in birds of >2-6 weeks of age i.e. 24.24% and no sample was found to be positive in birds of 0-2 week of age. However, statistical analysis showed that there was no significant association among flocks of different age with respect to detection of MS (p- 0.170) (Table 1).

District: There was no significant difference among samples from different districts for detection of *M. synoviae (*p- 0.257) (Table 1). The maximum percentage of samples positive for MS were from the district of Bhiwani i.e. 50.00% and no samples was found to be positive in Sonepat.

Zone: There was no significant difference between zones of Haryana with respect to detection of MS (p- 0.295) (Table 1). Western zone (26.67%) showed more positive samples as compared to the eastern zone (15.71%)**.**

Flock Size: On the basis of poultry flock size from which samples were collected, flock size was categorized into three *i.e.* Small (<10,000), Medium (>10,000-30,000) and large (>30,000). There was no significant difference between the farm size with respect to detection of *M. synoviae* (p- 0.132) (Table 1). The maximum percentage was reported in large farms (>30,000) i.e. 33.33%, followed by medium farms (10,000-30,000) i.e. 25.00% and the least number of samples were positive in small farms (<10,000) i.e. 15.71%.

Duration of respiratory problem: There was no significant difference between duration of start of respiratory problem (0-7 days, 8-21 days and >21 days) with respect to cases positive for MS (p- 0.159) (Table 1). Birds suffering from respiratory problem from 0-7 days showed the maximum reported cases for *M. synoviae i.e.* 22.08% while least number of cases of *M. synoviae* infection were reported in birds suffering from >21

days i.e. 6.25%.

Use of Antibiotic during last 1 month: There was no significant association between use of antibiotic with respect to detection of *M. synoviae* (p- 0.210) (Table 1). The cases of MS were reported more in the flocks where antibiotics were given *i.e.* 21.79% as compared to the flocks where antibiotics were not given in the last 1 month *i.e.* 9.09%.

Location of farm: There was no significant association between location of farm with respect to detection of *M. synoviae* (p- 0.923) (Table 1). The maximum percentage of samples positive for MS were seen in urban areas *i.e.* 33.33% and least number of cases were reported in semi urban areas *i.e.* 14.29%.

No. of farm in 1 sq. km area: There was no significant association between number of farm in 1 sq. km area with respect to detection of *M. synoviae* (p- 0.375) (Table 1). The maximum cases were reported in farms with 1-5 farms/ km² area *i.e.* 20.22% and no cases were reported in >10 farm/ sq. km area.

Ventilation facilities: There was no significant association between ventilation facilities respect to detection of *M. synoviae* (p- 0.570) (Table 1). Maximum number of cases were reported in farms with "only exhaust" type of ventilation *i.e.* 25.00% and least number of cases were reported in "only fan/cooler" type of ventilation *i.e.* 12.5%.

Distance from nearby road: The results were not significant. All the farms from where *M. synoviae* was detected were located within 1 km from the road.

Discussion

Present study was conducted to study association of different factors in *M. synoviae* infection in poultry. In present study a total of 100 tissue samples (trachea, lungs, airsacs) were collected from flocks with history of respiratory problem and respective post mortem lesions of respiratory disease complex. These tissue samples were analysed by PCR for confirmation of the pathogen. Out of 100 samples, 19 were found positive for the *M. synoviae* infection by PCR. For studying association of different factors, questionnaire was designed and data was analysed and interpreted. Various factors studied included spatial and temporal distribution, correlation with age of poultry flock, ventilation strategies being adopted in farm, duration of respiratory distress, density of poultry farms in nearby vicinity of poultry farm, previous history of antibiotic usage etc. In present study, although differences in morbidity pattern of the diseases were observed in all above mentioned variables, however statistical analysis of the data concluded that the statistical association between these factors and occurrence of *Mycoplasma synoviae* infection could not be established, which may be due to small sample size as required. Some of the other studies showed association between age and prevalence of *Mycoplasma synoviae* [6, 7]. Other studies showed a different association in contrast to our studies with respect to ventilation [8,9]. Similar to our studies, Moreira *et al*. (2015) [10], showed no statistical association between flock size and disease prevalence. For establishment of association of these epidemiological factors, further studies with increased sample size with larger area of study and for a longer period is needed. Present study using small sample size with random area of selection for a shorter period unable to establish any significant association of these factors with occurrence of *Mycoplasma synoviae* infection.

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