

Seroprevalence and Risk Factors of *Mycoplasma gallisepticum* Infection in Iranian Broiler Breeder Farms

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Abstract: This study was aimed to determine the risk factors (age, size of flock, locale, sex and strain) and seroprevalence of *Mycoplasma gallisepticum* (MG) in Iranian broiler breeder farms. In addition Correlation between seroprevalence in breeder with chronic respiratory disease in their progeny was analyzed. The study was conducted from May 2002 to October 2008 that was based on rapid Serum Plate Agglutination (SPA) and enzyme linked immunosorbent assay (ELISA) tests. To find a correlation between MG in breeders with airsacculitis in their progeny, data from broiler slaughterhouses were registered. According to the results, the highest (21.4%) and lowest (0%) prevalence of MG infection was found in 2003, 2008 respectively ($p < 0.05$). The prevalence was highest (18.5%) in winter and lowest (6.8%) in summer. Ross, Cobb, Arian, Hubbard and Arbor Acres had 23.2, 8, 11.4, 14 and 6.9, respectively infection. The prevalence was recorded highest at 10-20 weeks of age (28%), but in above 60 weeks it was lowest (3.4%). MG infection was higher (56.21%) in female than in male (43.79%). No significant difference was seen in flocks up to 30,000 population (11.7%), 30,000-40,000 (19%) and upper 40,000 (23%). The prevalence of mycoplasmosis in foothills was relatively higher (9.4%) than coastal area (7.2%), however not significantly different. The correlation between MG in breeder and chronic respiratory disease in broiler wasn't significant (respectively $p = 0.743$, $p = 0.103$) according to this, vertical infection couldn't be an important cause of condemnation for broiler in slaughterhouse.

Key words: Broiler breeder, Iran, *Mycoplasma gallisepticum*, risk factors, seroprevalence

INTRODUCTION

Mycoplasmas are very small prokaryotes devoid of cell walls, bounded by a plasma membrane only. *Mycoplasma gallisepticum* (MG) infections are commonly known as chronic respiratory disease (CRD) of chickens and infectious sinusitis of turkeys (Ley, 2008). Clinical symptoms of MG infections in these avian species include rales, coughing, nasal discharge, sinusitis, and severe air sac lesions, and consequences of MG include mortality, carcass condemnation, and reduced egg production, hatchability, feed efficiency, and weight gain. Due to the substantial performance and production losses, MG has been described as the most economically important of the 4 pathogenic *Mycoplasma* species affecting poultry (Evans *et al.*, 2005). MG can be diagnosed by studying its different properties such as morphological, cultural characteristics, biochemical and serological properties of the causal agent (Ley, 2008). There are two major diagnostic methods, MG detection and MG serology, both of which are widely used. MG serology methods, such as Serum Plate Agglutination (SPA), Enzyme Linked Immunosorbent Assay (ELISA) and Hemagglutination Inhibition (HI) tests are widely used in the laboratory; however, each method is limited by sensitivity and specificity. SPA is used as the

screening test because it's rapid, has high sensitivity, and low specificity, as well as being inexpensive. ELISA has been proved to have good sensitivity and more specificity compared to SPA. HI has high specificity but the disadvantages are low sensitivity and it is not commercially available (Kleven, 1998). Control of MG is very dependent on serologic monitoring. Some factors such as: age, size of flock, locale, etc., may effect on severity of this disease. The aim of the present study was to determine the seroprevalence and risk factors (age, size of flock, locale, sex and strain) of *Mycoplasma gallisepticum* (MG) in Iranian broiler breeders.

MATERIALS AND METHODS

Study area and selection of bird: The study was conducted at the commercial farms of seven provinces of Iran, from May 2002 to October 2008. Two hundred seventy broiler breeder farms were followed for this study and three samples for every thousand were obtained randomly.

Blood collection and serum preparation: In live birds, 2 mL blood were collected from wing vein by using fresh disposable plastic syringe (5 mL) and collected blood was kept in room temperature for about 1-2 h. A clean

Table 1: Relationships between *Mycoplasma gallisepticum* infection and various risk factors

Risk factor	Level of risk factor	No. of flocks	Positive flocks(%)	Significance of difference (p-value)
Age (weeks)	10-20	71	28.0	0.092
	20-30	143	9.1	
	30-40	187	7.5	
	40-50	203	7.4	
	50-60	153	4.9	
Season	Above 60	213	3.4	0.284
	Spring	250	9.7	
	Summer	260	6.8	
	Autumn	265	6.9	
Breed	winter	221	18.5	0.000
	Ross	255	23.2	
	Cobb	100	8.0	
	Arian	61	11.4	
	Hubbard	64	1.4	
Flock size	Arbor acres	86	6.9	0.292
	Up to 30,000	162	11.7	
	3,000-40,000	72	19.0	
Year	Above 40,000	85	23.0	0.000
	2002	63	14.2	
	2003	177	21.4	
	2004	223	10.3	
	2005	255	3.9	
	2006	181	1.6	
	2007	85	1.7	
2008	83	0.0		

straw color serum was seen around the clotted clump and the serum was poured into a labeled and stored at -20°C until used.

Serum plate agglutination (SPA) test: The SPA test was conducted with crystal violet stained *M. gallisepticum* commercial antigen (Nobilis®MG) obtained from Intervet Company Ltd. (The Netherlands). Following the manufacturer's instruction, 0.03 mL antigen and 0.03 mL serum was placed side by side with pipette in a glass plate and mixed well by stirring with glass rod, followed by rocking. Results were read within 2 min. In positive cases granules were formed slowly which could be seen during rocking. In the negative case, no such granules were formed. All SPA results were recorded. The positive sample in 1/8 dilution, were tested with ELISA.

Enzyme Linked Immunosorbent Assay (ELISA): Sera were tested with commercial test kit, FlockChek® (IDEXX Laboratories, Inc., USA), following the manufacturers' direction. Briefly, diluted sera were added onto a MG antigen-coated plate, incubated, washed, and peroxidase labeled, anti-chicken antibody (conjugated antibody) was added. After incubation, the plate was again washed before adding a substrate, and adding the stop solution. The plate was read in a ELISA reader. The optical density of the negative, positive controls, and samples were calculated and interpreted according to the manufacturers' recommendation. According to the interpretation of FlockChek® ELISA, titer levels 0-1075, and levels equal or higher than 1076 were negative, or positive, respectively.

Statistical analysis: To find a correlation between MG in breeders with airsacculitis in their progeny, data from broiler slaughterhouses were registered. For Statistical test SPSS 13 (chi square test and Pearson correlation) was used.

RESULTS

Sera samples were collected during seven years. As showed in Table 1, the highest (21.4%) and lowest (0%) Prevalence of MG infection was found in 2003 and 2008 respectively ($p < 0.05$). The prevalence was highest (27%) in winter and lowest (6.8%) in summer. Ross, Cobb, Arian, Hubbard and Arbor Acres had 23.2, 8, 11.4, 14 and 6.9, respectively. The prevalence of MG infection decreased with the increase of age. The prevalence of MG was recorded highest at 10-20 weeks of age (28%), whereas the prevalence was lowest (3.4%) at above 60 wk. The Prevalence of MG infection was higher (56.21%) in female than in male (43.79%) indicating that female birds were more susceptible than male birds, but the cause was not established. The statistical analysis further revealed that there was significant difference in the prevalence of MG infection between the sex of birds ($p < 0.05$). No significant difference was seen in flocks up to 30,000 population (11.7%), 30,000-40,000 (19%) and upper 40,000 (23%). Farms were separated in two different zones (Foothills with less humidity Compared with Coastal area). Regional variation of prevalence of *M. gallisepticum* was observed in the present study. The prevalence of Mycoplamosis in Foothills was relatively higher (9.4%) than Coastal area (7.2%), however not significantly different. This variation was probably due to different humidity in these areas.

The correlation between *Mycoplasma galisepticum* in breeder and chronic respiratory disease in broiler wasn't significant ($p = 0.743$ and $p = 0.103$, respectively)

DISCUSSION

The results show that occurrence of *Mycoplasma gallisepticum* have a significant relationship with the year of sampling. A higher occurrence also can be seen in 2003 ($p = 0.000$). Furthermore, remarkable difference can be observed among *Mycoplasma gallisepticum* occurrence rate in different strains (Ross, Cobb, Arian, Hubbard, and Lohman). The prevalence of MG was significantly higher in Ross strain. However, age of flock, season, zone of study, and size of flock do not have an impressive effect in appearance of *Mycoplasma gallisepticum* infection but this problem is worse in the greater sizes of flocks. For this reason, lower incidence observed in flocks has a size up to 30,000 birds in each farm. Serological investigation showed the highest infection rate (23%) in large scale flocks (>40,000 birds) in comparison (11.7%) to small (up to 30,000 birds) flocks. Similar report was demonstrated by Talha (2003) who recorded 36% MG infection in a

flock containing 300 chickens in comparison to 33% in a flock containing 250 chickens. Highest infection rate in large scale flocks probably due to faulty in management and biosecurity.

In the present study seasonal variation for prevalence of MG infection was observed. The prevalence was highest (18.5%) in winter and lowest (9.7%) in summer which was in agreement with the result of Sikder *et al.* (2005). It might be due to the influence of cold weather. The statistical analysis by one way ANOVA method (F-test) showed no significant variation between the prevalence of seasons. It was also found that the prevalence of MG infection was higher (56.21%) in female than in male (43.79%), indicating that female birds were more susceptible than male birds to MG infection but the fact was not established. The statistical analysis by one way ANOVA method (F-test) revealed that there was no significant difference in the prevalence of MG infection between the sex of birds. Prevalence was also decreased with the increase of age.

The highest prevalence (21.4%) of MG infection was found in the present study in 2003, which was supported by the previous investigation of Biswas *et al.* (1992). They reported 13-32% seroprevalence of MG infection in the selected farms of the selected area. Also, the present findings were in close agreement with the previous results reported by Alam *et al.* (2003) and Talha (2003) in Bangladesh, Kelly *et al.* (1994) in Zimbabwe, Chrysostome *et al.* (1995) in Benin, Shah-Majid (1996) in Malaysia, Pandey and Hasegawa (1998) in Zambia. Bencina *et al.* (1987), Wieliczki *et al.* (2000) and Godoy *et al.* (2001) reported 56.54, 57.15 and 59.10% seroprevalence of MG infection in chickens, respectively. The prevalence of MG infection was lower in the present study than latter studies. This may be due to quarantine and health strategy and elevation of farmer's knowledge about biosecurity.

According to the age, the highest prevalence of MG infection was 28% in 10-20 weeks age group whereas lowest prevalence was 3.4% in above 60 weeks. Similar report was demonstrated by Sikder *et al.* (2005) who reported highest MG infection (71.42%) at 18 weeks of age and lowest (55.17%) at 63 weeks of age. This finding also supports the report of Sarkar *et al.* (2005) who recorded 73.80% MG infection at 20 weeks of age in comparison to 45.16% at 55 weeks of age. Similar report was demonstrated by Talha (2003) who reported the prevalence of MG infection significantly decreased with the increase of age. Highest infection in the young chickens is probably due to the vertical transmission of the organisms.

In consequence, *M. gallisepticum* is prevalent Iran. Additionally, it was proved that the occurrence of *M. gallisepticum* have a consequential relationship with the strain of chickens and sampling year. Future studies on the current topic are therefore recommended.

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