

Molecular detection of *Mycoplasma Synoviae* in broiler flock with respiratory injury in Ahvaz slaughterhouse

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Abstract

Mycoplasmosis is one of the most important diseases in the poultry industry. *mycoplasma synoviae* is one of the important disease-causing factors in chickens, which causes respiratory tract infection and synovitis. and leads to significant economic losses to the poultry industry all over the world. In this study, mycoplasma infection of broiler flocks in Ahvaz slaughterhouse was investigated using molecular method. For this purpose, 200 samples of lung and trachea tissues and joint fluid of hock joint were collected from 20 broiler flocks with macroscopic symptoms and 20 samples from the mentioned parts from apparently healthy broiler chickens of each flock in the winter season. Tissue DNA extraction was performed. The polymerase chain reaction (PCR) was performed to detect *Vlha* gene of *mycoplasma synoviae*. Of the 200 samples, 55 lungs and 45 tracheas from flocks with macroscopic symptoms and 4 lung and trachea samples from apparently healthy birds were positive. In the molecular examination of joint samples, no positive cases were observed for the presence of *mycoplasma synoviae*. The PCR products of positive samples were sent to Gene FanAvaran Company for sequencing. The sequence of a part of the *Vlha* gene of this isolate was compared with other isolates in the gene bank and it was about 99% similar to the previous Iranian isolates (IRG11/C/09, IRG6/C/08, IRG1/C/08). According to the results of the present study, *mycoplasma synoviae* is common among broiler flocks under investigation in Ahvaz slaughterhouse. Therefore, the policy makers of the health system of the poultry industry should prevent the economic losses caused by this disease with preventive measures.

Key words: *Mycoplasma Synoviae*, broiler poultry, PCR, Ahvaz

Introduction

Mycoplasmosis is one of the most significant poultry infections that causes serious economic losses. This disease is caused by four known mycoplasma pathogens, including *Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae*

(MS), *Mycoplasma meleagridis* (MM), and *Mycoplasma iowae* (MI). Although *Mycoplasma galisepticum* and *Mycoplasma synoviae* are very critical (Heleili et al, 2011). *Mycoplasma synoviae* is from the genus *Mycoplasma*, one of the species of

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the class Mollicutes, in the order Mycoplasmatales. This bacterium has one serotype, but there is heterogeneity between its strains and its tissue tendencies, so their pathogenicity is different. The transmission of the disease may be vertical from the flocks of chickens broodstock or horizontal transmission due to contact with environmental contamination. In vertical transmission, infection is transmitted to eggs through the ovaries and ducts, and in horizontal transmission, infected chickens which are apparently healthy can spread the disease by coughing. In susceptible birds, the respiratory system is contaminated by air born particles. MS infection often occurs as a subclinical infection of the upper respiratory tract. It may cause air sac disease, which is described as severe inflammation of the air sac. Co-infection of MS or MG with respiratory viral infections such as Newcastle disease, infectious bronchitis or both can aggravate the respiratory condition. MS infection is commonly known as infectious synovitis, which is an acute to chronic infectious disease in chickens and turkeys that primarily involves the synovial membrane of joints and tendon sheaths (Ferguson-Noel et al, 2020; Sadrzadeh, 2015). In MS infection, the joints and cushions of the feet soles swell as well as the pallor of the crown and lameness. Synovial mycoplasma has a wider host range than other mycoplasmas. So, chickens, turkeys and guinea fowls can acquire contamination and get sick (Gharibi et al, 2018).

Mycoplasma infection may often persist at a subclinical level in the herd. For this reason, accurate and quick diagnosis is significant to control the spread of the disease. Although pathogen isolation is considered as the gold standard test for diagnosing MG and MS, this method is relatively difficult, because avian mycoplasmas require specific growth conditions and a lot of time. Nowadays, the polymerase chain reaction is used to identify and analyze the DNA of

mycoplasmas with specificity, sensitivity and rapid response (Galluzzo et al, 2022).

The variable hemagglutinin lipoprotein expressed by *Mycoplasma synoviae* is believed to play a critical role in disease pathogenesis through binding and immune evasion. Haemagglutinins are the most important surface proteins responsible for the colonization and virulence of avian mycoplasmas (Pourbakhsh et al., 2013). Tracking mycoplasma synovium and determining its prevalence in broiler, laying and brood stocks in Ahvaz by molecular and serological methods have also been done by other researchers (Gharibi et al, 2018; Mayahi et al, 2017).

In the present study, the molecular detection of *Mycoplasma synoviae* was performed on the tissue samples of broiler slaughterhouse, while in the previous research, most of the samples were swabs or serum. Therefore, the purpose of this study was to identify and determine the prevalence of synovial mycoplasmas in broiler flocks in the Ahvaz abattoir.

Material and Methods

In order to carry out this study, samples were taken from 20 flocks of broiler slaughtered in Ahvaz slaughterhouse which had respiratory injuries (hyperemia, hemorrhage and edema in lung and trachea samples) from December to March of 2020. In each flock, for every 10,000 birds, 10 samples of lung tissues, trachea and synovial fluid of hock joints of broilers with respiratory injuries and 1 sample of apparently healthy broilers were collected. The samples were transferred to the bacteriology laboratory at Shahid Chamran University of Ahvaz. Using a sterile scalpel, pieces of lung and trachea tissues of diseased and apparently healthy chickens were placed in 1.5 ml microtubes, as well as hock joint fluid samples taken by swab in microtubes. It was dissolved in a sterile buffer of PBS and samples were stored in a -70 °C until molecular studies were carried out.

Molecular detection

In order to determine the presence of mycoplasma in lung, trachea and joint tissue samples by PCR method, DNA extraction of each of the samples separately performed by using a commercial DNA extraction kit (Raha Bioantibody Company, Iran) and according to the company's instructions. 20 mg of homogeneous tissue were placed in 1.5 ml microtubes and the extracting process was performed. Finally, the extracted DNA was stored at -20°C. Then, the PCR test was performed using the Amplicon master mix and two specific forward and reverse primers related to the Vlha gene (Table 1) of *Mycoplasma synoviae*, which amplified a 392 base pair region of the Vlha gene (Jeffery et al, 2007).

The PCR was performed in a final volume of 25 microliters containing 12.5

microliters of mastermix 2X (Amplicon, Denmark) with a concentration of 1.5 mM MgCl₂, 1 microliter of each of the primers at a concentration of 10 pM, 3 microliters of template DNA and 7.5 microliters of sterile distilled water and using an Eppendorf thermocycler device (Eppendorf Master Cycler 5330, Germany), The temperature schedule is shown in (Table 2). It should be noted that sterile distilled water was used as a negative control and the PCR positive sample of *Mycoplasma synoviae*, which was confirmed by sequencing, was used as a positive control in all stages of the PCR reaction. Finally, the PCR products were electrophoresed in 1% agarose gel with a safe stain for 20 minutes at 90 volts, then exposed to UV light. The DNA marker used was 100bp (manufactured by Cinagen Company, Iran).

Table1: Sequences of primers and sizes of PCR product of the oligonucleotide primers used for the detection of *Mycoplasma synoviae*

Reference	Amplicon size (bp)	Sequence	Primer	Organism
Jeffery et al., 2007	392bp	TACTATTAGCAGCTAGTGC AGTAACCGATCCGCTTAAT	Vlha	<i>Mycoplasma synoviae</i>

Table2: Thermal program used in this study to perform PCR

Number of cycles	Time	Temperature(°C)	steps	Cycles
1	5min	95	initial denaturation	First
35	45seconds	95	Secondary denaturation	Second
	30seconds	55	annealing	
	30seconds	72	extension	
1	10min	72	final extension	Third

Phylogenetic analysis

In order to confirm the positive samples of *Mycoplasma synoviae* in PCR, Vlha gene nucleotide sequence determination was used. For this purpose, 40 microliters of the PCR product of the positive sample along with 30 microliters of reverse primers related to the Vlha gene were sent to Gene Technologies Company for determination. Upon receiving the results of the sequence determination, the relevant sequence was

analyzed by the Mega 10 software and finally the nucleotide sequence of the obtained sequences with the nucleotide sequence available in the NCBI gene bank (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) was compared.

Results

Based on the molecular results of 200 lung and tracheal tissues from broilers with

respiratory problems (hyperemia, bleeding and edema in lung and trachea samples) slaughtered at the Ahvaz slaughterhouse. *Mycoplasma synoviae* infection were identified in 55 (27.5%) lung samples and 45 (22.5%) tracheal samples. Also 15 samples (7.5%) were simultaneous infected in the tissues of the lungs and trachea. In the molecular analysis of 20 samples of lungs and trachea of apparently healthy chickens, infection with *Mycoplasma synoviae* was detected in 4 samples (20%) of trachea and 4 samples (20%) of lung, but contamination was not observed in simultaneous cases. Furthermore, in the molecular examination of all joint samples studied, no positive cases were found for the presence of synovial mycoplasmas. Figure 1 illustrates an example of positive cases of *Vlha* gene amplification with a molecular weight of 392 base pairs.

Determining the sequence of PCR products and preliminary Blast analysis showed that the target sequence had the most similarity (98.82%) to isolates IRG11/C/09, IRG6/C/08, IRG1/C/08. The sequence was registered in the gene bank

and received accession number (ON569257). The phylogenetic relationship was calculated by the software and finally the phylogeny tree was obtained using the Neighbor joining method and Mega10 software. The topological stability of the tree was evaluated by 1000 bootstrap replications.

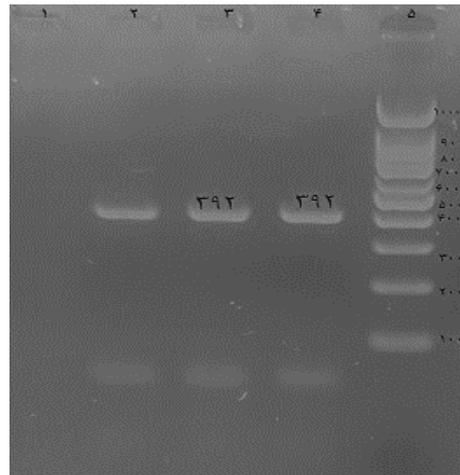


Figure1: Electrophoresis of PCR products related to *Vlha* gene amplification on 1% agarose gel stained with safe stain; column1: negative control; column2: positive control; column3,4 *Mycoplasma Synoviae*-specific 392 bp band; column5: 100bp molecular weight marker.

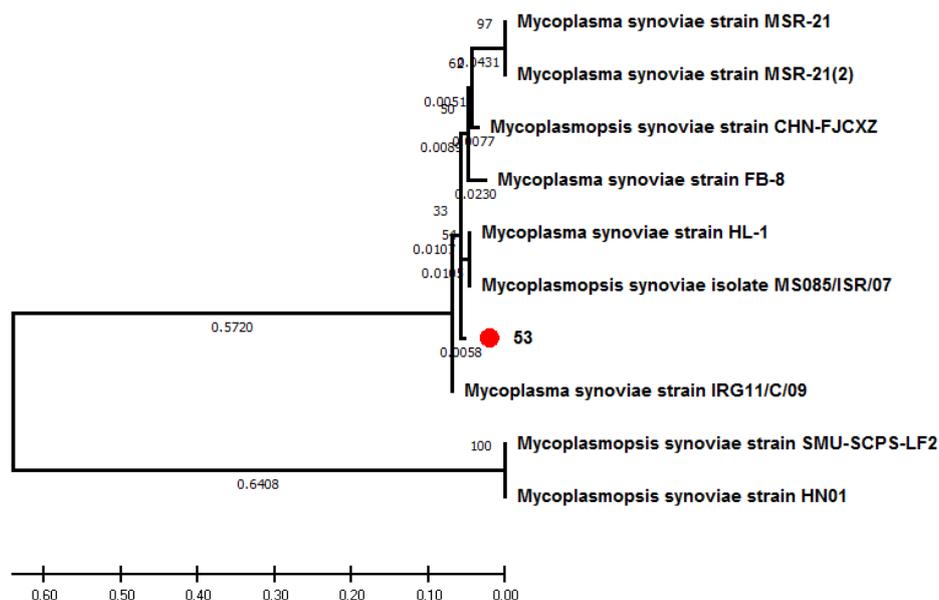


Figure 2: phylogenetic tree constructed by neighbor-joining method based on the nucleotide sequence of a part of the *Vlha* gene of the mycoplasma synovial isolate obtained in this study and the sequence available in the gene bank.

Discussion

Mycoplasma infection is of great importance in the world due to the occurrence of significant economic losses in breeding farms and meat ancestors of chicken and turkey. International Poultry Disease Control Centers have made great efforts to eradicate mycoplasma infection in the farms of ancestors and brood stocks, but in many countries, including Iran, mycoplasmosis and the losses arising from it still remain as a serious problem (Akbari, 1996).

Considering the importance of the problems caused by *Mycoplasma synoviae*, including subclinical respiratory and joint infections, including bursitis, tenosynovitis in chickens and turkeys. As well, the imposition of treatment costs on companies and the failure to respond appropriately to treatment and the loss of desired performance are very significant in this respect. In infected poultry, direct identification of *Mycoplasma synoviae* based on DNA in tissues or among laboratory isolates seems necessary (Mettifogo et al, 2015; Feberwee et al, 2005). Today, PCR is used for the identification and analysis of mycoplasma DNA with specificity, sensitivity and rapid response (Galluzzo et al, 2022). In a study, the type of *Mycoplasma synoviae* was investigated by PCR and Vlh gene detection, and the sensitivity of this method was considered very high in the diagnosis of *Mycoplasma synoviae* (Ansari et al, 2010).

In the molecular analysis of this research, out of a total of 200 lung and trachea tissue samples obtained from 20 slaughterhouse herds with respiratory symptoms, 55 lung samples and 45 trachea samples were positive. Of these, 15 samples were simultaneously contaminated in the tissues of the lungs and trachea. The presence of *Mycoplasma Synoviae* in the respiratory organs of birds has been reported by other researchers. In the examination of lung tissue samples of ostriches killed in Kerman province, the contamination rate 24.5% of

Mycoplasma Synoviae was reported (Tebyanian et al. 2014). Also, *Mycoplasma Synoviae* was detected in 14% of the respiratory tissues of slaughterhouse ostriches (Moumivand et al, 2017). In another study, the prevalence of *Mycoplasma Synoviae* was reported in 31.2% of tracheal swab samples, air sacs and cleft palate in poultry flocks with respiratory problems in Mazandaran province by PCR method (Hosseini Aliabad et al, 2012). Also, *Mycoplasma Synoviae* infection was reported in 12 herds (20%) in the PCR study on the mixed samples of tracheal swabs and lung, trachea, and air sac tissues of 60 broiler flocks with respiratory lesions in western Maharashtra, India (Bagal et al, 2019).

In the present study, the detection rate of synovial mycoplasma in tracheal tissue was 22.5%. In similar studies, mycoplasma contamination of synovium was reported in 26% of tracheal, palatal, and synovial joint swab samples from a broiler flock after death by PCR method (Rajkumar et al., 2018). In another study, 27.9% of tracheal swabs of broilers and laying hens were found to be infected with *Mycoplasma synovium* by Multiplex-PCR method (Mettifogo et al, 2015). Also, *Mycoplasma synovium* contamination was observed in 23.25% of laying hens tracheal swab samples by Duplex Real Time PCR method (Galluzzo et al, 2022). In another study, they reported the prevalence of synovial mycoplasma to be 33.33% by examining tracheal and cleft palate samples of industrial turkeys in different regions of Iran (Rasoulinejad et al, 2018). The percentage of contamination of the trachea tissue obtained from these studies is in close agreement with the present study. Several factors such as ammonia in the breeding environment, dust, herd density and distance play an important role in respiratory diseases (Kleven and Ferguson-Noel, 2008). On the other hand, in another study, synovial mycoplasma was reported

in 72% of tracheal swab samples of laying hens in Khuzestan province by PCR method. The reason for the high prevalence of MS in this research is the lack of vaccination of the studied farms (Gharibi et al, 2018). In another study, synovial mycoplasma was reported in 55% of tracheal swab samples, palatopharyngeal cleft and air sacs of industrial poultry farms in Tehran, Markazi and Qazvin provinces by PCR method (Maghami et al, 2013). Also, in another study, synovial mycoplasma was detected in 75% of tracheal swab samples obtained from laying hen flocks in Germany by PCR method (Koehn et al, 2009). The higher prevalence of synovial mycoplasma in the mentioned studies compared to the results of the present study can be due to the method of sampling (sampling with a swab or taking a part of the tissue), the number of samples, age, region, sample season, vector (Seifi and Shirzad, 2012) and the occurrence of immunodeficiency diseases (Sadrzadeh, 2015).

In the present study, mycoplasma contamination of synovium was observed in a number of apparently healthy samples. Also, in a study, synovial mycoplasma was

detected by PCR method in 33% of the palate swab samples of apparently healthy chickens and chickens with respiratory problems (Rajkumar et al, 2018). In another study, with the molecular analysis of tracheal swabs of 21 broilers (17 herds with respiratory problems and 4 apparently healthy herds), it was reported the prevalence of synovial mycoplasma in 8 herds (38.1%), and in this study, synovial mycoplasma in 2 apparently healthy herds were positive (Ghaniei, 2016). The detection of mycoplasma in apparently healthy herds is one of the factors that should be considered in monitoring and screening. The herds were positive for the presence of bacteria in the absence of clear macroscopic signs, and therefore, it should be considered from a managerial and clinical point of view.

According to the results of the present study, synovial mycoplasma is common among broilers under investigation in Ahvaz slaughterhouse. Therefore, the policy makers of the health system of the poultry breeding industry should prevent the economic losses caused by this disease with preventive measures.

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Conflict of interest

The authors declare that they have no conflict of interest.

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ردیابی مولکولی مایکوپلازما سینوویه در گله‌های ماکیان گوشتی با جراحات تنفسی در کشتارگاه اهواز

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چکیده

بیماری مایکوپلازما سموز طیور یکی از بیماری‌های مهم در صنعت طیور است. مایکوپلازما سینوویه از عوامل مهم ایجاد کننده‌ی بیماری در ماکیان است که موجب عفونت دستگاه تنفسی و سینوویت می‌گردد، که منجر به خسارات اقتصادی قابل توجهی به صنعت طیور در سراسر جهان می‌شود. در این مطالعه، عفونت مایکوپلازما از گله‌های طیور گوشتی کشتارگاه اهواز با استفاده از روش مولکولی مورد بررسی قرار گرفت. بدین منظور تعداد ۲۰۰ نمونه از بافت‌های ریه و نای و مایع مفصل خرگوشی از ۲۰ گله مرغ گوشتی دارای علائم ماکروسکوپی و ۲۰ نمونه از قسمت‌های مذکور از مرغ‌های گوشتی سالم هر گله در فصل زمستان جمع‌آوری شد. استخراج DNA بافت با استفاده از کیت تجاری شرکت رها زیست پادتن طبق دستورالعمل کیت استفاده شد. سپس آزمایش PCR با استفاده از پرایمرهای اختصاصی مربوط به ژن *Vlha* استفاده شد. نتایج حاصل از PCR برای ۵۵ نمونه ریه و ۴۵ نمونه نای از گله‌های دارای علائم ماکروسکوپی و ۴ نمونه ریه و نای از پرند‌های به ظاهر سالم مثبت تشخیص داده شد. در بررسی مولکولی نمونه‌های مفصل مورد مثبتی از نظر وجود مایکوپلازما سینوویه مشاهده نگردید. نمونه مثبت مایکوپلازما سینوویه جهت تعیین توالی به شرکت ژن فن‌آوران فرستاده شد. توالی بخشی از ژن *Vlha* این جدایه با سایر جدایه‌های موجود در بانک ژن مورد مقایسه قرار گرفت و حدود ۹۹ درصد با جدایه‌های ایرانی قبلی شباهت داشت (*IRG1/C/08*, *IRG6/C/08*, *IRG11/C/09*).

کلمات کلیدی: مایکوپلازما سینوویه، مرغ گوشتی، PCR، اهواز

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