MESP Journals www.mespj.com ISSN:2980 - 4521
Academic International Journal of Veterinary Medicine (AIJVM)

ISOLATION AND HISTOPATHOLOGICAL EXAMINATION OF *MYCOPLASMA GALLISEPTICUM* IN AN INFECTED LAYER CHICKEN IN A DIFFERENT REGION OF KARBALA PROVINCE

Dhiaa Jaber Hamzah[1*], Haider A. Muhammid[2], Mediha Hadj Ayed[3]
[1 A.] Department of Life Sciences, Faculty of Sciences- Sfax , University of Sfax, Tunisia .
[B.] Department of pathology and poultry diseases, College of veterinary medicine , University of Al_Qadisiyah, Iraq .
[2] Department of Microbiology, Karbella University, Iraq
[3] Institute Superior agronomique of Chott- Mariem ,University of Sousse ,Tunisia
[*] Corresponding author Dhiaa Jaber Hamzah., Email: Dhiaa.hamzah@qu.edu.iq

ABSTRACT

"Chronic respiratory disease" in hens and "infectious sinusitis" in turkeys are both caused by the same pathogen, "*Mycoplasma gallisepticum"* (MG). It was spread through eggs and led to processing performance, decreased feed efficiency and egg production, and expensive control measures; double tracheal swabs were obtained from 120 commercial chickens with a mean of 5.5 weeks between February 2020 and August 2021 and tested for Mycoplasma gallisepticum at Karbala city (Iraq) from chicken detailed history, and all complaint concerning the caseous exudate accumulation in the trachea was inoculation in the modified Frey's broth (PPLO) medium for Mycoplasma culturing and histological analysis, sterile scissors removed necropsy chicken parts like the trachea and lung were inoculated with 10% formalin for histological evaluation. The results were found under an X10 dissecting microscope; colonies resembling a fried egg confirmed the incidence of *Mycoplasma gallisepticum* in 39 out of 120 layer fowl poultry tested, a histopathological slice of trachea exhibits epithelial layer sloughing and necrosis as well as poultry lung histopathology demonstrates severe fibrinous bronchopneumonia and constriction of pulmonary airways and pulmonary hemorrhage.

Keywords: **Mycoplasma gallisepticum, Culturing, Histopathological examination**

How to cite: Dhiaa Jaber Hamzah, Haider A. Muhammid, Mediha Hadj Ayed .Isolation and histopathological examination of mycoplasma gallisepticum in an infected layer chicken in a different region of karbala province. Acad. Int. J. Vet. Med. 2022; 1(2):12-22

INTRODUCTION

One of the main obstacles to poultry farming in the world is outbreaks of diseases that cause morbidity and mortality and, therefore it, affects production. "*Mycoplasma gallisepticum"* Mycoplasma gallisepticum, known as MG, is a respiratory pathogen that is highly contagious and has the potential to cause distress in poultry. It is one of the contagious elements that contribute to the economic lost of poultry producers. In 1926, the majority of cases of mycoplasma infection were ascribed to 'turkeys,' whereas later cases were attributed to 'hens.' In 1936, the "*Mycoplasma gallisepticum* (MG) chicken sepsis infection" was called 'chronic respiratory disease' (CRD) in chickens and infectious sinusitis in turkeys (1). It causes disease symptoms in laying hens, broilers, and poultry flocks, such as tingling and

sneezing, in the respiratory system, coughing, and discharge from the chicken's eyes and nose. (2).

"Mycoplasmosis is a disease that is particularly cost-effective in poultry. Deaths in the poultry business, particularly those referred to as "chicken meat losses," occur all around the world. In broilers, it will result in a loss in weight gain, a drop in feed "conversion efficiency," and an increase in "increased mortality and conviction rates.". In breeders and pigs, sickness leads to a decrease in disease. Laying eggs increase and decrease fetal mortality, Hatchability and quality of chicks (3), "Chickens of all ages are susceptible to infection Mycoplasma disease, but young birds are more susceptible to adults, in addition, The cost of medicines and vaccinations makes this disease one of the most common challenge and most expensive disease problem in the poultry industry" (4).

Previous studies have defined substitute indicators used in the laboratory for regular bacterial culture that led to the detection of "*Mycoplasma gallisepticum*" (5,6). These indicators include "serological testing" for CRD infection to control the occurrence of antibodies in contradiction of "Mycoplasma gallisepticum" (7) and molecular testing for Mycoplasma gallisepticum (8).

The drive of this study was to examine the impact and presence of MG infection on tracheal mucosa in chickens and the underlying histological mechanisms involved in a different region of Karbala city.

MATERIALS AND METHODS:

Tracheal swabs collection: double tracheal swabs were obtained from 120 commercial chickens (range, 4-6 weeks; mean, 5.5 weeks) between February 2020 till august 2021 and were verified for the occurrence of "*Mycoplasma gallisepticum"* at Karbala city (Iraq) from detailed chicken history was obtained, and all complaint concerning the caseous exudate accumulation in trachea and inoculation in the modified Frey's broth (PPLO) medium HiMedia /India and the media was transported to the research laboratory on dry ice, which was located in the microbiology department of the 'veterinary medicine college at Karbala University, this was done so that culture and histological examinations could be performed.

Culture technique: A tracheal swab was placed in modified Frey's broth along with an aliquot of the vortex in 1 ml of sterile deionized water. The mixture was then cultured at 37 ºC with 5% carbon dioxide and high relative moisture for 24 hours (9). The color of Frey's broth shifted subtly from pink to orange-yellow over the course of each day, and its gradual transformation was interpreted as a positive sign for the culture; following an incubation period of one week, cultured broths with unaltered colors were transferred to fresh 'Frey's broth.' If the color continued to be unaltered after one week, an additional passage was performed (10).

Streaks of positive broths were then placed on a medium consisting of 'avian Mycoplasma modified Frey medium' and cultured for at least two weeks at a temperature of 37 ºC in an atmosphere containing 5% carbon dioxide. Under a dissection microscope, the plates were inspected to look for characteristic colony formations. Once the colonies had reached a sufficient size, a digitonin test was utilized in order to differentiate "Mycoplasma colonies from Acholeplasma colonies" (11), the composition of this medium depends on (12) with some modification supplements consist of [PPLO agar 165 ml, BBL tryptone soya broth 2g, $MgSo_4.H_2o$ 0.04 g, Yeast extract 2g, Agar 2g, PH=6.0] After thoroughly combining and heating to 100 $^{\circ}$C in a water bath for 1 hour, autoclaving, cooling to 57 $^{\circ}$C, and adding the following supplements, the mixture was ready for use. [2 ml yeast extract (25%), 20 ml horse

serum], L-cystine (2%) 2ml, L-arginine (30%) 2ml, glutamine 2ml, DNA(0.2%) 2ml, putericine dihydrochloride 2ml, Glucose 3 g followed by Ampicillin 2ml, ceftriaxone 2ml, Flucomnazole 2ml and phenol red (0.4%) and each component was diluted to 1 ml and placed onto a petri dish..

Ethical Declaration

This study was conducted with the approval of the Laboratory Animal Ethics Committee of the veterinary medicine college im Kerbala University.

Histological examination:

After the physical inspection, organs (including trachea and lungs) from necropsy chickens were removed by sterile scissors for the purpose of the histological examination. These organs were then fixed in 10% formalin. After that, the organs were processed for a histological inspection by having various concentrations of ethyl alcohol, xylene, and paraffin applied to them successively. They manipulated the waxy molds and sliced the specimens with a shredder of five microns before mounting them on glass slides and staining them with hematoxylin and eosin (13).

RESULT

Clinical Signs of infected layer chicken:

Infected layer chickens in the intentional field revealed different types of clinical signs summarized by frothiness about the eyes and, congestion of mucous membranes (32.5%), rapid and difficult respiration (91.6%). In addition, that other chickens were suffering from lethargy (76.6%), weight loss (15%), and nasal discharge (75%) the table (1) shows this investigation. Necropsy findings revealed the presence of fibrous exudate on the internal organs due to the inflammation of the internal organ figure (1).

Table (1): Clinical signs of infected chicken with *Mycoplasma gallisepticum*

Clinical signs	No. of infected Chicken	%
Frothiness about the eyes with congestion of mucous membranes	39	32.5%
Rapid and difficult respiration	110	91.6%
Lethargy	92	76.6%
Weight loss	18	15%
Nasal discharge and rales	91	75%
Total	120	-

Figure 1: Macroscopic examination of internal chicken organ A: fibrin exudate in side internal organ of chicken B: hemorrhagic of the trachea

Mycoplasma modified Frey medium: Of the 120 infected layer chicken studied, 39 were positive for *M. gallisepticum*, and colonies resembling a fried egg indicated the presence of *M. gallisepticum*, under an **X**10 dissecting microscope figure 2.

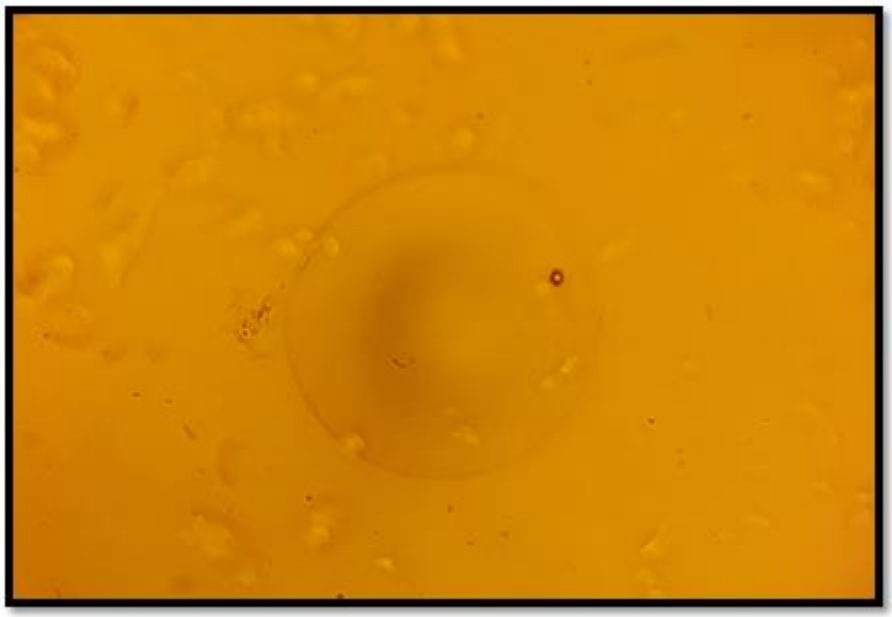

Figure 2: shows fried egg colony of Mycoplasma gallisepticum Histological examination :

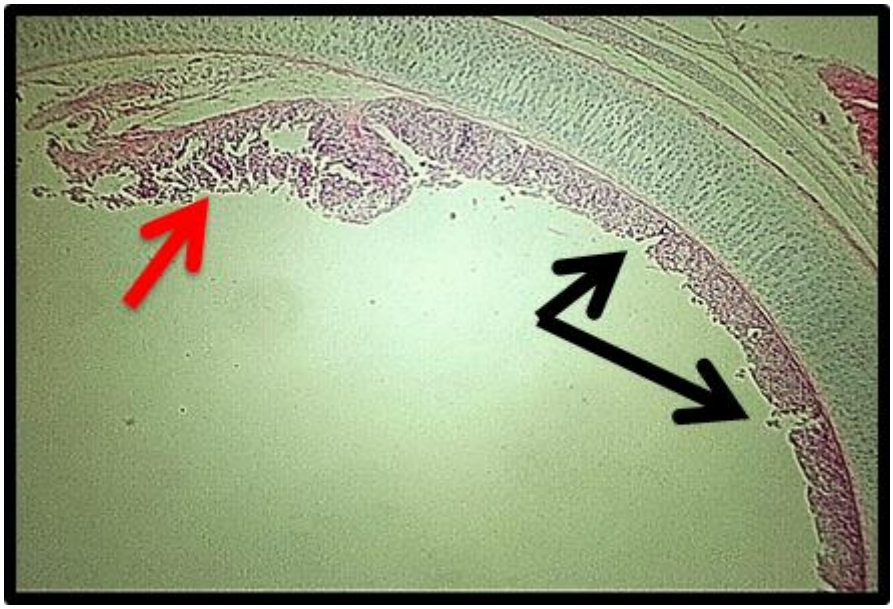

Figure 3: Histopathological section in the trachea shows sloughing and damage in the epithelial layer of tissue (red arrow), and a necrotic process can be seen in some areas of the epithelial layer (black arrow). The section is stained with hematoxylin and eosin (H&E) stain. The figure is captured using a digital camera with a light microscope under 10 X magnification.

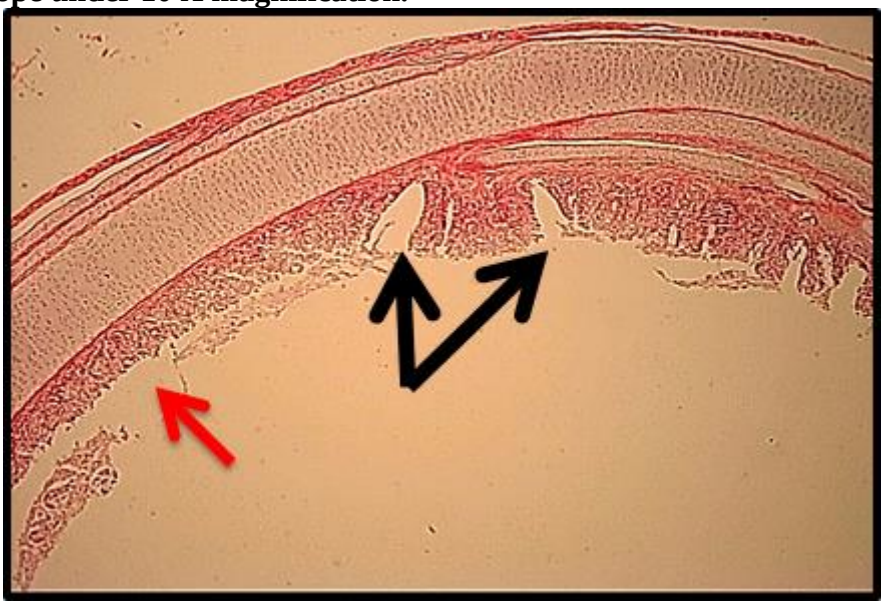

Figure 4. The histopathological section in the trachea shows sloughing and damage in the epithelial layer of tissue (red arrow), and the necrotic process can be seen in some areas of the epithelial layer (black arrow). The section is stained with hematoxylin and eosin (H&E) stain. The figure is captured using a digital camera with a light microscope under 10 X magnification.

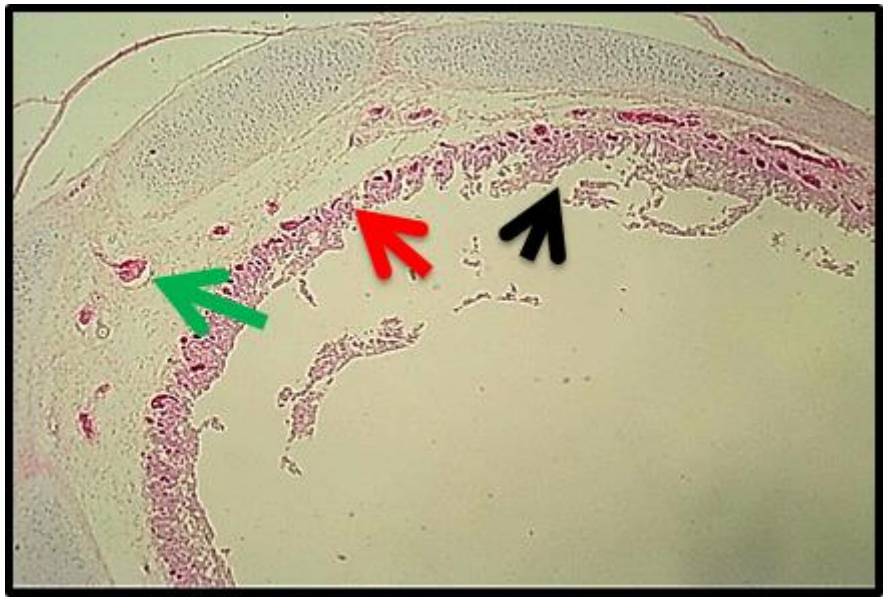

Figure 5. The histopathological section in the trachea shows sloughing and damage in the epithelial layer of tissue (red arrow), and the necrotic process can be seen in some areas of the epithelial layer (black arrow). Multiple blood vessel congestion areas have localized in the sub-epithelial layer (green arrow). The section is stained with hematoxylin and eosin (H&E) stain. The figure is captured using a digital camera with a light microscope under 10 X magnification.

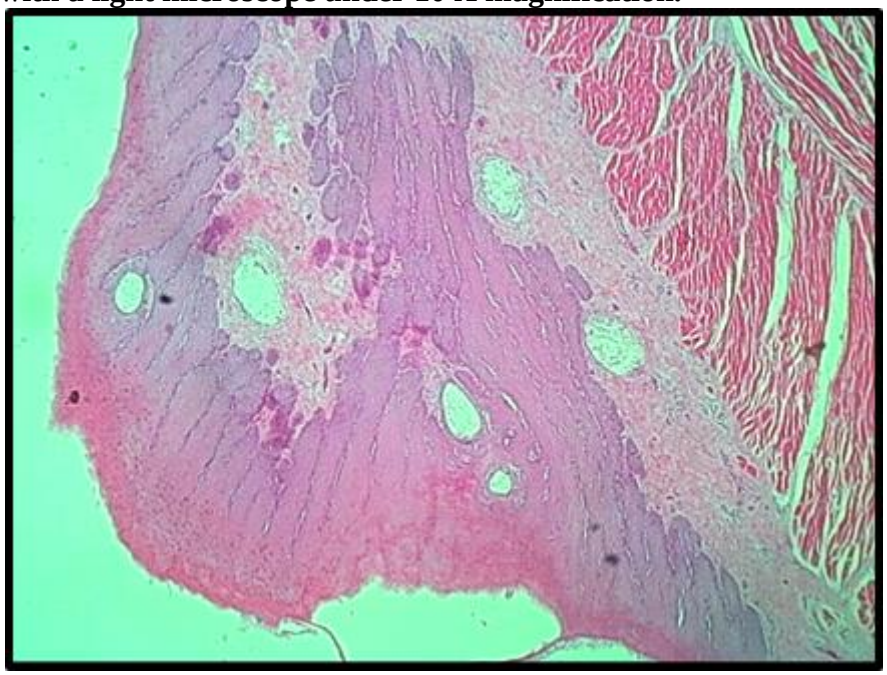

Figure 6. The histopathological section in poultry lung shows narrowing of pulmonary bronchi (Black arrows) with severe fibrinous bronchopneumonia (yellow arrow). The section is stained with hematoxylin and eosin (H&E) stain. The figure is captured using a digital camera with a light microscope under 10 X magnification.

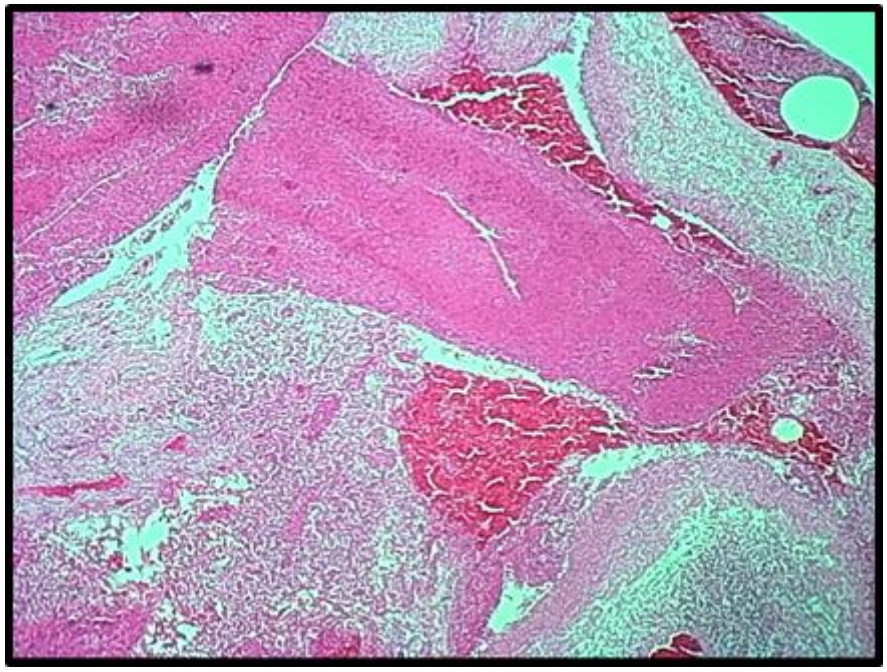

Figure 7. The histopathological section in poultry lung shows narrowing of pulmonary bronchi (Black arrows) with severe fibrinous bronchopneumonia (yellow arrow). Hemorrhage can be seen in the pulmonary tissue at a different area of the histopathological section (green arrow). The section is stained with hematoxylin and eosin (H&E) stain. The figure is captured using a digital camera with a light microscope under 10 X magnification.

On the farmhouse, the occurrence of MG culture medium was 32.5%. It was distributed around Karbala city with four breed flocks as the following ; the numbers for the Al-Hur Poultry flock were 26.6%; the numbers for the Al Husayniyah Poultry flock were 16.6%; the numbers for the Ain al-Tamr Poultry flock, 53.3% and the numbers for the Al-Hindiyah Poultry flock were 33.3% (Table 2) figure 8.

Table 2: Number and Percentage of MG in Karbala poultry farms

Karbala city (no.)	Culture	
	positive (%)	**Negative(%)**
Al-Hindiyah (30)	10 (33.3)	20 (66.7)
Ain al-Tamr (30)	16 (53.3)	14 (46.7)
Al Husayniyah (30)	5 (16.6)	25 (83.4)
Al-Hur (30)	8(26.6)	22 (73.4)
Total (120)	39 (32.5)	81 (67.5)
Statistical analysis	X^2= 9.839, P > 0.05	

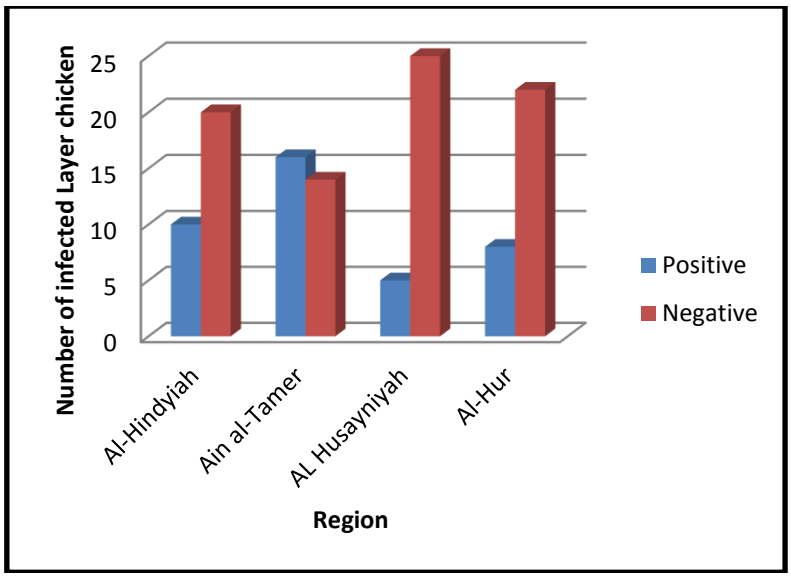

Figure 8: Positive and Negative Number of MG in Karbala poultry farms

DISCUSSION

Cultural isolation and identification are a time-consuming and laborious but reliable way of diagnosing MG; it is highly helpful for molecular strain typing since it delivers a direct confirmation of the presence of bacteria. Similar to the findings of (8), it was observed that isolating "MG with PPLO broth and agar medium" with supplements worked well for isolating "Mycoplasma." Frey's medium-modified medium, with "Pleuropneumonia" like organism (PPLO) medium, have all been used by various researchers to isolate M.gallisepticum and evaluated for their suitability for growth (14, 15, 16).

The suspected colonies showed fried eggs shaped on solid media (17, 18, 19).Also appeared another form of the colony with less center on solid media under stero micrscop (14) In the present study differentiation of Mycoplasma from L form bacteria and fungi was done by Diene's staining , all suspected colonies were positive with diene's staining similar criteria for identification of Mycoplasma spp. was used by (20, 21 and 22).

"Mycoplasma gallisepticum" was found to have a prevalence of 32.5% (39/120) in this study, which is higher than the prevalence of 15.38% and 14.43% found in previous studies by (23 and 24), who isolated Mycoplasma at different localities from Baghdad, and the prevalence of Mycoplasma in other countries (Singapore, Pakistan, and Egypt) by (25, 26 and 16). It's possible that the reasons for the poor isolation rate in this study are attributable to the fact that the samples were collected from treated birds or chronic instances (27) perhaps there could be anti-Mycoplasma compounds, antiserum, or other sorts of inhibitors present, which would lead to a increased likelihood of isolation (28)

Histopathological investigation of the diseased trachea revealed that the increased thickness of the tracheal mucous membrane was caused by hyperactivity of the mucous glands, these changes have been associated with congestion, necrosis, and mild to moderate inflammation. The lesions also revealed modest desquamation and degeneration of the trachea's pseudostratified columnar epithelium. Subsequently, inflammatory cells penetrated the tracheal mucosa and submucosa (29). Additionally, deciliation and epithelial hyperplasia were observed in the tracheal mucosa. On the other hand it was a severe congestion of the sub-mucosa was also found in some locations, and necrosis, blood-filled vacuoles, and disintegration in basal cells were

observed at varying intensities. Some histological studies have shown hyperplasia of the epithelium and the presence of mononuclear inflammatory cells (30).

The microorganisms primarily bind to the cilia and mucosa of respiratory epithelial cells, leading to cilia loss and tracheal mucosal injury that can persist over time and cause chronic respiratory illness (31).

An examining the bronchial tree and parenchymal cells of sick chickens' lungs under a microscope revealed pathological changes, inflammation of the lung parenchyma was mild to severe in a few of instances (32). There was mononuclear inflammatory cell infiltration and multifocal necrosis in the lung lesions. These were identified in lung parenchymal cells and were linked to airway narrowing, hemorrhaging, and necrosis in the epithelium of the affected location. Parabronchial edema and bleeding were found to have bronchial hyperplasia as its root cause (33). Lung parenchymal interstitial broncho-pneumonia was characterized by the presence of fibrin-induced septal thickness, lymphocyte infiltration, and epithelial disruption in the affected area, the parenchymal tissues had emphysema, and the air capillaries had exudates (34).

On the farm, the frequency of MG culture medium was 32.5%; it was dispersed around Karbala city with four breed flocks our result were found Ain al-Tamr Poultry flock: 53.3% more prevalence than other poultry farms, One of the reasons for the difference may be due to the type of area and methods of breeding for poultry fields, the number of domestic animals near the fields, the large number of service workers and wild birds (35).

REFERENCES

1. Prabhu, M., Malmarugan, S., Sweet line Anne, N., Parthiban, S., Balakrishnan, G., & Johnson Rajeswar, J. (2021). Detection of Mycoplasma gallisepticum infection in Turkey and chicken farms of Tamilnadu, India. Int. J. Curr. Microbiol. App. Sci, 10(01), 3151-3158
2. Weisbroth, S. H., & Kohn, D. F. (2020). Bacterial, mycoplasmal, and mycotic infections. The laboratory rat, 451-539.
3. Osman, K. M., Aly, M. M., Amin, Z. M., & Hasan, B. S. (2009). Mycoplasma gallisepticum: an emerging challenge to the poultry industry in Egypt. Revue scientifique et technique (International Office of Epizootics), 28(3), 1015-1023.
4. Nunoya, T., Yagihashi, T., Tajima, M., & Nagasawa, Y. (1995). Occurrence of keratoconjunctivitis apparently caused by Mycoplasma gallisepticum in layer chickens. Veterinary Pathology, 32(1), 11-18.
5. Kang, M. S., Gazdzinski, P., & Kleven, S. H. (2002). Virulence of recent isolates of Mycoplasma synoviae in turkeys. Avian Diseases, 46(1), 102-110.
6. Parker, T. A., Branton, S. L., Jones, M. S., Peebles, E. D., Gerard, P. D., Willeford, K. O., ... & Maslin, W. R. (2002). Effects of an S6 strain of Mycoplasma gallisepticum challenge before beginning of lay on various egg characteristics in commercial layers. Avian diseases, 46(3), 593-597.
7. Ganapathy, K., Saleha, A. A., Jaganathan, M., Tan, C. G., Chong, C. T., Tang, S. C., ... & Bradbury, J. M. (2007). Survey of campylobacter, salmonella and mycoplasmas in house crows (Corvus splendens) in Malaysia. Veterinary Record, 160(18), 622-624.
8. Vogl, G., Plaickner, A., Szathmary, S., Stipkovits, L., Rosengarten, R., & Szostak, M. P. (2008). Mycoplasma gallisepticum invades chicken erythrocytes during infection. Infection and immunity, 76(1), 71-77.
9. Luo, H., He, J., Qin, L., Chen, Y., Chen, L., Li, R., ... & Wu, Y. (2021). Mycoplasma pneumoniae lipids license TLR-4 for activation of NLRP3 inflammasome and autophagy to evoke a proinflammatory response. Clinical & Experimental Immunology, 203(1), 66-79.
10. Hamzah, D. J., Ayed, M., & Muhammed, H. A. (2022). Evaluation of culturing and molecular assay for detection of Mycoplasma gallisepticum in chicken suffering from chronic respiratory disease. Cellular and Molecular Biology, 68(4), 86-92.

11. Boonyayatra, S., Fox, L. K., Gay, J. M., Sawant, A., & Besser, T. E. (2012). Discrimination between Mycoplasma and Acholeplasma species of bovine origin using digitonin disc diffusion assay, nisin disc diffusion assay, and conventional polymerase chain reaction. Journal of veterinary diagnostic investigation, 24(1), 7-13.
12. Naher, H. S., AL-Hamadanib, A. H., & Muhammid , H. A. (2014). Evaluation of Culturing Method and Mycoplasma System Plus Kit in Diagnosis of Genital Mycoplasma Isolated from Infertile Women.Photon Journal of Microbiology,107 :207-211.
13. Luna, (1968) Manual of histologic staining methods of the Armed Forces Institute of Pathology. 3rd Edition, McGraw-Hill, New York.
14. OIE.(2008). Manual of Diagnostic tests and Vaccines for Terrestrial Animals. Avian mycoplasmosis (Mycoplasma gallisepticum, M. synoviae), pp. 482-496 .
15. Ongor, H. R.; Kalin., M.; Karahan., B.; Çetinkaya and Akan, M. (2009). Detection of mycoplasma species in turkeys by culture and polymerase chain reaction. Rev. sci. tech. Off. Int. Epic. 28(3): 1103-1109.
16. Rauf, M.; Chaudhary Z. I.; Younas M.; Anjum, A. A.; Khan. H. M. and Ali, M. A.(2014). Detection of Mycoplasma gallisepticum in experimentally inoculated layer birds by immunohistochemistry. j. anim. plant. 24(2): 385-391.
17. Kleven S. H. and Garcia,M. (2003).Epidemiology of Mycoplasma infections based on analysis of gene polymorphism, FEMS Microbiology Letters 222 (1):101
18. Kleven, S.H., and Ferguson-Noel, N. (2008). Mycoplasma synoviae Infections. In: Saif, Y.M., Fadly, Feberwee, A., De Vries, T.S., Landman, W.J.M. A.M., Glisson, J.R., McDougald, L.R., Nolan, L.K., , D.E. (Eds.),Diseases of Poultry. 12th Ed, Blackwell Publishing, Iowa State University Press, USA, Ames, pp.845-856.
19. Ehtisham, S.; Rahman, S.U.; Siddique M. and Qureshi A.S. (2011). Involvement of Mycoplasma synoviae in respiratory distress cases of broilers.Pak. Vet. J., 31(2): 117-119.
20. Osman,K.M.; Aly., M. M. ; Amin, Z. M. and Hasan ,B. S. (2009) Mycoplasma gallisepticum: an emerging challenge to the poultry industry in Egypt. Rev. Sci. Tech. 28(3): 1015-23.
21. Heleili, N. B.; Mamache and Chelihi, A. (2011). Incidence of Avian Mycoplasmosis in the region of Batna, Eastern Algeria. Veterinary World, 4(3):101-105.
22. Khalifa, K. A.;Abdelrahim., E, S.; Badwi, M. and Mohamed, A. (2013). Isolation and Molecular Characterization of Mycoplasma gallisepticum and Mycoplasma synoviae in Chickens in Sudan. Journal of Veterinary Medicine:1-4.
23. AL-Delaify, S.J.M. (1986). Study on Isolation and characterization of avian Mycoplasma in Iraq. M.Sc.Thesis. Collage of Veterinary Medicine.Baghdad University.
24. Karem, E.A.R. (1989). Immunization of chicken against Mycoplasma gallisepticum. M.Sc. Thesis. Collage of Veterinary Medicine. Baghdad University.
25. Tiong, S. K.; Liow., T. M. and R. J. S. Tan (1979) Isolation and identification of avian mycoplasmas in Singapore. British Poultry. Sci., 20: 45 –54
26. Reda, L. M., and Abd E-Samie, L.K. (2012) Some Studies on the Diagnosis of Mycoplasma Gallisepticum in Chicken. Nat. Sci. 2012;10 (12): 247-251.
27. Hyman, H.C.; Levisohn, S.; Yogev, D. and Razin, S. (1989). DNA probes for Mycoplasma gallisepticum and Mycoplasma synoviae: application in experimentally infected chickens. Vet Microbiol. 20:323–337.
28. Aimeur, R.; Bouaziz, O.; Kabouia, R. and Bererhi, H (2010). Prévalence de Mycoplasma gallisepticum et de Mycoplasma synoviae dans les élevages avicoles de l'Est Algérien. Rev. Med. Vet. 16: 141-145.
29. Abed, A. A., Al-Iedani, A. A., & Neamah, A. J. (2021). Chicken Immune Profile against Mycoplasma gallisepticum Infection. Al-Qadisiyah Journal of Veterinary Medicine Sciences, 20(1): 33-36.
30. Wu, Z., Ding, L., Bao, J., Liu, Y., Zhang, Q., Wang, J., ... & Li, J. (2019). Co-infection of Mycoplasma gallisepticum and Escherichia coli triggers inflammatory injury involving the IL-17 signaling pathway. Frontiers in microbiology, 10, 2615.
31. Wu, C., Zhong, L., Zhang, L., Li, W., Liu, B., Huang, B., ... & Wu, Y. (2022). Study on the mechanism of Mycoplasma gallisepticum infection on chicken tracheal mucosa injury. Avian Pathology, 51(4), 361-373.

32. Vitula, F., Peckova, L., Bandouchova, H., Pohanka, M., Novotny, L., Jira, D., ... & Pikula, J. (2011). Mycoplasma gallisepticum infection in the grey partridge Perdix perdix: outbreak description, histopathology, biochemistry and antioxidant parameters. BMC Veterinary Research, 7(1), 1-12.
33. Qadir, M. F., Khan, A., Saleemi, M. K., Gul, S. T., Khan, A., & Mujahid, Q. (2021). Epidemiological and pathological status of Mycoplasma gallisepticum in layer chicks at Faisalabad, Pakistan. Pakistan Journal of Agricultural Sciences, 58(1.(
34. Zhenikhova, N. I., Drozdova, L. I., Sadovnikov, N. V., Kundryukova, U. I., & Korch, M. A. (2021). Peculiarities of a respiratory mycoplasmosis course of birds. In E3S Web of Conferences (Vol. 282, p. 03006). EDP Sciences.
35.Sawicka, A., Durkalec, M., Tomczyk, G., & Kursa, O. (2020). Occurrence of Mycoplasma gallisepticum in wild birds: A systematic review and meta-analysis. PloS one, 15(4), e0231545.