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SEROLOGICAL INVESTIGATION OF MYCOPLASMA GALLISEPTICUM INFECTION IN COMMERCIAL BROILER FLOCKS IN NINEVEH PROVINCE, IRAQ

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Abstract. The poultry industry plays an important role in the country's economy and early detection and diagnosis of infections of the disease helps minimise the huge losses that poultry farmers face as a result of spread of the disease throughout the flock. The study aimed to evaluate the serological diagnosis of the Mycoplasma infection in broiler chicks in Nineveh Governorate by the enzyme linked immunosorbent assay (ELISA) and the pathological histological changes in some organs. A study was conducted from August 2025 to March 2026 in broiler farms in Nineveh Governorate. Samples of 220 blood samples were taken from 16 broiler farms. The overall prevalence of infection in the farms examined in this study was 55/220 (33.38%) based on the results of the indirect enzyme linked immunosorbent assay (ELISA). The study results further revealed that poultry farms located east of the city (44.55%) had the highest prevalence of *M. gallisepticum* infection while those located south of the city had 48%. The prevalence was lower in poultry farms to the north and west of the city, at 18/55 (29.33%) and 8/55 (13.18%), respectively. of the city, 11/55 (48 %) and 26/55 (44.55%), respectively. Pathological histological changes showed acute bronchitis and acute pneumonia with inflammatory exudate in bronchial wall and between alveoli. The acute inflammation of the air sac is characterized by the presence of serous exudate in the air space, infiltration by polymorphonuclear inflammatory cells, and thickening of the wall. *M. gallisepticum* is more prevalent in Nineveh Governorate and is an effective, inexpensive method of controlling the infection in broiler chickens using ELISA.

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

Introduction

The bacterium *Mycoplasma gallisepticum* can cause respiratory disease in chickens and other birds. It is part of the class of bacteria called Mollicutes, which are bacteria that do not have a cell wall [1]. It is one of the most lethal pathogens responsible for poultry diseases and which causes immense losses in the poultry industry [2]. Chronic respiratory disease (CRD) or mycoplasmosis is the name given to the disease caused by *M. gallisepticum*. Clinical signs of the disease are coughing, sneezing, nasal discharge, swelling of the eyes and loss of appetite and activity. It may also decrease the fertility of the bird and egg production. Affected birds may not exhibit any clinical signs but still be able to shed the bacteria [3].

The disease can spread from infected to susceptible birds by direct contact between birds [4]. It may also be transmitted horizontally to the young chickens from infected chickens through the egg. *M. gallisepticum* primarily affects chickens, but can also infect turkeys, quails and pheasants. The incubation period is from a few days to weeks [5]. The disease is global, and its occurrence may differ from region to region and by the type of poultry production system.

The incidence can vary between 10% and 100% on commercial poultry farms depending on the biosecurity measures and vaccination programmes used [5].

These are all factors that make an animal susceptible to infection by *M. gallisepticum*: poor biosecurity, overcrowding, stress and co-infection with other pathogens. Clinical signs, serology and bacterial isolation from infected tissues are used to diagnose *M. gallisepticum* infection. Other tests, such as enzyme-linked immunosorbent assay (ELISA) and the polymerase chain reaction (PCR) are also helpful in making the diagnosis of this disease [6]. Control measures for *M. gallisepticum* infection include vaccination, biosecurity measures and antibiotic usage. Vaccination is the best way to control *M. gallisepticum* in commercial poultry farms [7].

Materials And Methods

Ethical approval

Birds were handled humanely and placed in the laboratory in a sterile environment. At the time of autopsy organs were retrieved. The researcher obtained all necessary ethical approvals from the relevant authorities before starting the study.

Animals and Experimental Design

This study was carried out on chickens in Nineveh province from August 2025 to March 2026. Blood samples were taken from 220 birds from 16 broiler flocks distributed over Nineveh province. Birds with signs of conjunctivitis, sneezing, sinusitis, weight loss and feed conversion ratio/FCR were sampled. Blood samples were drawn from wings and jugular veins and stored in sterile blood tubes with no anticoagulants. Blood samples were centrifuged for four minutes at 4,000 revolutions per minute (rpm). The serum was extracted and was stored in 2 mL tubes in freezer at -20 °C until further use. Indirect ELISA was used to analyze the samples according to the kit manufacturer's instructions.

Afterwards, Pathological anatomy and sampling of some organs was performed in the affected birds by cutting the jugular vein. The organs were thoroughly washed in water and were then immersed in neutral buffered formalin and kept until the histological section was done. Pathological changes of some of the organs were studied by taking tissue sections from the dead birds and staining with haematoxylin and eosin.

Performance of Indirect Enzyme-Linked Immunosorbent Assay

All samples were treated using the manufacturer's instructions in the ELISA kit supplied by BioChek (UK) Ltd. and all negative and positive controls were processed in the same way.

The principle of ELISA

ELISA is based on coating of a microplate with the antigen of *M. gallisepticum*. Then a sample of hen's serum is placed on the plate and if *M. gallisepticum* antibodies are present in the serum, they will attach to the antigen. The anti-M. gallisepticum antibody is then bound by a secondary antibody that is conjugated to an enzyme and that binds to the anti-*M. gallisepticum* antibody. The plate is washed and a substrate is added, which turns into a coloured product when acted on by the enzyme. The intensity of the colour produced depends on the concentration of *M. gallisepticum* antibody in the sample.

Statistical Methods

The P-value for all study data was calculated by statistically analysing it using GraphPad Prism software (*version 8*). A chi-square test was employed to obtain the P-value and mean value of the values. One-way ANOVA was used to determine which differences between groups were statistically significant.

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Results

ELISA Technique

The data from the indirect enzyme-linked immunosorbent assay test from the study are presented in Table 1 and Figure 1. The overall infection rate for all the fields examined was 55/220 (33.38%). The results also revealed that the highest prevalence (26/55, 44.55%) of M. gallisepticum infection was found in the poultry fields in the east side of the city, and second highest prevalence (11/55, 48%) of M. gallisepticum infection was observed in the poultry fields in south side of the city. The infection rate was also lower in the field to the north and west of the city (8/55 and 18/55 respectively) and there was a statistically significant difference ($P > 0.05$) in the infection rate between the eastern and the north/west fields. The analysis showed that there was a significant difference in infection between the southern and western fields ($P > 0.05$). The percentage of infected poultry was also lower in poultry fields to the north and west of the city (18/55, 8/55, respectively) and this difference between the percentage of infected poultry in the east and north versus in the west of the city was statistically significant ($P < 0.05$). Results of the study indicated that significant difference occurred in infection rate for the south and west fields.

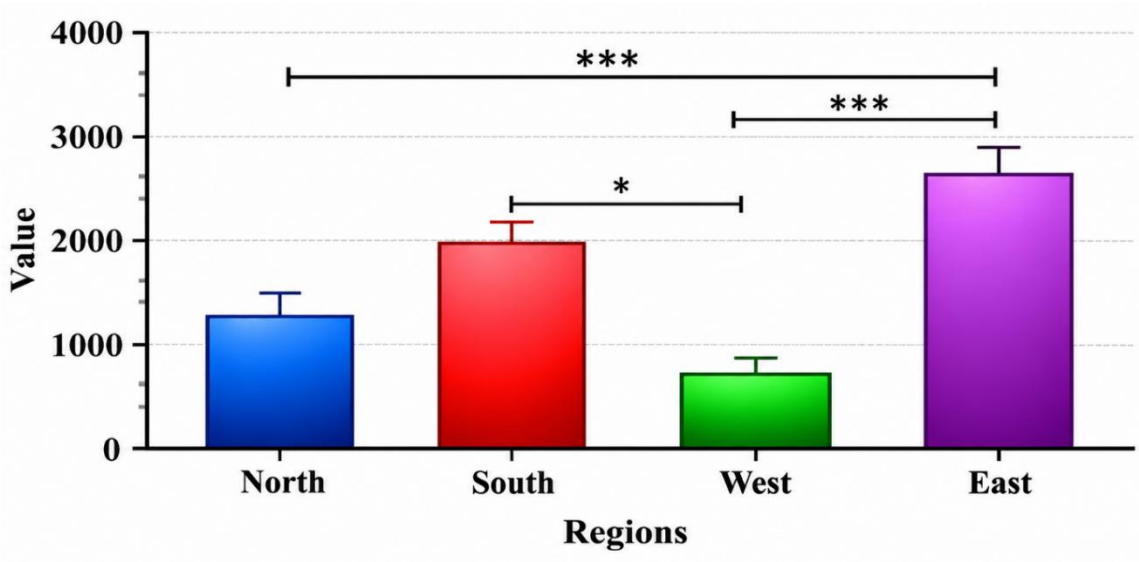


Figure 1. Illustrates the mean MG antibody titers across the studied regions, with significant differences among groups (*p < 0.05; ***p < 0.001).

Table 1. Comparison of mean MG antibody titres across the different study regions.

Groups field	Samples	Total of Infec. samples	Percentage	Mean ±SE
A1 (North)	55	18	29.33% b	1244.36±253.87 b
A2 (South)	55	11	48% a, b	2063.27±252.62 a, b
A3 (West)	55	8	13.18% c	789.8±59.72c
A4 (East)	55	26	44.55% a, b	2834.51±262.03 a

Different lowercase letters indicate statistically significant differences between groups at $p < 0.05$.

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Histopathological Findings

The histological findings included high levels of inflammation causing the lumen to be blocked with increased inflammatory secretions, infiltration of inflammatory cells, severe vascular congestion and haemorrhage, thickening of cartilage and muscular atrophy in the trachea caused by a mycoplasma infection. Moreover, there was serous fibrinous inflammatory exudate, infiltrations of polymorphonuclear and the mononuclear inflammatory cells, necrosis of chondrocytes and cartilaginous thickening (Figure 1, 2, 3, 4).

Lung tissue also revealed significant inflammation of the lungs with secretion in alveoli and bronchi with vascular congestion (Fig 5, 6). There was also necrosis of the epithelial cells lining the bronchi and fibrosis was increased. The histopathology of the air sac (Figure7) revealed marked airsaculitis, inflammatory secretions in the lumen which were serous and fibrinous, inflammatory cell infiltrate consisting of polymorphonuclear inflammatory cells, and thickened wall.

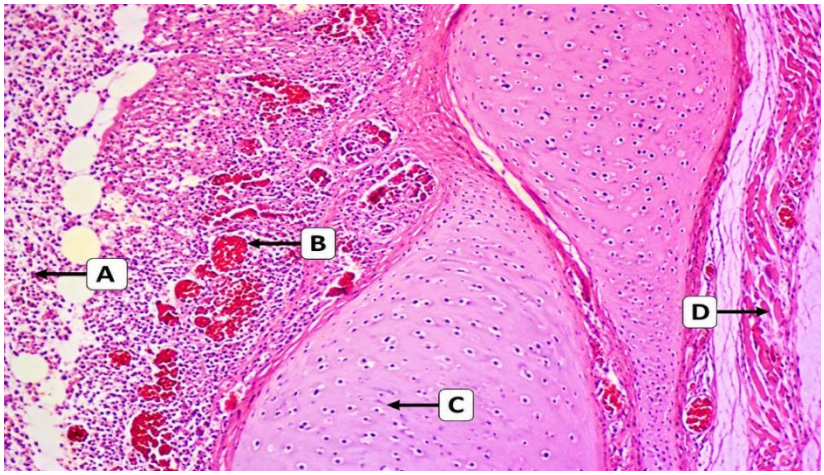


Figure 1. Severe mycoplasmal tracheitis showing inflammatory exudate (a), congestion and hemorrhage (b), cartilage thickening (c), and muscle atrophy (d). H&E, 100×.

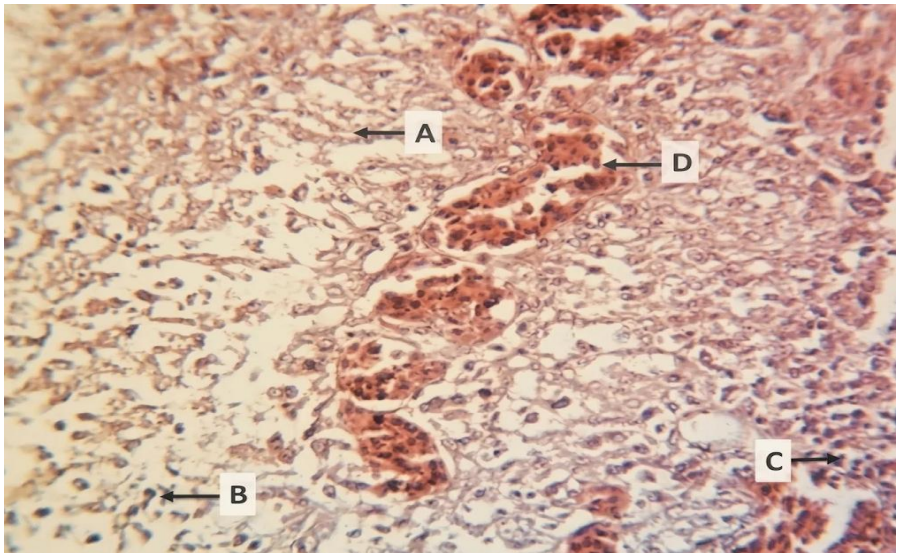


Figure 2. Tracheitis showing serofibrinous exudate (a), polymorphonuclear (b) and mononuclear (c) cell infiltration, and severe vascular congestion (d). H&E, 400×.

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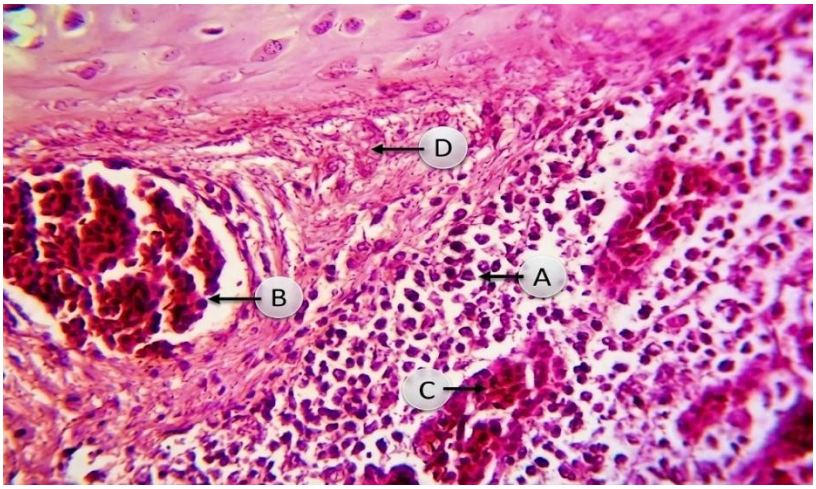


Figure 3. Severe tracheitis showing inflammatory infiltration (a), vascular congestion (b), hemorrhage (c), and chondrocyte necrosis (d). H&E, 400×.

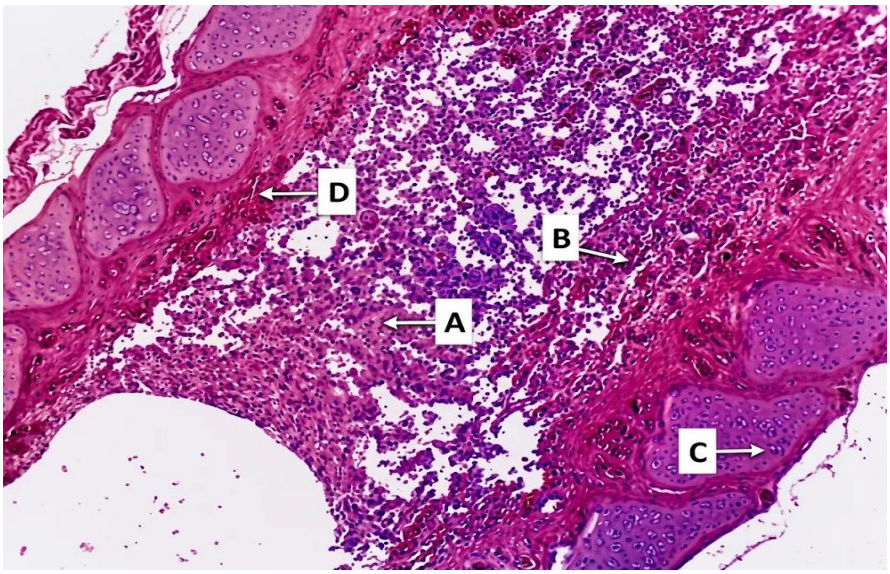


Figure 4. Severe tracheitis showing obstructive exudate and cellular infiltration (a), vascular congestion (b), hemorrhage (c), and cartilage thickening (d). H&E, 40×.

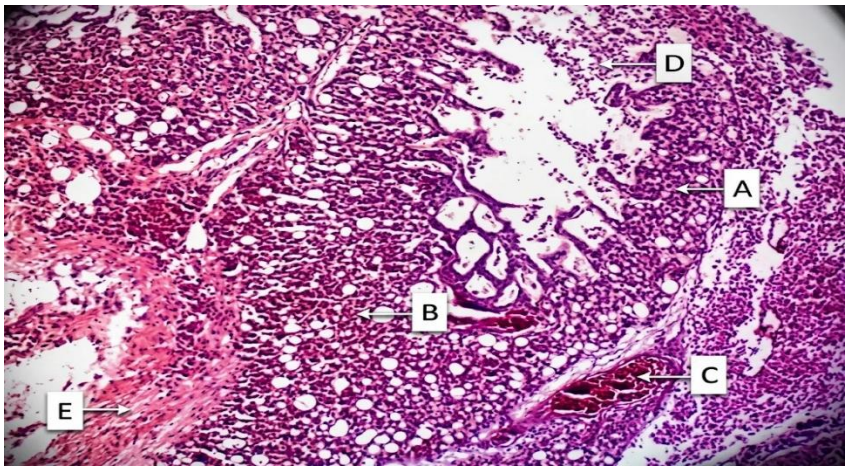


Figure 5. Severe pulmonary inflammation showing bronchial and interalveolar exudates (a, b), vascular congestion (c), bronchial epithelial necrosis (d), and fibrosis (e). H&E, 100×.

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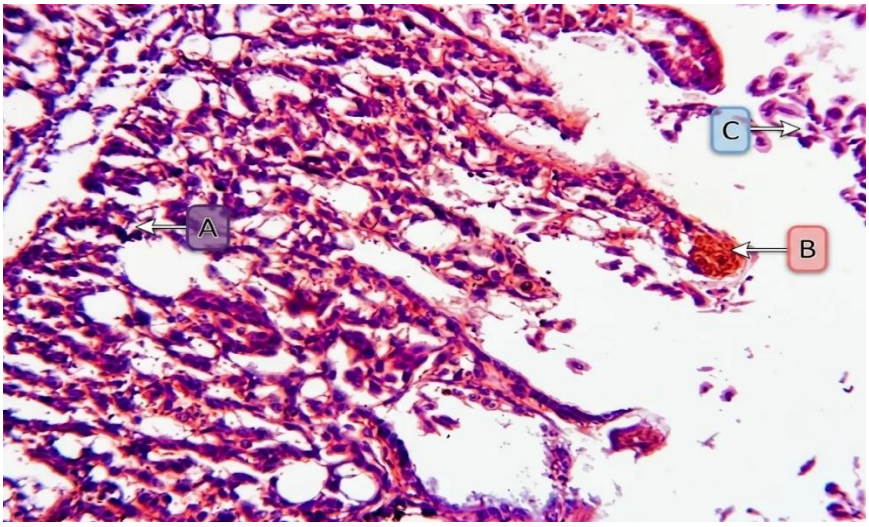


Figure 6. Severe pneumonia showing bronchial inflammatory exudate (a), bronchial epithelial necrosis (b), and hemorrhage (c). H&E, 400×.

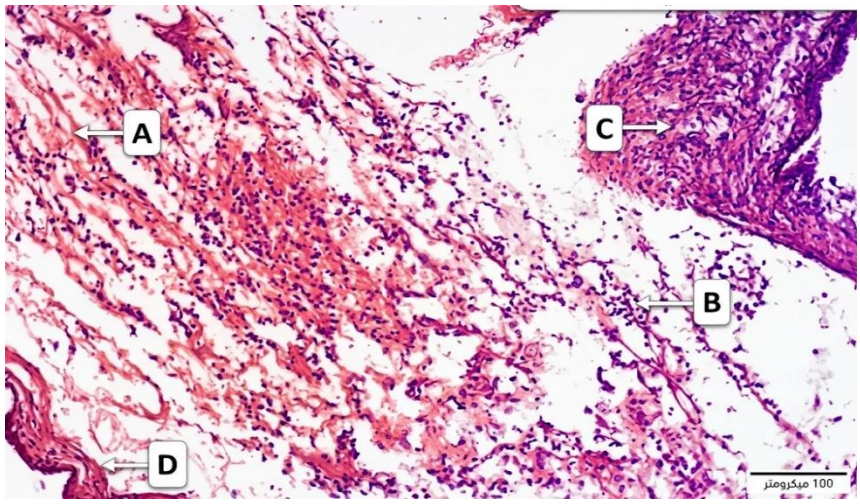


Figure 7. Severe airsacculitis showing serofibrinous exudate (a), inflammatory cell infiltration (b), and air sac wall thickening (d). H&E, 100×.

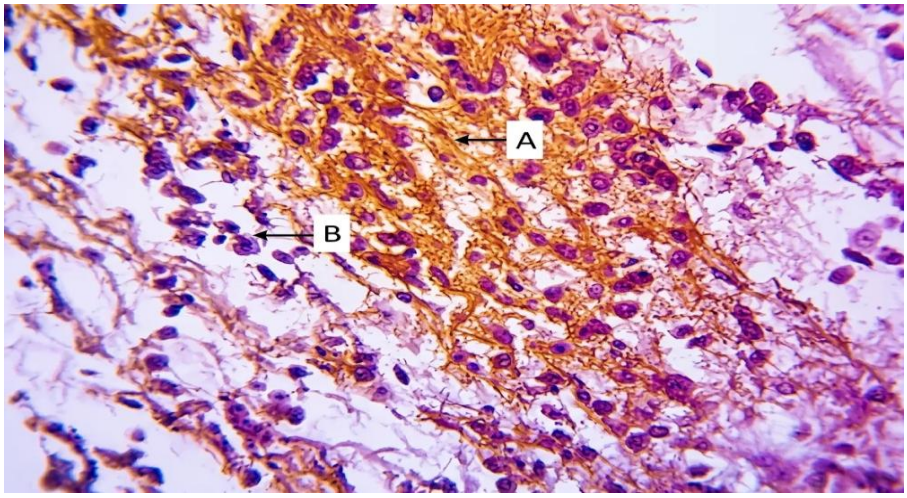


Figure 8. Severe airsacculitis showing serofibrinous exudate (a) and polymorphonuclear inflammatory cell infiltration (b). H&E, 400×.

Discussion

Chronic respiratory disease (CRD) is associated with Avianae infected with *M. gallisepticum*. It is the most common cause of respiratory disease in chickens and other birds and may lead to a decrease in egg and meat production [13]. The seroprevalence of the presence of *M. gallisepticum* in unvaccinated broilers was measured using an indirect ELISA technique. ELISA, in combination with clinical signs, history, histopathology and other laboratory tests, can aid in the diagnosis of *M. gallisepticum* infection if the infection is suspected (14).

ELISA has some limitations that should be taken in to account when interpreting the results. There can be cross reactivity with other species of mycoplasmas, non-specific binding to serum proteins that can result in false positives, and, weak antibody responses or getting a sample too early in the infection or immunization schedule can result in false negatives.

The total infection rate in the studied fields was 33.38% (55/220) as seen from the results of the study (see Table 1 and Figure 1). It also revealed that there were more cases of *M. gallisepticum* infection in the east side of the city (26/55 = 44.55%) than in the south side of the city (11/55 = 48%). The high infection rate in the eastern and southern areas might be attributed to a number of factors such as seasonality, poor ventilation, bird pollution, there being no restrictions on the movement of technical personnel, and poor management and visitors. This is in line with previous research [15]. The investigation also showed that the average ELISA titres in the investigated fields varied from 787.8 (68.72) to 2713.71 (232.03). The overall seropositivity of the results indicate a possible risk of infection, with *M. gallisepticum* spread likely to be from infected to susceptible birds by direct contact. Survivability in the environment is continued for several days after the bacteria have excreted in the respiratory secretions, faeces and eggs of infected birds. A flock can be infected in several ways, such as by new birds, contaminated water, feed or equipment [16]. In addition, this study's results yield a new method for making a direct discrimination between clinical mycoplasma titres.

ELISA will only detect the antibodies and not the bacteria. If a test is negative, this does not mean that the bird is not infected with the bacterium M. gallisepticum. The incidence of the infection was lower in fields to the north and west of the city (18/55 and 8/55 respectively) and there was statistically significant difference (P>0.05) between incidence of infection in fields east of the city and in fields north and west of the city. Good biosecurity practices such as quarantines for new birds and limiting exposure to birds from other sources might have helped prevent and control M. gallisepticum and could have contributed to this. Other vaccines are also available to help prevent infection, but not always completely effective. If a bird is suspected of being infected, it is important to contact a veterinarian to confirm the diagnosis and provide treatment for the bird and to take appropriate steps for disease prevention [17].

The results of the serological examination were confirmed by histopathological examination of the respiratory tract, which was used to determine if there was any histopathological change to the respiratory tract due to *M. gallisepticum*. The presence of *M. gallisepticum* is usually accompanied by tracheitis, bronchitis and pneumonia, which consists of infiltration and inflammation of the respiratory tract with lymphocytes and plasma cells [18]. The bacteria can also become lodged in the air sacs (alveolitis). This can cause the wall of the air sacs to thicken, develop fibrin and fuse together and with other organs [19] as has already been demonstrated. In general, the infection of birds with *M. gallisepticum* leads to significant histopathology, decreased productivity and poor welfare [20]. Tracheal infection with *M. gallisepticum* can result in histopathological alterations of chicken organs like the trachea,

lungs, air sacs and heart [20]. There are several changes that can be seen in the trachea, such as: inflammation of the tracheal mucosa; the loss of cilia from the tracheal epithelium; the presence of cellular debris and mucous plugs in the lumen and thickening of the tracheal wall (oedema and infiltration of inflammatory cells) [21].

In the lungs it causes bronchial pneumonia characterised by infiltrations of inflammatory cells in the air spaces and in the alveolar walls, consolidation of the lung tissue due to fibrin deposition and necrosis, infiltration of the interstitial tissue due to oedema and blood cells infiltrating around blood vessels and the trachea [22]. It also invades the myocardium at one point leading to its infiltration, fibrosis and necrosis. The alveolitis in the alveoli is associated with the accumulation of air sacs with inflammatory cells, thickening of the walls of the alveoli due to oedema, adhesion of the alveoli to other organs and accumulation of secretion and fibrin in the alveoli [24]. The effect of histopathology changes induced by infection with *M. gallisepticum* can be a significant influence on the health and productivity in chickens. The role of early detection/manage infected birds to prevent the spread and reduce impact of infection [25].

Conclusion

ELISA can be used to detect the antibodies of M. gallisepticum in broiler chickens which is beneficial. It has tremendous potential to play a major role in controlling *M. gallisepticum* infection and, therefore, is cost effective for the poultry industry. The conclusion was that the indirect ELISA gave significantly different results in different fields in Nineveh Province, and that the infection rate is higher in the east and south of the Province than in the north and west.

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