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Detection of Antibodies for *Mycoplasma gallisepticum* by ELISA in Broiler

Dhuha Kahtan Taha

Department of Microbiology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq.

Abstract

THE POULTRY industry has become a critical component of the country's economy, and as a result, detecting and diagnosing disease infections before they spread in the herd limits the massive losses that poultry breeders face. This study was to look for serological diagnosis of mycoplasma disease in broiler using enzyme linked immune sorbent assay ELISA, as well as the histopathological changes in some organs. Between November 2021 and March 2022, this study was carried out broiler fields in Nineveh province. One hundred eighty blood samples were collected from 12 broiler fields. Results revealed the total infection rate of the examined fields was 55/180 (30.56%). In addition, percentage of *M. gallisepticum* infection was highest in the broiler fields located east of the province 21/45 (46.66%), followed by the percentage of infection in the fields located south of the province 18/45 (40%). While the infection rate was lower in the fields on the north and west of the province, 11/45 (24.44%) and 5/45 (11.11%), While the infection rate was lower in the fields to the north and west of the province, 11/45 (24.44%) and 5/45 (11.11%), respectively. Histopathological changes recorded severe tracheitis, severe inflammation in lung with purulent mucoid secretions in the bronchial and between the alveoli. The air sac showing also severe airsaculitis serous fibrin inflammatory secretion in the lumen with inflammatory cell of polymorphnuclear inflammatory cells infiltration and wall thickening. ELISA is a cost-effective tool for diagnosis of *M. gallisepticum* infection in broiler chickens, with high incidence in Nineveh province.

Keywords: Antibodies, *Mycoplasma gallisepticum,* ELISA, Broiler

Introduction

The bacterium *Mycoplasma gallisepticum* can cause respiratory disease in chickens and other birds. It belongs to the Mollicutes class of bacteria, which lack a cell wall [1] .It is one of dangerous pathogen of poultry diseases that causes significant economic losses in the poultry industry [2].

Chronic respiratory disease (CRD) or mycoplasmosis is the name given to the disease caused by *M. gallisepticum*. Coughing, sneezing, nasal discharge, swollen eyes, and decreased appetite and activity are all clinical symptoms of the disease. It also can reduce adult bird fertility and egg production. Even if they show no symptoms, affected birds can be carriers of the bacteria even if they showing any symptoms [3].

The disease can be transmitted directly contact between infected and susceptible birds [4]. It also can be passed down from infected chickens to their offspring via the egg. *M. gallisepticum* primarily affects chickens, but it can also infect turkeys, quails, and pheasants. The incubation period ranged from several days to weeks [5]. The disease is found all over the world, and its prevalence varies depending on geographic location and poultry production system. Its prevalence in commercial poultry farms can range from 10% to 100%, depending on biosecurity measures and vaccination programs in place [5].

Poor biosecurity, overcrowding, stress, and co-infection with other pathogens are all predisposing factors for *M. gallisepticum* infection. Clinical signs, serology, and bacterial isolation from infected tissues are used to diagnose *M.*

*Corresponding author: Dhuha Kahtan Taha, E-mail: duhataha@uomosul.edu.iq, Tel.: +964 774 091 7361
Orcid ID: http://orcid.org/0000-0002-0060-8918
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gallisepticum infection. Enzyme linked immune-sorbent assay (ELISA) and polymerase-chain-reaction (PCR) are also used as a good tools for diagnosis of this disease [6]. Biosecurity procedures and using of antibiotic are used to control *M. gallisepticum* infection. In commercial poultry farms [7]. Understanding the epidemiology of *M. gallisepticum* infection is critical for implementing effective control measures and reducing the disease's impact on poultry production [8].

The enzyme linked immune-sorbent assay (ELISA) is a common serological test used to detect antibodies in broiler chickens [9].

ELISA is a well-established technique for detecting of antibodies to *M. gallisepticum* and several commercial ELISA kits are available for this purpose. These kits have high sensitivity, specificity, and are simple to use, making them ideal for routine flock examination [10].

Infection with *M. gallisepticum* can result in a variety of histopathological effects in chickens and other birds. Birds' respiratory tracts, conjunctiva, and reproductive tracts can be infected with the bacteria, causing inflammation and tissue damage of conjunctiva (11).

In chickens, *M. gallisepticum* can cause salpingitis and oophoritis in the reproductive tract, resulting in decreased egg production and fertility. Inflammation and infiltration of lymphocytes and plasma cells into the oviduct and ovary characterize these conditions.(12)

This study was aimed to detection of antibodies against *M. gallisepticum* in broiler flocks in in Nineveh province in addition to show the main the histopathological changes in some organs in infected birds.

Material and Methods

Ethical approval

The birds were dealt with humanely and were placed in the laboratory under sterile conditions. When the autopsy was performed, the organs were collected. The researcher obtained all ethical approvals from the college of veterinary medicine in Mosul University in to start the study.

Animals and experimental design

Between November 2021 and March 2022, this study was carried out on chickens in the fields of Nineveh province. One hundred eighty blood samples were collected from 12 broiler flocks spread across the Nineveh province. Birds with conjunctivitis, sneezing, sinusitis, poor weight gain, and low feed conversion efficiency had their samples collected. Blood was collected from wing and jugular veins and placed in sterile tubes without anticoagulants. Blood samples were separated using centrifugation at 4.000 rpm for 4 minutes. Serum obtained and placed in 2-mL tubes then stored at -20 °C until further using. The samples were examined by indirect ELISA, as directed by the kit manufacturer.

After that, the jugular vein of the affected birds was cut for the purpose of conducting pathological anatomy and collecting some organs. They were washed well with water and then placed in neutral buffered formalin until the histological section was performed, tissue sections were made, and tissue slices were stained with the routine dye hematoxylin-eosin to observe the pathological changes in some of the organs of the affected birds.

Performance of indirect enzyme-linked immunosorbent assay

BioChek (UK) Ltd. provided the ELISA kit. All samples in addition to control positive and negative were carried out according to manufacturer's instructions of the kit.

The principle of ELISA

ELISA works by coating a microplate with *M. gallisepticum* antigen. The chicken serum sample is added to the plate, and any *M. gallisepticum* antibodies present in the sample bind to the antigen. The plate is then treated with a secondary antibody conjugated to an enzyme, which binds to anti-*M. gallisepticum* antibodies. After washing the plate, a substrate is added, which is converted by the enzyme to produce a coloured-product. The colour intensity is proportional to the amount of *M. gallisepticum* antibody in sample.

Statistical analysis

To calculate the P-value, all study data were statistically analyzed using GraphPad Prism (version 8). The chi square test was used to determine the P-value and mean of values, while one-way ANOVA was used to determine which differences were statistically significant between groups.

Results

ELISA

The results of the indirect enzyme linked immune-sorbent assay test in the study was shown in (Table 1 and Figure 1), the total infection rate of the examined fields was 55/180 (30.56%). result also revealed that the percentage of *M. gallisepticum* infection was highest in the poultry fields located east of the city 21/45 (46.66%), followed by the percentage of infection in the poultry fields located south of the city 18/45 (40%).

While the infection rate was lower in the poultry fields to the north and west of the city, 11/45 (24.44%) and 5/45 (11.11%), with a statistically significant at (P > 0.05) in the percentage of infection between the fields to the east and those to the north and west. The result revealed statistically significant at (P > 0.05) in the infection rate between fields in the south and west.

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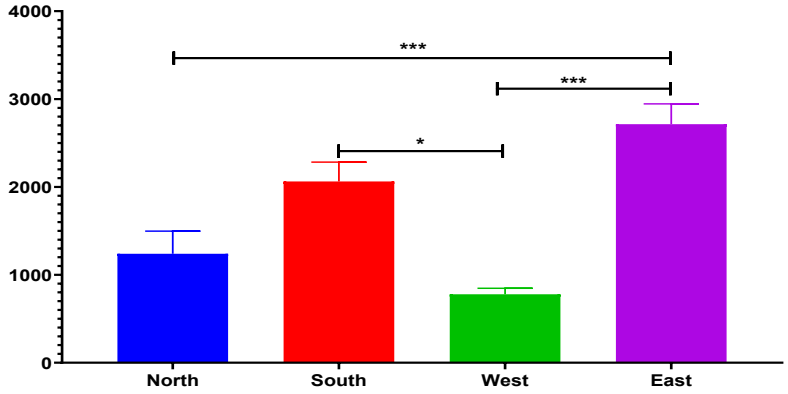

Fig. 1. Shows MG mean titers between fields regions. Vertical bars signify that the means and significant statistical differences seen among groups are (*p<0.05, ***p<0.001).

TABLE 1. Mean titers between fields regions of MG infection.

Groups of study	Total No. of Samples	Total No. of positive samples	Percentage 100%	Mean Titers ±SE
G1 (North field)	45	11	24.44% [a]	1239.36±257.78 [a]
G2 (South field)	45	18	40% [a, c]	2063.27±218.72 [c, a]
G3 (West field)	45	5	11.11% [a]	778.8±68.72[b]
G4 (East field)	45	21	46.66% [b, c]	2713.71±232.03 [c]

Small letters: mean presence of significant difference between groups under (P <0.05).

Histopathological results

Histopathological examination showed presence of severe tracheitis caused by mycoplasma infection which characterized by increased inflammatory secretions blocking the lumen with inflammatory cell infiltration, severe vascular congestion and hemorrhage, cartilage thickening, and muscular atrophy, in addition to serous fibrinous inflammatory secretion, infiltration of polymorph nuclear and mono morph nuclear inflammatory cells, and chondrocyte necrosis and cartilaginous thickening (Fig. 1,2,3,4).

In (Figs. 5, 6), Histopaths of lung tissues also showed severe lung inflammation with secretion in the bronchial and between alveoli, vascular congestion, necrosis of the epithelial cells lining the bronchi, and an increase in fibrous. Whereas (Fig.7) histopath of the air sac showed severe airsaculitis serous fibrin inflammatory secretion in the lumen with inflammatory cell of polymorph nuclear inflammatory cells infiltration and wall thickening.

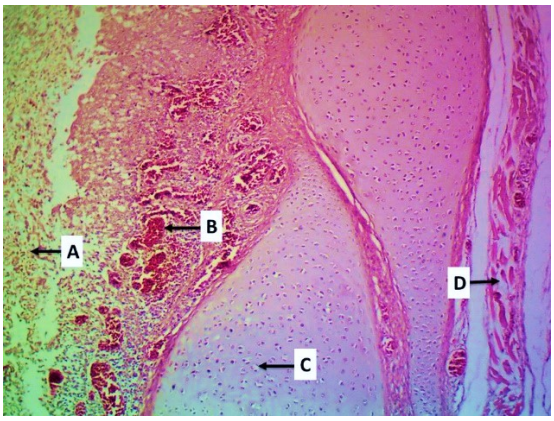

Fig. 1. Trachea showing severe tracheitis caused by mycoplasma infection is characterized by increased inflammatory secretion blocking the lumen with inflammatory cell infiltration (a), severe vascular congestion and hemorrhage (b), cartilage thickening (c), and muscular atrophy (d). H. &E. stain 100.X.

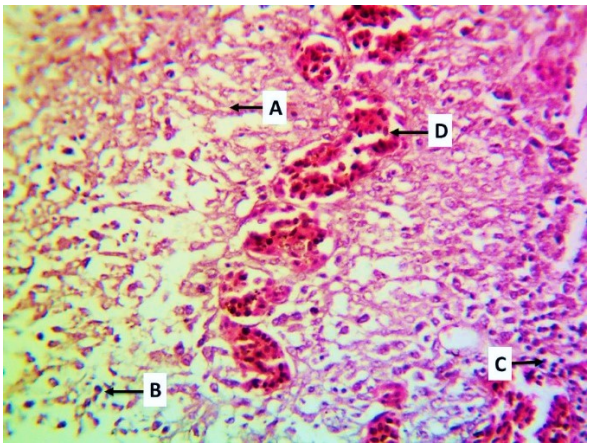

Fig. 2. Trachea showing tracheitis characterised by serous fibrinous inflammatory secretion (a), infiltration of polymorph nuclear cells (b) and monomorph nuclear cells (c) inflammatory cells, and severe blood vessel congestion (d). 400. X H.&E. stain.

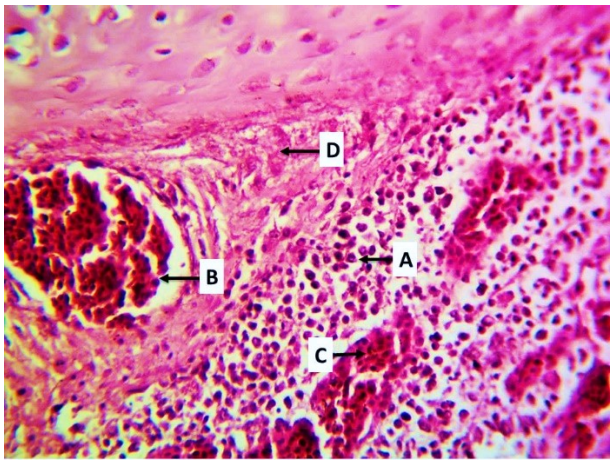

Fig. 3.Trachea showing severe tracheitis infiltration of inflammatory cells (a), vascular congestion (b), hemorrhage (c), and chondrocyte necrosis (d). 400X, H. &E. stain.

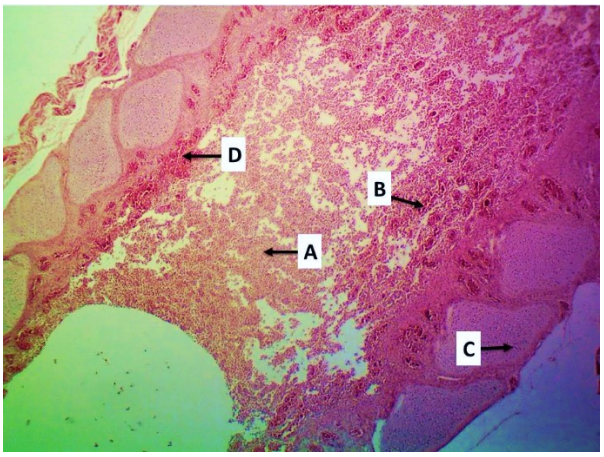

Fig. 4. Trachea showing severe tracheitis characterized by a highly inflammatory exudate that obstructs the lumen with inflammatory cell infiltration (a), vascular congestion (b), hemorrhage (c) and cartilaginous thickening (d). H. &E. stain, 40X.

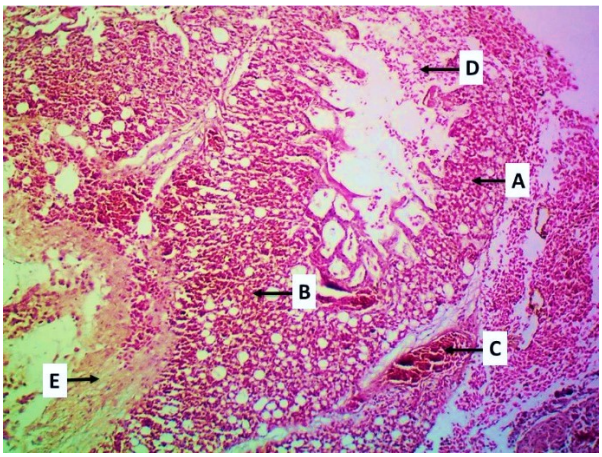

Fig. 5. Lung tissue showing severe inflammation characterized by present of secretion in the bronchial-wall (a) and between-alveoli (b), vascular cong. (c), necrosis of the epithelial-cells lining the bronchi (d) also an increase fibrous-tissue (e). Stain of H. &E., 100X.

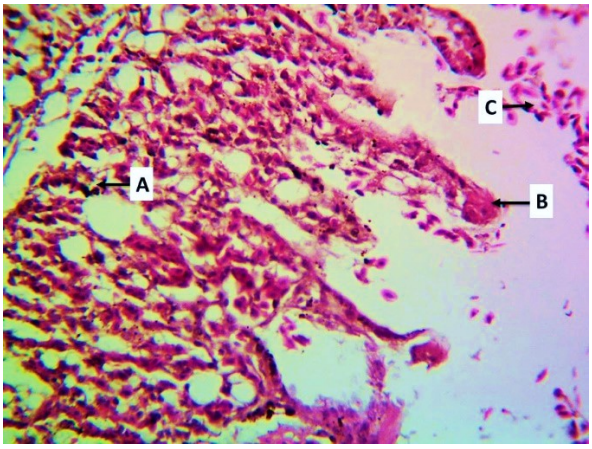

Fig. 6. Lung tissue showing severe pneumonia with inflammatory exudate in the bronchial wall (a), epithelial cells necrosis in lining the bronchi (b), and hemorrhage. (c). stain of H. &E. 400X.

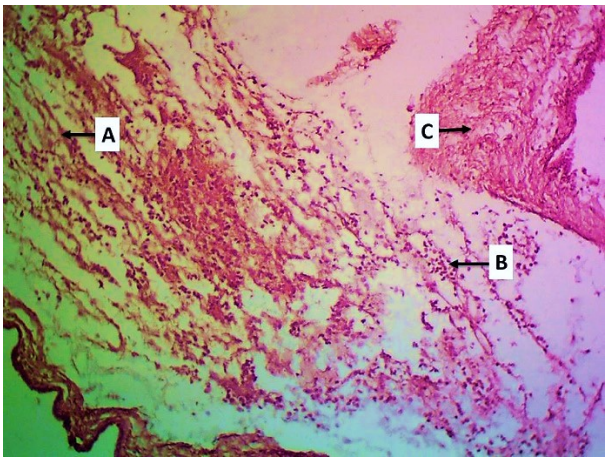

Fig. 7. Air sac showing severe serous fibrin inflammatory secretion in the lumen (a) with inflammatory cell infiltration (b) and wall thickening (d). H. &E. stain. 100. X.

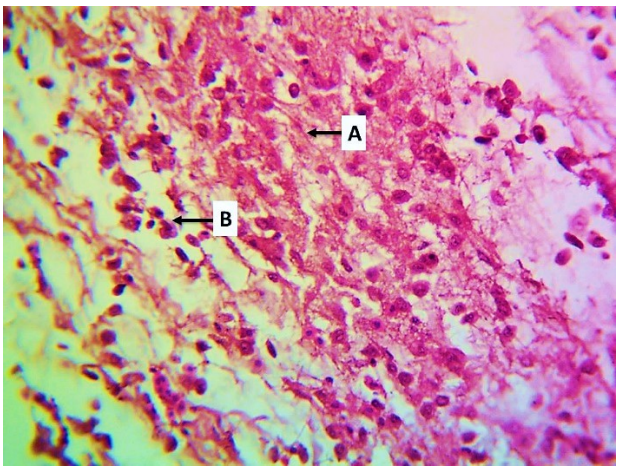

Fig. 8. Air sac showing severe Airsaculitis characterized by serous fibrin inflammatory secretion in the lumen (a) with infiltration of polymorph nuclear inflammatory cells (b). H. &E. stain, 400X.

Discussion

Infection with *M. gallisepticum* causes chronic-respiratory- disease (CRD) in avian species. It was the most common cause of respiratory- disease in chickens and other birds, in addition it may lead to decreasing in egg and meat production [13]. In this study, indirect ELISA was used to assess the seroprevalence of *M. gallisepticum* in broilers that did not receive their vaccine.

When *M. gallisepticum* infection is suspected, ELISA results associated with clinical signs and history with histopathology, and other laboratory tests can interpret the disease) [14].

There are some limitations of ELISA considered when interpreting results. Cross-reactivity with other mycoplasma species or non-specific binding to serum proteins can result in false positives.

According to the study's findings (Table 1 and Figure 1), the total infection rate of the examined fields was 55/180 (30.56%). The study's findings also revealed that the percentage of *M. gallisepticum* infection was higher in the fields located east of the city, 21/45 (46.66%), than in the fields located south of the city, 18/45 (40%). The high infection rate in the eastern and southern regions may be due to a combination of factors, including seasonal effects, poor ventilation, birth pollution, a lack of restrictions on technical staff movement, poor management, visitors, and other people, in addition to other biosecurity measures [14], which is consistent with previous findings [15].

The investigation also revealed that the average ELISA titer ranged from (787.8 68.72 to 2713.71 232.03) in the investigated fields. Because *M. gallisepticum* is primarily spread through direct contact between infected and

susceptible birds, the general prevalence of seropositive results indicates the possibility of infection. The bacteria can survive in the environment for several days after being excreted in infected birds' respiratory secretions, faeces, and eggs. The disease can enter a flock via new birds, equipment, feed, or contaminated water [16]. Furthermore, the findings of this study provide a tool for direct serodiscrimination of clinical mycoplasma titers.

ELISA can only detect antibodies and not the presence of bacteria. As a result, a negative results of ELISA result does not always indicate that the bird is free of *M. gallisepticum* infection. The infection rate was lower in fields located north and west of the city, 11/45 (24.44%) and 5/45 (11.11%), respectively, with a statistically significant at ($P > 0.05$) in the infection rate between fields located east and those located north and west. The prevention and control of *M. gallisepticum* through good biosecurity measures, such as quarantine of new birds and limiting contact between birds from different sources, may have a role in the emergence of this result [15,17]

Histopathological changes in the respiratory tract were studied to confirm the results of the serologic examination, as *M. gallisepticum* can cause tracheitis, bronchitis, and pneumonia, which are characterised by inflammation and infiltration of lymphocytes [18].

Alveolitis, or inflammation of the air sacs, can also be caused by the bacteria. As previously demonstrated [19], this can result in thickening of the air sac walls, secretion and fibrin accumulation, and adhesion of the air sacs to each other and to other organs. In general, *M. gallisepticum* infection in birds can cause significant histopathological effects, resulting in decreased productivity and welfare .[20].

Infection with *M. gallisepticum* can cause histopatholological changes in chicken organs such as the trachea, lung, heart, and air sacs. Inflammation of the tracheal mucosa, loss of cilia on the tracheal epithelium, accumulation of mucus and cellular debris in the lumen, and thickening of the tracheal wall due to edema and inflammatory cell infiltration are some of the specific changes that may occur in the trachea [21]. In the lung, it causes bronchial pneumonia, which is characterized by inflammatory cell infiltration in the air spaces and alveolar walls, lymphocytosis around the trachea and blood vessels, lung tissue consolidation due to fibrin deposition and necrosis, interstitial tissue thickening due to edoema, and inflammatory cell infiltration [22].

Focal myocardial lymphatic infiltration, fibrosis, and myocardial fibre necrosis) . [23].

Alveolitis in alveoli is characterised by alveolitis, secretion and fibrin accumulation in the air sacs, adhesion of the air sacs to each other and to other organs, edema-induced thickening of the air sac walls, and inflammatory cell infiltration [24].

Histopathological changes caused by *M. gallisepticum* infection can have a significant impact on chicken health and productivity. Early detection and management of infected birds is critical to preventing the spread of infection and minimizing its impact [25,26,27].

Conclusion

It was concluded that were significant difference results achieved by indirect ELISA between fields of different sides in Nineveh province which established that fields located in east and south of the province have high incidence of infection in comparison with north and west regions of the same province. Histopathological changes caused by *M. gallisepticum* infection can have a significant impact on chicken health and productivity.

Conflicted interest: No Conflicted interest.

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Author contribution: The researcher designed the research idea and prepared the practical part of the research, while conducting statistical analysis, writing, and final review of the research**.**

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الكشف عن الأجسام المضادة لـ Mycoplasma gallisepticum بواسطة ELISA في دجاج التسمين

ضحى قحطان طه

فرع الاحياء المجهرية - كلية الطب البيطري - جامعة الموصل - الموصل - العراق.

الخلاصة

أصبحت صناعة الدواجن مكونًا حاسمًا في اقتصاد البلاد، ونتيجة لذلك، فإن اكتشاف عدوى الأمراض وتشخيصها قبل انتشارها في القطيع يحد من الخسائر الهائلة التي يتكبدها مربو الدواجن. تهدف هذه الدراسة إلى النظر في فعالية في التشخيص المصلي لمرض المايكوبلازما في افراخ اللحم في محافظة نينوى باستخدام اختبار مقايسة الممتز المناعي المرتبط بالإنزيم(ELISA) ، وكذلك التغيرات النسيجية المرضية في بعض الأعضاء. أجريت هذه الدراسة بين تشرين الثاني 2021 وآذار 2022 في حقول افراخ اللحم في محافظة نينوى. تم جمع 180 عينة دم من 12 حقلا لفروج اللحم. وفقًا لنتائج اختبار مقايسة الممتز المناعي المرتبط بالإنزيم غير المباشر في الدراسة، كان معدل الإصابة الإجمالي للحقول المفحوصة 180/55 (30.56٪). كما أوضحت نتائج الدراسة أن نسبة الإصابة *M. gallisepticum* كانت الأعلى في حقول الدواجن الواقعة شرق المدينة 45/21 (46.66٪)، تليها نسبة الإصابة في حقول الدواجن الواقعة جنوب المدينة 45/18) 40 .(٪ .(بينما كان معدل الإصابة أقل في حقول الدواجن شمال وغرب المدينة، 45/11 (24.44٪) و45/5 (11.11٪)، بينما كان معدل الإصابة أقل في حقول الدواجن شمالاً وغرباً. من المدينة، 45/11 (24.44٪) و45/5 (11.11٪)، على التوالي. سجلت التغيرات النسيجية المرضية التهاب القصبات الحاد والالتهاب الرئوي الحاد الذي يتميز بإفراز التهابي في جدار الشعب الهوائية وبين الحويصلات الهوائية. يُظهر كيس الهواء التهابًا حادًا في إفراز الليفين المصلي في التجويف مع تسلل الخلايا الالتهابية للخلايا الالتهابية متعددة الأشكال وسماكة الجدار. ELISA .هي أداة فعالة من حيث التكلفة للسيطرة على عدوى *M. gallisepticum* في فروج اللحم، مع ارتفاع معدل الإصابة في محافظة نينوى.

الكلمات المفتاحية: الاجسام المضادة، *Mycoplasma gallisepticum,* ELISA، فروج اللحم.