MOLECULAR DIAGNOSIS OF *MYCOPLASMA GALLISEPTICUM* IN TURKEY IN MOSUL CITY

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ABSTRACT

Mycoplasma gallisepticum cause respiratory infection in Turkey which lead to economic losses in poultry industry. The study included the diagnosis of *M. gallisepticum* of Turkeys. Seventy samples were collected from the trachea of newly slaughtered turkeys in different regions of Mosul city for the period between September 2020 to February 2021. The samples were placed i n sterile bags and transferred directly to PCR laboratories at College of Veterinary Medicine under cooling conditions. The samples were kept under freezing conditions until the examination was performed. DNA was extracted according to the provided Company (gSYNC ™ Geneaid kit). A PCR test was performed using two pairs of primers (MY- GA) provided by the Korean company BIONEER for the purpose of diagnosing the genus of the mycoplasma in general and the type *M. gallisepticum* in particular, and then electrophoresis was performed using agarose 2%, (Biometra, Germany). The results showed that 64.3% of the samples were positive for the mycoplasma genus and 28.8% for the species *M. gallisepticum.*

Key words: *Mycoplasma gallisepticum*, PCR, Turkey

Introduction

Mycoplasma gallisepticum is an important offender that infect the respiratory system of the avian species especially turkeys and chicken causing chronic and sinus infection, respectively (Quinn *et al.*,2011). This lead to losses for poultry keepers represented in reduced feed conversion, decrease production of eggs, and increase vaccination and medication costs (Raviv and Ley, 2013). Infection with MG can occur vertically or horizontally, which plays potent role in decreasing the hatchability rates of the fertile eggs of turkeys with embryonic death (OIE, 2008; Ferguson-Noel and Noormohammadi, 2013).

The observed symptoms of mycoplasmosis in turkey are more severe than in chickens such as shortness of breath and sever coughing fits, swelling of the paranasal sinuses depression, emaciation and nasal discharge (Ley, 2008). In addition to some reports which indicate to neurological symptoms caused by MG in turkey (Wyrzykowski *et al*., 2013; Eissa *et al*., 2011). The culture methods which is used for the diagnostic of avian pathogenic mycoplasmas is complicated because it is fastidious bacteria, slow growing and the incubation period consume 14-21 days (Zakeri, 2016). Therefore, rapid diagnostic methods are required to check the safety of birds from mycoplasma or to eliminate the pathogen from the flock (Beko *et al*., 2019; Ghanem *et al*., 2018; Kanci *et al*., 2018).

Recent publications were focused about rapid test including serological tests as ELISA and immunoflorecent conjugated antisera (Qasem *et al*., 2015; Ul-Haque *et al*., 2017) and molecular examination like PCR, RAPD amplification reactions (Ferguson *et al*., 2005; El-Gazzar, 2020; Rasoulinezhad *et al*., 2017; Felice *et al.,* 2020; Gracia *et al*., 2005). *M. gallisepticum* possess some of the virulence factors, therefore, recently studies put high light to use the analysis of gene targeted sequencing (GTS) for the genes of surface protein (MG) as a classifying method that introduced to diagnosis MG strains and differentiation between them, as well as the revealing cyt adhesin protein mgc2 gene responsible for production of cyt adhesin protein, which play role in the anchoring the pathogen to the surface of the host (Eissa *et al*., 2011; Gracia *et al*., 2005; Ammar *et al*., 2014). Since there are lack or scarcity studies on Mycoplasma in turkeys in Iraq, especially in Mosul city, so our study aimed to diagnose the *M. gallisepticum* using PCR.

Materials and Methods

Sample collection

Seventy tracheal samples were collected from newly slaughtered turkeys in Mosul city at the period between September 2020 to February 2021. Sample were transferred to specialized laboratories to be molecularly diagnosed and to determine infection, under cooled conditions.

Genetic processes

DNA extraction: DNA was extracted from tracheal samples according to manufacturer information (gSYNC™ Geneaid extraction kit). Thermodynamic DNA template was designed by Mohammad *et al*. (2019), AL-Jumaa (2017) for detection of *Mycoplasma. spp.* and *M. gallisepticum* we used couple of Korean company supplied templates (BIONEER)as in Table 1 (Aghabalaei *et al*., 2012; Malekhosein *et al*., 2017). The amplification protocol PCR reaction thermal cycler was performed as in Table 2, 3 and 4. A UV transillumination system was used to visualize the Ethidium bromide-stained gel (Biometra, Germany) (Garcia *et al*., 2007).

This result is closely to study of Sokkar *et al*. (1986) who found that 32 % of the bacteriologically examined samples of turkeys in Egypt were *M. gallisepticum* and disagreement with the studies of El-Gazzar (2020), Rasoulinezhad *et al*. (2017), Beko *et al*. (2019), who isolated MG in backyard turkey farms at percentage 48.38%, 45.45%, 13.8%, respectively. These difference in results may be due to the difference in climate from one country to another, as dry weather leads to the

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movement of aerosols to greater distances, which leads to an increase in the chance of exposure to infection horizontally (Ongor *et al*., 2009; Rasoulinezhad *et al.,* 2017), or vertically through the egg (Matucci *et al*., 2020).

Mycoplasma was diagnosed by PCR test (Fig. 1 and Fig. 2) showed the emergence of high isolation rates that explains the efficiency of this method in diagnosis. The results were confirmed previously by Demirbilek *et al*. (2020) and Bharathi *et al*. (2018).

Because serological tests doubtable due to the MG strain with low pathogenicity may not have an immune response or antibody production, also electrophoretic analysis unreliable resulted from antigenic instability in MG therefore, the authors recommended PCR test with high accuracy to detect MG infection because it gives a faster result than isolation and high sensitivity, which makes it the most used in the diagnosis of *M. gallisepticum* disease especially in endemic countries (Ferguson *et al*., 2005; Gracia *et al*., 2005).

Table 1: Primers used for the *M. spp.* and *M. gallisepticum*

Primer	Sequence (5'-3') reference
MY.f	GGG-AGC-AAA-CAC-GAT-AGA-TAC-CCT 21
MY.r	TGC-ACC-ATC-TGT-CAC-TCT-GTT-ACC-CT
GA.f	GAG-CTA-ATC-TGT-AAA-GTT-GGT-C 22
GA.r	GCT-TCC-TTG-GGG-TTA-GCA-AC

Table 2: PCR thermocycler program for Mycoplasma

Cycle	Temp. C°	Time
	95	5min.
30	95	20sec.
	59	0.5 min
	72	0.5 min
1	72	5min.

Table 3: PCR thermocycler program for *M. gallisepticum*

Cycle	Temp. C°	Time
1	95	5min.
30	95	20sec.
	53	0.5 min
	72	0.5min
1	72	5min.

Table 4: The numbers and percentages of Mycoplasma isolated from trachea samples of turkey

Samples	No.	*Mycoplasma* spp	%
Trachea	70	45	64.3
Samples	No.	*M. gallisepticum*	%
Trachea	45	13	28.8

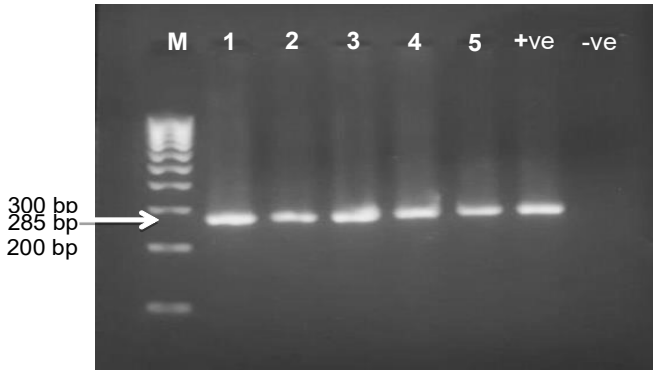

Fig. 1: Electrophoresis and ethidium staining showing as a result of PCR procedures for *Mycoplasma* spp.*,* M: Represent marker (1-5) form positive result for *Mycoplasma spp.* with band size 285 bp; +ve: Positive control, -ve: Negative control

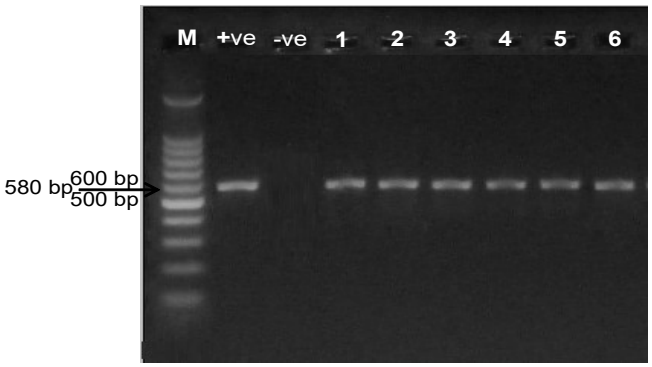

Fig. 2: Electrophoresis and ethidium staining showing as a result of PCR procedures for *Mycoplasma gallisepticum*, M: Represent marker.(1-6): Form positive result for *M. gallisepticum* with band size 580 bp, +ve: Positive control, -ve: Negative control

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