Archives of Razi Institute, Vol. 73, No. 2 (2018) 79-85

Original Article

Molecular Detection of *Mycoplasma synoviae* from Backyard and Commercial Turkeys in Some Parts of Iran

Rasoulinezhad [1], S., Bozorgmehrifard [1,*], M. H., Hosseini [2], H., Sheikhi [1], N., Charkhkar [1], S.

1. Department of Poultry and Obstetrics, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Department of Clinical Science, Faculty of Veterinary Medicine, Karaj Branch, Islamic Azad University, Alborz, Iran

Received 21 January 2017; accepted 09 April 2017
Corresponding Author: mhbfard@yahoo.com

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'infectionau *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. 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, Zanjan, 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 µl 10 x PCR buffer, 1 µl of 10 mM dNTP, 0.5 µl of (20 µM) *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 µl of Taq DNA polymerase (5 U/µl), 2 µl of 50 mM $MgCl_2$, 39.75 µl of deionized distilled water, and 1 µl 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 ^oC for 3 min as the first step. In the second step, we performed 40 cycles each including three cycles of denaturation at 94 ^oC for 30 s, annealing at 55 ^oC for 30 s, and extension at 72 ^oC for 60 s. Eventually, final extension was conducted at 72 ^oC 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 µL per sample, containing 2.5 µL of 10X PCR buffer, 2 µL of $MgCl_2$ (50 mM), 0.2 µL of 10 mM dNTPs, 0.1 µL of each primer, 0.1 µL of Taq DNA polymerase (5U/µL), 19 µL of deionized distilled water, and 1 µL 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).

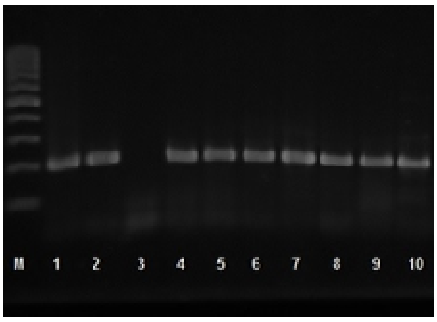

Figure 1. Electrophoresis gel image of *Mycoplasma synoviae* 16S rRNA bands amplified in polymerase chain reaction (205 bp). Lane M: Marker 100 bp, Lanes 1 and 2: Positive controls (Bioproperties, Australia), Lane 3: Negative control (distilled water), Lanes 4-10: Samples.

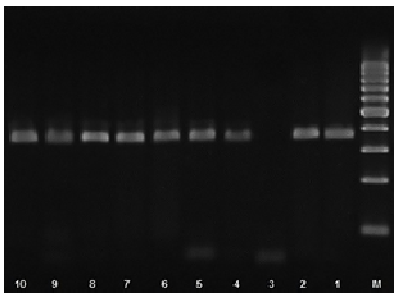

Figure 2. Electrophoresis gel image of Mycoplasma synoviae vlhA bands amplified in polymerase chain reaction (350-400 bp). Lane M: Marker 100 bp, Lanes 1 and 2: Positive controls (Bioproperties, Australia), Lane 3: Negative control (distilled water), Lanes 4-10: Samples.

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 poults and mandatory control programs of Iran Veterinary Organization for the import of pathogen-free poults 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 poults 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 Haghighi-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. 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 poults 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 phylogenic 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.

References

Bradbury, J.M., and S. Levisohn, 1996. Experimental infections in poultry. In: Molecular and Diagnostic Procedures in Mycoplasmology, Volume II – Diagnostic Procedures, Vol. II.J.G. Tully, ed. Academic Press, San Diego, California 361–370.

Bradley, L.D., Snyder, D.B.,van Deusen, R.A,. 1988. Identification of species specific and interspecies-specific polypeptides of Mycoplasma gallisepticum and mycoplasma synoviae. Am J Vet Res 49, 511-515.

Cordy, D.R., Adler, H.E., 1965. Brain and muscle lesions caused by Mycoplasma gallisepticum in turkey poults. Am J Vet Res 26, 186e190.

Christensen, N.H., Yavari, C.A., Mc Bain, A.J., Bradbury, J.M., 1994. Investigations into the survival of mycoplasma gallisepticum, Mycoplasma synoviae and Mycoplasma iowae on materials founds in poultry house environment. Avian Pathol 23, 127-143.

Dhondt, A.A., DeCoste, J.C., Ley, D.H.,Hochachk, W.M., 2014. Diverse wild bird host range of Mycoplasma gallisepticum in eastern North America. Plos One 9, 1l103553.

Feberwee, A. & Landman. W., 2012. The successful implementation of mycoplasma gallisepticum monitoring and control programmes in dutch commercial poultry: a declining seroincidence during an eleven year period. Abstract of the 19th congress of the international organization for mycoplasmology, (p.153).

Feberwee, A., de Vries, T.S., Landman, W.J., 2008. Seroprevalence of Mycoplasma synoviae in Dutch commercial poultry farms. Avian Pathol 37, 629-633.

Ghafouri, S. A., Bozorgmehrifard, M. H., Karimi, V., Nazemshirazi, M. H., Noormohammadi, A., Hosseini, H., 2011. Identification and primary differentiation of Iranian isolates of Mycoplasma synoviae using PCR based on amplification of conserved 5′ end of vlhA gene. J Vet Res 66, 117-122.

Ghaniei, A., 2016. Molecular characterization of Mycoplasma synoviae isolated from broiler chickens of

West Azarbaijan province by PCR of vlhA gene. In Veterinary Research Forum (Vol. 7, No. 3, p. 197). Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Gross, W.B., 1990. Factors affecting the development of respiratory disease complex in chickens. Avian Dis 34, 607–10.

Haghbin, N. H., Pourbakhsh, S. A., Charkhkar, S., Sheikhi, N.,Ashtari, A., 2010. Isolation and detection of Mycoplasma synoviae from seropositive rapid reaction broiler breeder flocks by polymerase chain reaction and culture methods.Vet Res 6, 31-35.

Haghighi-Khoshkhoo, P., Akbariazad G., Roohi, M., Inanlo, J.,Masoumi, M., Sami-Yousefi, P., 2011. Seroprevalence of Mycoplasma gallisepticum and Mycoplasma synoviae infection in the commercial layer flocks of the Centernorth of Iran. Afr J Microbiol Res 5, 2834-2837.

Jeffery, N., Gasser, R. B., Steer, P. A., Noormohammadi, A. H., 2007. Classification of Mycoplasma synoviae strains using single-strand conformation polymorphism and high-resolution melting-curve analysis of the vlhA gene single-copy region. Microbiology 153, 2679-2688.

Khan M.I., 2002. Multiplex PCR of avian pathogenic Mycoplasmas. Pp. 201-223 in PCR Detection of Microbial Pathogens. Humana Press, Totowa, New Jersey.

Kleven, S.H., 2003. Mycoplasma synoviae infection. In: Saif, Y.M., Barnes, H.J., Glisson, J.R., Fadly, A.M., McDougald, L.R., Swayne, D.E. (Eds.), Diseases of Poultry. Iowa State Press, Ames, Iowa.

Kleven, S.H., Fulton, R.M., Garcia, M., Ikuta, V.N., Leiting, V.A., Liu, T.,et al., 2004. Molecular characterization of Mycoplasma gallisepticum isolates from Turkeys. Avian Dis 48, 562–569.

Kleven, S.H., 2008. Mycoplasma synoviae infection. In: Swyne, Y.M., Barnes, H.J., Glisson, J.R., Fadly, A.M., McDougald, L.R., Swayne, D.E. (Eds.), Diseases of Poultry. Iowa State Press, Ames, Iowa.

Landman, W.J., 2014. Is Mycoplasma synoviae outrunning Mycoplasma gallisepticum? A viewpoint from the Netherlands. Avian Pathol 43, 2–8.

Landman, W.J. &Feberwee, A., 2001. Field studies on the association between amyloid arthropathy and Mycoplasma synoviae infection, and experimental reproduction of the condition in brown layers. Avian Pathol30, 629–39.

Levisohn, S., Kleven, S.H., 2000. AvianMycoplasmosis (Mycoplasmagallisepticum) Revue Scientifique et Technique 19, 425-442.

Ley, D. H., 2012. Mycoplasmagallisepticum infection. Pages 807–834 in Diseases of Poultry. 12 ed. Y. M. Saif, ed. Blackwell Publishing Professional, Ames, IA.

Ley, D. H., Berkhoff, J.E., Levisohn, S., 1997. Molecular Epidemiologic Investigations of Mycoplasma gallisepticum conjunctivitis in songbirds by random amplified polymorphic DNA analysis. Emergin Infect Dis 3, 375-380.

Loria, G.R., Ferrantelli, E., Giardina, G., Li Vecchi, L., Sparacino, L., Oliveri, F., et al., 2008. Isolation and characterization of unusual Mycoplasma spp. from captive Eurasian Griffon (Gyps fulvus) in Sicily. J Wildlife Dis 44, 159-63.

McBride, M.D., D.W. Hird, T.E. Carpenter, K.P. Snipes,C. Danaye-Elmi, and W. W. Utterback., 1991. Health survey of backyard poultry and other avian species located within one mile of commercial California meat-turkey flocks. Avian Dis. 35, 403–407.

Michiels, T., Welby, S., Vanrobaeys, M., Quinet, C., Rouffaer, L., Lens, L., et al., 2015. Prevalence of Mycoplasma gallisepticum and Mycoplasma synoviae in commercial poultry, racing pigeons and wild birds in Belgium. Avian Pathol Cavp2015-0116.R.

Marois, C., Savoye, C., Kobisch, M., Kempf, I., 2002. A reverse transcription-PCR assay to detect viable mycoplasma synoviae in poultry environmental samples. Vet Microbiol 89,17-28.

Marois, C., J.P. Picault, M. Kobisch, and I. Kempf., 2005. Experimental evidence of indirect transmission of Mycoplasma synoviae. Vet Res 36, 759–769.

Mohammed, H.O., Carpenter,T.E.,Yamamoto, R., McMartin, D.A.,1986. Prevalence of Mycoplasma gallisepticum and M. synoviae in commercial layers in southern and central California. Avian Dis 30, 519–526.

Nagai, S., Kazama, S.,Yagihashi, T., 1995. Ribotyping of Mycoplasma gallisepticum strains with a 16S ribosomal RNA gene probe. Avian Pathol 24, 633–642.

Nascimento, E.R., Yamamoto, R., Khan, M.I., 1993. Mycoplasma gallisepticum F vaccine strain-specific polymerase chain reaction. Avian Dis37, 203–211.

Noormohammadi, A. H., Markham, P. F., Kanci, A., Whithear, K. G., Browning, G. F., 2000. A novel mechanism for control of antigenic variation in the haemagglutinin gene family of Mycoplasma synoviae. Mol Microbiol35, 911-923.

Osorio, C., Fletcher, O.J.,Abdul-Aziz, T., Gonder, E.,Tilley, B., Ley, D.H., 2007. Pneumonia of turkey breeder hens

associated with Mycoplasma synoviae. Avian Dis 51, 791–796.

Rezaie, M., 2008. 'Isolation and Molecular Identification of Mycoplasma Pathogen from turkeys of Eastern Azarbaijan Provinces', DVSc thesis, Science and Research Branch, Islamic Azad University, Tehran, Iran.

Stipkovits, L. & Szathmary, S., 2012. Review: Mycoplasma infection of ducks and geese. Poul Sci 91, 2812–
2819.

Swayne, D.E., Glisson, J.R., McDougald, L.R., Nolan, L.K., Suarez, D.L, Nair, V.,2013.13th Edition. Diseases of poultry-Wiley-Blackwell.

Wyrzykowski, B., Albaric, O., Moreau, S., Nguyen, F., Fleurance, R., Belluco, S.,et al., 2013. Retrospective Study of Mycoplasma gallisepticum Meningoencephalitis in Six Turkey Flocks in western France. J Compar Pathol 148,173–177.

Zhao, S and Yamamoto, R, 1993. Detection of mycoplasma synoviae by polymerase chain reaction. Avian Pathol 22, 533-542.