

Original Article

Molecular detection of *Mycoplasma synoviae* from backyard and commercial turkeys in some parts of Iran

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ABSTRACT

M. synoviae (MS) is an economically important pathogen and the major cause of airsacculitis and infectious synovitis in turkeys. Infection with this pathogen may remain asymptomatic but can render infected birds susceptible to secondary infections. This study was carried out for the molecular detection of MS infection in commercial and backyard turkey flocks in Tehran, Semnan, Isfahan, Qazvin, Zanjan, East Azerbaijan, Gilan, Mazandaran, and Golestan provinces of Iran. Sixty-hundred tracheal, choanal cleft or/and infraorbital sinus samples were collected from 18 commercial and 31 backyard turkey flocks. The polymerase chain reaction (PCR) technique was performed by using primers specific for detecting the 16S rRNA and *vlhA* genes of MS. The results showed that 51.61% of backyard and 33.33% of commercial farms were MS-positive. These findings suggested the molecular presence of MS, especially in northern and central regions of Iran. Further, the frequency of MS-positive samples was significantly lower in commercial farms than backyard farms ($P < 0.05$).

Keywords: *Mycoplasma synoviae*, Commercial, Backyard, Turkey, Molecular detection, Iran

Détection moléculaire de *Mycoplasma synoviae* chez des dindes domestiques et commerciales dans certaines parties de l'Iran

Résumé: *Mycoplasma synoviae* (MS) est un agent pathogène important sur le plan économique et la principale cause de l'airsacculite et de la synovite infectieuse chez les dindons. L'infection peut rester sans signes cliniques, mais peut rendre les oiseaux sensibles aux infections secondaires. Cette étude a été réalisée pour la détection moléculaire de l'infection au *Mycoplasma synoviae* (MS) dans les fermes commerciales de dindes dans les provinces iraniennes de Téhéran, Semnan, Ispahan, Qazvin, Zanjan, Azerbaïdjan oriental, Gilan, Mazandaran et Golestan. Un total de 600 échantillons de trachée, de fente choanale et / ou de sinus infra orbital ont été prélevés à partir de 18 troupeaux de dindes commerciales et 31 dindes de basse-cour. La réaction en chaîne par polymérase (PCR) a été réalisée avec des amorces spécifiques pour la détection des gènes ARNr 16S et *vlhA* de la SEP. Les résultats ont été comparés et ont montré que 51,61% des basses-cours et %33,33 des fermes commerciales étaient séropositives. Ces résultats suggèrent la présence moléculaire du MS, en particulier dans le nord et le centre de l'Iran. De même que les échantillons MS positifs obtenus des fermes commerciales étaient significativement plus bas que dans les basses cours ($0/05 > p$).

Mots-clés: *Mycoplasma synoviae*, Commercial, basse-cour, Turquie, Détection moléculaire, Iran

INTRODUCTION

Four *Mycoplasma* species are important pathogens of poultry. *Mycoplasma meleagridis* and *Mycoplasma iowae* are specific pathogens of turkey, causing airsacculitis, skeletal abnormalities, and reduced hatchability. *Mycoplasma gallisepticum* (*MG*) is an economically important pathogen of poultry (Levisohn and Kleven, 2000); *Mycoplasma synoviae* (*MS*) has been considered less important than *MG* in poultry, but its significance has been highlighted in several studies (Feberwee et al., 2008; Landman, 2014). *MS* infection may be accountable for subclinical disorders, especially if it occurs concomitant with Newcastle disease in turkeys. Infectious synovitis in turkeys results in systemic *MS* infection (Ley, 2008; Kleven, 2003). Furthermore, *MS* infection can incur great economic losses due to decreased egg production and growth retardation and lameness (Landman and Feberwee 2001; Kleven, 2008). *MS* can be transmitted horizontally or vertically via susceptible birds, humans, and fomites because of the possible resistance of *MS* in the environment (Christensen et al., 1994). With the promotion of biosecurity, preventive programs such as serological and/or bacteriological tests have been adopted. In serologic tests, inter-species cross-reactions and nonspecific reactions are preventive factors (Bradley et al., 1988; Kleven, 2003). In addition, *Mycoplasma* growth inhibition in culture hinders successful isolation (Zhao and Yamamoto, 1993). However, polymerase chain reaction (PCR) is a more rapid, effective, sensitive, and cost-effective method than the standard culture technique, which could be adopted as an alternative to traditional culture. This method provides data regarding the actual number of *MS*-contaminated broiler chickens (Bayatzadeh et al., 2011). Another study showed that 15 of 30 samples were culture-positive (approved by using the standard *MS*-specific antiserum), and 25 of 30 samples were positive by PCR. These observations highlight the higher sensitivity of PCR compared to culture (Haghbin et al., 2010). Initially, *MS*-specific PCRs were setup on the 16S rRNA gene. However, currently

other genes like variable lipoprotein hemagglutinin (*vlhA*) are being applied (Noormohammadi et al., 2000). Out of 21 field samples in West Azarbaijan Province, 8 (38.0%) samples were positive using two sets of primers (Ghaniei, 2016). In Iran, the epidemiological investigation of avian mycoplasmosis like *MS* infection has been mainly focused on poultry farms. To the best of our knowledge, there are limited studies on the molecular prevalence of *MS* derived from backyard and industrial turkey flocks in Iran. In one study by Rezaie et al. (2010), 8 (19.5%) commercial turkey flocks were found positive via the agglutination test with the specific *MS* antiserum in East Azerbaijan Province, but PCR showed no *MS*-positive results. In this study, we sought to gain insight into the presence of *MS* in turkey flocks in some regions of Iran by using the PCR technique.

MATERIALS AND METHODS

Sample collection. Overall, 600 tracheal, choanal cleft or/and infraorbital sinus swab samples were obtained from 49 farms (31 backyard turkey farms and 18 commercial turkey farms [20 swabs per house]). The swab samples were collected randomly from Tehran, Semnan, Isfahan, Qazvin, Zanzan, East Azerbaijan, Gilan, Mazandaran, and Golestan provinces in Iran during February-August 2016. The samples were immediately transported to laboratory.

DNA extraction. DNAs were extracted from the swab samples suspended in 1 ml of PCR-grade phosphate-buffered saline (PBS). The suspensions were centrifuged for 30 min at 14,000 *g* at 4 °C. The supernatant was carefully removed with a Pasteur pipette, and the pellets were suspended in 25 µl of PCR-grade water. The tube and the contents were boiled for 10 min and then placed on ice for 10 min before centrifugation at 14,000 *g* for 5 min. The supernatant was used as a DNA template for PCR.

Detection of *MS* 16s ribosomal RNA gene by PCR. The PCR assay for the detection of *MS* 16s ribosomal RNA gene was carried out on all the swab samples. The PCR reaction was performed in 50 µl reaction

volume consisting of 5 μ l 10 x PCR buffer, 1 μ l of 10 mM dNTP, 0.5 μ l of (20 μ M) *MS*-F (5'-GAG-AAG-CAA-AAT-AGT-GAT-ATC-A-3') and *MS*-R (5'-CAG-TCG-TCT-CCG-AAG-TTA-ACA-A-3') primers, 0.25 μ l of Taq DNA polymerase (5 U/ μ l), 2 μ l of 50 mM MgCl₂, 39.75 μ l of deionized distilled water, and 1 μ l of template DNA (Kleven *et al.*, 2004). Afterwards, 205-bp fragments of *MS* 16s rRNA gene were amplified. The thermal cycle included three steps as follows. The primary denaturation was performed at 94 °C for 3 min as the first step. In the second step, we performed 40 cycles each including three cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 60 s. Eventually, final extension was conducted at 72 °C for 5 min as the third step (Kleven *et al.*, 2004). The PCR products were electrophoresed on 1.5% agarose gel for 1 h at 100 V and visualized by Ethidium bromide staining. *MSH* (Bioproperties, Australia) was used as positive control and distilled water as negative control in all the PCR reactions.

Detection of variable lipoprotein haemagglutinin (*vlhA*) gene by PCR. The second specific *MS* primers, *MS*-Link-F: (5'-TACTATTAGCAGCTAGTGC-3') and *MS*-Cons-R: (5'- AGTAACCGATCCGCTTAAT-3') were used for amplifying the *vlhA* gene (Jeffery *et al.*, 2007). The 350 to 400-bp fragments of *MS vlhA* gene were amplified. *vlhA* PCR was performed in a mixture with the total volume of 25 μ L per sample, containing 2.5 μ L of 10X PCR buffer, 2 μ L of MgCl₂ (50 mM), 0.2 μ L of 10 mM dNTPs, 0.1 μ L of each primer, 0.1 μ L of Taq DNA polymerase (5U/ μ L), 19 μ L of deionized distilled water, and 1 μ L of extracted DNA as template. After denaturation at 95 °C for 1 min, the reaction was performed in 40 cycles including denaturation (95 °C for 20 s), annealing (60 °C for 40 s), primary extension (72 °C for 10 s), and a final extension (72 °C for 5 min). All the amplification reactions were carried out in a T100™ Thermal Cycler (Bio-Rad, US). After Ethidium bromide-stained gel electrophoresis (1%

agarose gel in 1X Tris–acetic acid–EDTA buffer), DNAs were visualized by UV transillumination system.

Statistical analysis. Data analysis was performed using MedCalc software, version 15. Inter-group comparison was conducted using Test of Proportions based on Z-statistics.

RESULTS

This study was performed in 49 turkey farms (commercial farms n: 18 and backyard turkey farms n: 31) in Tehran, Semnan, Isfahan, Qazvin, Zanjan, East Azerbaijan, Gilan, Mazandaran, and Golestan provinces of Iran. Twenty-two turkey farms were positive for *MS*. In other words, the 16s rRNA and *vlhA* genes of *MS* were successfully amplified in PCR reactions in 44.89% of the turkey farms (figures 1 and 2).

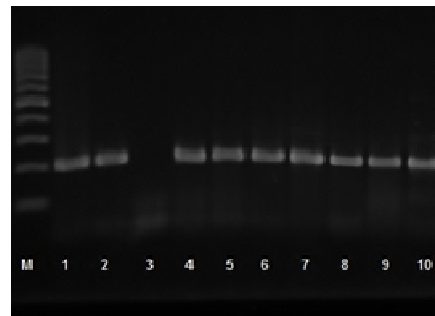


Figure 1. Electrophoresis gel image of *Mycoplasma synoviae* 16S rRNA bands amplified in polymerase chain reaction (205 bp). Lane M: Marker 100 bp, Lanes 1 and 2: Positive controls (Bioproperties, Australia), Lane 3: Negative control (distilled water), Lanes 4-10: Samples.

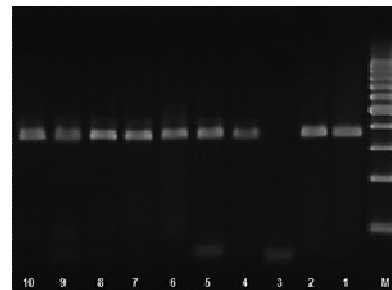


Figure 2. Electrophoresis gel image of *Mycoplasma synoviae* *vlhA* bands amplified in polymerase chain reaction (350-400 bp). Lane M: Marker 100 bp, Lanes 1 and 2: Positive controls (Bioproperties, Australia), Lane 3: Negative control (distilled water), Lanes 4-10: Samples.

The numbers of *MS*-positive commercial and backyard turkey farms were 6 (33.33%) and 16 (51.61%), respectively (Table 1). The frequency of *MS*-positive samples was significantly lower in the commercial farms relative to the backyard farms ($P < 0.05$, Test of Proportions).

Table 1. Results in backyard and commercial turkey farms in several provinces of Iran

| Province | Number of commercial turkey farms | Number of backyard turkey farms | Total Number of turkey farms | Number of positive commercial turkey farms | Number of positive backyard turkey farms | Total of positive turkey farms |
|-----------------|-----------------------------------|---------------------------------|------------------------------|--|--|--------------------------------|
| Qazvin | 1 | 6 | 7 | 1(16.6%) | 4(25%) | 5(22.7%) |
| Gilan | 2 | 3 | 5 | - | - | - |
| Isfahan | 5 | 1 | 6 | 2(33.3%) | 1(6.2%) | 3(13.6%) |
| Golestan | 1 | 8 | 9 | - | 6(37.5%) | 6(27.2%) |
| Mazandaran | 1 | 6 | 7 | - | 4(25%) | 4(18.1%) |
| Semnan | 1 | 2 | 3 | - | - | - |
| East azerbaijan | 2 | - | 2 | 1(16.6%) | - | 1(4.5%) |
| Zanjan | 3 | 4 | 7 | - | 1(6.2%) | 1(4.5%) |
| Tehran | 2 | 1 | 3 | 2(33.3%) | - | 2(9%) |
| Total | 18 | 31 | 49 | 6 | 16 | 22 |

DISCUSSION

Some studies have reported *MS* infection in poultry, but there is not enough data regarding the molecular presence of *MS* in turkey farms in Iran. Previous studies have shown that molecular methods are highly beneficial for determining the actual prevalence rate of *MS* in avian flocks, hence we used the PCR technique for this purpose (Nascimento *et al.*, 1993; Marois *et al.*, 2002). At first, *MS*-specific PCRs were set up with the 16S rRNA gene, but the recent approach of distinguishing *MS* strains is based on the *vlhA* gene.

The *vlhA* gene is an immuno-dominant surface lipoprotein with a protected and variable region (Noormohammadi *et al.*, 2000). Two sets of *MS*-specific primers (16S rRNA and *vlhA*) were applied for the evaluation and verification of the results. This study showed quite similar PCR results. Ghaniei (2016) used two sets of PCRs (16S rRNA and *vlhA*) for the detection of *MS* isolates. His results revealed no difference between PCRs. However, another study showed that the results of the two sets of primers were not the same (Ghafouri *et al.*, 2011). Our findings demonstrated that the prevalence of *MS* in backyard turkey farms was significantly higher than that of commercial turkey farms. It is known that eradication programs for European breeder flocks as the main supplies of commercial poult and mandatory control programs of Iran Veterinary Organization for the import of pathogen-free poult can account for the lower prevalence of this pathogen in Iranian industrial farms. The lower prevalence of *MS* in industrial farms proves the role of biosecurity principles (during the breeding period). Marois *et al.* (2005) presented that after placing poult in a polluted environment, they became infected and remained carriers. The higher prevalence of *MS*-positive cases in commercial farms in Isfahan, Qazvin, and Tehran provinces may be due to the dry climate, which can increase transmission distance of aerosols, enhancing susceptibility to infections (Bradbury *et al.*, 1996). Another reason for positive results in commercial turkey farms in this area is the proximity of *MS*-positive multi-age layer farms to turkey flocks (Mohammed *et al.*, 1986). The seroprevalence of *MS* in layer flocks has been reported by Haghghi-khoshkhoo *et al.* (2011). They demonstrated that 42.5% of flocks were positive in the center and north of Iran. In another study, 24 out of the 43 swab samples taken from suspected commercial broiler flocks of three provinces (i.e., Tehran, Markazi, and Qazvin) were found positive by PCR (Bayatzadeh *et al.*, 2011). Although *MS* has been detected in a broad range of wild and domestic avians, preference for Galliformes such as backyard fowls. These avians can

spread airborne infections via direct or indirect contact (Swayne et al. 2013; Michiels *et al.* 2015). There were more positive backyard flocks in north of Iran (i.e., Mazandaran and Golestan provinces), which might be due to extensive backyard turkey rearing without considering the biosecurity principles and supplying poult from local infected sources. Furthermore, multiple factors such as atmospheric ammonia, dust, flock density, and distance play an important role in contracting respiratory diseases (Kleven, 2008). The prevalence of *MS* infection in broiler breeder flocks was reported by Haghbin *et al.* (2012) in the same area. This report showed that backyard flocks function as a reservoir for commercial poultry farms. The results exhibited that some positive commercial farms (Isfahan and Tehran provinces) were suffering from lameness, decreased feed intake, weight loss, and increased mortality at the same time. Although respiratory signs were not observed, previous respiratory symptoms had been reported in these commercial flocks. In the same vein, Osorio *et al.* (2007) isolated *MS* from trachea and choanal clefts of turkeys with pneumonia and reported increased mortality. The role of biosecurity principles during the breeding period must be considered to diminish economic losses sustained by commercial turkey farms. These findings revealed the molecular presence of *MS* in commercial and backyard turkey farms, especially in northern and central regions of Iran, which could be due to the high density of backyard and multi-age layer farms in those areas. Good management and biosecurity practices during the breeding period are necessary to ensure that *MS* infections are not introduced by diverse sources to *MS*-free flocks. The management of this pathogen can help reduce the incidence of this infection and prevent economic losses in these regions. Based on our results, the evaluation of the prevalence of *MS* in turkey farms in other parts of Iran and future phylogenetic studies on this topic are highly recommended.

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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