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MYCOPLASMA GALLISEPTICUM BASED MOLECULAR AND PHYLOGENETIC STUDIES OF INFECTED CHICKEN FARMS IN IRAQ

Abbas Hadi Jasim Al-Mahmoudi, Hasan Ali Hammadi, Hayder Naji Ayyez, Israa Najm Abdullah Al-Ibadi*, Hala Mohsin mutter and Ahmed Jasim Neamah

College of Veterinary Medicine, University of Al-Qadisiyah, Iraq.

Abstract

The current survey was focused on detecting the local strains of *Mycoplasma gallisepticum* infecting chicken in Iraqi farms from Al-Diwaniyah province. The study involved the use of tracheal and air sac swabs in a polymerase chain reaction (PCR) method and partial gene sequencing (four positive PCR products) that targeted *16S rRNA* gene. The results found that 90% of the samples were positive to the PCR, and the local strains closely aligned to strains from South Africa. In conclusion, the current findings demonstrate the presence of *M. gallisepticum* in the tested farms with similar identity to those strains detected from South Africa.

Key words: Airsacculitis, chronic respiratory disease, *Mycoplasma gallisepticum*, partial gene sequencing

Introduction

Mycoplasma is a very small prokaryote that belongs to class molicutes which characterized by its small genome size and lack the cell wall (El-Aziz, *et al*., 2014). There are four pathogenic *Mycoplasma* species in poultry. *M. gallisepticum* is consider as one of the important pathogens in multifactorial disease complex, and it causes reduction of carcasses, a decrease in feed conversion ratio, and depletion to the financial resources via vaccination and drug programs. *M. gallisepticum* affects turkeys and chickens causing infections in body parts such as airsacs (airsacculitis) and sinuses (infraorbital sinusitis) in a major disease commonly known as chronic respiratory disease (CRD) of a-wide-range-clinical-sign poultry illness (El-Aziz, *et al.,* 2014).

Some reports referred to the occurrence of neurological clinical signs induced by *M. gallisepticum* infections in turkeys (Wyrzykowski B, *et al*., 2013). Some bacterial and viral infections may predispose the incidence of CRD in chickens such as *Escherichia coli*, Newcastle disease (vaccine strains), or infectious bronchitis virus (El-Aziz, *et al*., 2014).

M. gallisepticum can be diagnosed using three main lines, isolation of the microorganism, serological identification, and molecular detection. Using cultivation processes may add financial and time unnecessary expenditures (Liu, T., *et al*., 2001). Although serological approaches are commonly used for successful clinical phase identification of *M. gallisepticum*, subclinical phases of the CRD cannot be detected properly by those techniques. Interestingly, improved diagnosis of *M. gallisepticum* have been introduced by using molecular techniques such as polymerase chain reaction (PCR) methods and nucleic acid based sequencing approaches (Khalifa, K.A., *et al*., 2013).

The present study on *M. gallisepticum* was focused on determining the molecular characterization and sequencing of the microorganism isolated from diseased chickens in Iraq.

***Author for correspondence :** E-mail : esraa.najm@qu.edu.iq

Materials and Methods

Collection of blood samples

Samples from trachea and air sacs of flocks suffered a respiratory problem in Al-Diwaniyah province, Iraq, were collected with sterile cotton swabs kept in ice-cooled 2-ml-PBS-contained tubes until they were transported to the processing laboratory.

DNA extraction

The DNA of *M. gallisepticum* was subjected to extraction procedures using the clinical swab samples that were PBS-twirled and tube-side-wall-pressed for

eluting the clinical materials. The process was conducted by utilizing a DNA purification kit and its instructions purchased from Geneaid Company, Korea.

DNA harvests were evaluated by a NanoDroop 2000 (Thermo Scientific, German), and the range of the concentration was 6 to 20ng/µl**.** A deep freezer at -20ÚC was used to store the DNA for later analyses.

Polymerase chain reaction

The primers for amplifying the target gene, *16S rRNA*, piece (643bp), were sense primer 8F (5-ATTAGAGGCGAACGGGTGAG-3) and antisense (5-CGCCTCTGGTGTTCTTCCAT-3) which designed in this study. The PCR reaction was conducted in a thermocycler (Bioneer, Korea) using the following criteria: initial denaturation 94°C for 5 min followed by 35cycles of (94°C for 30 sec. ; 58°C for 30 sec and 72°C, 1 min) then 72°C for 7 min extension after the last cycle. PCR mixture was 50 µl contained: 10 mMTris/ HCl, pH 8.3; 50 mMKCl; 2.5 mM MgCl2; 200 mM each of deoxynucleoside triphosphates (dNTPs); 15pM each primer; and 2U Taq polymerase. DNA at 5µl as a template was employed to be a part of the total volume of the PCR reaction of 50µl. For electrophoresis, a 1.5% agarose gel was recruited for running the PCR product screening which was done by taking images of the gel bands by using a Gel documentary (Bionead, Korea).

DNA sequencing and phylogenetic analysis

The purified PCR product amplicon fragment was sequenced by Bioneer Company, Korea, targeting four PCR positive samples. The accession numbers, MH986181.1, MH986182.1, MH986183.1 and MH986184.1, were obtained from the deposition of the resulted sequences in the GeneBanck (NCBI).

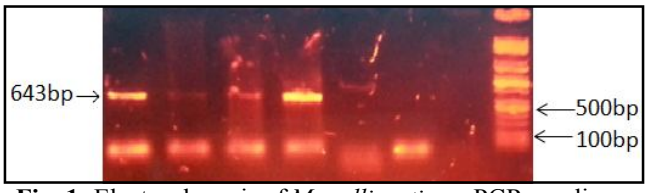

Fig. 1: Electrophoresis of *M. gallisepticum* PCR amplicon.

Results

Samples from four farms were positive for *M. gallisepticum* which successfully amplified the target gene by PCR in 90% of collected clinical specimens in which the amplicon size was 643 bp Fig. 1.

According to nucleotide phylogenetic tree of the *16S rRNA* gene, the four amplicons were arrangrd closely with a *M. gallisepticum* strain, B878-14-M, and with other NCBI BLAST isolates table 1 and Fig. 2. This means that there was 100% matching with the *16S rRNA* gene sequence of this strain and the other world strains deposited in the GenBank. The GenBank accession numbers of our study strains are MH986181.1, MH986182.1, MH986183.1 and MH986184.1 Fig. 3.

Discussion

M. gallisepticum is a well-known pathogenic microorganism that causes, in chickens, chronic respiratory disease, and a severe disease in turkeys. For the prevention of the related diseases, attenuated vaccines of this pathogen are present for chickens; however, they are either not efficient or not safe for turkeys. It has been *in-vitro*-shown that *M. gallisepticum* can infect nonphagocytic cells to stay inside those cells for a minimum of 48hrs for surviving, multiplying, and spreading systemically to other organs launching from the respiratory system (Vogl G, *et al*., 2008) (Wijesurendra DS, *et al*., 2015).

The PCR outcomes revealed successful amplification of the 643bp-region of the *16SrRNA* gene in 90% of the samples. Targeting the same gene, (Rasoulinezhad S, *et*

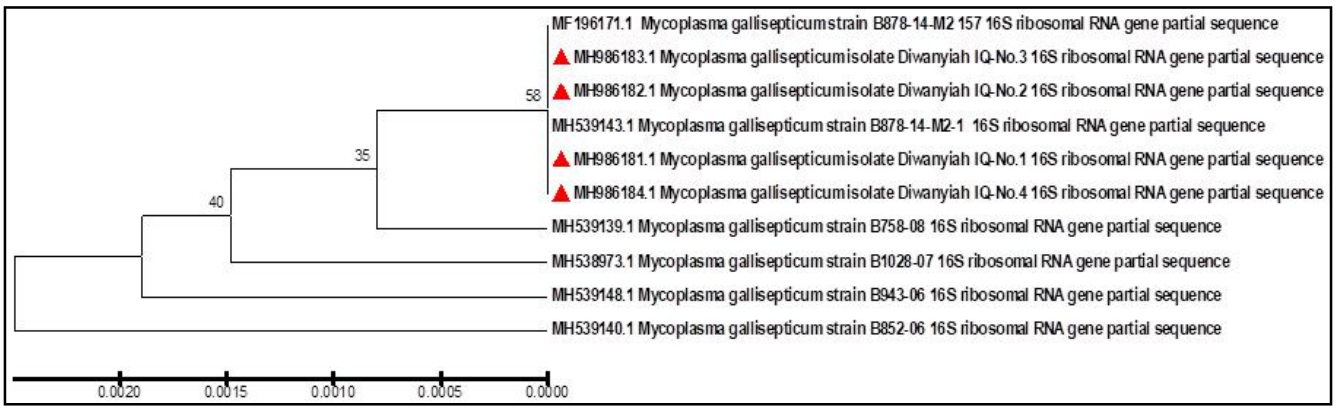

Fig. 2: *Mycoplasma gallisepticum 16S rRNA* partial gene sequencing based phylogenetic tree analysis constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA v6.0). The local strain No.1 was aligned with a world strain, B878-14-M2-1 (MH539143.1).

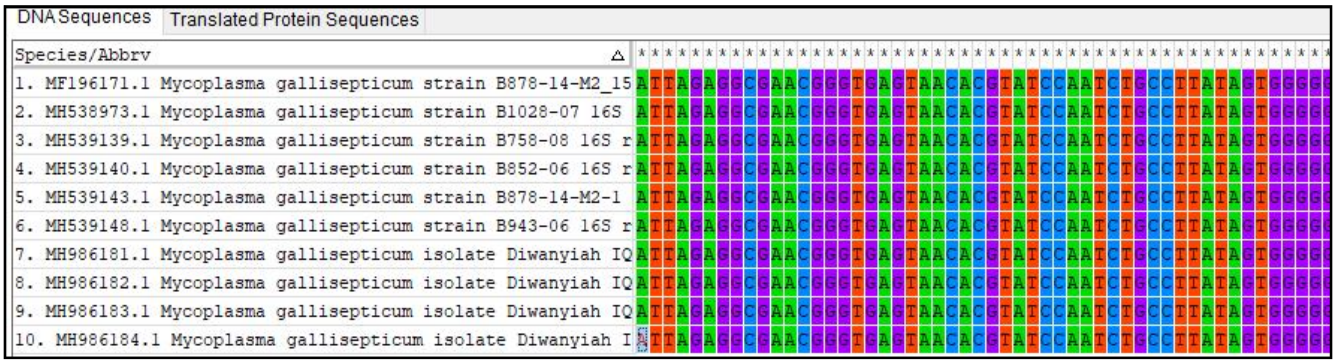

Fig. 3: *Mycoplasma gallisepticum 16S rRNA* partial gene sequencing based multiple sequence alignment analysis (MSAA) employing (MEGA v6.0, the MSAA tool). The MSAA similarity (*) and nucleotide substitution between the local and NCBI strains.

Table 1: Homology sequence identity between local and NCBI strains.

Name of isolate	Genbank submission accession number	NCBI-BLAST Homology Sequence identity			Identity (100%)
		NCBI BLAST identity isolate	accession number	Country	
Mycoplasma gallisepticum isolate No.1	MH986181.1	*Mycoplasma gallisepticum* strain B878-14-M2-1	MH539143.1	South Africa	99%
Mycoplasma gallisepticum isolate No.2	MH986182.1	*Mycoplasma gallisepticum* strain B878-14-M2-1	MH539143.1	South Africa	99%
Mycoplasma gallisepticum isolate No.3	MH986183.1	*Mycoplasma gallisepticum* strain B878-14-M2-1	MH539143.1	South Africa	99%
Mycoplasma gallisepticum isolate No.4	MH986184.1	*Mycoplasma gallisepticum* strain B878-14-M2-1	MH539143.1	South Africa	99%

al., 2017) has detected *M. gallisepticum* in 48.38% of samples collected from backyard birds; however, they have identified this microorganism in only 16.66% of samples obtained from commercial farms. Moreover, (Wijesurendra DS, *et al*., 2015). Development of a Mycoplasma gallisepticum infection model in turkeys. Avian Pathol. 44(1): 35–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/254310018) have also revealed the presence of *M. gallisepticum* in farms of infected chickens using a PCR method by which they have also been able to detect vaccine strains which is important in making a better diagnosis of the related diseases. Moreover, (Vogl G, *et al*., 2008) have been able to identify *M. gallisepticum* inside erythrocytes of infected chickens using a nested PCR. Those practical studies confirm the current findings and the ability of the present used PCR method in detecting the microorganism in the infected chickens.

The phylogenetic analysis revealed that the current strains were closely aligned to two isolates from South Africa. The migrating birds around the world may play a big role in spreading the infectious agent from an area to another, and this was validated in eastern North America in which wild birds have been found to carry *M. gallisepticum* (Dhondt AA, *et al*., 2014).

References

Dhondt, A.A., J.C. DeCoste, D.H. Ley and W.M. Hochachka (2014). Diverse wild bird host range of *Mycoplasma gallisepticum* in eastern North America. *PLoS One.,* **9(7):** e103553. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25061684.

García, M., N. Ikuta, S. Levisohn and S.H. Kleven (2005). Evaluation and Comparison of Various PCR Methods for Detection of *Mycoplasma gallisepticum* Infection in Chickens. *Avian Dis.,* **49(1):** 125–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15839425.

Khalifa, K.A., E.S. Abdelrahim, M. Badwi and A.M. Mohamed (2013). Isolation and molecular characterization of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in chickens in Sudan. *J. Vet. Med.,* **10:** 1155/2013/208026.

Liu, T., M. Garcia, S. Levisohn, D. Yogev and S.H. Kleven (2001). Molecular variability of the adhesin-encoding gene *pvpA* among *Mycoplasma gallisepticum* strains and its application in diagnosis. *J. Clin. Microbiol.,* **39:** 1882-1888.

Rasoulinezhad, S., M.H. Bozorgmehrifard, H. Hosseini, N. Sheikhi and S. Charkhkar (2017). Molecular detection and phylogenetic analysis of *Mycoplasma gallisepticum* from backyard and commercial turkey flocks in Iran. *Vet. Res. forum an Int. Q. J.,* **8(4):** 293–8. Available from: http://

www.ncbi.nlm.nih.gov/pubmed/29326787.

Vogl, G., A. Plaickner, S. Szathmary, L. Stipkovits, R. Rosengarten and M.P. Szostak (2008). *Mycoplasma gallisepticum* invades chicken erythrocytes during infection. *Infect Immun.,* **76(1):** 71–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17954728.

Wijesurendra, D.S., A. Kanci, K.A. Tivendale, B. Bacci, A.H. Noormohammadi and G.F. Browning (2015). Development of a *Mycoplasma gallisepticum* infection model in turkeys. *Avian Pathol.,* **44(1):** 35–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25431001.

Wyrzykowski, B., O. Albaric and S. Moreau (2013). Retrospective study of *Mycoplasma gallisepticum* meningoencephalitis in six turkey flocks in western France. *J. Comp. Pathol.,* **148(2):** 173-177.