*Egypt. J. Vet. Sci.* **Vol. 54,** No. 2, pp. 253-261 (2023)

Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

# Seroprevalence of *Mycoplasma gallisepticum* in Commercial Broiler Chickens in Duhok Governorate

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IN the present study, indirect ELISA conducted to estimate the prevalence of MG antibodies in serum samples collected from 20 broiler flocks in Duhok governorate. Three hundred and sixty blood samples from these flocks, 126,144 and 90 serum samples were collected from broilers aged 4, 5 and 6 weeks, respectively. All samples were examined in Duhok College of veterinary medicine (Kurdistan/Iraq), from October 2021 to January 2022. The positive serum samples to MG were found in 84/126 (66.66%), 80/144 (55.55%), 32/90 (35.36%) of broilers examined at 4, 5 and 6 weeks, respectively. The overall serum positive reactors to MG were 52.48%. There was a decreasing tendency in seroprevalence with examination at 4, 5 and 6 weeks of age. Out of seven broiler flocks (A-G) examined at 4 weeks of age, three (A, B and C) show high mean ELISA MG antibodies' titer (≥5000), and only significant ($P<0.05$) in flock (A) (14015.3±5891.8), in comparison with all remaining five flocks (B-G). Those flocks (H-O) examined at 5 weeks of age, two flocks (H, I) show significantly ($P<0.05$) high mean ELISA MG antibodies' titer (≥5000) (9192.3±5944.9 and 7036±2279.3) respectively when compared with remaining six flocks (J-O). The 5 flocks (P-T) of broiler chickens examined at 6 weeks of age, only one flock (P) show significantly ($P<0.05$) high mean antibody ELISA MG titers (≥5000) (9357.6±6709.9). The remaining flocks (D-G), (J-O) and (Q-T) had MG antibody titers (≤5000).

**Keywords:** Broiler; Broilers, ELISA, *Mycoplasma gallisepticum*

## Introduction

Avian Mycoplasmosis (AM) is a contagious disease in poultry [1]. The pathogens of mycoplasma are gram negative and they belong to class Mollicutes, order-I Mycoplasmatales, family-I Mycoplasmataceae and genus Mycoplasma. *Mycoplasma gallisepticum* (MG) are small size bacteria, without having cell wall and having a triple layers of plasma membrane [2, 3]. (MG) is the most common pathogenic mycoplasmas in broiler and layer production. MG causes polymicrobial " Chronic respiratory disease of domestic poultry, particularly in the presence of Management stress and/or other respiratory pathogens, resulting in loss of production and downgrading of meat and layer type birds, resulting in increased condemnations in the processing as well as significant economic losses in large commercial and noncommercial egg production processes [4-6].

The disease spread vertically via infected eggs from certain unseen carriers. The agent is transmitted horizontally by the infected progeny by coughing or contaminated feed, inhalation of

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(*Received* 19/09/2022; *accepted* 11/11/2022)
DOI. 10.21608/EJVS.2022.163806.1397

contaminated dust, airborne droplets and feathers, water, and the surroundings[7, 8]. Direct or indirect contact through the movement of birds, people, or fomites from infected to susceptible flocks allows for easy flock-to-flock transmission [9, 10]. Young birds are more vulnerable to mycoplasmosis than older birds, which can afflict birds of all ages [11].

Coryza, conjunctivitis, sneezing, sinusitis, mortality, low weight gain, and reduced feed consumption in broiler chickens, as well as reduced egg production in layer chickens are all symptoms of the disease [12, 13]. The upper and lower respiratory tracts can both have lesions. Nasal passageways, infra orbital sinuses, trachea, and bronchi may all have catarrhal exudates [14, 15]. The air sacs' thickness may grow twenty times compared to normal thickness, and they may include mucous or caseous materials [16].

Infectivity and severity of MG vary, and infections can sometimes go undetected. Individuals or flocks that become infected remain infected for the rest of their lives, acting as carriers or reservoirs for infection [17].

MG infection occurrence has increased in poultry probably as a result of the concentration of huge chicken populations in narrow geographic areas and poor biosecurity, as well as the fact that MG infection is more prevalent in young birds than in adults [18, 19].

In Duhok governorate, where the chicken industry is already huge and growing, effective biosecurity strategies are needed to combat chronic respiratory diseases. However, clinical symptoms and conventional culture procedures are insufficient to diagnose MG infections in chicken flocks, but the serological methods of agglutination and ELISA techniques are widely used to monitor avian mycoplasmosis in poultry farms [20-22]. The sensitivity of diagnostic tests and the fast detection of the infectious agent are especially crucial during *Mycoplasma gallisepticum* infection [23]. Serology is the best tool for detection of clinical and subclinical MG infection in poultry flock [24]. Enzyme-linked immunosorbent assay is one of the most widely used serological tests that are broadly used for finding of *Mycoplasma gallisepticum* (MG) infections [17, 25], since ELISA and HI tests only identify IgG antibodies that arise after 7-10 days (DPI) and last for 180 days [26, 27].

Keeping chicken farms free of MG requires retaining alternatives from mycoplasma-free sources. This program necessitates strong biosecurity and an efficient monitoring mechanism. Medication can assist to prevent clinical signs and lesions [28]. The eradication of organisms from breeding flocks and their progeny has been the mainstay of MG control, with periodic serological monitoring, followed by culture and/or PCR monitoring, confirming the flocks' Mycoplasma-free status [12, 29, 30].

The goal of this study was to determine the seroprevalence of *Mycoplasma gallisepticum* in commercial broiler chicken in the Duhok governorate in Iraq's Kurdistan region.

## Material and Methods

From 20 *Mycoplasma gallisepticum* unvaccinated commercial broiler flocks in Duhok province, Kurdistan /Iraq (Table 1), 360 blood samples were collected from broilers of 4, 5 and 6 weeks of age, suffered from signs of conjunctivitis, sneezing, sinusitis, mortality, poor weight gain as well as reduced feed efficiency, during October 2021 to January 2022 for monitoring the prevalence of *M. gallisepticum* antibodies by indirect ELISA.

Birds bled from the wing or jugular vein and blood samples were collected aseptically and processed for serological study. Collected blood centrifuged (Hettich Centrifuge, Germany, EBA-20) for 4 minutes at 4000 rpm. After centrifugation, sera were transferred into 2 mL tubes and stored in a freezer at -20 $C^{o}$ until processing.

The ELISA kit used was from IDEXX American Company and test was performed according to the manufacturer's instructions. In the Beginning, ELISA test reagents and serum samples were brought to room temperature, then 1 microliter of serum sample separately placed in a clean 2 ml Eppendrorf tube, and 500 microliter of dilution sample was added to each of them.

After 30 minutes, 100 microliters of negative control added to the first and second wells of ELISA microplate, and 100 microliters of positive control were added to the third and fourth wells, followed by 100 microliters of diluted samples in the remaining wells of the microplate. After 30 minutes, all wells of the plate were washed four times with a special microplate washer from BioTek Company. Double distilled water used as a washing solution. Then 100 microliters

**TABLE 1. Distribution of broiler flocks in Duhok governorate used for blood samples collection from broilers at 4,5 and 6 weeks of age for estimation of MG antibody titers using (IDEXX®) ELISA test during 2021-2022.**

| Regions | October | | | November | | | December | | | January | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Flocks tested/ Weeks | | | Flocks tested/Weeks | | | Flocks tested/Weeks | | | Flocks tested/Weeks | | |
| | 4 w | 5 w | 6 w | 4 w | 5 w | 6 w | 4 w | 5 w | 6w | 4w | 5w | 6 w |
| Sheladiz | | | | A* | | | | | P | | H | |
| Kani Golan | | | | | | | B | | | | | |
| Zakho | | | | C | | | | | | | | Q |
| Sndore | | | | | | | | | | | I | |
| Msirik | | | | | | T | | | | | | |
| Bade | | | | | | | | | | | J | |
| Bagera | | | | | | | | | | D | | |
| Bardarash | | | | | | | | | R | | | S |
| Sumel | E | | | | | | | K | | | M | |
| Sarsink | | | | | | | | | | F | | |
| Piromora | | | | | | | | | | | N | |
| Kevla | | | | | O | | | | | | | |
| Mangesh | | | | | | | | L | | | | |
| Glbesh | | | | | | | G | | | | | |

*A= the letters of studied broiler flocks.

of conjugate added to all of the wells. After 30 minutes washing has been done again for four times.

The wells then filled with 100 microliters of substrate. Then 100 microliters of stop solution were added to the well, and detection of antibody by indirect ELISA test using Elx800 reader with a 650-nm filter (biotek™) used to read the titration microplates.

*Statistical analysis*

To get the P-value, all of the data collected throughout the study statistically analyzed. ANOVA used to estimate the P-value and average values using SPSS (version-20) software analysis. The total prevalence of MG infection estimated by dividing the total number of ELISA positive samples by the total number of animals tested. If the S/P ratio is less than 0.5, the sample is regarded negative, and if the S/P ratio is greater than 0.5, the sample is declared positive.

## Results

The results of MG seroprevalence in commercial broilers flocks gained by using indirect ELISA illustrated in (Tables 2, 3 and 4). From tables, it is evident that there was no significant ($P< 0.05$) variation in prevalence among investigated twenty flocks examined at 4, 5 and 6 weeks of age. Out of 126 serum samples tested for MG at 4 weeks old broiler chicken, 84 (66.66%) were found positive. Flocks tested for MG aged 5 weeks, show that 80 samples were positive out of 144 (55.55%), while in those flocks examined for MG at 6 weeks of age, 32/90 (35.36%) were positive for MG. The overall seroprevalence in positive serum samples tested (196/360) was (52.48%).

Those flocks examined at 4$^{th}$ week of their life (A-G) (Table 2), had mean ELISA titers ranging from 14015.3±5891.8 to 583.2±1047.9. Flock A shows significant ($P< 0.05$) higher MG mean titers in comparison with other flocks. (B, C, D, E, F, G). A lower seropositivity was found in (C-G) flocks (83.3% - 16.6%), and mean titers of (6019±1350 to 583.2±1047.9) compared with other flocks.

Flocks examined at 5 weeks of age (H-O) flocks (Table 3), show positive seroprevalence of

55.55%, Highest seroprevalence reported in flock H (100%) with a significant (P< 0.05) higher mean titer in flocks (H and I) (9192.3±5944.9 and 7036±2279.3) respectively.Compared with other remaining flocks (J-O) with seroprevalence of (66.6%-22.7%) and mean titer between (2246±1795.9 - 191±552.9).

Seroprevalence of flocks examined at their 6 weeks of age (Table 4), of Five flocks (P-T) were positive at a rate of 35.36%, being highest in flock P (100%) with a significant (P< 0.05) higher mean titer of (9357.6±6709.9) when compared with other flocks. A lower seroprevalence was reported (38.8%-5.5%) in flock (Q-T), with a significant (P< 0.05) low mean antibody titers (2305±2865-132.5±394.2) compared with (P) group.

## Discusion

(MG) is an infectious agent that causes chronic respiratory diseases in chickens. MG infections are a significant issue in the poultry industry because they lead to large economic losses in the poultry production [31-33]. In this study, Indirect ELISA was employed for the determination of MG seroprevalence in non-vaccinated with MG vaccine broilers that should to be zero, but the results of the investigation showed that all

**TABLE 2. Seroprevalence, Mycoplasma gallisepticum antibody titers and CV percentage of broiler flocks examined at 4 weeks of age.**

| Broiler flocks | ELISA S/P value | | | | | | | ELISA Mean± S. D | All Titers > 5000 | CV percentage |
|---|---|---|---|---|---|---|---|---|---|---|
| | Positive | | | | Negative | | | | | |
| | | No. | % | Total Prevalence | | No. | % | Total Prevalence | | | |
| A | | 18 | 100 | | | 0 | 0 | | 14015.3±5891.8[a*] | + | 42 |
| B | | 18 | 100 | | | 0 | 0 | | 6462±1202.5[b] | + | 18.6 |
| C | ≥ 0.50 (+) | 15 | 83.3 | (+)66.66%[a*] | ≤ 0.50 (-) | 3 | 16.7 | (-)33.34% | 6019±1350[b] | + | 22.4 |
| D | | 14 | 77.7 | | | 4 | 22.3 | | 2442±5324[c] | - | 218 |
| E | | 9 | 50.0 | | | 9 | 50.0 | | 1396.2±1228[c] | - | 87.9 |
| F | | 7 | 38.8 | | | 11 | 61.2 | | 649±964[c] | - | 148.5 |
| G | | 3 | 16.6 | | | 15 | 83.4 | | 583.2±1047.9[c] | - | 131.9 |

*a-c= Different letters within one column means significant (p≤ 0.05).

**TABLE 3. Seroprevalence, Mycoplasma gallisepticum antibody titers and CV percentage of broiler flocks examined at 5 weeks of age.**

| Broiler flocks | ELISA S/P value | | | | | | | ELISA Mean± S. D | All Titers > 5000 | CV percentage |
|---|---|---|---|---|---|---|---|---|---|---|
| | Positive | | | | Negative | | | | | |
| | | No. | % | Total Prevalence | | No. | % | Total Prevalence | | | |
| H | | 18 | 100 | | | 0 | 0 | | 9192.3±5944.9a* | + | 64 |
| I | | 17 | 94.4 | | | 1 | 5.6 | | 7036±2279.3a | + | 32.3 |
| J | ≥ 0.50 (+) | 12 | 66.6 | | ≤ | 6 | 33.4 | | 2246±1795.9b | - | 79.9 |
| K | | 10 | 55.5 | (+)55.5%[a] | 0.50 | 8 | 44.5 | (-)44.45% | 1725.7±1410b | - | 84 |
| L | | 10 | 55.5 | | (-) | 8 | 44.5 | | 1436.2±1540bc | - | 107.2 |
| M | | 5 | 27.7 | | | 13 | 72.3 | | 869.6 ± 892.6c | - | 102.6 |
| N | | 4 | 27.7 | | | 14 | 72.3 | | 686±801.9c | - | 116.7 |
| O | | 4 | 22.2 | | | 4 | 22.2 | | 191±552.9d | - | 289.4 |

*a-d= Different letters within one column means significant (p≤ 0.05).

**TABLE 4. Seroprevalence, *Mycoplasma gallisepticum* antibody titers and CV percentage of broiler flocks examined at 6 weeks of age.**

| Broiler flocks | ELISA S/P value | | | | | | | | ELISA Mean± S. D | All Titers > 5000 | CV percentage |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Positive | | | | Negative | | | | | | |
| | | No. | % | Total Prevalence | | No. | % | Total Prevalence | | | |
| P | ≥ 0.50 (+) | 18 | 100 | (+)35.36%[a] | ≤ 0.50 (-) | 0 | 0 | (-)63.88% | 9357.6±6709.9a* | + | 71.7 |
| Q | | 7 | 38.8 | | | 11 | 61.2 | | 2305±2865b | - | 127.9 |
| R | | 4 | 22.2 | | | 14 | 77.8 | | 837.2±952c | - | 113.7 |
| S | | 2 | 11.1 | | | 16 | 88.9 | | 530±1607.4cd | - | 318.3 |
| T | | 1 | 5.5 | | | 17 | 94.5 | | 132.5±394.2d | - | 297.2 |

*a-d= Different letters within one column means significant (p≤ 0.05).

collected samples from six out twenty broiler flocks tested had high antibody titers (more than 5000), i.e., suspect infection reflected by the overall prevalence of positive sera (52.58%) (Tables 2, 3 and4). The Six broiler flocks (A, B, C) of 4 weeks of age; (H, I) of 5 weeks of age, and (P) of 6 weeks of age had the highest positive seroprevalence (titers ≥5000) to *M. gallisepticum* in 90% or more of the serum samples in these flocks. Furthermore, the findings of this study provide a novel tool for simple serological distinction of clinical titers of mycoplasmosis [34].

Surveys in Ethiopia show some similarity to our findings about the overall seroprevalence of *M. gallisepticum* of 49.4% (254/514) [35]. Another close result to our finding of 57.7 % was recorded by some authors [36]. Similar prevalence was obtained in China by Sun et al. [37] of 50% in 2012 and 57% in 2013.

On the other hand, Lower prevalence was recorded in Iran (33%) [38], and also in Nakompathion [39] ranged from 18-40%. It was found that Low seroprevalence was recorded in broilers of 6 weeks old compared with those obtained from broilers examined at five or those tested in younger ages of 4 weeks. This may be attributed to the intensive treatment practiced during mycoplasmal infections in broiler flocks.

Broiler parent stock should be monitored for MG infection on a regular basis with vaccination when needed. The author suggest vaccination of broiler chicks in Duhok province should be carried out in birds as young as 2–4 weeks of age, using autogenous strain, since the sequences results of the isolated *Mycoplasma gallisepticum* in this study revealed and for the first time a new gene in NCBI (national center for biotechnology information) in Duhok governorate (Kurdistan region/Iraq) with Accession NO. LC721221) (not yet published data).The evidence from MG seroprevalence in the current finding indicates that infection is widespread in Duhok province. So, biosecurity measures including vaccination and sero-monitoring should be implemented for establishing *M. gallisepticum*-free especially in breeding flocks, that should be managed and kept under good biosecurity condition to prevent introductions of the organism vertically or horizontally, serological monitoring in order to consistently affirm infection-free is the most effective management approach.

## Conclusion

In conclusion, the current study finding indicates a significant seroprevalence of MG antibody titer in Duhok governorate. Data from an indirect ELISA test indicate that birds aged six weeks had less antibody titer to MG infection than birds aged four weeks. In commercial farms, Autogenous vaccines and sero-monitoring should be implemented.

*Acknowledgments*

The authors thank Duhok University and the farmer owners for allowing us to obtain blood samples from their chicks.

*Conflicts of Interest*

The authors of this study have revealed no conflicts of interest.

*Funding statement*

No funding

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## الانتشار المصلي للإصابة بالمايكوبلازما غاليسيبتيكوم في فروج اللحم في محافظة دهوك

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في هذه الدراسة تم تطبيق تقنية الادمصاص المناعي المرتبط بالانزيم (ELISA) غير المباشر لتقدير مستوى الاضداد ل MG في عينات مصل الدم التي تم جمعها من ٢٠ قطيع لافراخ فروج اللحم في محافظة دهوك . تم جمع ٣٦٠ عينة دم من هذه القطعان بواقع ١٢٦و ١٤٤ و٩٠ عينة مصل من الافراخ بعمر ٤ و ٥ و ٦ اسابيع على التوالي. تم فحص جميع العينات في كلية الطب البيطري /جامعة دهوك (كردستان / العراق) للفترة الممتدة من تشرين الأول ٢٠٢١ إلى كانون الثاني ٢٠٢٢. سجلت عينات مصل دم ايجابية لـ MG في ١٢٦/٨٤ (٦٦,٦٦٪) ، ١٤٤/٨٠ (٥٥,٥٥٪) ، ٣٢ / ٩٠ (٣٥,٣٦٪) من افراخ فروج اللحم بعمر ٤ و ٥ و ٦ أسابيع على التوالي ، وكان إجمالي المصول الموجبة لـ MG ٥٢,٤٨٪. وكان هناك اتجاه متناقص في مستوى الاضداد مع كل زيادة أسبوعية من عمر الافراخ. فمن بين سبعة قطعان (G- A) تم فحصها في عمر ٤ أسابيع ، أظهرت ثلاثة منها (A ، B و C) متوسط عالي للاضداد وبمعيار (اكثرمن ٥٠٠٠) ، وبصورة معنوية (P<٠,٠٥) في القطيع(A) ليصل الى ( ١٤٠١٥,٣) ± ٥٨٩١,٨ ، بالمقارنة مع جميع القطعان الخمسة المتبقية (G-B). اما القطعان (O- H) والتي تم فحصها في عمر ٥ أسابيع ، أظهر قطيعان هما (H ، I) وبشكل ملحوظ (P<٠,٠٥) متوسط عالي من الأجسام المضادة لل MG وبمعيار (اكثرمن ٥٠٠٠) ٩١٩٢,٣±٥٩٤٤,٩) و ٧٠٣٦±٢٢٧٩,٣) على التوالي عند مقارنتها مع ٦قطعان متبقية(J-O). تم فحص خمسة قطعان (T-P) من فروج اللحم بعمر ٦ أسابيع ، أظهرمنها قطيع واحد (P) وبشكل ملحوظ (P<٠,٠٥) ارتفاع في متوسط الاضداد لل MG وبمعيار (اكثرمن ٥٠٠٠) (٦٧٠٩,٩±٩٣٥٧,٦).. القطعان (G-D) و (O J-) و (T -Q) اظهرت متوسط لاضداد MG أقل من (٥٠٠٠).

# الانتشار المصلي للإصابة بالمايكوبلازما غاليسيبتيكوم في فروج اللحم في محافظة دهوك

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