

Incidence and risk factors of *Mycoplasma synoviae* infection in broiler breeder farms of Iran

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Abstract

Mycoplasma synoviae (MS) is an important pathogen of poultry worldwide, causing respiratory tract infection and infectious synovitis in chickens and turkeys. The aim of this study was to determine the incidence and risk factors (age, size of flock, locale, sex and strain) of *Mycoplasma synoviae* in broiler breeder farms in Iran. The study was based on Rapid Serum Plate Agglutination (SPA) and enzyme linked immunosorbent assay (ELISA) tests. The prevalence was highest (44%) in winter and lowest (34%) in summer. Ross, Cobb, Arian, Hubbard and Arbor Acres strains had 40%, 44%, 32%, 27% and 45% infection, respectively. The prevalence was recorded highest in above 60 weeks of age (47.8%), but at 10-20 weeks it was lowest (14.2%). No significant difference was seen in flocks up to 30,000 population (41.9%), 30,000-40,000 (52%) and upper 40,000 (57%). The prevalence of *Mycoplasmosis* in foothills was significantly ($p < 0.05$) higher (41%) than coastal area (34.5%). The results showed that occurrence of MS have a significant relationship with the age and zone of sampling.

Key words: Broiler breeder, *Mycoplasma synoviae*, *Mycoplasmosis*, Risk factors, Seroprevalence,

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Introduction

Mycoplasmas are very small prokaryotes devoid of cell walls, bounded by a plasma membrane only (Kleven, 2008). *Mycoplasma synoviae* (MS) is one of the most important pathogenic chickens *Mycoplasmas*. MS infection occurs as a subclinical upper respiratory infection. It may cause air sac lesions when combined with Newcastle disease (ND), infectious bronchitis (IB), or both. Less frequently, MS becomes systemic and results in infectious synovitis, an acute to chronic infectious disease of chickens and turkeys, involving primarily the synovial membranes of joints and tendon sheaths, and bursitis (Kleven and Ferguson-Noel, 2008). Lameness and respiratory disorder caused by this infection results in decreased growth rate and loss of egg production and causing economic loss in intensive production. As the vertical transmission plays a major role in spread of MS in chickens the most effective method of control is regular flocks monitoring and eliminating of positive breeder flocks. Reliable and rapid diagnosis is needed to prevent dissemination of infection (Lockaby *et al.*, 1998; Kleven and Ferguson-Noel, 2008).

Provisional diagnosis and monitoring of *M. synoviae* infection is usually made using serological assays, while definitive diagnosis is made by isolation and identification of the organism. The most used serological tests are serum plate agglutination (SPA), hemagglutination inhibition (HI), and enzymelinked immunosorbent assay (ELISA) (Kleven, 1998). SPA reactors must generally be confirmed by the HI or ELISA tests. The HI test is less sensitive but more specific than the SPA test. It is however, a longer procedure and the reagents are not commercially available. In general the ELISA test is more sensitive than the HI test and more specific than the SPA test (Kempf and Gesbert, 1998; Kempf *et al.*, 1994). Culturing of MS could be costly, time-consuming and inconclusive (Ewing *et al.*, 1998). In Iran, serum plate agglutination (SPA) is used for flocks monitoring as screening test. SPA is used as the screening test because it's rapid, has high sensitivity, and low specificity, as well as being inexpensive (Kleven, 1998). ELISA has been proved to have good sensitivity and more specificity compared to SPA. Control of MS 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 risk factors (age, size of flock, locale, sex and strain) and seroprevalence of *Mycoplasma synoviae* (MS) in broiler breeders in Iran.

Materials and Methods

Ethical approval: Use of birds in this study was approved by the Institutional Animal Ethical Committee.

Samples: The study was conducted at the commercial farms of Iran, from May 2002 to October 2008. 350 broiler breeder farms were followed for this study and three samples for every thousand were obtained randomly.

Blood collection and serum preparation: Blood samples were aseptically collected from the wing veins using 5-mL sterile disposable syringes and needles. Blood was allowed to clot in the syringe and was kept for about 1 hour at room temperature. After this, serum of each sample was separated, centrifuged, and transferred to sterile microtubes kept at -20°C until used.

Serum Plate Agglutination (SPA) Test: All serum samples were inactivated by heating at 56°C/30 minutes to destroy nonspecific inhibitory substances and tested for MS by SPA, using a commercial antigen for the diagnosis of *Mycoplasma synoviae* by serum plate agglutination (Synovitest-Laboratorio BioVet-Brazil), according to the manufacturer's instructions. In short, 0.02mL of the serum to be tested was mixed with 0.02mL of the commercial antigen (1: 1) in a glass plate. After that, the plate was placed under a light source, and samples that showed agglutination (presence of clots) were considered positive. Positive sera were diluted 1: 5 and 1: 10 with 0.5M phosphate-buffered saline (PBS), pH 7.2. Both dilutions were tested again by SPA as described above. Sera were considered positive when clots were observed in dilutions up to 1: 10.

Enzyme-Linked Immunosorbent Assay (ELISA): Sera that were positive in SPA were analyzed for antibodies against MS using a commercially available ELISA antibody test kit (*Mycoplasma synoviae* antibody Test Kit- IDEEX Laboratories, Inc., Maine, USA) according to the manufacturer's instructions. Briefly, samples were diluted five-hundredfold (1: 500) with the diluent, and 0.1mL of each sample was dispensed in a well of a plate previously coated with MS antigen. Plates were incubated for about 30 minutes at room temperature. After that, plates were washed with deionized water, and 0.1mL of the conjugate

was placed in each well (Goat antichickens: horse radish peroxidase conjugate HRPO). Plates were incubated for about 30 minutes and washed again. Finally, 0.1mL of the substrate solution (tetramethylbenzidine or TMB) was dispensed into each well and incubated for 15 minutes at room temperature. The reaction was blocked with 0.1mL of stop solution. Absorbance was measured at 650 nm. Results were expressed as serum-to-positive ratios (S/P ratios) relative to a standard positive control. Serum samples, with S/P ratios greater than 0.5 (titers greater than 1,076) were considered positive.

Statistical analysis: Results were analyzed using SPSS 13 (chi square test and Pearson correlation).

Results

The choice of the SPA test to study the seroprevalence of *M. synoviae* was based on previous research in which the specificity and sensitivity of this laboratory test was compared with that of culture, polymerase chain reaction (PCR) and various commercial enzyme-linked immunosorbent assay kits. The SPA test was found to perform equally as well as the tested enzyme-linked immunosorbent assay kits (Feberwee *et al.*, 2005). Sera samples were collected during seven years. The highest (45.7%) and lowest (0%) Prevalence of MS infection was found in 2003 and 2008 respectively (Table 1).

Table-1. Relationships between *Mycoplasma synoviae* infection and various risk factors

Risk factor	Level of risk factor	Positive flocks(%)	Significance of Difference(p value)
Age (weeks)	10-20	14.2	0.000
	20-30	26.3	
	30-40	34.0	
	40-50	36.4	
	50-60	45.0	
	Above 60	47.8	
Season	Spring	38.9	0.123
	Summer	34.0	
	Autumn	36.1	
	Winter	44.0	
Breed	Ross	40	0.000
	Cobb	44	
	Arian	32	
	Hubbard	27	
	Arbor acres	45	
Flock size	Up to 30,000	41.9	0.112
	3,000-40,000	52.0	
	Above 40,000	57.0	
Year	2002	41.2	0.138
	2003	45.7	
	2004	35.9	
	2005	34.7	
	2006	40.0	
	2007	27.5	
	2008	0	
Zone	Coastal area	34.5	0.027
	Foothills	41.0	

The prevalence was highest (44%) in winter and lowest (36.1%) in summer. Ross, Cobb, Arian,

Hubbard and Arbor Acres had 40%, 44%, 32%, 27% and 45% respectively. The prevalence of MS was recorded highest at above 60 wk (47.8%), whereas the prevalence was lowest (14.2%) at 10-20 weeks of age ($p < 0.05$). Farms were separated in two different zones (foothills with less humidity compared with coastal area). The prevalence of Mycoplasmosis in Foothills was significantly ($p < 0.05$) higher (41%) than coastal area (34.5%). No significant difference was seen in flocks up to 30,000 population (41.9%), 30,000-40,000 (52%) and upper 40,000 (57%).

Discussion

M. synoviae can be transmitted vertically and horizontally (Stipkovits and Kempf, 1996). Results showed that the prevalence of MS infection increased with the increase of age. Culling of *M. synoviae*-positive flocks reduces the risk of vertical transmission of this mycoplasma to the offspring, which is reflected in the low seroprevalence of *M. synoviae* in rearing breeder stock (14.2%). Similar report was demonstrated by Feberwee *et al.* (2008) who reported lowest MS infection (6%) at rearing age. As the risk of vertical transmission is minimized by culling *M. synoviae*-infected flocks, horizontal transmission is expected to be the most important transmission route for infected rearing breeder flocks. Most *M. synoviae*-seropositive submissions originated from breeder flocks above 60 weeks of age (47.8%). This finding is in agreement with data of other research groups. Feberwee *et al.* (2008) reported Most *M. synoviae*-seropositive from layer type breeder flocks ≥ 51 weeks of age (60% of positive flocks). The prevalence study of Hagan *et al.* (2004), which was based on the detection of *M. synoviae* antibodies in eggs, reported a prevalence of 78.6% in commercial layer flocks in East England. In another study (Mohammed *et al.*, 1986), a *M. synoviae* prevalence of 87% was found in commercial layer flocks in Southern California. The infection was associated with older flocks that had been moulted or frequently medicated. The high prevalence and persistence of *M. synoviae* infections in layer stock can be explained by the frequent occurrence of multiple age housing and lower biosecurity standards in this sector (Stipkovits and Kempf, 1996; Kleven and Ferguson-Noel, 2008).

No relation between a special breed with *M. synoviae* status could be found which was in agreement with the result of Dufour-Gesbert *et al.* (2006). The prevalence of MS was significantly higher in age above 60 week. However, season and size of

flock do not have an impressive effect in appearance of *Mycoplasma synoviae* 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 (57%) in large scale flocks ($> 40,000$ birds) in comparison (41.9%) to small (up to 30,000 birds) flocks. Similar report was demonstrated by Dufour-Gesbert *et al.* (2006).

In the present study seasonal variation for prevalence of MS infection was observed. The prevalence was highest (44%) in winter and lowest (34%) in summer which was in agreement with the result of Heleili *et al.* (2011), Sikder *et al.* (2005), Sarkar *et al.* (2005) and Hossain *et al.* (2010). 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.

Regional variation of prevalence of *M. synoviae* was observed in the present study. The prevalence of Mycoplasmosis in Foothills was significantly ($p < 0.05$) higher (41%) than coastal area (34.5%). Density of flocks was higher in foothills than coastal area, probably this factor influence in prevalence of MS in these regions. As in Hagan's study (2004), infection was more frequent on sites with several houses. This risk factor is also observed for other diseases or infections (Refregier-Petton *et al.*, 2001; Skov *et al.*, 1999). Indeed Goodwin described that, for another mycoplasma disease, i.e. enzootic pneumonia in pigs, the geographical position of an uninfected herd in relation to infected herds was the dominant factor for breakdowns (Goodwin, 1985). This study confirmed the high prevalence of *M. synoviae* infection in broiler breeder farms in Iran. Furthermore, the results show that occurrence of *M. synoviae* have a significant relationship with the age of flocks and zone of sampling.

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Conflict of interest

Authors declare that they have no conflict of interest.

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