Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Isolation and Molecular Detection of *M. gallisepticum* and F Strain like Isolates from Chicken Flocks Suffering from Respiratory Problems in Egypt

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Abstract

THE STUDY aimed to assess the possibility of molecular typing utilizing a gene exclusive to *M. gallisepticum (MG)* that encodes *a phase-variable putative adhesion protein (PvpA)*. Based on size differences and nucleotide divergence of the C-terminus-encoding region, the *pvpA* PCR-RFLP assay was utilized to distinguish between vaccine F strains and *M. gallisepticum* isolates. The study found a high *M. gallisepticum* infection rate (66.6%, 100%, and 75.75%) in layer, broiler breeder, and broiler flocks, respectively, and a *pvpA* PCR-RFLP clarified that isolations were closely related to the vaccine F strain. The three *M. gallisepticum* isolates and the F-vaccine were evaluated for their pathogenicity and ability to spread in infected and in-contact chickens utilizing clinical symptoms, air sac lesions, PCR, and serological testing at 14 and 28 days after the experimental infection. Isolates sampled at the same time, in the same geographic area, and causing similar pathologic effects under field conditions revealed identical RFLP patterns and were closely situated in the phylogenetic development tree. The study found that in-contact birds in both vaccinated and infected groups showed positive PCR results at 14 days post-infection, indicating shedding and lateral transmission, and the immunity to *M. gallisepticum* field isolates and vaccination against the *M. gallisepticum* F strain was weak until 35 days post-infection. The study suggests that *a pvpA* PCR-RFLP assay can efficiently differentiate vaccine strains from field isolates, eliminating the need for culture isolation, and can also aid in the molecular epidemiology of *M. gallisepticum* epidemics by using sequence analysis or RFLP.

Keywords: *Mycoplasma gallisepticum; pvpA gene;* PCR-RFLP assay; Experimental infection; serology test.

Introduction

Mycoplasma gallisepticum is a significant issue in the poultry industry, leading to chronic respiratory disease (CRD) in chickens and infectious sinusitis (IS) in turkeys and causing notable decreases in global flocks of layer, broiler, and breeder chickens [1, 2]. *M. gallisepticum* control involves eradicating the organism from poultry flocks and maintaining the free status of breeders from *Mycoplasma* and their progeny through biosecurity implementation. Poultry expansion in restricted areas has led to re-evaluating control strategies for *Mycoplasma* infections. In areas where complete eradication is challenging, live vaccine vaccination is used as an alternative control strategy [3, 4]. *M. gallisepticum* has three types of live vaccines that are used in numerous countries around the world: F-vaccine (*Schering Plough, Kenilworth, N.J.*), ts-11-F-vaccine (*Bioproperties, Inc., Australia, marketed in the United States by Merial Select Laboratories, Gainesville, Ga.*), and 6/85-F-vaccine (*Intervet America, Millsboro, Del.*). The rise in vaccination usage necessitates the development of robust tools to identify contamination sources and distinguish vaccine strains

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(Received 15 December 2024, accepted 16 August 2025)
DOI: 10.21608/ejvs.2025.344630.2561

from the circulated infectious biological isolates, thereby enhancing disease epidemiology and control strategies [5].

Many reports by Armour and Ferguson-Noel [6], El Gazzar, Laibinis [7], Ghorashi, Noormohammadi [8] in Egypt, found that Mycoplasma gallisepticum is a pest of the poultry industry [25] discovered by sequencing the mgc2 gene of four closely related isolates close together and placed in a group that has the vaccine strain 6/85 and strain ts11, also Abdelhassieb, Attia [9] discovered by sequencing lipoprotein gene of three closely related isolates close together in one clade and close related with Avipro vaccine CP028147 which is a type of F strain vaccine, other selected strains circulating in Egypt and other selected strains from countries around the world. So, we need to differentiate between the field strain and the vaccine strain.

Randomly amplified polymorphic-DNA (RAPD)-based PCR, or arbitrarily primed PCR, is the most widely used method to identify *M. gallisepticum* strains [10-12]. These techniques are used to recognize vaccine strains in herds inoculated with *M. gallisepticum* and for epidemiological investigations, in accordance with different investigations conducted by different investigators [13]**.** The RAPD reaction requires pure cultures of the target *Mycoplasma* because of the randomized primer and weak-strictness cases, making it expensive, time-consuming, and technically complicated to isolate *Mycoplasmas*, especially when non-pathogenic *Mycoplasma* species outcompete virulent species. Technical factors like target DNA/primer ratio can significantly affect the reproducibility of RAPD patterns in cases where multiple *M. gallisepticum* subtypes exist [14]. Over the past decade, significant advancements have been made in the molecular biology of *Mycoplasmas*, with the identification of numerous protein surfaces in virulent (highly pathogenic) *Mycoplasmas* [15, 16]. Researchers have identified that the high phenotypic genetic diversity of, *Mycoplasma*, significantly contributes to their pathogenicity (infectiousness) and chronic host infection [16-19].

In the course of host infection, *M. gallisepticum* surface topology changes along with the molecular properties of multiple surface protein molecules, such as the putative cytadhesin protein *PvpA* that was recently described [15, 20-22]. The immune system of chickens recognizes *PvpA*, a phase-variable protein *PvpA* is a phase-variable protein that is recognized by the chicken immune system [21, 23]. The immune system of chickens recognizes *PvpA*, a phase-variable protein, as described by Yogev, Menaker [23]. The exposure surface of the C-terminal of the *PvpA* protein contains 28% proline and 52 amino acids of immediate repetitive sequencing, DR-1 and DR-2, as per Boguslavsky, Menaker [22]. *The pvpA* gene's size variation was described in *M. gallisepticum* strains due to deletions in the encoding segment of the proline-rich C-terminus protein region. The eliminations were observed between two direct repetitive sequences, suggesting that molecular genetic sequences of the different strains of *M. gallisepticum* utilizing the C-terminus-encoding protein region of the pvpA gene may be a beneficial focus for epidemiology surveillance of *M. gallisepticum* isolates [2]. The assay's sensitivity was enhanced by designing a semi nested set of primers.

The technique used amplified samples that were typed using restriction enzymes and further sequenced, utilizing RFLP patterns to identify *M. gallisepticum* in the clinical diagnostic samples that were obtained. The assay expedites *M. gallisepticum* isolate diagnosis and characterization, serving as a molecular typing tool for understanding *M. gallisepticum* outbreak epidemiology. The primary objectives of our research were to study the incidence of *MG* infection in the surrounding three governorates' poultry farms in Egypt and to assess the genetic relatedness of them along with vaccine strain targeting the distinctive *pvpA* gene using a restriction fragment PCR length polymorphism (PCR-RFLP assay) and evaluate the pathogenicity (infectiousness) of these isolates and the transmissibility of the F vaccine.

Material and Methods

Sample collection

For PCR a total of 49 clinical samples (using tracheal swabs) were collected from diseased chickens in various governorates, including twelve laying flocks, four broiler breeder flocks, and thirty-three broiler flocks. Four tracheal swabs per flock were submerged in Frey's broth medium and stored at -20 C. The collected clinical samples were approved by the Institutional Animal Care and Use Committee under approval number **(**2023/1/112**).**

Detection of M. gallisepticum from clinical samples by PCR:

DNA Purification:

Swabs from 49 clinical samples (1–1.5 ml) were centrifuged for 10 min at 5000 xg, and the fluid supernatant was discarded. Then, 180 µl of digestion solution and 20 µl of proteinase K were added to the pellets and vortexed for 15 seconds. The solution in micro-centrifuge tubes was put in a water bath at 56°C for 30 min., and then 20 µl of RNAase was added and vortexed for 15 seconds. The tubes were left for 10 minutes (at room temperature). Then 200 µl of lysis solution was added and vortexed for 15 seconds, and then 400 µl of 50% ethanol were added to the tubes and vortexed for another 15 seconds. The purification columns were assembled on the collection tubes, and the solution was transferred to the columns and then centrifuged for 1 minute at

6000 x g. The solution and collection tube were discarded, and new collection tubes were put in. 500 µl of wash buffer was added, followed by centrifugation for 1 min. at 8000 x g, and then the solution in the collection tube was discarded. 500 µl of wash buffer II was added and centrifuged for 3 min. at a maximum speed of ≥12000 x g. The collection tubes were discarded, and the columns were placed over 1.5-ml microcentrifuge tubes. After adding 200 µl of elution buffer, the mixture was incubated for two minutes (at room temperature). The tubes were centrifuged at 8000 xg for 1 minute. The micro-centrifuge tubes containing the DNA were frozen at -20°C.

Primer selection

The primers were chosen from *the pvpA gene* sequence of the R strain, as per Boguslavsky, Menaker [22] study. Using conserved sequences from typical *M. gallisepticum* strains, semi-nested PCR primers were created. The primers are used to locate the direct repeat area within the C-terminus-encoding region of the *pvpA gene*. About the R-strain sequence (2), are the primer locations. Primers 1 *(pvpA1F)*, which is situated at nucleotide locations 415 to 437 (59GCCAM TCCAACTCAACAAGC TGA39), AccuOligo®, and BIONEER were used in the first reaction. Primers 2 *(pvpA2R)*, which is situated at nucleotide positions 1059 to 1081 (59GGACGTSGTCCTGGCT GGTTAGC39), were also employed. Primers 3 *(pvpA3F)*, which are situated at nucleotide positions 583 to 604 (59GGTAGTCCTAAGTTATTAGGTC39), and 2 *(pvpA2R)*, which were used for the first amplification, were used in the seminested reaction.

Determination of coliform count

The Violet Red Bile Lactose Agar (VRBL) medium (HIMEDIA/Indin) was utilized, and the same protocols as in the APC were followed, International Commission on Microbiological Specifications for Foods [24]. The counting of the pink-red colonies larger than 0.5 mm in diameter was done after a 24-hour incubation period at 37°C. The number of coliforms per gram of sample was calculated by multiplying the number of counted colonies by the dilution factor.

PvpA gene PCR

Two amplifications were performed with seminested primers in two steps: amplification of the pvpA gene, followed by amplification of the C-terminus-encoding region of the *pvpA* gene. The first amplification was carried out in a 50 µl reaction volume as follows: Nuclease-free water (23 µl), 1 µl template DNA, 0.5 µl outer forward primers (pvpA1F), 0.5 µl reverse primers (pvpA2R), and 25 µl Master mix (Fermentas # K1081). The initial amplification was conducted at 94°C for 3 minutes, subsequently followed by 20 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute. The last extension stage was then 72°C for 10 minutes **[25]** . The second amplification was performed using one microliter as a template for the previous one. The second amplification was carried out in a 50 µl reaction volume as follows: 25 µl Master Mix, 0.5 µl forward primer *(pvpA3F)*, 0.5 µl reverse primer *(pvpA2R*), 1 µl template DNA (product of the first amplification), and 23 µl nuclease-free water. The second amplification was carried out at similar temperatures and periods as the initial amplification for 40 cycles [25].

Restriction fragment length polymorphism (RFLP):

RFLP testing was carried out on 35 out of 37 positive PCR samples and the F-vaccine to determine their genetic relationship. Thirty µl reaction mixtures (17 µl of nuclease-free water #R0581, 2 µl of 10× Fast Digest® green buffer, 1 µl of FastDigest® PvuII enzyme, and 10 µl of PCR result) were used for the restriction enzyme digestion. After that, the mixes were incubated for ten minutes at 37 °C. The digested PCR products were electrophoresed on a 1.5% agarose gel with 1X TBE buffer at a voltage of 80 volts for one hour. UV light was used to visualize the gels.

Data analysis

Total Lab Software Analysis (version 1.1) was used to examine the RFLP data. Additionally, as shown in (Fig. 1), cluster analysis was carried out using the PAST software (version 1).

Culturing of M. gallisepticum from poultry flocks tested PCR- MG- positive:

The only positive samples (37 samples) for the PCR test were used for culturing *M. gallisepticum* in Frey's broth medium and Frey's agar [26].

Experimental infection:

The aim was to test the pathogenicity of three selected isolates and study the F vaccine's horizontal transmissibility rate. Vaccine: The F strain vaccine (Schering Plough Animal Health, USA) was used for vaccination by eye dropping. *M.* gallisepticum isolates inoculum preparation: The 3 *M.* gallisepticum isolates selected for experimental infections were propagated in Frey's broth, and the inoculum was titrated to contain 10^8 CFU/ml.

Experimental design:

Six groups were formed from the 220 one-day-old male grandparent chicks that were free of *M. synoviae and M. gallisepticum:*

- Groups 1, 2, and 3 had 120 chicks (each group had 40 chicks), of which 20 were infected by the isolate numbers 23, 36, and 37, in that order. The remaining twenty chicks in each group,

meanwhile, continued to communicate with their infected companions inside the same chick battery.

- Group 4 consisted of 40 chicks that were organized as follows: 20 were given a single dose of the F-strain vaccine, and the remaining 20 stayed together in the chick battery.
- Group 5 had 40 chicks, with 20 given a double dose of the F-strain vaccine and the remaining 20 remaining in contact in the same battery.
- Group 6 comprised twenty chicks housed in a different battery as a non-vaccinated and non-infected control group.

Both infection and vaccination were performed on the first day of age via the intranasal and intraocular routes. The chicks were kept under daily observation for 5 weeks. The infectivity and transmissibility of the F vaccine and *M. gallisepticum* isolates were determined by PCR testing and by serological testing. Six infected, vaccinated, or contact chicks and eight contacts from each group (1-6) were tested by PCR on the 14th and 28th days of age. Also, 5 blood samples from each of the infected, vaccinated, and contact chickens in each group on the 35th day of age were tested for *M. gallisepticum* antibody ELISA (ProFlok®, Synbiotics).

Results and Discussion

Detection of M. gallisepticum from clinical samples by pvpA gene PCR:

The molecular mass of *M. gallisepticum* isolates and the F-strain vaccine (350–410 bp) were determined by amplifying the *pvpA* C-terminal-encoding region. The results of PCR testing revealed that 37 flocks out of 49 were positive for *M. gallisepticum* infection (75.5%) and were distributed as follows: 66.6% in layer flocks, 75.75% in broiler flocks, and 100% in broiler chicken breeder flocks. The results of the PCR are shown in (Figs. 1 and 2). From the literature in Egypt**,** El-Hamid, Abd El-Halim [27] recorded 45.8% as an overall incidence of *M. gallisepticum* in chicken flocks, and the details were: 75% in layer, 75% in broiler breeders, and 26.6% in broiler flocks. This average incidence of *M. gallisepticum* infection has risen in Egypt during the last 4 years, and this further supports the Egyptian endemic disease prevalence due to the expansion of chicken in the absence of a nationwide control strategy. The lateral transmission may be due to an increased number of small-scale farms in close contact and poor biosecurity. According to Gharaibeh and Al Roussan [28]**,** the use of the *M. gallisepticum* live vaccine in both commercial layer and broiler breeder herds may also contribute to the spreading of infection with pathogenic vaccine strains that have undergone mutations. Most isolates sampled in the same month and geographic area showed similar clinical pathologic effects under field conditions (data not shown). The isolates displayed RFLP patterns with over 95% genetic resemblance, strongly placed in the phylogenic tree (Fig. 3 and 4**).** Isolates No. 1, 3, 4, 5, 6, and 19 showed over 92% genetic resemblance to the F-strain vaccine.

The isolates numbers 9 and 28 of *M. gallisepticum* were classified in two different clusters even though these isolates were obtained from the same flock; isolate No. 9 was obtained from a 6-day-old flock, while isolate No. 28 was recovered from a 25-week-old flock. The genetic similarity between the two isolates was 38%. This difference may be explained by *M. gallisepticum* superinfection, or it may be that the same strain has undergone a genetic mutation in *the pvpA* gene's C-terminal encoding region [28].

Regarding the experimental infection, experimental infection of one-day-old grandparent male chicks by *M. gallisepticum* isolates (No. 23, 36, and 37) and the F vaccine revealed that neither *M. gallisepticum* isolates nor the F vaccine caused distinctive pathogenic effects. The 3 *M. gallisepticum* isolates and F vaccine caused mild pathogenic effects in chickens (infected and contact) in the form of conjunctivitis and air sacculitis.

This may be attributed to the absence of complicating agents such as *E.coli, infectious bronchitis , and Newcastle disease* viruses and also to the absence of environmental stressors that increase the severity of *M. gallisepticum* infection [26, 29]. Similarly, Throne Steinlage, Ferguson [30] mentioned that the *M. gallisepticum* isolates did not extensively colonize the air sacs of infected birds, and no lesions were recorded in the air sacs. Also, in the studies of Feberwee, Mekkes [31], Feberwee, Landman [32], no diagnostic clinical symptoms were noticed following the *M. gallisepticum* challenge strain application, and only air sacculitis in the inoculated and in-contact chicks were noted.

Speaking about the lateral transmission during the experimental infection, swabs taken at 2 weeks post-infection at 14 days of age showed that 100% of the infected birds with isolate 23 and 36 (groups 1 and 2, respectively) and 75% of the contact birds were PCR positive for *M. gallisepticum* The number of positive contact birds decreased at 28 days of age from 75% to 25% in group 1 and to 50% in group 2. This may indicate a decrease in *M. gallisepticum* organisms excreted by chickens after 28 days post-inoculation. This can be explained by the findings of Feberwee, Landman [32], which revealed a rapid increase in *M. gallisepticum* shedding by the challenged birds from day 3 to 14 post-challenge, followed by a decrease in

shedding. Also in the study of Pakpinyo, Pitayachamrat [33], they found that all infected birds tested positive by PCR 2 weeks post-infection, decreased to 33% at 5th week post-infection, and reached zero at 7th week post-infection.

Birds infected with isolate 37 (group 3) were negative until the age of 14 days, but at the 28th day of age, they became PCR positive, while at the same age, only 50% of the in-contact birds became PCR positive **(Fig. 3).** The same result was found by Feberwee, Mekkes [31], where two inoculated chickens discharged a comparatively small *M. gallisepticum* concentration, while in-contact infected birds, this low excretion level.

In vaccinated groups with either a single (group 4) or double dose (group 5), all birds (100%) tested positive by PCR at the 14th and 28th days of PV, indicating the colonization and replication of the vaccinal strain. But only 50% of the contact birds in group 4 and 75% of the contact birds in group 5 were PCR positive at 14 days of PV post-vaccination, which indicated also shedding and lateral transmission of the vaccine F strain to the contact birds. The study by Pakpinyo, Pitayachamrat [33] revealed that vaccinated birds with the F strain vaccine were positive by PCR at 2 weeks of post-vaccination , but the number of positives decreased at 3 weeks to 66% and reached zero at 5 weeks of post-vaccination.

In all groups, the immune response to *M. gallisepticum* isolates and the F-strain vaccination was very poor at 5 weeks of PI or PV. Except one chicken out of five that produced extremely low antibody titers, the dose-vaccinated group did not exhibit any reactions until five weeks into the trial. In contrast, the double-dose-vaccinated group had two birds that produced extremely low titers and one bird out of five that was positive. The infected groups showed no response except for one contact bird out of five in Group 1, which was positive. The results are shown in Table 1.

The fact that Pakpinyo, Pitayachamrat [33] reported that after 5 weeks, there was a significant increase in the positive reactor numbers in the poultry vaccinated with the *MG* live vaccine may help to clarify this. Infected birds in Group 1 showed a response of only 1/5, as confirmed by McMartin, Khan [34] study, which found that antibodies to *M. gallisepticum* appeared gradually 6 weeks post-challenge in 5–10% of the population, while 90%–95% developed antibodies not before 7–11 weeks. Five weeks after the vaccination or infection, the current study was stopped.

Conclusion

The results of the present study revealed that the F strain vaccine can be transmitted directly by contact, and according to the study of [26], the *M. gallisepticum* organisms of the F strain vaccine are maintained in the upper respiratory tract over the lifetime of the birds, so it's probable that with the usage of live vaccines as *ND, IB, ILT*, or complications with secondary infection with respiratory viruses, the F strain vaccine may induce pathogenic effects, causing severe outbreaks with some morbidity and mortality, especially in broilers, due to these concurrent infections and environmental stress factors [26].

Declarations statement

Ethics approval and consent to participate

This study has been carried out with ethical permission from the Faculty of Veterinary Medicine, Alexandria University, and approved by the Institutional Animal Care and Use Committee (**ALEXU-IACUC**). The collected clinical samples were approved by the Institutional Animal Care and Use Committee under approval number (**2023/1/112**). All methods were performed in accordance with relevant guidelines and regulations by the Basel Declaration and the International Council for Laboratory Animal Science (ICLAS). The study was carried out in compliance with the ARRIVE guidelines.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of interests

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

Funding statement

The current study has not received any funds from any organizations or institutions.

Author contributions

All authors have read and approved the manuscript. **E.H.F., A.H.S., O.M.A., E.A.R., and R.G.T.** designed the in vivo experiments. **G.A., N.E., A.B.A., and R.G.T.** reviewed the manuscript. **E.H.F.** wrote the manuscript. **A.H.S., E.A.R., R.G.T., and G.A.** performed the data analysis.

Consent for publication: Not Applicable.

Consent to participate: Not applicable.

Acknowledgment

We wish to thank all staff members of the laboratory branches of the microbiology department and the poultry and fish department for providing facilities during this study.

Figure legends

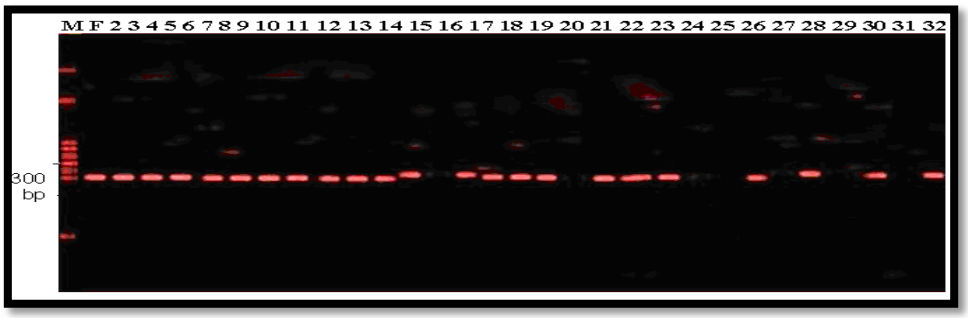

Fig. 1. PCR of field samples (lanes 2-32): lane M: 100 bp DNA marker (Fermentas# SM0243), lane F: F strain vaccine.

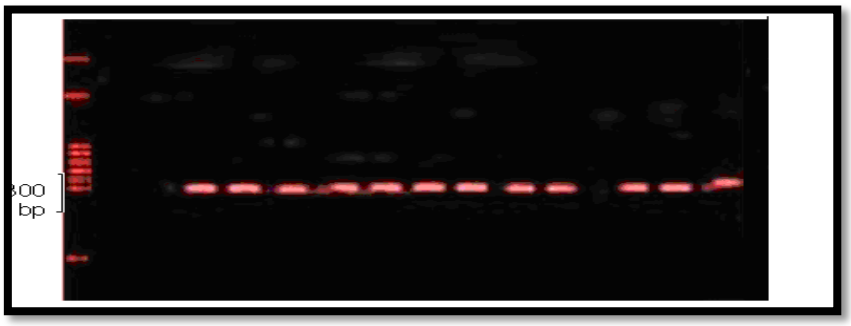

Fig. 2. PCR of field samples: lane 1: 100 bp DNA ladder (Fermentas# SM0243), lane 2 forward, field sample (33-47) lane.

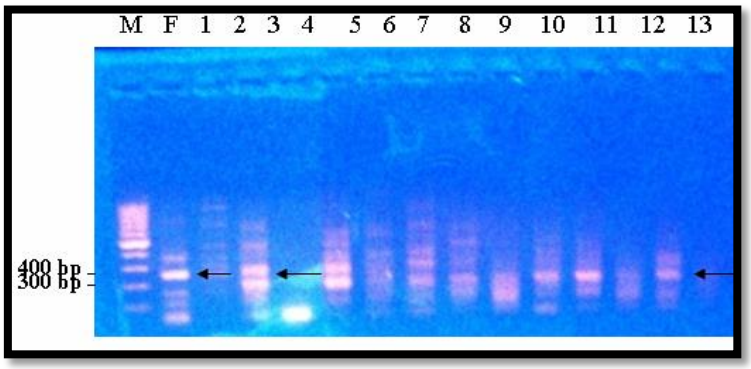

Fig. 3. PCR of experimental samples: lane 1, DNA marker; lane 2, F vaccine; Lane 3 forward, experimental sample.

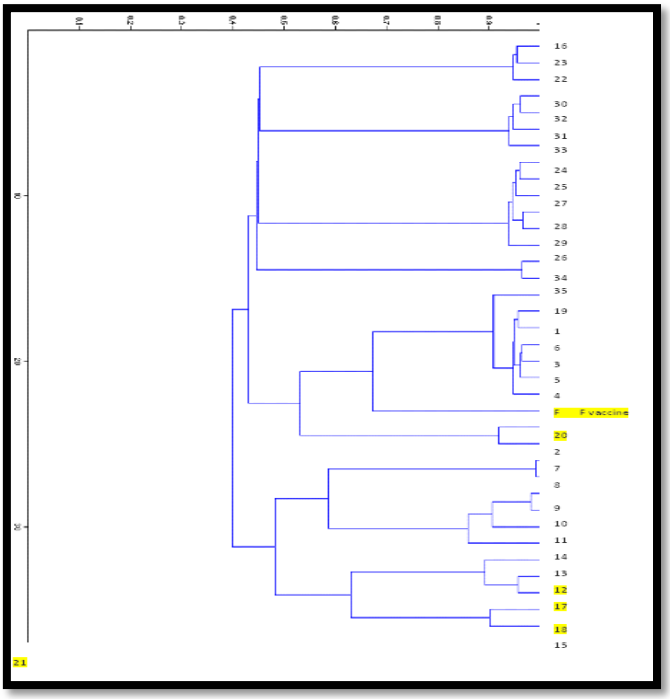

Fig. 4. Phylogenic analysis of the C-terminus-encoding portion of *pvpA* of *MG* F vaccine and 35 positive PCR samples based on RFLP results.

TABLE 1. Shedding of *MG* from experimental chickens by PCR and ELISA test

Group	Number of positives / total tested samples					
	2wks PI (PCR)		4 wks. PI (PCR)		5 wks PI (ELISA)	
	Infected	Contact	Infected	Contact	Infected	Contact
1	3/3	¾	0/3	1/4	0/5	1/5
2	3/3	¾	3/3	2/4	0/7	0/5
3	0/3	0/4	3/3	2/4	0/7	0/5
4 (One dose vaccine)	3/3	¾	3/3	2/4	1/6 low titer	0/5
5 (Double dose vaccine)	3/3	2/4	3/3	2/4	1/5 high titer 2/5 low titer*	0/5
6	0/2		0/2		0/7	

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عزل واكتشاف جزيئي لعزلات M. Gallisepticum وسلالة F من قطعان الدجاج التي تعاني من مشاكل في الجهاز التنفسي في مصر

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الملخص

يؤثر الميكوبلازما غاليسيبتيكوم، وهو مسبب مرضي كبير في الدجاج والديك الرومي، بشكل كبير على صناعة الدواجن العالمية، مما يتسبب في خسائر اقتصادية كبيرة. ونظرًا لزيادة استخدام التطعيمات الحية وإعادة ظهور أوبئة الميكوبلازما غاليسيبتيكوم بين الدواجن، تدرس هذه الدراسة تأثير سلالة اللقاح الحي F على تفشي الأمراض التنفسية في الدجاج. أدى تحديد الميكوبلازما غاليسيبتيكوم في القطعان إلى زيادة الطلب على التقييمات الجينية الجزيئية واختبارات التمييز بين السلالات، باستخدام طفرات شظايا طول محدودة من الحمض النووي للجينوم (RFLP) كأدوات قوية. هدفت الدراسة إلى تقييم إمكانية التصنيف الجزيئي باستخدام جين حصري للميكوبلازما غاليسيبتيكوم يشفر بروتين التصاق مفترض متغير الطور (PvpA). بناءً على الاختلافات في الحجم وتباعد النوكليوتيدات في منطقة ترميز الطرف الطرفي C، تم استخدام اختبار pvpA PCR-RFLP للتمييز بين سلالات اللقاح F وعزلات MG. وجدت الدراسة معدل إصابة مرتفع بـ MG (66.6٪ و 100٪ و 75.75٪) في قطعان البياض وأمهات الدجاج اللاحم والدجاج اللاحم على التوالي، وأوضح اختبار تفاعل البوليميراز المتسلسل لـ pvpA-RFLP أن العزلات كانت وثيقة الصلة بسلالة اللقاح F. تم تقييم عزلات M. gallisepticum الثلاثة واللقاح F من حيث قدرتها على التسبب في الأمراض وقدرتها على الانتشار في الدجاج المصاب والملامس باستخدام الأعراض السريرية وآفات الأكياس الهوائية وتفاعل البوليميراز المتسلسل والاختبارات المصلية بعد 14 و 28 يومًا من الإصابة التجريبية. أظهرت العزلات التي تم أخذ عينات منها في نفس الوقت، في نفس المنطقة الجغرافية، والتي تسببت في تأثيرات مرضية مماثلة في ظل ظروف الحقل أنماط RFLP متطابقة وكانت تقع بشكل وثيق في شجرة التطور النشوئي. وجدت الدراسة أن الطيور المخالطة في كل من المجموعات الملقحة والمصابة أظهرت نتائج تفاعل البوليميراز المتسلسل إيجابية بعد 14 يومًا من الإصابة، مما يشير إلى تساقط المرض وانتقاله جانبيًا، وكانت المناعة ضد عزلات MG الميدانية والتطعيم ضد سلالة MG F ضعيفة حتى 35 يومًا بعد الإصابة. تشير الدراسة إلى أن اختبار تفاعل البوليميراز المتسلسل-pvpA RFLP يمكن أن يميز بكفاءة بين سلالات اللقاح والعزلات الميدانية، مما يلغي الحاجة إلى عزل الميكوبلازما غاليسيبتيكوم ، ويمكنه أيضًا المساعدة في علم الأوبئة الجزيئي لأوبئة M. gallisepticum باستخدام تحليل التسلسل أو RFLP.

الكلمات الدالة: الميكوبلازما غاليسيبتيكوم؛ جين pvpA؛ اختبار PCR-RFLP؛ عدوى تجريبية؛ اختبار مصل الدم.