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# Detection of *Mycoplasma gallisepticum* in broiler chickens by PCR

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**Abstract**

**Background:** *Mycoplasma* is a significant microorganism of poultry, which can cause respiratory infections and synovial inflammation, bringing about huge financial misfortunes to poultry workmanship worldwide.

**Aim**: The goal of existing research was to determine the infection rate of *Mycoplasma gallisepticum* (MG) from chronic respiratory disease cases among broilers fields in Mosul/ Iraq using the polymerase chain reaction (PCR) technique.

**Methods:** All 92 lungs samples were collected from broilers with classical respiratory signs in different regions of the Nineveh governorate for 3 months from February to April 2021.

**Results:** PCR tests were performed using two couple primers, one for the qualitative amplification of 16S rRNA genes (285 base pairs) in *Mycoplasma* spp. and the second couple for the detection of *M. gallisepticum* (580 base pairs). Among the samples obtained from broilers, 87 (94.7%) were positive for *Mycoplasma* and 79 (85.9%) were positive for *M. gallisepticum*.

**Conclusion:** Our results showed that MG infection in broiler chickens leads to serious clinical symptoms and severe lesions. The rate of *Mycoplasma* isolation in this study is high despite the short lifespan of broiler chickens.

**Keywords:** *Mycoplasma gallisepticum*, Broiler, Chronic respiratory disease, PCR.

## Introduction

*Mycoplasma* belongs to the class Mollicutes, which contains more than 100 species, and is distinguished from bacteria by a phenotype of small size and complete lack of cell wall (Yassin *et al*., 2018). *Mycoplasma* spp. requires certain conditions to grow, and sometimes it takes up 3 weeks until the colonies appear clearly on the culture medium (Manimaran *et al*., 2019). The major pathogenic species of *Mycoplasma* in poultry are MG, *M. meleagridis*, *M. synoviae*, and *M.* iowae, and the most prevalent one is MG. Other types of birds are also infected by *Mycoplasma* spp. such as house finches, quails, guinea fowl, geese, starlings, etc. (Hamad *et al*., 2019a; Matucci *et al*., 2020). MG infection usually causes chronic respiratory disease (CRD) in chickens (Yadav *et al*. 2021). CRD clinical signs include nasal secretions, coughing, sneezing, tracheal thrombosis, and conjunctivitis, other less common diseases of MG are keratoconjunctivitis, arthritis, salpingitis, and encephalopathy (Ferguson-Noel *et al*., 2020). CRD is the predominant infection of broiler in Iraq and in the recent past, MG outbreaks took a heavy toll on poultry workmanship (Abed *et al*., 2021; Basit *et al*., 2021).

There is a confusion in the differential diagnosis depending on clinical and autopsy findings with other infectious respiratory diseases. Isolation and identification of MG *in vitro* can be reliable, but because it is very delicate the results are not precise (Rauf *et al*., 2013).

Accurate diagnosis based on cultural, biochemical, and serological tests is a routine but is time-consuming practice (Rauf *et al*., 2013). Recently, the detection of MG infection by PCR is recommended as a reliable test (Demirbilek *et al*., 2020). In comparing between conventional isolation techniques and PCR to identify the tracheal sample from the white leghorn layer infected with MG, it has shown that the molecular diagnosis was more accurate (80.51%) than isolation technique (39.28%) (Rauf *et al.*, 2013). Therefore, the existing study aimed to detect MG in broiler chickens in Mosul city, Iraq using PCR as molecular tools.

## Materials and Methods

### *Broilers' specimens*

Ninety-two lung specimens were summed from diseased broilers that showed classical signs and P.M. lesions for CRD during the period between February to April 2021 from fields in Mosul city. The specimens were collected aseptically and subjected to DNA extraction.

### *DNA extraction*

DNA has extracted from broilers' specimens (Kilic *et al*., 2013; Hamad *et al*., 2019a, 2019b), for that 25 mg of each lung was cut and summed in the disposable container and kept at −80°C to be used later (Santos *et al*., 2010). DNA extraction kit was supplied by gSYNC™ Geneaid extraction kit, Korea. According to the kit instructions, the specimen was ground in a 1.5 ml tube, then GST buffer (200 µl) and proteinase-K (20 µl) were supplemented. Samples were swirled

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fully for 15 seconds then incubated at 60°C nightlong. Digested specimens were centrifugated at 16,000 × g for 120 seconds, the floating was gathered in a novel 1.5 ml tube, and 200 µl of GSB was supplemented to the floating, then swirled again for 10 seconds and blended utterly with 200 µl of absolute ethanol by a vortex. The blend was moved to the GS column and centrifugated at 16,000 × g for 1 minutes, after that 400 and 600 µl of W1 and W2 buffers were appended, respectively, to the GS column with centrifugation, and lasting, 100 µl of warmed eluted buffer was appended to the tubes after thoroughly dehydrating for eluting the purified DNA. The resulting product was kept at −20°C till used up.

***DNA amplification***

For the diagnoses of MG by PCR technique, two pairs of PCR primers were used Table 1 and consisted of universal primer pair for the genus of *Mycoplasma* depending on 16S rRNA sequences and the second pair was specific for MG according to (Aghabalaei and Hedaiati, 2012; Malekhoseini *et al*., 2017), and the efficacy of these primers was confirmed by (Hamad *et al*., 2019 a, 2019b; Al-dabbagh *et al*., 2021). primers were synthesized by Bioneer Co., Korea.

PCR reaction was operated in whole size 25 µl as in Kit and consisted of: 5 µl from extracting DNA (template), 1 µl of every primer, 2 µl of $MgCl_2$, 6 µl of PCR water, and 10 µl of 2.5× prepared Mastermix solution. The control positive in both PCR runs was supplied by Hamad *et al*. (2019a and 2019b). Thermocycler programs were explained in Table 2. Electrophoresis was done using 10 µl of the amplified DNA in a 2% agarose gel. The bands were distinguished at 245–312 nm through the UV transilluminator (Biometra, Germany).

***Ethical approval***

Not needed for this study.

**Table 1.** Primers are used to detect MG.

| Primer | Sequence (5′-3′) Product | M.W pb | References |
|---|---|---|---|
| MYCO. –F | GGGAGCAAACACGATAGATACCCT | 285bp | Aghabalaei and Hedaiati, 2012 |
| MYCO. –R | TGCACCATCTGTCACTCTGTTACCCTC | | |
| GALLI. –F | GAGCTAATCTGTAAAGTTGGTC | 580bp | Malekhoseini *et al*., 2017 |
| GALLI. –R | GCTTCCTTGGGGTTAGCAAC | | |

**Table 2.** PCR thermocycler program for *Mycoplasma* (genus) and MG.

| Step | Temp. ºC | Time | No. of Cycles |
|---|---|---|---|
| **Program for *Mycoplasma*** | | | |
| Initial denaturation | 95 | 5 minutes | 1 cycle |
| Denaturation | 95 | 20 seconds | 35 cycles |
| Annealing | 59 | 30 seconds | |
| Extension | 72 | 30 seconds | |
| Final extension | 72 | 5 minutes | 1 cycle |
| **Program for MG** | | | |
| | | | |
| Initial denaturation | 95 | 5 minutes | 1 cycle |
| Denaturation | 95 | 20 seconds | 35 cycles |
| Annealing | 53 | 30 seconds | |
| Extension | 72 | 30 seconds | |
| Final extension | 72 | 5 minutes | 1 cycle |

**Table 3.** The percentage of *Mycoplasma* and MG in broiler lungs.

| Samples | No. Lungs | No. of +ve samples | Total (%) | Percent of *M. gallisepticum* |
|---|---|---|---|---|
| Total no. | 92 | 87 | 94.7 | |
| *Mycoplasma* | 92 | 87 | 94.7 | |
| MG | 92 | 79 | 85.9[a] | 90.8[b] |

[a]Rate of MG from the total lungs samples.
[b]Rate of MG from the positive Mycoplasma's samples.

## Results

The clinical signs of the diseased broilers were recorded and included gauntness, sternutation, coughing, meager growing, mouth-gasping, diminished feed ingesting, and other miscellaneous signs. When necropsy was carried out on diseased broilers, revealed the deposit of caseous materials on air sacs, heart, liver, and other organs (Fig. 1).

The PCR results demonstrated that 87 out of 92 samples were definite as *Mycoplasma* (94.7%), and 79 samples were assured as MG (85.9%), which that MG exist in (90.8%) of Mycoplasma's positive samples (Table 3 and Figs 2 and 3).

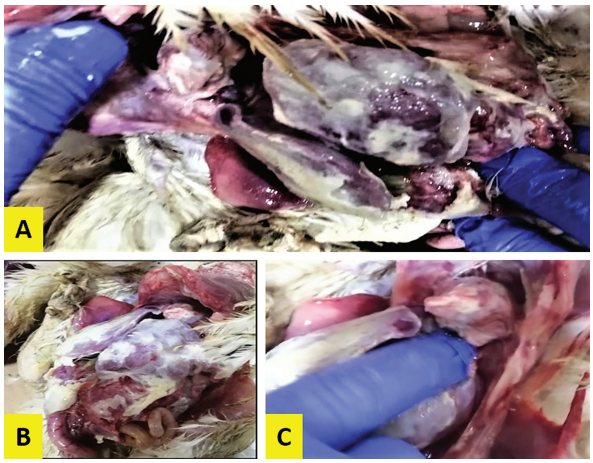


**Fig. 1.** Fibrin deposits on the internal organs of the diseased broilers.

## Discussion

CRD is considered one of the main illnesses that affect poultry workmanship and cause great economic losses, especially if they are accompanied by secondary infections (Chandhar *et al*., 2018). MG is responsible for this disease, and the annual global economic losses incurred by these organisms to the poultry industry have been estimated at more than $780 million (Basit *et al*. 2021). Economic losses in poultry are represented by reduced weight and feed conversion efficiency and may lead to decreased egg production and increased fetal mortality (Ali *et al*., 2020).

The clinical signs that appeared on the infected chickens under this study, were upper and lower respiratory tract infections because the MG mostly colonized the mucosal surface of the host respiratory tract and proliferated in the lung, trachea, and air sacs (Manimaran *et al*., 2019). The observed signs were limited to coughing, mouth-gasping, nasal secretions, and general gauntness. Such signs were described by researchers (Islam *et al*., 2011; Feizi *et al*., 2013; Karthik *et al*., 2018).

Several researchers have described lesions seen during postmortem examination (Chandhar *et al*. 2018; Basit *et al*. 2021), which are hemorrhagic secretions in the trachea and bronchi, as well as cheesy secretions in the air sacs, heart, liver, and lungs, and congestion in these organs, the same observations were observed in the current study.

The samples that were used to isolate MG were taken from the lungs because it was found to be feasible and beneficial according to the study (Hamad *et al*., 2019a).

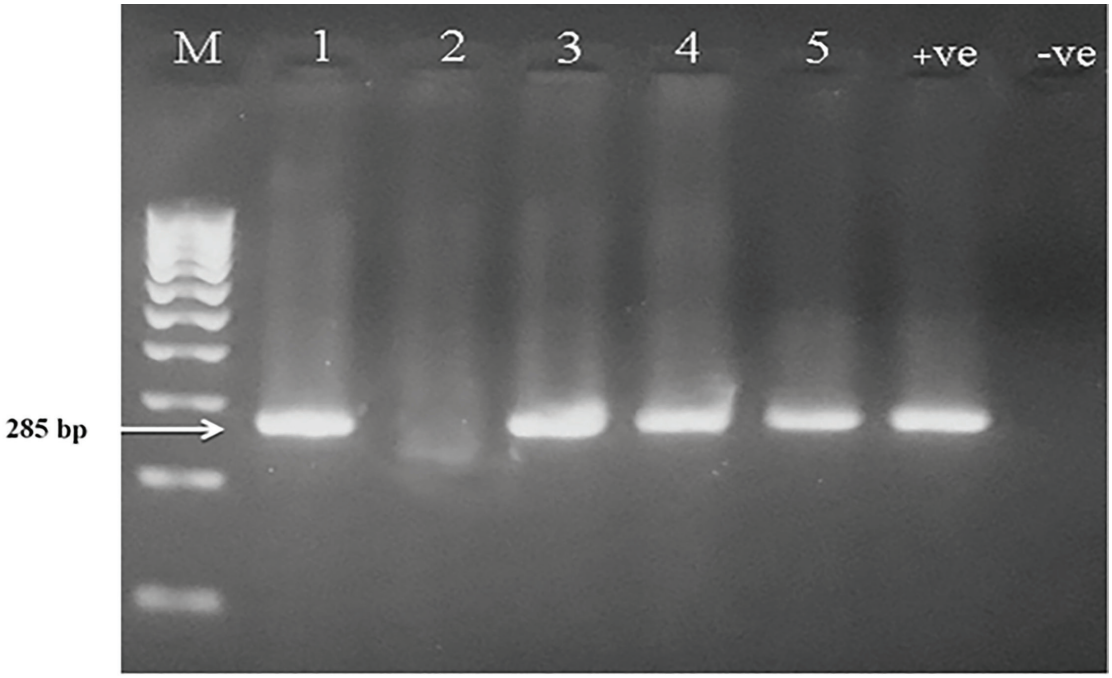


**Fig. 2.** PCR results of genus *Mycoplasma* on 2% agarose gel. (M): Ladder; (Lanes 1, 3, 4, 5): positive for *Mycoplasma* at 285 bp; (Lane 2): negative sample; (+ve): positive control for *Mycoplasma*; (−ve): negative control.

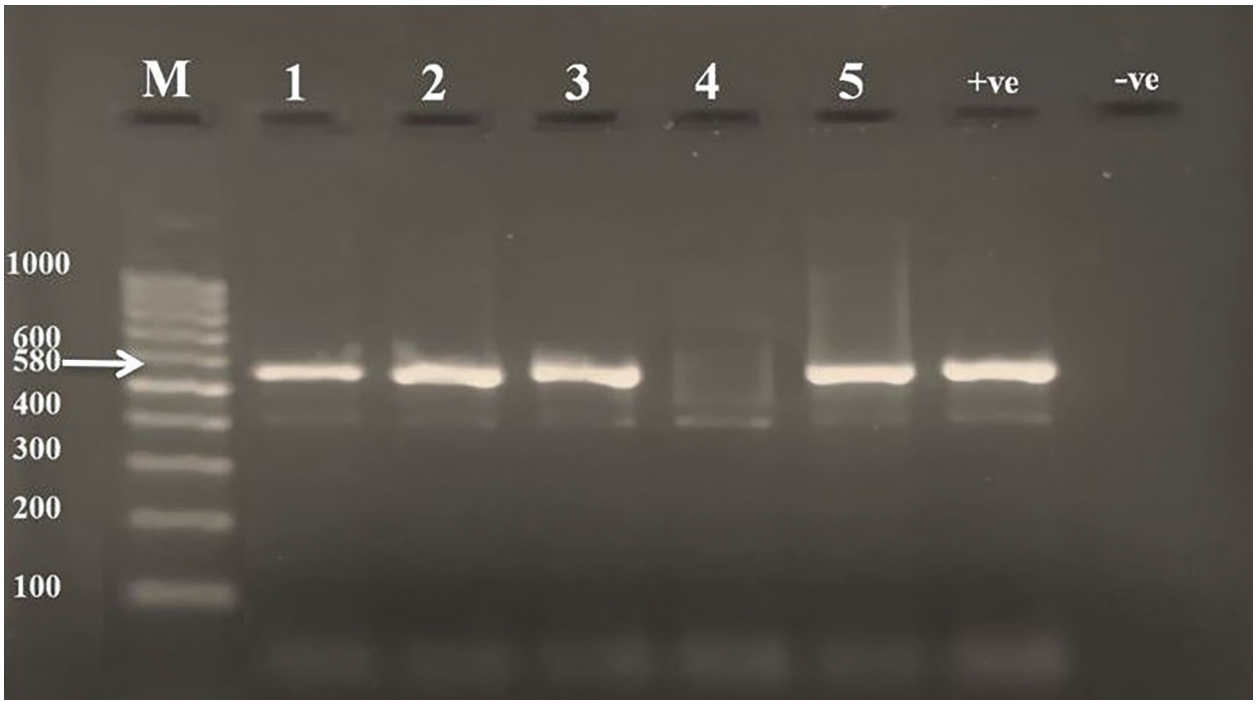


**Fig. 3.** PCR results of *M. gallisepticum* on 2% agarose gel. (M): Ladder; (Lanes 1, 2, 3, 5): positive samples of MG at 580 bp molecular weight; (Lane 4): negative sample; (+ve): positive control for MG; (−ve): negative control.

This is inconsistent with the findings of Mukhtar *et al*. (2012), who isolated MG from the air sacs and trachea of commercial laying chickens, but they could not perform the isolation from the lungs, so they considered the trachea to be an important organ for detecting MG.

*Mycoplasma* culture techniques are tiring, costly, time-consuming (Demirbilek *et al*., 2020), and because *Mycoplasma* spp. belongs to the group of fastidious organisms, it needs special nutritional requirements (Prajapati *et al*., 2018). Its growth may take 3 weeks or more, during this time, *Mycoplasma* saprophytic species may grow, which are characterized by fast growing, such as *M. gallinarum* and *M. gallinaceum* (Bibak *et al*., 2013). In serological techniques, the probability of obtaining nonspecific outcomes is increasing due to *M. synoviae* and MG cross-reaction (Manimaran *et al*., 2019), so the serological methods are used for flock monitoring in MG control programs (Qasem *et al*., 2015).

The diagnosis of *Mycoplasma* need a fast, more specific, and sensitive method such as polymerase chain reaction (Khalifa *et al*., 2013, Rauf *et al*., 2013), in addition to the above reasons, this pathogen has many strains; therefore, diagnosing diseases of these organisms by conventional methods is ineffective (Qasem *et al*., 2015).

Based on the PCR results, *Mycoplasma* was recorded in 94.7% of the examined samples from broiler (Table 3 and Fig. 2). This percentage was much higher than previous studies recorded in Iraq and other countries including 58% in Kuwait (Qasem *et al*., 2015), 75% in northern Pakistan (Abbas *et al*., 2018), 36.6% in Iraq (Jafar and Noomi, 2019), and higher than in other birds like starlings 78.8 % (Hamad *et al*., 2019a) and in turkeys 64.3% (Al-dabbagh *et al*., 2021). The increase in the percentage may be due to the time of samples collection, the differences in weather, or it may be due to a lack of biosafety and biosecurity in the respective study area, as well as the professionalism in samples collection.

The infection rate of MG (85.9%) in the running study had disagreed with the outcomes of other researchers (Ching *et al*., 2016; Michiels *et al*., 2016; Chandhar *et al*., 2018; Jafar and Noomi, 2019; Marouf *et al*., 2020) who reported less than what was recorded in the existing study on (Table 3 and Fig. 3). The ratios were, respectively: 2.7%, 63.5%, 13.33%, 78.4%, and 50%. These differences in MG infection ratios might be due to sampling size and type, stage of infection (chronic or acute), and age of birds since MG affects younger birds more seriously than adult birds (Chandhar *et al*. 2018).

According to the species diagnosis, several samples (15.1%) appeared negative for MG, while manifested positive for the genus of *Mycoplasma*, and that revealed possibility of the existence of other *Mycoplasma* spp. employed as the causative agent of CRD (Hamad *et al*., 2019a; Ferguson-Noel *et al*., 2020).

## Conclusion

The results of the current study showed that the MG infection in broilers leads to occasional serious clinical symptoms and gross lesions and can lead to decreased performance of broiler breeds. The rate of MG isolation in this study is high despite the short lifespan of broiler chickens. This leads to the suggestion that the area is constantly vulnerable to infection with these organisms.

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***Conflict of interest***

The authors declare that there is no conflict of interest.

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