



Original article

A picture of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in poultry in Egypt: Phenotypic and genotypic characterization

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ABSTRACT

Objectives: The study reveals a picture for *M. gallisepticum* (MG) and *M. synoviae* (MS) in poultry industry of Egypt.

Methods: A total of 628 samples were collected from different species of diseased and apparently healthy poultry species from 15 Egyptian governorates. The isolates identified based on *mgc2* and 16 s rRNA specific primers for MG and MS respectively. Sequencing for two isolates using *mgc2* gene.

Results: Two hundred and five samples were microscopic fried egg colony. Using digitonin test, 160 samples were identified as genus *Mycoplasma*. Out of the 160 isolates, 86 were identified by PCR as MG, while 39 were identified as MS. Sequencing reveals that one isolate MGC2-EGY1 was related to the Egyptian strain MG-EIS6-T-10, while, the other isolate MGC2-EGY2 was related to the Indian strain MG-MGS-9B.

Conclusion: The study suggested that strictly applied programmes for the prevention and control of avian mycoplasmosis and confirmed the use of PCR on a large scale to help disease eradication programmes to limit economic losses in poultry farms.

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1. Introduction

Mycoplasmas are a big problem for all bird population. Mycoplasmas are very small bacteria that lack cell walls and belong to various genera within the class *Mollicutes*; these bacteria are able to cause serious and chronic diseases (Zakeri, 2016). *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) are the most important avian pathogens, as they induce significant

economic losses in the poultry industry. The rapid detection of *Mycoplasma* species is an essential step for adequate and fast control (Osman et al., 2009). Culturing of *Mycoplasma* can be quite difficult due to its fastidious nature, while serology is much faster. Non-specific reactions and cross-reactions, misinterpretations due to recent vaccination against *Mycoplasma* and cost are all disadvantages of the current diagnostic techniques (Ferberwee et al., 2005; Erfan and Marouf, 2019). Therefore, a rapid, accurate, inexpensive and definitive diagnostic technique for mycoplasmas is of great importance. PCR methods are accepted, faster and less costly than culture methods (Yuan et al., 2003; Abdel Halium et al., 2019).

The present study give a picture for MG and MS in different Egyptian governorates from both apparently healthy and diseased birds based on different *Mycoplasma* diagnostic techniques.

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2. Materials and methods

2.1. Animal ethics

All animal samples related procedures were approved ethically by institutional animal care and use committee (Vet.CU-IACUC) at Faculty of Veterinary Medicine, Cairo University. Approval document number is Vet.CU-10102019085.

2.2. Samples

A total of 628 samples were collected from chickens, turkeys, quails, ducks, parrots and pigeons during the period from January 2016 to December 2018 from fifteen different Egyptian governorates (Cairo, Giza, Fayoum, BaniSuef, Behera, Dakahlia, Gharbia, Monofia, Qualiobia, Sharkia, Alexandria, Ismaielia, Kafr El-Sheikh, El Minya, and Elwahas). The sample group included 278 apparently healthy and 350 diseased birds (coughing, nasal and ocular discharge, swelling in the infraorbital sinus, poor productivity, slow growth, leg problems, stunting, in-appetence, reduced hatchability and chick viability and abnormal feathers. Postmortem lesions were variable and included air sacculitis, pericarditis, perihepatitis, sinusitis and occasionally arthritis). Samples from chickens were taken from different breeds: 68 breeders (46 apparently healthy and 22 diseased), 100 layers (58 apparently healthy and 42 diseased) and 378 broiler (226 apparently healthy and 152 diseased). The collected samples included 190 tracheal swabs, 200 trachea, 168 lung tissues, 20 fertile eggs, 4 joints and 46 yolk sac samples of baby chicks. All samples were collected under complete hygienic conditions, transferred directly to the laboratory using transport media in an ice box and examined immediately (OIE, 2004). All previously data were listed in Tables 1 and 2.

2.3. Microbiological analysis of *Mycoplasma* spp.

All samples were inoculated into Frey's broth medium and incubated at 37 °C with 5–10% CO₂ in a humid atmosphere for 5–7 days. Any observable growth (yellow or straw-coloured broth changes) was subcultured on solid medium immediately and incubated at the same previously mentioned condition (OIE, 2004). The plates were examined daily for fried egg colonies for up to 20 days. The suspected colonies were subjected to a digitonin sensitivity test as described by Erno and Stipkovits (1973). The suspected positive digitonin sensitivity tests were serologically identified by growth inhibition (GI) tests using specific mycoplasmal antisera for MG and MS (Clyde, 1964).

2.4. Molecular identification

DNA of suspected isolates were extracted by QIAamp DNA Mini Kit was carried out by PCR using primers targeting the *mgc2* and 16SrRNA genes for the detection of MG and MS, respectively. The sequence of *mgc2* primer (F:CGC-AAT-TTG-GTC-CTA-ATC-CCC-AACA, R: TAA-ACC-CAC-CTC-CAG-CTT-TAT-TTC) Producing amplified product of 300 bp (Lynsyansky et al., 2005) with a cycling condition of (primary denaturation at 94 C/5 min followed by 94 C/30 s, then annealing step at 58/30 s followed by extension step at 72 C/30 s) for 35 cycles and finalized by final extension at 72 C/7 min. The sequence of 16S rRNA primer (F:GAG-AAG-CAA-AAT-AGT-GAT-ATC-A, R: CAG-TCG-TCT-CCG-AAG-TTA-ACA-A) Producing amplified product of 210 bp (OIE, 2008) with a cycling condition of (primary denaturation at 94 C/5 min followed by 94 C/30 s, then annealing step at 55/30 s followed by extension step at 72 C/30 s) for 35 cycles and finalized by final extension at 72 C/7 min.

2.5. Sequencing and phylogenetic analysis

Two isolates were confirmed by *mgc2* sequencing using Big dye Terminator V3.1 cycle sequencing kit.(Perkin-Elmer, Foster city, CA) cat-number 4336817. Nucleotide sequence alignment was carried out using BioEdit software and dnastar, Lasergene, megaalign. Sequence distance carried out using BioEdit software and dnastar, Lasergene, megaalign. The phylogenetic relationship was performed using MEGA5 software.

3. Results and discussion

3.1. Microbiological analysis

In the present study, a total of 205 samples revealed characteristic fried egg appearance of *F. Mycoplasmataceae* with an incidence of 32.6%. The recovery rate of *Mycoplasma* isolates (digitonin-sensitive) was 78% (160/205), resembling 25.4% of the total collected samples (160/628). The nearly results were documented by Mohamed et al. (1987), Levisohn and Kleven (2000), Nicholas and Ayling (2003), Feberwee et al. (2009) and Raviv and Ley (2013). The rate of culture results was higher than detected by Bibak et al. (2013) (10.66%) and Senthilnathan et al. (2015) (15.5%) and lower than that reported by Heleili et al. (2011) of 60.33%; however, this rate closely agreed with that obtained by Poubakhsh et al. (2010). The obtained results indicate that fried egg colony-producing isolates are highly prevalent in poultry

Table 1
Number of samples from different sources and types of examined samples from apparently healthy and diseased poultry.

| | Samples | Apparently healthy | Diseased | Total |
|--------------------|--------------------|--------------------|----------|-------|
| Sources of samples | chickens | 148 | 320 | 468 |
| | One day old chicks | 68 | 10 | 78 |
| | Egg | 14 | 6 | 20 |
| | Turkey | 18 | 4 | 22 |
| | Quail | 14 | 2 | 16 |
| | Duck | 6 | 2 | 8 |
| | Parrot | 2 | 4 | 6 |
| | pigeon | 8 | 2 | 10 |
| | Total | 278 | 350 | 628 |
| | Type of samples | Tracheal swabs | 122 | 68 |
| trachea | | 54 | 146 | 200 |
| lung | | 48 | 120 | 168 |
| Fertile Egg | | 14 | 6 | 20 |
| yolk sac | | 40 | 6 | 46 |
| Joint | | | 4 | 4 |
| Total | | 278 | 350 | 628 |

Table 2

Number of samples and results of culture and PCR (MG and MS) for examined samples collected from 15 different Egyptian Governorates.

| Governorates | Total examined No | Positive culture | | PCR results | | | |
|--------------------|-------------------|------------------|--------------|-------------|--------------|-----------|--------------|
| | | NO | % | MG | % | MS | % |
| 1-Cairo | 112 | 52 | 46.4% | 35 | 31.2% | 10 | 8.9% |
| 2-Giza | 80 | 32 | 40% | 18 | 22.5% | 7 | 8.7% |
| 3-Al fayoum | 24 | 5 | 20.8% | 0 | 0% | 0 | 0% |
| 4-Beni seuf | 20 | 8 | 40% | 0 | 0% | 0 | 0% |
| 5-Behera | 40 | 18 | 45% | 3 | 7.5% | 0 | 0% |
| 6- AL Dakahlia | 110 | 40 | 36.3% | 16 | 14.5% | 0 | 0% |
| 7-AL Garbia | 16 | 2 | 12.5% | 2 | 12.5% | 6 | 37.5% |
| 8-AL Monofia | 46 | 6 | 13% | 0 | 0% | 0 | 0% |
| 9-AL Qaliobia | 16 | 8 | 50% | 3 | 18.7% | 2 | 12.5% |
| 10- AL Sharkia | 20 | 8 | 40% | 0 | 0% | 2 | 10% |
| 11-Alexandria | 16 | 1 | 6.25% | 1 | 6.25% | 1 | 6.25% |
| 12-Ismaileia | 18 | 6 | 33.3% | 2 | 11.1% | 3 | 16.60% |
| 13- Kafr EL Shiekh | 48 | 8 | 16.6% | 0 | 0% | 0 | 0% |
| 14- EL Menia | 22 | 1 | 4.5% | 0 | 0% | 0 | 0% |
| 15- EL Wahat | 40 | 10 | 25% | 6 | 15% | 6 | 15% |
| Total | 628 | 205 | 32.6% | 86 | 53.7% | 39 | 24.3% |

respiratory diseases, which agrees with the results of Ammar et al. (2016) and Pflaum et al. (2016).

3.2. Serotyping records

Serotyping of digitonin-sensitive isolates revealed that MG and MS were detected in 56.2% and 31.2%, respectively. The rates of MG and MS among different types of birds and the collected samples are illustrated in Table 3. MG was higher than the previously recorded rate of 47.7% by Abd El-Ghany (2008) and lower than in Abd El-Gawad and Rania (2005) in the Dokki Layer breed (67.5%) and Rauf et al. (2013) (79.8%). The serological identification of MS in this study was higher than that in Eissa et al. (2000) and Tawfik et al. (2016), which were 13.33% and 10%, respectively.

3.3. Molecular detection, sequencing and phylogenetic analysis

One hundred and sixty isolates was performed via PCR using *mgc2* (300 bp) supported by Lynsyansky et al. (2005) and 16S rRNA genes (210 bp) according to OIE (2008) for the detection of MG and MS respectively. The investigation revealed 86 samples were positive for MG (53.7%) (Fig. 1) and 39 samples were positive for MS (24.3%) (Fig. 2). These results closely agreed with that reported by Gondal et al. (2015) and Elbehiry et al. (2016), 50% and 51.92%, respectively; however, these results disagree with those of Davinder et al. (2013) and Kamble et al. (2015), 35.3% and 66.4%, respectively.

The nucleotide and amino acid sequences of the MG *mgc2* gene for two pathogenic strains were deposited into GenBank under the accession number MG742314 for isolate EGY1-2017 and

Table 3Recovery rate of *M.gallisepticum* and *M.synoviae* among different types of birds and their samples using Digitonin sensitivity and PCR.

| Sources and Types of samples | | Digitonin sensitive | | | PCR | | |
|------------------------------|-------------------------|---------------------|-----------------------|-----------------------|----------------|-----------------------|-----------------------|
| | | No. of samples | Positive MG | Positive MS | No. of samples | Positive MG | Positive MS |
| Sources | Chickens & fertile eggs | 149 | 85 (57%) | 49 (32.8%) | 149 | 84 (56.3%) | 38 (25.5%) |
| | Turkeys | 3 | 2 (66.6%) | 1 (33.3%) | 3 | 2 (66.6%) | 1 (33.3%) |
| | Quails | 2 | 2 (100%) | 0 (0%) | 2 | 0 (0%) | 0 (0%) |
| | Ducks | 2 | 0 (0%) | 0 (0%) | 2 | 0 (0%) | 0 (0%) |
| | Parrots | 2 | 1 (50%) | 0 (0%) | 2 | 0 (0%) | 0 (0%) |
| | Pigeons | 2 | 0 (0%) | 0 (0%) | 2 | 0 (0%) | 0 (0%) |
| | Total | 160 | 90 (56.2%) | 50 (31.2%) | 160 | 86 (53.7%) | 39 (24.3%) |
| Types | Tracheal swabs | 28 | 10 (35.7%) | 3 (10.7%) | 28 | 9 (32.1%) | 1 (3.5%) |
| | Trachea | 63 | 46 (73%) | 13 (20.6%) | 63 | 44 (69.8%) | 10 (15.8%) |
| | Lungs | 54 | 28 (51.8%) | 23 (42.5%) | 54 | 27 (50%) | 20 (37%) |
| | Fertile Eggs | 6 | 2 (33.3%) | 4 (66.6%) | 6 | 2 (33.3%) | 3 (50%) |
| | Joints | 2 | 0 (0%) | 1 (50%) | 2 | 0 (0%) | 1 (50%) |
| | Yolk sac | 7 | 4 (57.1%) | 6 (85.7%) | 7 | 4 (57.1%) | 4 (57.1%) |
| | Total | 160 | 90 (56.2%) | 50 (31.2%) | 160 | 86 (53.7%) | 39 (24.3%) |



Fig. 1. Amplified PCR product of molecular size of 300 bp using primers targeting *mgc2* gene of *M. gallisepticum*. Lane 1 showing 100 base pair ladder.

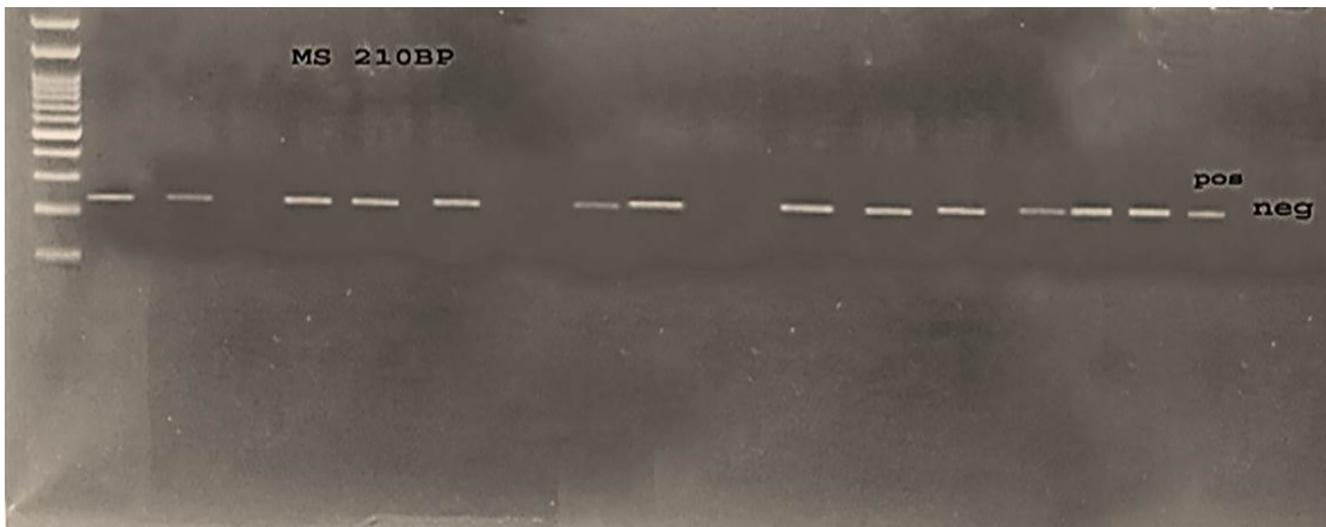


Fig. 2. Amplified PCR product of molecular size 210 bp using primers targeting 16SrRNA gene of *M. synoviae*.

MG742315 for isolate EGY2-2017. The distance between the *mgc2* genes of the MG isolates indicated that the percent identity of both the nucleotide and amino acid sequences was between 85% and 95.4%. The phylogenetic analysis of the nucleotides of the *mgc2* genes of the two isolates and selected samples from Gen Bank revealed two main clusters. MGC2-EGY1 was in one cluster close to HQ591357.1MG-EIS6-T-10, which was related to an Egyptian strain isolated from a local turkey breed in 2011. The other isolate, MGC2-EGY2, was in another cluster close to KP279742.1 MG-MGS-9B (Fig. 3), which is related to an Indian strain isolated from chickens in 2014 (Loolmani et al., 2014).

3.4. Incidence of MG and MS among the samples

Eighty-four positive samples of MG were isolated from chickens and fertile eggs, and 2 samples were isolated from turkey, while quail, duck, parrot and pigeon samples were negative for *mgc2* gene of MG (Table 3). The highest percent of PCR detection for MG was from tracheal samples (69.8%). According to the different types of chicken, breeder, layer and broiler, the incidence of MG was 52.5% (21/40), 57.6% (15/26) and 60% (46/77), respectively.

A total of 39 samples were positive for MS with a recovery rate of 24.3%. Thirty-eight samples from chickens and eggs and one sample from a turkey were positive, while quail, duck, parrot and pigeon samples were negative for MS. Among the different samples of selected avian species, yolk sac samples showed the highest incidence of MS (57.1%) (Table 3). According to the different types of chickens, the incidence of MS was 27.5% (11/40), 30.7% (8/26) and 19.4% (15/27) from breeder, layer and broiler, respectively.

The isolation of MG from different samples agreed with Davinder et al. (2013) and Gondal et al. (2015) who concluded that tracheal, lung and air sac tissues may be used for the rapid screening of MG in poultry. MS was identified with the highest rate in yolk sac samples, followed by egg samples, which agreed with the findings of Dijkman et al. (2016).

From the obtained results, it was concluded that the highest incidence of MG was in broilers as recorded by Kapetanov et al. (2010), Heleili et al. (2011) and Feiziet al. (2013) in commercial broiler flocks, indicating that these farms do not consider biosecurity and hygienic conditions. Additionally, Muhammad et al. (2018) analysed 181 samples from broiler, layer and breeder chickens for mycoplasmal infection and detected 152 positive *Mycoplasma* by PCR. The agreement value between the tests was 67%, thus

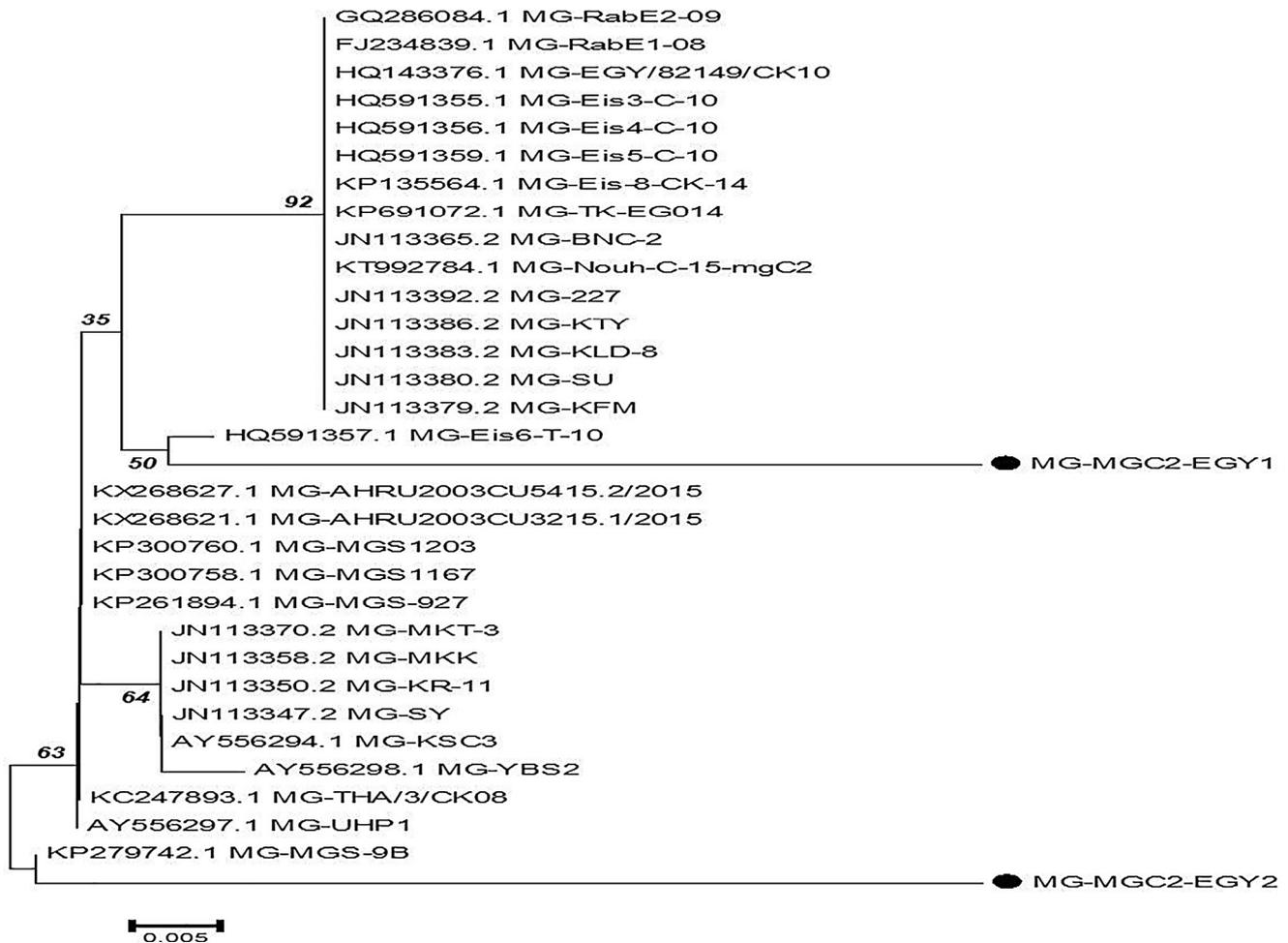


Fig. 3. Phylogenetic tree of *mgc2* gene sequence for 2 isolated field *M. gallisepticum* strains with other reference strain.

confirming that PCR is the most sensitive and reliable tool for the diagnosis of avian mycoplasmosis in field samples.

The investigation found that MS is the predominant *Mycoplasma* spp. in layer flocks; these findings disagreed with Bayatzadeh et al. (2011) and Michiels et al. (2016), who recorded MS infections was higher in broiler chickens. Additionally, regarding the MG and MS positive yolk sac samples, only one isolate was positive for both mycoplasmas, indicating the possibility of both infections at the same time.

The incidence of both MG and MS in the governorates examined in this study are shown in Table 2.

4. Conclusion

The obtained findings suggested that strictly applied programmes for the prevention and control of avian mycoplasmosis, including chemotherapy and/or vaccination, are urgently needed to reduce the economic losses of mycoplasmal infection in Egyptian poultry flocks. Additionally, molecular methods for the accurate diagnosis of avian mycoplasmosis (MG and/or MS) should be applied on a large scale to help disease eradication programmes to limit economic losses in poultry farms

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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