Vet. Med. J., Giza. Vol. 59, No. 3 (2011): 183 - 195

# Molecular characterization of *Mycoplasma gallisepticum* isolated from Chicken and Turkey

**Eissa , S. I.* ; Hassan, A. M.*; Metwally, A. M.**; Hashem, Y. M.* and Abd El-Aziz, E. E.***

* Animal Health Research Institute, Dokki- Giza – Egypt.
** Cairo Poultry Company, Giza, Egypt

**Received:** 06/04/2011
**Accepted:** 17/04/2011

## SUMMARY

*Mycoplasma gallisepticum* (*MG*) infection in chicken and turkey is still one of the important reasons causing economic losses in poultry. The current study concerned with rapid detection and molecular characterization of *MG* isolates. The all samples positive by culture were positive by PCR and rt- PCR. Five isolates (four from chicken and one from turkey) were sequenced for *mgc2* gene. The present molecular study proved that four wild-type *MG* strains. (Eis 3- C-10, Eis 4- C-10, Eis 5- C-10 were recovered from chicken and one (Eis 6- T-10) was recovered from turkey. While Eis 7- C-10 (vaccinal F- strain) was isolated from commercial layer flock vaccinated with F-strain vaccine. We concluded that *mgc2* gene was able to distinguish between *MG* wild - type and vaccinal strains.

## INTRODUCTION

*Mycoplasma gallisepticum* (*MG*) is commonly involved in chronic respiratory diseases in chickens and infectious sinusitis in turkeys (Kleven et al., 1998 and Stipkovits and Kempf, 1996). In layers and breeders, the disease causes drop in egg production, increase in embryo mortality and increased medication costs as additional factors that make this one of the costliest disease problems confronting commercial poultry production worldwide (Bradbury, 2001 and Ley, 2003).

*MG* is relatively fastidious organism, and might require up to 3 weeks for detectable growth. In some cases, the isolation of avian mycoplasmas is impaired by overgrowth of saprophytic mycoplasmas that inhabit the upper respiratory tract of avian species as well as contaminant bacteria and fungi that may not be successfully inhibited by mycoplasma- selective media (Kleven, 2003).

Recently real- time (rt-PCR) has revolutionized the diagnosis of infection diseases. The combination of excellent sensitivity and specificity, ease performance, speed and inherent quantitative nature has made rt PCR technology an good alternative to conventional PCR testing methods (Raviv and Kleven, 2009; Jarquin et al., 2009 and Kahya et al. ,2010)

More recently, the use of gene – targeted sequencing analysis (GTS) of *MG* surface protein genes as a typing method have been introduced as a approach to identify and differentiate *MG* strains. Amplification of the target sequence in *mgc2* gene was performed using primers located at nucleotide positions 476 and 775 of *mgc2* gene which encodes a second cytadhesin protein known to play a role in the attachment process and identified as genome CDS MGA -0932 (Hnatow et al., 1998 and Garcia et al., 2005). Therefore, the present work aimed to detect *MG* in commercial layers and breeders, as well as to carry out molecular characterization of the isolated strains.

Due to the increased use of *MG* live vaccines. More powerful tools are required to trace the source of infection and to differentiate vaccine strains from circulating field isolates (Ferguson et al., 2005).The present work is concerned with detection and characterization of *Mycoplasma gallisepticum* in Chicken and Turkey, where 15 Egyptian flocks were examined by culture and PCR.

## MATERIALS AND METHODS

### Sampling and sample preparation:

Two hundred tracheal swabs were collected aseptically from 10 chicken flocks (healthy or showing respiratory manifestation) either layers or broiler-breeders. In addition, 50 swabs were taken from sinuses of 5 turkey flocks (Table 1). Different age ranges (from 15 up-to 46 weeks) during the period from April 2010 till December 2010. The swabs were pooled (samples of each flock pooled together) to be used for isolation and DNA extraction.

### Isolation of mycoplasma

All swabs were propagated in Frey's medium broth (Frey et al., 1968) for isolation of mycoplasma. When color of the cultures was changed from red to orange or yellow, the cultures were tested for presence of *MG* by Polymerase Chain Reaction test (PCR).

### Control positive for *MG*:

PG31 reference strain was used as control *MG* in every PCR experiment.

### Reference sequences:

Eighteen GenBank published *MG* field and vaccinal *mgc2* sequences were selected including 9 USA strains sequences, one from Pakistan, 4 from Israel and one from Australia, in addition, 3 vaccinal strains (Table 1).

Table (1): Field and vaccinal *MGc2* sequences were selected to be used in sequence analysis and phylogeny

| # | Strain | GenBank Acc. No. | Type |
|---|---|---|---|
| 1 | EgPk1UAF08 | FJ395202 | Pakistani strain |
| 2 | UHP1 | AY556297 | Israeli strain |
| 3 | OR2 | AY556296 | Israeli strain |
| 4 | BRT14 | AY556291 | Israeli strain |
| 5 | YBS2 | AY556298 | Israeli strain |
| 6 | K4669ATK98 | AY556303 | US strain |
| 7 | R-strain | AY556228 | US strain |
| 8 | S6 | AY556229 | US strain |
| 9 | Au96022 | AY556301 | Australian strain |
| 10 | K435 | AY556237 | USA |
| 11 | K503 | AY556234 | USA |
| 12 | K703 | AY556235 | USA |
| 13 | K730 | AY556236 | USA |
| 14 | K2101 | AY556238 | USA |
| 15 | K5054 | AY556282 | USA |
| 16 | F- strain | AY556230 | vaccine |
| 17 | ts11 | AY556232 | vaccine |
| 18 | 6/85 | AY556231 | vaccine |

**DNA extraction for PCR:**

The DNA extraction was carried out using QIA amp ® DNA Mini kit (QIAGEN, GmbH, Hilden, Germany) according to the manufacturer's instructions. DNA was extracted from cultures grown in Frey's broth culture after 48 hours incubation. Briefly, pooled broth cultures were centrifuged at high speed (12.000 r. p. m. for 3 min.). Then the produced pellets were re-suspended in PBS, washed twice and then reconstituted in PBS. The cell suspension was heated directly at 100 C, and then cooled. After that they were centrifuged at high speed. The supernatant containing mycoplasma DNA were kept at -20 C till used for PCR.

**PCR Amplification according to (Garcia *et al.*, 2005)**

By using Oligonucleotide primers encoding *mgc2* gene. These primers are specific for *mgc2* of *MG*. They have specific sequence and amplify a specific product (300 bp). Primers were prepared in Germany (GACT) by Sigma Company. The primer sequences were as follows: the forward primer, *mgc2*-F [5'-CGC AAT TTG GTC CTA ATC CCC AAC A-3'] and the reverse primer; *mgc2*-R [5-TAA ACC CAC CTC CAG CTT TAT TTC C-3']. PCR procedure was done according to (Garcia *et al.*, 2005) as follows: the amplification was performed by heating the sample for 3 minute at 95 C, then

35 cycles of denaturation for 20 sec at 94 C, annealing for 40 sec. at 58 C, and extension for 1 min at 72 C, a final extension step at 72 C held for 10 min. The analysis of PCR products was performed by using 10 ul of amplified PCR product, mixed with 2µl loading buffer and electrophoresed through 1.5 % agarose gel and DNA was visualized by UV fluorescence after ethidium bromide staining and then photographed.

**DNA extraction for rt PCR:**

DNA was extracted from the samples using QIA amp ® DNA Mini kit (QIAGEN GmbH, Germany) for extraction of DNA of Mycoplasma from the tracheal samples, following the manufacturers' recommendations. Extracted DNA was kept at -20C° until testing.

**Running of *MG* by rt PCR according to (Jarquin *et al.*, 2009). :**

The same primer sets used for conventional PCR were used in SYBR green rt-PCR reaction. The primer set was synthesized by Sigma Chemical Company. A 25 ul total volume reaction mixture consisted of 12.5 ul Quanti Fast SYBR Green PCR, 0.5 uM of each primer, 2ul of DNA template, and water to volume. Cycling parameters were as follows: initial denaturation at 95C for five min. followed by 40 cycles of denaturation at 95C for 10 seconds, combined annealing /extension at 60C for 30 seconds. A standard curve was created and the threshold cycle number (Ct).

**Sequencing and sequence analysis:**

The amplified *mgc2* fragments (4 from chickens and 1 from turkeys) were purified using Gene Jet PCR purification kit; Fermentas (cat no. KO701), and submitted to Macrogen Company (24, Gasan-dong, Geumchun-gu, Seoul 153-781, Korea),for *mgc2* sequencing. Identification of homologies between nucleotide and amino acid sequences of the detected *MG* strains and other strains published on GenBank was done using BLAST 2.0 and PSI-BLAST search programs (National Center for Biotechnology Information "NCBI" http:// www.ncbi.nlm.nih.gov/), respectively. The scores designated in the BLAST search have a well-defined statistical interpretation, making matches easier to distinguish from random background hits (Altschul et al., 1997). The obtained nucleotide sequences' comparisons and their multiple alignments with reference *MG* as well as the deduction of amino acid sequences (*MGc2*) were done using the BioEdit sequence alignment editor (Hall, 1999), ClustalW software for multiple sequence alignment (Thompson et al., 1994), ClustalV (Higgins and Sharp, 1989) and MegAlign (DNASTAR, Lasergene®, Version 7.1.0, USA) (*Kumar et al., 2004*). The phylogenetic trees were constructed using MegAlign for tree reconstruction of sequences by Neighbor-joining method based on ClustalW. Bootstrapping values were calculated using a random seeding value of

111 (Thompson et al., 1994). ClustalV was used when end gaps were faced. Sequence divergence and identity percents were calculated by MegAlign. The structural character of *mgc2* protein sequence was identified by Protean (DNASTAR, Lasergene®, Version 7.1.0. USA).

## RESULTS

### Isolation and PCR results:

Four chicken flocks and one turkey flock were *MG* positive. There are 5 positive flocks by culture method which, further, confirmed and identified as *MG* positive by PCR (Table 1 and Photo1).

**Table (1):** Isolation rate and PCR results of the examined flocks

| Host | No. of tested flocks | Culture | | PCR | |
|---|---|---|---|---|---|
| | | No. of positive | % | No. of positive | % |
| Chicken | 10 | 4 | 40 | 4 | 40 |
| Turkey | 5 | 1 | 20 | 1 | 20 |
| Total | 15 | 5 | 33.3 | 5 | 33.3 |

**Photo. (1):** PCR amplification of *mgc2* gene

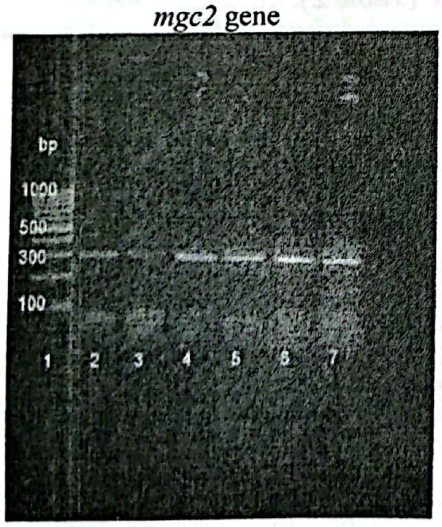


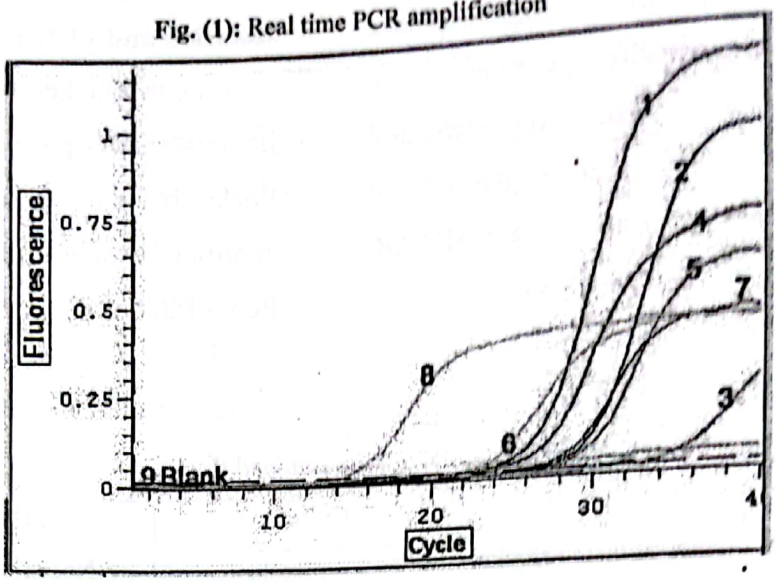

Photo. (1): shows amplification of the isolated five *MG* strains, with PCR product at 300 bp.
Lane 1: 100 bp DNA Ladder (Fermentas)
Lane2: Control Positive (PG31 reference stra
Lane 3-6 : *MG* Field isolates from Chicken;
Lane 7: *MG* Field isolates from Turkey.

### SYBR Green rt PCR results:

The results of MG rtPCR for five positive tested flocks showed amplification signals with SYBR Green dye as shown in Fig. (1). Amplification curves crossed the

threshold with Ct between (14.43- 27.36) indicating positive results.

Fig. (1): Real time PCR amplification




**Sequence results:**

The positive PCR products representing the 5 *MG* positive flocks were selected for sequencing of *mgc2* gene. Sequencing of the PCR products of *mgc2* gene were conducted in both directions and a consensus sequence of 307 bp was used for nucleotide and deduced amino acid analysis. The original sequence was trimmed to remove ambiguous nucleotide sequences usually exist in the beginning of the sequencing reaction. Five *mgc2* sequences were submitted to GenBank database (Table 2).

**Table (2):** the Accession number and the name of the strains submitted to GenBank database

| Accession No. | Designation | Host |
|---|---|---|
| HQ 5911355 | Eis 3 -C -10 | Chicken |
| HQ 5911356 | Eis 4 -C -10 | Chicken |
| HQ 5911357 | Eis 6 -T -10 | Turkey |
| HQ 5911358 | Eis 7 -C -10 | Chicken |
| HQ 5911359 | Eis 5 -C -10 | Chicken |

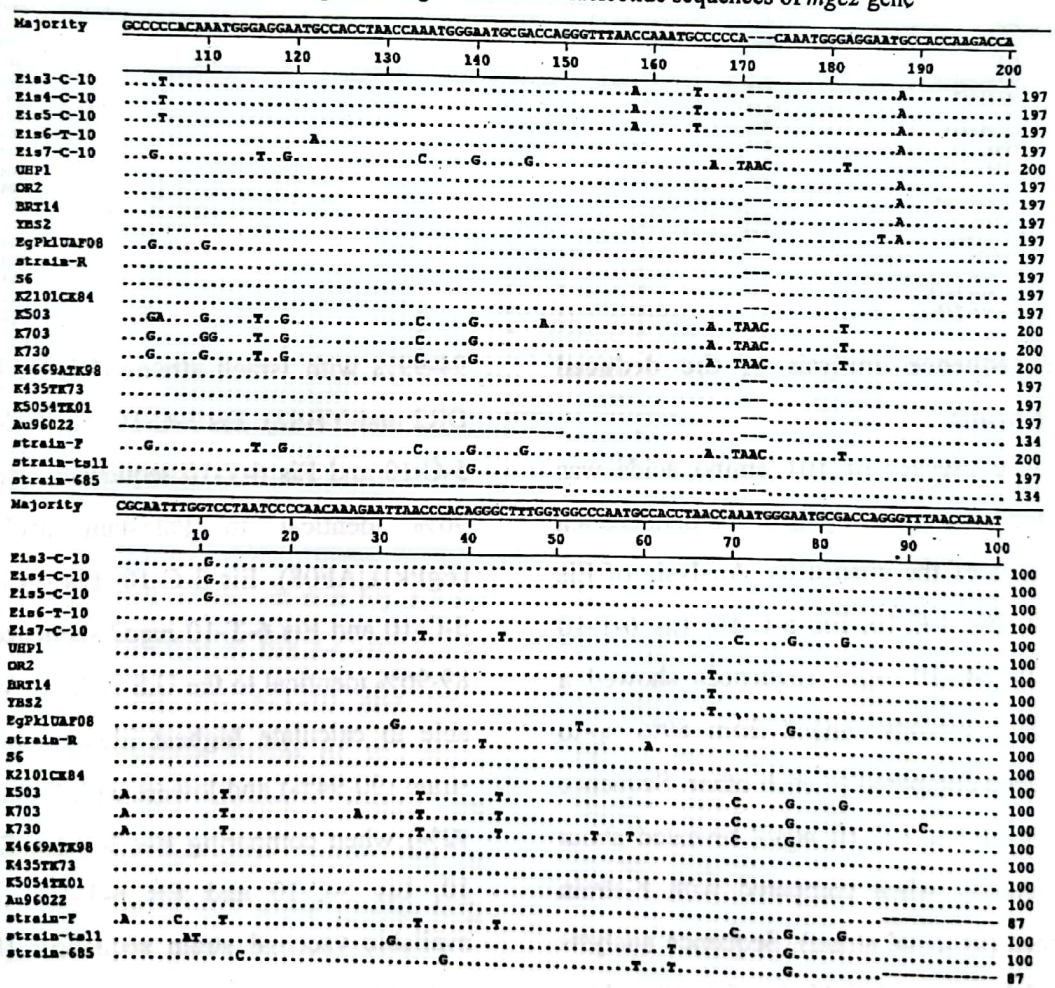
***mgc2* nucleotide sequence analysis:**

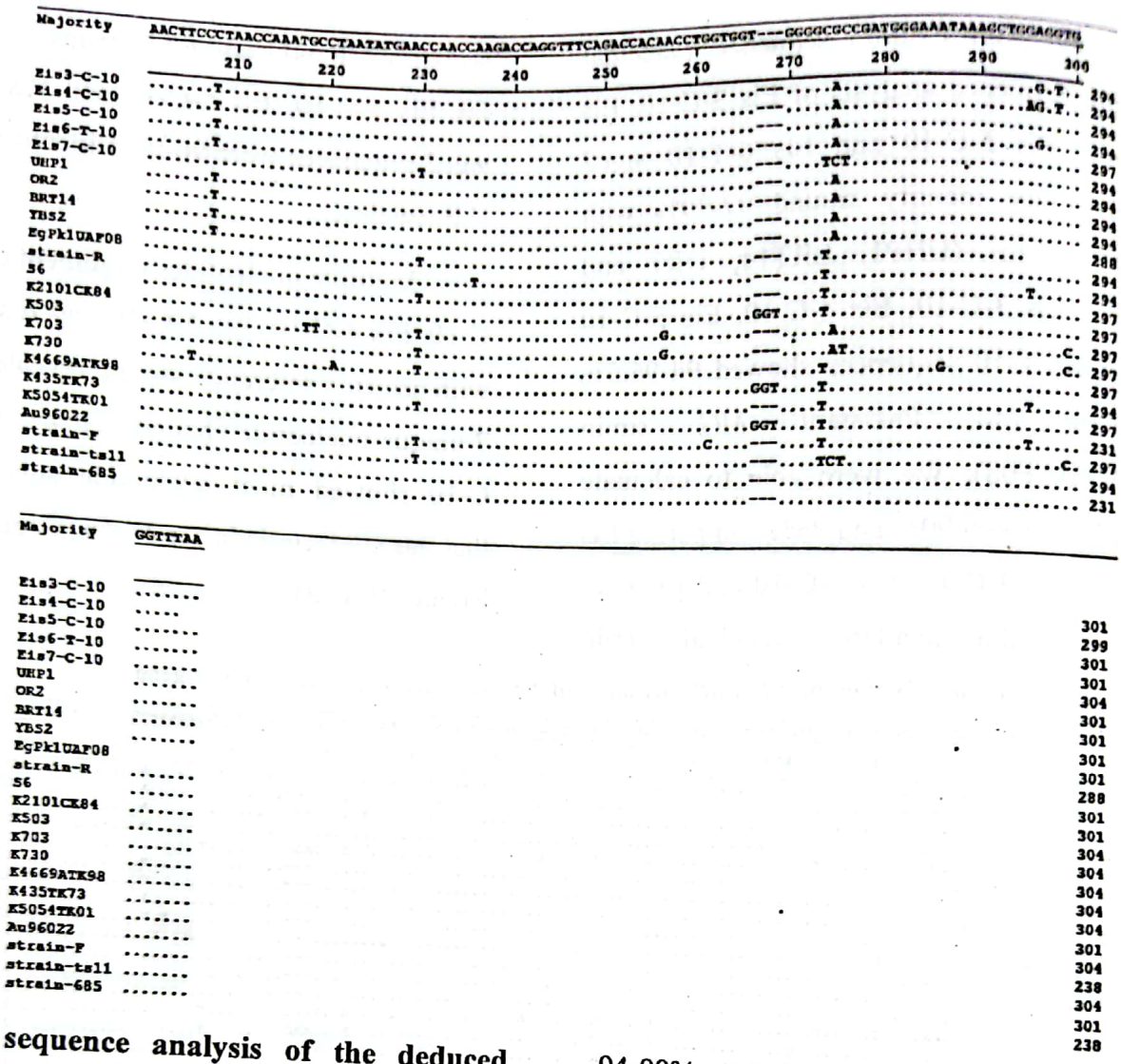
Analysis of Eis 3-C-10, Eis 4-C-10, Eis 5-C-10, Eis 6-T-10and Eis 7-C-10 *mgc2* sequences showed a variable similarity ranged from 91% up-to 99.7% when compared to each other. Sequence analysis of Eis 7-C-10 *mgc2* revealed about 99% homology when

compared with F-strain sequence (vaccinal strain). Sequence analysis of Eis 3-C-10, Eis 4-C-10, Eis 5-C-10 and Eis 6-T-10 *mgc2* demonstrated identity around 97-99% with Israeli strains (UHP1, BRT14, OR2 and YBS2). Eis 3-C-10, Eis 4-C-10, Eis 5-C-10 and Eis 6-T-10 sequences showed 94-96.5% similarity to Pakistani *MG* strain (EgPk1UAF08). We were able to calculate identity between 90% and 96% comparing Eis 3-C-10, Eis 4-C-10, Eis 5-C-10 and Eis 6-T-10 with the available vaccinal strain sequences (F, ts11 and 6/85 strains). Eis 3-C-10, Eis 4-C-10, Eis 5-C-10 and Eis 6-T-10 *mgc2* sequences were 96-98% identical to the U.S isolates.

Multiple nucleotides alignment of Eis 3-C-10, Eis 4-C-10, Eis 5-C-10 and Eis 6-T-10 with selected reference strains revealed up-to 7 unique nucleotide changes. However Eis 7-C-10 showed most nucleotide substitutions that are characteristic for F strain and related isolates (Fig. 2).

**Fig. (2)** ClustalW multiple sequence alignment of the nucleotide sequences of *mgc2* gene



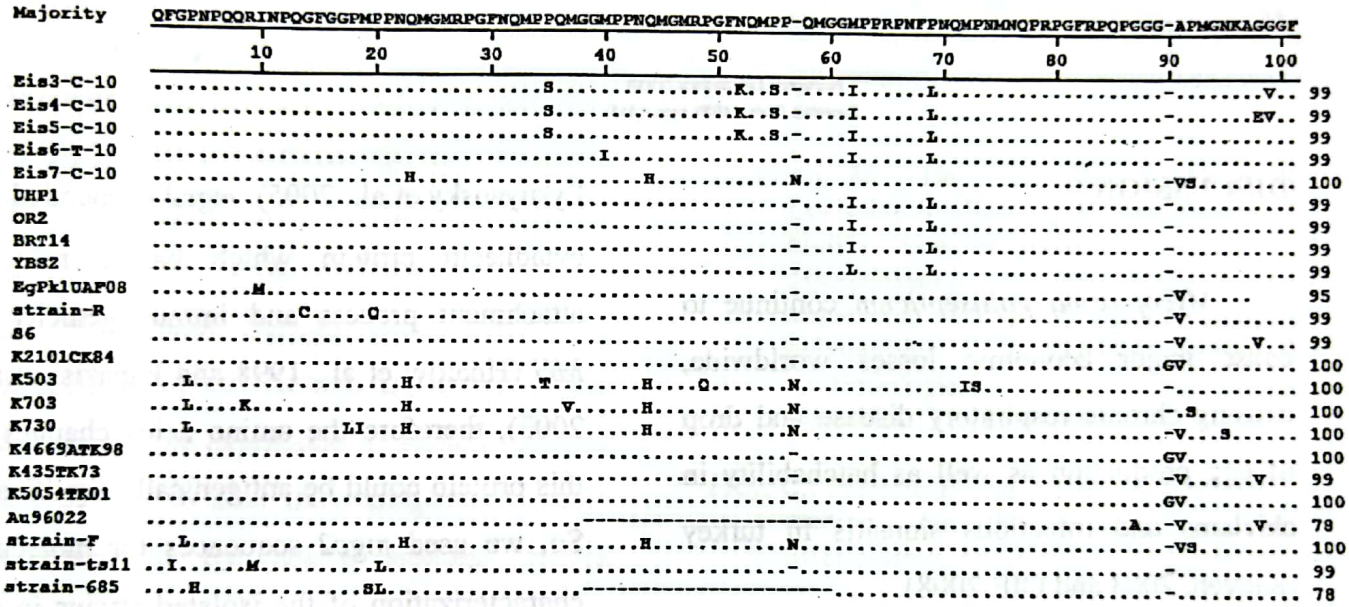



***mgc2* sequence analysis of the deduced amino acids:**

A consensus of 101 amino acids was used for sequence analysis of the deduced a.a sequences of the consensus. Analysis of Eis 3-C-10, Eis 4-C-10, Eis 5-C-10, Eis 6-T-10 and Eis 7-C-10 *mgc2* sequences showed a variable homology ranged from 89% up-to 99% when compared to each other. Sequence analysis of Eis 7-C-10 *mgc2* revealed about 99% identity when compared with F-strain sequence (vaccinal strain). Sequence analysis of Eis 3-C-10, Eis 4-C-10, Eis 5-C-10 and Eis 6-T-10 *mgc2* demonstrated identity around 94-99% with Israeli strains (UHP1, BRT14, OR2 and YBS2). Eis 3-C-10, Eis 4-C-10, Eis 5-C-10 and Eis 6-T-10 sequences were 92.6-96% identical to Pakistani *MG* strain (EgPk1UAF08). Eis 3-C-10, Eis 4-C-10, Eis 5-C-10 and Eis 6-T-10 *mgc2* sequences were 89-96% identical to the U.S isolates. We were able to calculate highest identity with ts11 strain (90-94%) and lowest with F-strain (88-92%) when comparing Eis 3-C-10, Eis 4-C-10, Eis 5-C-10 and Eis 6-T-10 with the available vaccinal strain sequences (F, ts11 and 6/85 strains).

Amino acids sequence analysis of Eis 3-C-10, Eis 4-C-10, and Eis 5-C-10 *mgc2* in comparison with selected reference strains (Fig. 3) showed 3 unique a.a substitutions (P35S, N52K and P55S). In addition to another sole a.a mutation (G99V) in Eis 3-C-10 and Eis 4-C-10 isolates, (G98E) in Eis 4-C-10 and (M40I) in Eis 6-T-10 were observed as well. These characteristic a.a mutations produced marked changes in the antigenic index at 2 regions (a.a49:55 and aa94:99) as predicted by Protean® software (data not shown). We observed two mutations (M62I, P69L), shared Israeli strains, in Eis 3-C-10, Eis 4-C-10, Eis 5-C-10 and Eis 6-T-10 *mgc2* sequences, which lead to increase in the antigenic indices at these locations. However, Eis 7-C-10 revealed 5 out of 6 a.a mutations characteristic (Q23H, Q44H, 57N, A91V, P92S) for F-strain (Fig. 3).

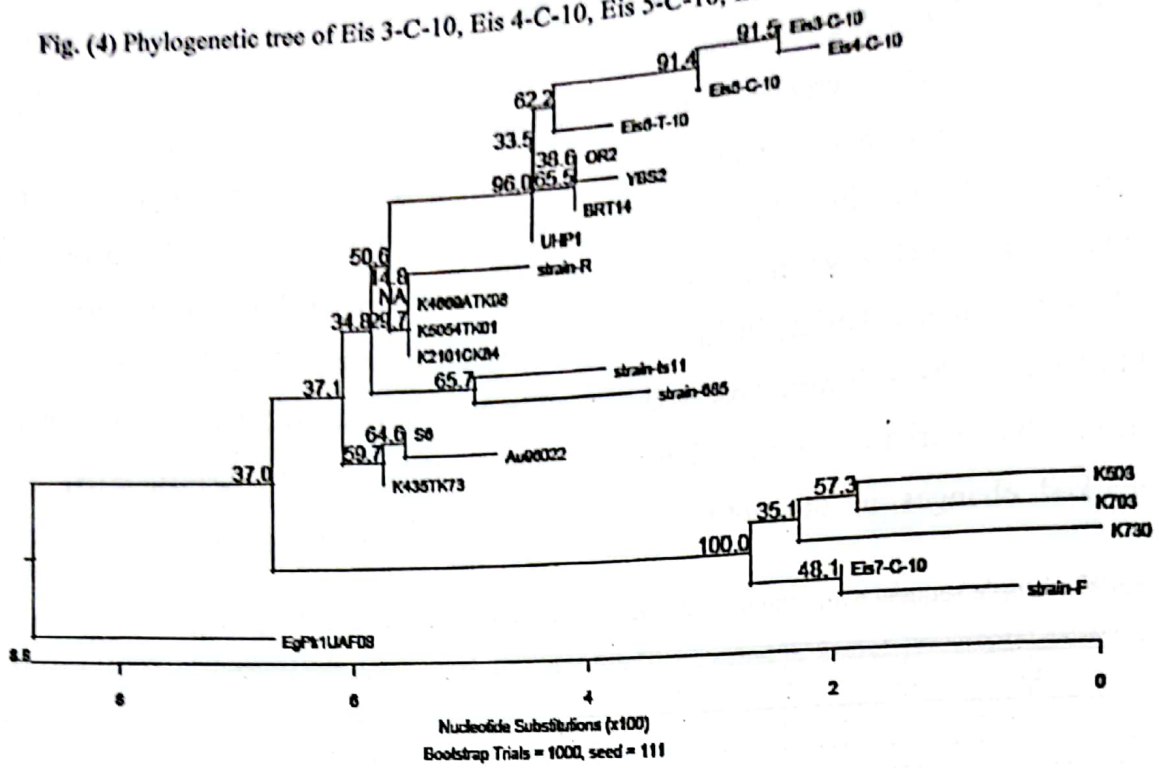
**Fig. (3):** ClustalW multiple sequence alignment of the deduced amino acid sequences of *mgc2* region




**Phylogenetic analysis:**

Phylogenetic tree of Eis 3-C-10, Eis 4-C-10, Eis 5-C-10, Eis 6-T-10 and other reference and vaccinal strains of *MG mgc2* revealed that these Egyptian strains are isolated together in same cluster but in different branches of high bootstrapping, with Israeli strains in one group. While RabE3-09 exist in separate branch close to cluster of Egyptian and Israeli *MG* strains. However, Eis 7-C-10 is isolated with F-strain in separate cluster within group of F-strain related isolates (Fig. 4).

Fig. (4) Phylogenetic tree of Eis 3-C-10, Eis 4-C-10, Eis 5-C-10, Eis 6-T-10 *mgc2* and other reference *MG* strains




## DISCUSSION

*Mycoplasma gallisepticum* continue to cause major economic losses worldwide, causing chronic respiratory disease and drop of egg production as well as hatchability in chickens and infectious sinusitis in turkey (Kleven, 2003 and OIE 2008).

The success of control programs depend on accurate and timely diagnosis of infected flocks. Therefore, diagnostic assays with high sensitivity, specificity and fast turnaround time are required for the screening of *MG* in poultry flocks. The rt-PCR method has been used to identify the *MG* strains with higher sensitivity as well as accuracy (Mekkes and Feberwee, 2005 and Callison et al, 2006). Recently, *mgc2* sequence analysis has been developed to be used for characterization of mycoplasma strains (Garcia et al., 2005 and Lysnyansky et al., 2005). *mgc2* is encoded for cytadhesin protein which has a role in attachment process and immunogenicity of *MG* (Hnatow et al., 1998 and Papazisi et al., 2003), therefore the amino acids changes in this protein could be antigenically significant. So, we used *mgc2* sequences for molecular characterization of the isolated strains in our study.

In the present work consistent findings between PCR and rt-PCR result were observed, all isolated samples were PCR and rt-PCR positive. The present study concerned with detection of *MG* infection in chicken and turkey flocks.. Culture showed that only 40% of the tested flocks of chickens were positive and 20% in turkey, this was inconsistent with Khalifa, 2009, who mentioned that 66.6% of tested chicken flocks were positive for *MG*. In

the present molecular study, we established the presence of 3 field *MG* strains: Eis 3-C-10, Eis 4-C-10 and Eis 5-C-10 in chicken flocks, and one field strain (Eis 6-T-10) isolated from a turkey flock, as well as F-strain related isolate (Eis 7-C-10) which was isolated from another chicken flock.

Nevertheless the phylogentic analysis demonstrated that Eis 3-C-10, Eis 4-C-10, Eis 5-C-10, Eis 6-T-10 are closely related to Israeli strains, however sequence analysis of *mgc2* showed that they have particular and common nucleotide and deduced a.a changes. These characteristic a.a mutations acquired by of Eis 3-C-10, Eis 4-C-10, Eis 5-C-10 and Eis 6-T-10 isolates have resulted in specific changes in this region of the *mgc2* (cytadhesin protein). These changes could affect the antigenicity of such isolates where MG strains can change the expression of surface antigens and thereby to alter the ''antigenic profile'' presented to the host's immune system (Bencina, 2002). Also the sequence analysis revealed presence of F-strain related isolate (Eis 7-C-10) which was able to be circulating in the chicken flocks. Hence, the biosecurity measures about *MG* need to be more concerned.

In conclusion, our data demonstrate the success and continuous evolution of the *MG* in the Egyptian environment. There is an urgent need to develop dynamic mechanisms to combat the emerging *MG* mutants.

## REFERENCES


Altschul, F.; Madden, L.; Schaffer, A.; Zhang, J.; Zhang, Z.; Miller, W. and Lipman, J. (1997): Gapped BLAST and PSI-BLAST : a new generation of protein database search programs. Nuc. Acids Res. 25: 3389-3402.

Bradbury, *M.* (2001): Avian *Mycoplasma* in Poultry diseases. 5th ed. Jordan f., Pattison M, Alexander D. and Faragher T., eds.W,B saunders, London. pp.178-193.

Callison, A.; Ribet, *M.* ; Ikuta, N., Hilt, D.; Leiting, V.; Kleven, H.; Suarez, L. and Garcia, *M.* (2006): Development and validation of Real- Time Taqmqn polymerase chain reaction assay for the detection of *Mycoplasma gallisepticum* in naturally infected birds. Avian Dis.50:527-544

Ferguson, *M.* ; Hepp, D.; Sun, S.; Lkuta, N.; Levisohn S.; Kleven, H. and Garcia, *M.* (2005): Use of molecular diversity of *Mycoplasma gallisepticum* by gene-targeted sequencing (GTS) and random amplified polymorphic DNA (RAPD) analysis for epidemiological studies. Microbiol.151:1883-18993.

Frey, C.; Hanson, P. and Anderson, P. (1968): A medium for the isolation of avian *Mycoplasma*. Ann.Vet. Res.29:2164-2171.

Garcia, N.; Ikuta, S.; Levisohn, N. and Kleven, H (2005): Evaluation and comparison of various PCR methods for detection of *Mycoplasma gallisepticum* infection in chickens. Avian Dis.49:32-125.

Hall, A. (1999): BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nuc.Acids Symp. Ser.41:95-98.

Higgins, G. and Sharp, M. (1989): Fast and sensitive multiple sequence alignment on a microcomputer. Comput.Applic.Biosci.5:151-153.

Hnatow, L.; Keeler, R.; Tessmer, L.; Czymmek, K. and Dohms, J. (1998): Characterization of MGC2, a *Mycoplasma gallisepticum*

Cytadhesin with Homology to the *Mycoplasma pneumonia* 30-Kilodalton Protein P30 and *Mycoplasma genitalium* P32. Infect. Immun.66:3436-3442.

Jarquin, R.; Joseph, S.; Irene, H. and Steven, C.R. (2009). Development of a real-time polymerase chain reaction assay for the simultaneous detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* under industry conditions. Avian Dis., 53, 73-77.

Kahya, S.; Seran, T.; Aysegul, E. and Kamil, T. C.(2010). Real-Time PCR culture and serology for the diagnosis of *Mycoplasma gallisepticum* in chicken breeder flocks. Vet. Microbiol., 144, 319-324.

Khalifa, R. A. (2009). Application of real – time PCR and gene sequencing of field *Mycoplasma gallisepticum* isolates recovered from broiler breeders. Ph.D. Thesis, Fac. Vet. Med., Cairo University

Kleven, H. (2003): Changing expectations in the control of *Mycoplasma gallisepticum*. Introduction in diseases of poultry, 11th ed. Y.*M.* Saif, H.J. Barnes, J.R. Glisson, Iowa State University Press Ames, IA. pp.719-721.

Kleven, S. H., H.-H. Fan, and K. S. Turner (1998). Pen trial studies on the use of live vaccines to displace virulent Mycoplasma gallisepticum in chickens. Avian Dis. 42:300–306.

Kumar, S.; Tamura, K. and Nei, *M.* (2004): MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief. Bioinfor*M.* 5:150-163.

Ley, H. (2003): *Mycoplasma gallisepticum* infection. In: Diseases of Poultry, 11th ed.Swayne,D.E., eds. Lowa State University Press, Ames, Lowa. pp.722-744.

Lysnyansky, I.; Garcia, M . and Levisohn,S. (2005): Use of *mgc2*- Polymerase chain reaction- Restriction fragment length polymorphism for rapid differentiation between field isolates and vaccine strains of *Mycoplasma gallisepticum* in Israel. Avian Dis.49:238-245.

Papazisi, L.; Gorton, S.; Kutish, G.; Markham, F.; Browning, F.; Nguyen, K.; Swartzell, S.; Madan, A.; Mahairas, G. and Geary, J.(2003): The complete genome sequence of the avian pathogen Mycoplasma gallisepticum strain R low.Microbiol. 149, 2307 – 2316.

Mekkes, R. and Feberwea,A. (2005): Real-time Polymerase Chain Reaction for the qualitative and quantitative detection of *Mycoplasma gallisepticum*. Avian Pathol.34, 348-354.

OIE (2008): Avian mycoplasmosis (*Mycoplasma gallisepticum*, *M. synoviae* in Manual of diagnostic tests and vaccines for terrestrial animals. Chapter, 2, 3, 5.

Raviv, Z. and Kleven, H. (2009): The Development of diagnostic Real-time TaqMan PCRs for the four pathogenic avian *Mycoplasmas*. Avian Dis.53:103-107.

Stipkovits, L. and Kempf, L. (1996): Mycoplasmosis in poultry. Revue Technique, Office International des Epizooties. 15:1495-525.

Thompson, D.; Higgins, G. and Gibson, J.(1994): CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nuc. Acids Res.22:4673-4680.

# التوصيف الجزيئي للميكوبلازما جاليسبتكم المعزولة من الدجاج والرومي

صبري إسماعيل عيسي*، عبد الواحد محمود حسن*، أشرف متولي**، يسرية هاشم محمد*، عيد السعيد عبد العزيز*

*قسم بحوث الميكوبلازما - معهد بحوث صحة الحيوان – الدقي
** شركة القاهرة للدواجن – الجيزة– مصر

عدوي الميكوبلازما جاليسبتكم في الدجاج والرومي مازالت ضمن المسبب الرئيسي للخسائر الأقتصادية في الدواجن، الدراسة الحالية تهتم بالكشف السريع والتوصيف الجزيئي لمعزولات الميكوبلازما جاليسبتكم.كل المعزولات الإيجابية بالعزل كانت إيجابية باختبار أنزيم البلمرة المتسلسل واختبار انزيم البلمرة حقيقي الوقت.

تم إجراء أختبار التتابع النيوكليوتيدي بجين (mgc2 gene) لخمسة معزولات(٤ من الدجاج وواحدة من الرومي).

أثبتت الدراسة الجزيئية الحالية أن الأربع عترات المعزولة من قطعان مصابة، ثلاثة من الدجاج (عيسي10-3 عيسي ٤- ١٠، عيسي ٥ - ١٠ ) والرابعة من الرومي (عيسي٦-١٠) أنها عترات حقلية بينما العترة (عيسي ٧-١٠) والمعزولة من قطيع دجاج بياض تجاري كانت عترة مستخدمة في التحصين بلقاح أف .ولقد وجدأن كل العترات الحقلية المعزولة قد أكتسبت تغيرات في تتابع النيكلوتيدات أو الأحماض الأمينية أدت إلي تغيرات أنتيجنية مؤثرة.

خلصت هذه الدراسة أن جين (mgc2) قادر علي التفرقة بين العترات الحقلية والأخري المستخدمة في التحصين.