



# Serologic and Molecular Detection of *Mycoplasma gallisepticum* in Layer Flocks in South Marmara Region of Turkey

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

**Background:** Due to the economic impacts of *Mycoplasma gallisepticum* (MG) infection in poultry, it is essential to have a fast, reliable and accurate diagnostic test to diagnose the infection.

**Aims:** It was aimed to examine the presence of MG in the South Marmara Region of Turkey where extensive commercial layer flocks exist by RPA, ELISA and real-time PCR.

**Materials and Methods:** In the study, 981 sera and 160 tracheal swab samples (20 swabs per each flock) obtained from eight layer flocks were examined for the presence of MG-antibody by RPA, ELISA, and the presence of MG by real-time PCR, respectively.

**Results:** MG-seropositive flock rate was determined to be 100% by RPA. Twenty-three of the RPA positive sera in each flock LA, LB, LC, LD, LF, LG, and 17 RPA positive sera in flock LE (due to 17 positive RPA sera obtained) were examined for the presence of MG antibody by ELISA, and MG-seropositive flock rate was determined to be 87.5%. As a result of the examination of a total of 32 tracheal swab samples (20 swabs per flock/5 swabs=4 pooled samples, 8 flocksX4 pooled

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samples= 32 samples) for the presence of MG, real-time PCR positive flock rate was found to be 75%.

**Conclusion:** To decide the flock whether it is infected or not and the initiate effective preventive measures against MG infection as soon as possible; serology should be applied simultaneously with bacteriology and/or PCR to prevent time loss due to shortcomings of serological tests used as primary screening test such as cross reactions, sensitivity and specificity problems.

**Keywords:** *Mycoplasma gallisepticum*; RPA; ELISA; real-time PCR.

## 1. INTRODUCTION

*Mycoplasma gallisepticum* (MG) is an important avian respiratory pathogen, and causes chronic respiratory disease accompanied by catarrhal inflammation of the respiratory tract mucosa [1]. Since one of the ways of transmission is vertical, breeder flocks must be examined for *Mycoplasma*-free status on a regular basis, and surveillance systems are applied to prevent contamination of hatchery eggs, which are then passed on to layer flocks and broilers. MG is very important for the poultry industry as it causes a reduction in egg production consequently creates economic losses [1].

Diagnosis of avian mycoplasmosis is performed by primary screening tests such as, rapid plate agglutination test (RPA), haemagglutination-inhibition (HI), enzyme-linked immunosorbent assay (ELISA) and confirmation tests such as polymerase chain reaction (PCR) and culture [2, 3, 4, 5]. Serological tests are the primary screening tests for flock examination, however they sometimes lack the required specificity and sensitivity due to cross-reactions with other pathogenic mycoplasmas, false positive results due to oil-emulsion vaccines against other avian pathogens, and antigenic variations among *Mycoplasma* strains [6]. Delayed antibody response also makes early diagnosis difficult. For instance, RPA detects IgM antibodies found 7-10 days post-infection and persisted 70-80 days, and IgG antibodies are detected by haemagglutination-inhibition test and ELISA 3-4 weeks post-infection and IgG antibodies persisted up to 6 months [5, 6, 7, 8]. Culture is still a gold standard method to confirm active *Mycoplasma* infection [3].

Bacteriological culture is laborious and expensive, and requires serial passages. Moreover, a conclusive positive result confirming a *Mycoplasma* free flock takes up to 30 days. Furthermore, overgrowth by other contaminating bacteria and/or inhibitory effects of antibiotic therapy may make culture unsuccessful [9, 10, 11]. Recently, PCR has been proposed as a

reliable alternative method for the detection of MG infection by the World Organization for Animal Health (OIE) and National Poultry Improvement Plan (NPIP) [2, 3]. Various molecular techniques such as nucleic acid probes [12], recombinant DNA probes [13, 14], conventional PCR [15, 16, 17, 18] and real-time PCR [11, 19, 20, 21] were developed to detect MG.

Control of MG is one of the problems in the poultry industry. Increasing poultry production in small geographic areas, rearing multi-age poultry together and poor biosecurity render MG-free flocks impossible [22]. In order to monitor outbreaks effectively and develop effective control strategies, rapid and specific diagnosis of MG is essential [23, 24]. The primary goal of this study was to examine the presence of MG in the South Marmara Region of Turkey where extensive layer flocks by RPA, ELISA and real-time PCR (rPCR).

## 2. MATERIALS AND METHODS

### 2.1 Serological Examination

#### 2.1.1 Collection of serum samples

Eight flocks were selected randomly for serological test in South Marmara Region of Turkey. A total of 981 serum samples was collected aseptically from the wing vein of individual birds with 3 ml sterilized disposable plastic syringe without anticoagulant, and allowed to clot for 1 h in the syringe and then kept in the refrigerator at 4°C for 4-5 h. Subsequently the serum was decanted in centrifuge tube and centrifuge at 2,500 rpm for 5 min to have clear serum. The clear serum was then collected in a sterile eppendorf tube and then stored at 4°C for RPA and ELISA tests. Serum samples collected from the flocks were summarized in Table 1.

#### 2.1.2 Serology

RPA test and ELISA were applied to serum samples to detect anti-MG antibodies

(Anonymous, 2004). RPA test was done with MG Plate Test Antigen (MG Plate Test Antigen, Pendik Veterinary Control Institute, İstanbul, Turkey) according to the manufacturer's instructions. Briefly, each 30 µl MG antigen and undiluted serum were mixed and rotated for 2 minutes at room temperature. When agglutination occurred, it was evaluated as positive. The positive serum samples were additionally tested by ELISA.

ELISA was performed according to manufacturer's instructions (MG Antibody Test Kit; Idexx France). The OD value was measured at 650 nm. Calculation results and providing data summaries were done by IDEXX instrument and software systems. Calculations and interpretation of results were emphasized below according to manufacturer's instruction manual.

a) Calculations for Controls

$$NCX = \frac{NC1 A(650) + NC2 A(650)}{2}$$

$$PCX = \frac{PC1 A(650) + PC2 A(650)}{2}$$

b) Validity Criteria

$$PCX - NCX > 0.075 \quad NCX \leq 0.150$$

c) Samples

$$S/P = \frac{\text{Sample Mean} - NCX}{PCX - NCX}$$

$$\text{Log}_{10} \text{ Titer} = 1.09 (\log_{10} S/P) + 3.36^*$$

(\*Relates S/P at a 1:500 dilution to an endpoint titer)

The presence or absence of antibody to Mg is determined by relating the A(650) value of the unknown to the Positive Control mean. The Positive Control is standardized and represents significant antibody levels to Mg in serum. The relative level of antibody in the sample is determined by calculating the sample to positive (S/P) ratio. Endpoint titers are calculated using the equation described in the calculations section.

d) Interperetaion of results

$$\frac{S/P \text{ ratio}}{=} = \frac{(OD \text{ sample} - OD \text{ negative control mean})}{(OD \text{ positive control mean} - OD \text{ negative control mean})}$$

If this ratio was less than or equal to 0.5, the sample was considered negative. The sera with S/P ratios greater than 0.5 were considered positive. A positive result (titer greater than 1076) indicates vaccination or other exposure to Mg. The individual and mean ELISA titres were expressed as reciprocal of the calculated endpoint titres.

## 2.2 Molecular Examination

### 2.2.1 Collection of tracheal swab samples

A total of 160 tracheal swabs from 8 layer flocks, 20 per flock, in South Marmara Region were randomly collected and pooled. Each 20 tracheal swabs from the flocks were sampled as described by Zain and Bradbury [25]. Pools of 5 tracheal swabs were inoculated into tubes containing 5 ml Frey's broth (BBL, Becton-Dickinson, No. 212346), and transferred to the laboratory on ice within 5 hours. All the tracheal swab samples were examined for MG by rPCR. Each pool was considered as one sample for PCR as follows: After vortexing 5 minutes, the swabs were discarded from the tube. One ml of Frey's broth was transferred into sterile endpendorf tubes and used for DNA extraction .

### 2.2.2 DNA extraction

Clinical samples were extracted using a commercial DNA isolation kit (Roche; High Pure Template Preparation Kit, 11796828001, Germany) according to the manufacturer's instructions. All extracted DNA was stored at -20°C.

### 2.2.3 Real-time PCR parameters and volume

Real-Time PCR was applied according to *Mycoplasma gallisepticum* Real-Time PCR Detection Kit instructions (*Mycoplasma gallisepticum* Real-Time PCR Detection Kit, Biospeedy, Kat. No: BS-DTC-V-204-50, İstanbul). Negative control and positive control existed in the same kit. Three different real-time PCR volumes were described for each clinical samples, positive control and negative control. The total reaction volume was 11 µl including 5µl 2X qPCR mix, 3µl M.glspl-Oligo Mix, 1µl internal control, 2µl template for clinical samples. The total reaction volume was 10 µl including 5µl 2X qPCR mix, 3µl M.glspl-Oligo Mix, 2µl NTC, for the negative control sample. The total reaction volume was 10 µl including 10 µl PC-M.glspl for positive control. Real-time PCR parameters were as follows: Initial denaturation at 95°C for 5 min, 40 cycles of denaturation at 95°C for 15 s and annealing at 60°C for 50 s.

**Table 1. Information about the flocks and collected samples**

Flock name	Number of collected sera	Number of collected tracheal swabs	Age of chickens (week)
LA	117	20	24
LB	128	20	37
LC	77	20	20
LD	138	20	58
LE	147	20	60
LF	123	20	47
LG	97	20	33
LH	154	20	19
TOTAL	981	160	

### 3. RESULTS

#### 3.1 RPA Results

Six hundred twenty-seven out of 981 sera (63.91%) were positive for the presence of antibody against *M. gallisepticum*. The RPA test results per each flock, Flock LA to LH, were determined to be 79.48%, 79.68%, 75.32%, 31.88%, 11.56%, 67.47%, 87.6%, 94.15% (Table 2). The highest and the lowest MG positiveness were found in flock LH and flock LE with the rate of 94.15% and 11.56%, respectively. Comparing the flocks in terms of the ratio of antibodies against MG, the RPA test results were found to be relatively low with the ratio of 31.88% and 67.47%, in Flock LD and LF, respectively. The rate of antibody against MG in Flock LA, LB, LC and LG were determined with a range of 75.32% to 87.6% (Table 2). As a result, MG-seropositive flock rate was determined to be 100% (Table 3).

#### 3.2 ELISA Results

A total of 178 serum samples (23 per flock except for flock LE) were examined by ELISA. Twenty-three serum samples for each flock were selected from sera that were positive with RPA, but not for flock LE. Due to 17 RPA positive sera determined in flock LE, ELISA was performed with 17 sera for flock LE. The ratio of 16.85% positiveness was determined in the examined 178 sera. Flocks LA and LH were found to share first place with the rate of 39.13%, followed by LF, LB and LG with the rates of 21.73%, 13.04%, and 8.69%, respectively which were found at second, third and fourth place among 8 layer flocks. The ratios of MG antibody were determined 4.34% for each LC and LD while in LE no positiveness was determined. According to CV%, LF, LH and LA were determined to share first, second, and third places, with the rates of 93.1%, 81.5% and 80.1%, respectively while LE,

**Table 2. The results of RPA, ELISA, rPCR**

Code of Flocks	Age of flock (week)	Number of positive sera by RPA (%)	Number of positive sera by ELISA (%)	ELISA Mean Titer	ELISA CV%	Number of Positive tracheal swab samples* by Real-time PCR (%)
LA	24	93/117(79.48)	9/23 (39.13)	984	80.1	3/4 (75)
LB	37	102/128(79.68)	3/23 (13.4)	729	33.2	1/4 (25)
LC	20	58/77(75.32)	1/23 (4.34)	674	27.2	2/4 (50)
LD	58	44/138(31.88)	1/23 (4.34)	584	32.8	0/4 (0.0)
LE	60	17/147(11.56)	0/17 (0.00)	494	39.9	0/4 (0.0)
LF	47	83/123 (67.47)	5/23 (21.73)	624	93.1	1/4 (25)
LG	33	85/97(87.6)	2/23 (8.69)	602	32.7	1/4 (25)
LH	19	145/154(94.15)	9/23 (39.13)	838	81.5	3/4 (75)
TOTAL		627/981(63.91)	30/178(16.85)			11/32 (34.37)

\*20 swabs per flock/5 swabs=4 pooled samples, 8 flocksX4 pooled samples= 32 samples)

**Table 3. Flock rate positivity by RPA, ELISA, Real-time PCR**

RPA	ELISA	rPCR
Total Flock Positive (%)	Total Flock Positive (%)	Total Flock Positive (%)
8/8 (100)	7/8 (87.5)	6/8 (75)

LD and LG were found to share fourth, fifth, and sixth places with the rate of 39.9%, 32.8% and 32.7%, respectively. In LB and LC, CV% were found to be 33.2% and 27.2%, respectively (Table 2). MG-seropositive flock rate was determined to be 87.5% by ELISA (Table 3).

### 3.2.1 Results of real-time PCR with clinical samples

Of the tested 32 tracheal swab samples (20 swabs per flock/5 swabs=4 pooled samples, 8 flocksX4 pooled samples= 32 samples) 34.37% (11/32) were found to be positive by rPCR. Comparing the tracheal swabs in terms of MG load for each flock from highest to lowest, the rates were found to be 75%, 75%, 50%, 25%, 25%, 25%, 0.0% and 0.0% in LH, LA, LC, LB, LG, LF, LD and LE, respectively (Table 2). rPCR positive flock rate was found to be 75% (Table 3).

## 4. DISCUSSION AND CONCLUSION

There were several studies conducted on serology and molecular examination of MG in breeder flocks, layer flocks and broiler flocks in our country and the world [17, 19, 21, 26, 27, 28, 29, 30, 31]. In line with high prevalence of MG-positive flocks in our study, in Vietnam MG-PCR positive results have been reported as 58.1% [32]. In contrast to our results, Michiels et al. [33] declared pretty low prevalence in both layer (0.9%) and broiler (2.7%) flocks in Belgium. Osman et al. [34] reported that the prevalence of MG was found to be high in the layer flocks with the rate of 33.3%, 69.9% and 58.3% by PCR, RPA and ELISA, respectively in Egypt. In Kuwait, the prevalence of MG was declared to be 48% with ELISA and 58% with PCR [35]. The prevalences are varied from country to country for the reasons such as sample type (breeder, layer, broiler), applied test methods and official surveillance of MG in breeder flocks.

To the best of our knowledge, previous studies showed that MG was a great problem in Marmara Region of Turkey where our study was

conducted [19, 21]. In our study, while the rate of MG-seropositive layer flock was found to be 100% with RPA and 87.5% with ELISA, rPCR positive flock rate was detected to be 75% in South Marmara Region. However, in other studies in the same region the seropositive results and prevalence of MG by rPCR were relatively lower than our results. Kahya et al. [21] reported the flock rate base seropositivity with RPA and HI test as 48.8% and 32.3%, respectively, in breeder flocks exhibiting respiratory problems. In the same study, MG prevalence by rPCR was declared to be 29% by the same author [21]. Kahya et al. [36] reported MG-seropositive flock rate in the same region in breeder flocks as 68.8% with ELISA. Comparing the results, the respectively low rate of prevalences in Kahya et al's studies [21, 36] were attributed to sampling group (breeder flock) where strict biosecurity measures were applied. Commercial layer flocks, in contrast to breeder flocks are more exposed to disease agents due to inadequate biosecurity measures in the region such as intensive commercial poultry businesses in close proximity to each other in a narrow space. And also, high positiveness in our study was thought to be originated from the breeder flocks via vertical transmission, although the breeders were under official surveillance in the region. In the studies conducted in other regions of Turkey, while Dakman et al. [28] declared no MG positiveness in breeder flocks from Central Anatolia, Cengiz et al. [27] reported the rate of 53.8% MG positiveness in chicken flocks. According to RPA test results in this study, the prevalence of MG antibody was high in commercial layer hens of age 19-47 weeks that was in a range of 67.47%-94.15%, while the lowest prevalence of MG antibodies was found at age 58-60 weeks with the range of 11.56%-31.88%. Our findings were in line with the findings of the study conducted in 1995 [37]. The ELISA results coincided with the RPA test results at 19-24 weeks and 58-60 weeks in our study. Tendency of MG infection at younger age group than adult is a well known fact [1]. However, Demirbilek Kahya [38], determined 46 weeks as most MG positive period, followed by 40 weeks, 34 weeks, 27 weeks, and at least 20 weeks.

Screening of poultry flocks for infection with the pathogenic mycoplasmas is generally accomplished by RPA test (2). RPA test is quick, inexpensive and highly sensitive, however the greatest disadvantage of RPA is low specificity (false positive reactions) related to medium component, cross reactions between bacterial species, misinterpretation due to recent inactivated oil emulsion vaccination against other infections agents. Such positive reactions may persist 4-8 weeks or longer after vaccination [7]. Due to disadvantages of RPA, high sensitivity (false positive) and low specificity (false negative), flocks with RPA test reactors are generally confirmed as positive or negative with acceptable serological tests such as HI, ELISA and then confirmatory tests culture and/or PCR. In our study, as a result of examining 23 RPA positive sera for each flock except for LE and 17 RPA positive sera for flock LE by ELISA, the percentages of positiveness were found to be decreased in all eight flocks. It was expected because RPA restricted to the early stage of infection to detect IgM antibodies which were found 7-10 days post-infection and persist up 4-8 weeks or longer after vaccination while ELISA was considered suitable to detect IgG antibodies produced in the later stage of infection, 3-4 weeks post-infection and persisted up to 6 months [5, 7, 8]. ELISA detect antibodies about the same time after infection as HI test. Unfortunately, ELISA tends to have the same propensity toward giving false positive reactions as the agglutination test [7].

When we interpret the results of both serology and PCR, flocks LB, LC, LF and LG had high MG antibody rates determined by RPA with a range of 67-87.6% in contrast their low MG-antibody rate detected by ELISA (8.69%-21.73%). The low antibody rates determined by ELISA in flocks LB, LC, LF and LG were in contrast to their high MG-rPCR positivity rates (15%-40%). Our finding was found to be similar to Kahya et al's. [36] study, they found low MG antibody rate (42%-62%) by ELISA in contrast to high MG-rPCR positivity rate (70-100%). This situation could be explained by the possibility of a newly starting MG infection since high MG antibody rates by RPA (IgM-recently started infection) were in parallel to rates of MG-rPCR. While in flocks LD and LE MG-antibody rates were found to be low by both RPA (31.88% and 11.56%) and ELISA (4.34%-0.00%), MG-rPCR positivity rates were found to be 0.00% for both flocks. This suggests that either agglutination tests or ELISA have propensity to give false positive reactions as

described in the previous paragraph in detail. In flocks LA and LH, MG-antibody rates were found to be 79.48% and 94.15%, respectively by RPA, MG-antibody rate was detected as 39.13% in both flocks (with the high CV% values), and MG-rPCR positive rates were found to be 70% and 80%, respectively. This instance could be explained by a continuing infection.

Although the serological tests can be used as a primary screening tests with known shortcomings as cross reactivity, high sensitivity-low specificity (RPA) and high specificity-low sensitivity (ELISA), PCR and/or bacteriology should be used for confirmative and definite diagnosis. Consequently, to overcome the infection of layer flocks with MG, accurate and timely diagnosis of MG can be accomplished with serology and PCR and/or bacteriology at the same time to reduce the detection time. This prompt action gives a chance to producers to act quickly and prevent the spread of infection. Treatment with appropriate antibiotics to be followed after early diagnosis is one of the way to hinder the dissemination of the agent. In addition, we believe that it would be a precautionary measure to examine the pullets for MG before they are placed in the flocks. According to the results of our study, we thought that taking additional restrictive biosecurity measures in a region with dense and congested layer flocks existed would be meaningful in order to prevent economic loss and protect the poultry health.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## ETHICAL APPROVAL

All the samples were collected following approval of the ethical committee at Veterinary Control Central Research Institute, Ankara. (No:2019/13 Date:15.11. 2019).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

- Ley DH. *Mycoplasma gallisepticum* infection. In: Saif YM, Barnes HJ, Glisson JR, Fadly AM, McDougald LR, Swayne DE, eds. Diseases of Poultry 12<sup>th</sup> edn. Ames, IA: Blackwell Publishing. 2008;807-83.
- Anonymous. Standard test procedures for *mycoplasma* section 147.7. In: national poultry improvement plan and auxiliary provisions. Animals and animal products, Part 147, U.S. department of agriculture, animal and plant health inspection service; 2004.
- Manual of diagnostic tests and vaccines for terrestrial animals. Chapter 2.3.5. Avian mycoplasmosis (*Mycoplasma gallisepticum*, *M. synoviae*). Available: [https://www.oie.int/fileadmin/Home/eng/Health\\_standards/tahm/3.03.05\\_%20AVIAN\\_MYCO.pdf](https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.03.05_%20AVIAN_MYCO.pdf) (Accessed online: July, 2021)
- Kempf I. DNA amplification methods for diagnosis and epidemiological investigations of avian mycoplasmosis. Avian Pathol. 1998;27:7-14. Doi: <https://doi.org/10.1080/03079459808419268>
- Kleven SH. Antibody response to avian mycoplasmosis. Am J Vet Res, 1975; 36: 563-565.
- Glisson, JR, Dawe JF, Kleven SH. The effect of oil-emulsion vaccines on the occurrence of nonspecific plate agglutination reactions for *Mycoplasma gallisepticum* and *Mycoplasma synoviae*. Avian Dis. 1984;28:397-405.
- Kleven SH. Mycoplasmosis. In: a laboratory manuel for the isolation and identification of avian pathogens, 4th edn. Swayne DE, Glisson JR, Jackeood MW, Pearson JE, Reeds WM, eds. American Association of Avian Pathologists, Kennett Square, PA. 1998;74-80.
- Bradbury JM, Morrow CJ. Avian mycoplasmas. Pattison M, McMullin PF, Bradbury JM, Alexander DJ, eds. Poultry Disease, Saunders Elsevier, Philadelphia, USA. 2008;220-234.
- Kempf I, Gesbert F, Guittet M. Experimental infection of chickens with an atypical *Mycoplasma gallisepticum* strain: comparison of diagnostic methods. Res Vet Sci. 1997;63:211-213. DOI: [10.1016/s0034-5288\(97\)90022-9](https://doi.org/10.1016/s0034-5288(97)90022-9).
- Salisch H, Hinz KH, Graack HD, Ryll M. A comparison of a commercial PCR-based test to culture methods for detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in concurrently infected chickens. Avian Pathol. 1998;27:142-147. DOI: <https://doi.org/10.1080/03079459808419315>
- Mekkes DR, Feberwee. A Real-time polymerase chain reaction for the qualitative and quantitative detection of *Mycoplasma gallisepticum*. Avian Pathol. 2005;34:348-354. DOI: <https://doi.org/10.1080/03079450500179954>
- Razin S. Molecular biology and genetics of mycoplasmas (Mollicutes). Microbiol Rev. 1985;49: 419-455.
- Geary SJ, Intress R, Gabridge MG. Species specific biotinylated probe for the detection of *Mycoplasma gallisepticum*. Mol Cell. Probes. 1989;2:237-244.
- Hyman HC, Levisohn S, Yogev D, Razin S. DNA probes for *Mycoplasma gallisepticum* and *Mycoplasma synoviae*: application in experimentally infected chickens. Vet Microbiol. 1989;20: 323-337. DOI: [10.1016/0378-1135\(89\)90057-6](https://doi.org/10.1016/0378-1135(89)90057-6).
- Slavik MF, Wang RF, Cao WW. Development and evaluation of the polymerase chain reaction method for diagnosis of *Mycoplasma gallisepticum* infection in chicken. Mol Cell Probes. 1993; 7: 459-463. DOI: <https://doi.org/10.1006/mcpr.1993.1068>
- Wang H, Fadl JR, Khan MI. Multiplex PCR for avian pathogenic mycoplasmas. Mol Cell Probes. 1997;11:211-216. DOI: [10.1006/mcpr.1997.0108](https://doi.org/10.1006/mcpr.1997.0108)
- Garcia M, Ikuto N, Levisohn SS, Kleven SH. Evaluation and comparison of various PCR methods for detection of *Mycoplasma gallisepticum* infection in chickens. Avian Dis. 2005; 49:125-132. DOI: [10.1637/7261-0812204R1](https://doi.org/10.1637/7261-0812204R1).
- Marois C, Dufour-Gesbert F, Kempf I. Polymerase chain reaction for the detection of *Mycoplasma gallisepticum* in enviromental samples. Avian Pathol. 2002;31:163-168. DOI: [10.1080/03079450120118658](https://doi.org/10.1080/03079450120118658)

19. Carli KT, Eyigor A. Real-time polymerase chain reaction for the detection of *Mycoplasma gallisepticum* in chicken trachea. *Avian Dis.* 2003;47: 712-717. DOI: 10.1637/6041
20. Jarquin R, Schultz J, Hanning I, Ricke SC. Development of a real-time polymerase chain reaction assay for the simultaneous detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* under industry conditions. *Avian Dis.* 2009;53:73-77. DOI:https://www.jstor.org/stable/25599070
21. Kahya S, Temelli S, Eyigor A, Carli KT. Real-time PCR culture and serology for the diagnosis of *Mycoplasma gallisepticum* in chicken breeder flocks. *Vet Microbiol.* 2010;144:319-324. DOI: 10.1016/j.vetmic.2010.01.012.
22. Kleven SH. Changing expectations in the control of *Mycoplasma gallisepticum*. *Acta Vet Hung.* 1997;45:299-305.
23. Levisohn S, Kleven SH. Avian mycoplasmosis (*Mycoplasma gallisepticum*) In: Beard CW, McNulty S, eds. Diseases of poultry. Office International des Epizooties, Paris, France. 2000; 425-442.
24. Whithear KG. Control of avian Mycoplasmoses by Vaccination. *Rev Sci Tech.* 1996;15:1527-1533. DOI: 10.20506/rst.15.4.985
25. Zain ZM and Bradbury JM. The influence of type of swab and laboratory method on the recovery of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in broth medium. *Avian Pathol.* 1995;24:707-716. DOI: 10.1080/03079459508419109
26. Kesler K, Guler L, Orhan G. Investigation of *Mycoplasma gallisepticum* infection in layer flocks by rapid serum agglutination, culture and polymerase chain reaction methods. *Eurasian J Vet Sci.* 2013;29(2):76-8.
27. Cengiz S, Babacan O, Dinç G, Akan M. Diagnosis of *Mycoplasma gallisepticum* infection in chickens by polymerase chain reaction (PCR). *J Etlık Vet Mikrobiol.* 2011;22(2):45-48.
28. Dakman A, Günaydın E, Turkyılmaz MA, Gülec M, Coşar M, Ozdemir U. Damızlık tavuk işletmelerinde tespit edilen mikoplazma enfeksiyonları. *J Etlık Vet Mikrobiol.* 2009;20:27-34.
29. Tuzcu M, Özmen M, Karakoç SR, Tuzcu N, Yoldas A. Diagnosis of mycoplasmosis in chicks by pathological and real-time PCR methods. *Eurasian J Vet Sci.* 2012;28:82-86.
30. Feberwee A, Mekkes DD, Wit JJ, Hartman EG, Pijpers A. Comparison of culture, PCR and different serologic tests for detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* infections. *Avian Dis.* 2005;49:260-268.
31. Hess M, Neubauer C, Hackl R. Interlaboratory comparison of ability to detect nucleic acid of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* by polymerase chain reaction. *Avian Pathol.* 2007;36:127-133. DOI: 10.1080/03079450701203082.
32. Thu NV, Thuy LT, Phuong PT. Investigation of avian *Mycoplasma* infection in Vietnam by molecular tools. Proceedings of International Research on food Security, Natural Resource Management and Rural Development. October 8-10, Gottingen; 2003.
33. Michiels T, Welby S, Vanrobaeys M, Quinet C, Rouffaer L, Lens L, Martel A, Butaye P. Prevalence of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in commercial poultry, racing pigeons and wild birds in Belgium. *Avian Pathology.* 2016;45(2):244-252. DOI: 10.1080/03079457.2016.1145354.
34. Osman KM, Aly MM, Amin ZM, Hasan BS. *Mycoplasma gallisepticum*: an emerging challenge to the poultry industry in Egypt. *Rev sci Tech.* 2009;28(3):1015-23.doi:10.20506/rst.28.3.1940
35. Qasem JA, Al-Mouqati SA, Al-Ali EM, Ben-Haji A. Application of molecular and serological methods for rapid detection of *Mycoplasma gallisepticum* infection (*Avian mycoplasmosis*). *Pakistan J Biol Sci.* 2015;18(2):81-87. DOI: 10.3923/pjbs.2015.81.87.
36. Kahya S, Eyigor A, Temelli S, Carli KT. Detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* by real-Time PCRs and *Mycoplasma gallisepticum*-antibody detection by an ELISA in chicken breeder flocks. *KVFD.* 2015;21(3):361-366. DOI: 10.9775/kvfd.2014.12505
37. Nunoya T, Yagihashi T, Tajima M, Nagasawa Y. Occurrence of keratoconjunctivitis apparently caused by *Mycoplasma gallisepticum* in layer chickens. *Vet Pathology.* 1995;32:11-18.



38. Demirbilek SK, Ardicli Ö, Carli KT  
Comparison of *Mycoplasma gallisepticum*  
infection in different samples and ages of  
chicken breeder flocks. Brazilian J Poultry  
Sci. 2020;22(2):1-6. DOI: <https://doi.org/10.1590/1806-9061-2020-1271>

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