EVALUATION OF THE PREVALENCE *MYCOPLASMA GALLISEPTICUM* IN BROILER FARMS IN SAMARRA CITY.

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ABSTRACT

This study was aimed to evaluate the extent of the spread for *Mycoplasma gallisepticum* in the broiler chicken flocks of the Samarra city. 202 samples were collected from eight meat chicken fields in the city of Samarra for the period from September to December 2022, as these samples showed respiratory symptoms. The collected specimens were dissected in order to obtain both the trachea and the air sacs; After isolating the causative agent on Pleuropneumonia - like organisms (PPLOs) medium, mycoplasma infection reached 32.2%. The result appeared in the form of colonies with a shape similar to that of a "fried egg." The rates of mycoplasma infection at culture for each of the trachea were (41.1%), while in contrast to the air sac samples, which amounted to (22.1%). Polymerase Chain Reaction (PCR) results for the 16S rRNA gene also showed a positive result for *Mycoplasma gallisepticum*. The results were also disturbed by the appearance of respiratory signs, such as coughing and sneezing, along with the presence of ocular and nasal secretions. It was also noted that pathological changes were recorded in both the trachea and air sacs, represented by congestion of these organs.

Keywords: Trachea and air sacs, *Mycoplasma gallisepticum*, PCR

تقييم مدى انتشار بكتريا مايكوبلازما غاليسبتكم في مزارع الدجاج اللاحم في مدينة سامراء.

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المستخلص

هدفت هذه الدراسة إلى تقييم مدى انتشار بكتيريا مايكوبلازما غاليسبتكم في مزارع الدجاج اللاحم في مدينة سامراء. تم جمع 202 عينة من ثمانية حقول لفروج اللحم في مدينة سامراء للفترة من أيلول إلى كانون الأول 2022، حيث أظهرت هذه العينات أعراضاً تنفسية. تم تشريح العينات التي تم جمعها من أجل الحصول على كل من القصبة الهوائية والأكياس الهوائية؛ وبعد عزل العامل المسبب على وسط PPLOs بلغت نسبة الإصابة بالميكوبلازما 32.2%. وكانت نسبة الإصابة بالمايكوبلازما في المزرعة لكل من القصبات الهوائية 41.1%، في حين بلغت في مقابل عينات الأكياس الهوائية 22.1%. كما أظهرت نتائج PCR لجين 16 S rRNA نتيجة إيجابية لمرض مايكوبلازما غاليسبتكم .كما تأثرت النتائج بظهور علامات تنفسية مثل السعال والعطس.

الكلمات المفتاحية: القصبة والاكياس الهوائية، الميكوبلازما غاليسبتكم، PCR.

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INTRODUCTION

Avian Mycoplasmosis often refers to a number of pathogenic Mycoplasma infections, such as *Mycoplasma iowae*, *Mycoplasma synoviae*, *Mycoplasma*. *meleagridis*, and *Mycoplasma gallisepticum* (19,33).Mycoplasma is the smallest and most fragile type of bacteria found outside the host. It has a modest genome size 580-2200 kb. Due to their lack of certain genetic abilities, they are biologically incapable of manufacturing cell walls and many other necessary components for survival. This gives it a strong ability to antibiotics resist, such as penicillin and its derivatives, which change the structure of the cell wall and membrane added to the cultured agar. (25, 26). They often depend on host cells to carry out their biological functions; As a result, mycoplasmas are highly adept at adapting to survive on exposed surfaces and in host tissues, evading host defenses in a number of ways (6, 23). *Mycoplasma gallisepticum* is the most common illness in Iraq, and numerous researchers have successfully isolated it from broiler and layer farms that had respiratory indications of infection (4,10). Due to its lack of a cell wall, mycoplasmas is classified using phenotypic traits, serology, genotype sequences, and other related genes such as 16 s rRNA, mgc1 (GapA) , mgc 2 , pMGA and pvpA .(29**).** In hens and turkeys, *Mycoplasma gallisepticum* infection is known as infectious sinusitis or chronic respiratory disease, respectively. The chronic nature of *Mycoplasma gallisepticum* infections indicates that the host immune system was unable to adequately combat the*Mycoplasma* infection (20).*Mycoplasma gallisepticum* in hens identified by used serological checking technique such Enzyme-linked immunosorbent assay (ELISA) and serum plate agglutination (SPA), which evaluated and detected subclinical *Mycoplasma gallisepticum* colonies. (5). Because the live attenuated vaccine is highly immunogenic and effective in eliminating virulent (field) strains from chicken farms, it has been used extensively (4). Iraq used *Mycoplasma gallisepticum* vaccine that had been inactivated, called bacterins. The local isolate of *Mycoplasma gallisepticum* was found in broilers and layers after a week of infection. Its virulence traits increased its pathogenicity in the respiratory system and increased the possibility of an inflammatory response. (14).

MATERIALS AND METHODS

Clinical samples collection: Eight broiler chicken farms were the source of 202 samples 107 tracheal samples and 95 air sacs samples collected by taking part of trachea and air sac after postmortem. The chicken had obvious respiratory symptoms such as coughing, sneezing, face edema, and respiratory sounds. Each sample was collected from broiler farms in Samarra city between September and December 2022. The samples were divided into two groups: used for conventional laboratory culture work and DNA extraction so that isolated suspicious colonies could be confirmed by PCR. cloning assay for collected tissue samples. Assay for cloning obtained tissue samples. Every sample was delivered to the Tikrit university - College of Veterinary Medicine's Microbiology laboratory.

Isolation and identification of *Mycoplasma gallisepticum:* After inoculating 3 ml of PPLOs **(** Pleuropneumonia - like organisms) broth media with collected trachea with air sacs, the mixture was incubated at 37 ºC for three to five days until the change of color (27). After that, 20 microliters from the pigmented broth was applied in streaks the PPLO agar and then incubated at 37 ºC in the candle jar with low oxygen tension (8,16). During 21 days, inoculating at least two media plates from the same broth tube. After 6, 11, 16, and 21 days of incubation, the plates were inspected under a microscope to check of the present any fried egg form for Mycoplasma colonies matched .

***Mycoplasma* colonies staining method:** Diene's dye was used on several plates to examine the morphology and pigment-absorbing ability of the alleged Mycoplasma colonies as well as to boost confidence in the isolation procedure. (7)(21).

Molecular detection of isolates extraction

DNA and molecular analysis of *Mycoplasma gallisepticum*

Mycoplasma DNA used the previously authorized method (2) was followed to collect tissues from the birds' trachea, and air sacs. DNA was extracted using tissue extraction

kit's manufacturer's instructions (Anatolia, Turkey) primers based on the 1500 bp 16S ribosomal RNA gene were developed by (22) and used in conventional PCR technique Mycoplasma diagnosis. whereas the GapA gene primers (9). The procedure used to extract DNA from tissue samples involved heating the tissue pieces in a water bath for 10 minutes at 95 °C and cooling them for 10 minutes at -20 °C. The samples were then rapidly placed in phosphate buffer saline (PBS) and centrifuged at 13,000 x g for 20 minutes. The finished product was then twice laved in the PBS solution and liquid supernatant which conserved in 1.5 ml of the eppendorf tubes. The NanoDroop 2000 (Thermo Scientific, Germany) was used to measure DNA concentrations ranging from 30 to 100 ng /µl. DNA was kept in a deep -84°C freezer for a later experiment. Moicullar diagnosis of *Mycoplasma gallisepticum* 16 s RNA gene and GapA gene. The *Mycoplasma gallisepticum* 16s RNA and GapA genes were identified by used PCR using all positive cultures and tissue organ samples that were collected. With minor adjustments, the PCR reaction was carried out in accordance with (24). In brief: 25 µL reaction volume, 1 µL of each primer, 10 µL and 12.5 µL 2X PCR master mix, 1200 bp 16s RNA 27F, 5'-AGA GTT TGA TGG TCC CTC AG-'3 and 1492 R, 5'-GGT CTT TAC GTT ACT ACG T-'3 are sequences of Mycoplasma spp. (12,13). *Mycoplasma gallisepticum* 530 bp, Gap A 3F, 5'-TTC TAG CGC TTT CCT AGC AAA CCC-'3 and 4R, 5'-CTT GTG GAA CAG CAA CGT ATT CGC-'3, 20 µL distilled water, and two microliters of DNA template were used. (Abm , Canada). (9, 11). The following four phase comprised the conditions of the thermal cycler:-

-Denaturation phase: DNA separated completely at 94˚C for 3minutes.

-Primer-annealing phase: The primer was linked to the complementary sequence or annealed to the target sequence for thirty seconds at 55˚C. finally, the extension was completed at 72˚C for sixty seconds**.**

- DNA extension phase: The 35 cycles were divided into three sections, with each section undergoing denaturation at 94˚C for thirty seconds.

-Final DNA extension phase: The final extension occurring at 72˚C for five minutes (17). The results of PCR were separated by electrophoresis on a gel composed of 1.5% agarose for an hour at 80 V. After that, they were stained with ethidium bromide and photographed.

Clinical and gross manifestation: The study recorded the appearance of many respiratory clinical signs on the examined broiler chicken samples, such as coughing and sneezing, in addition to the presence of nasal secretions. The chicken was then dissected in order to observe pathological changes in the affected viscera.

RESULTS AND DISCUSSION

***Mycoplasma gallisepticum* isolation:** The culture of air sac, and tracheal samples for Mycoplasma on Fries agar revealed a 32.2% isolation for the Mycoplasma. Tracheal sample isolation rates were high (41.1%).in contrast to air sac samples 22.1% Table. This ratio is higher than the 23.4% reported by (15). Other studies (18,28) reported similar results. *Mycoplasma gallisepticum* claims that it was also isolated from cases of respiratory infections. Consequently, it could result in secondary respiratory infections, air saculitis, and subclinical infections (30).

Table 1. Mycoplasma isolated ratio from trachea and air sac samples by used biochemical test

The type of samples	Number of the samples	Number of the positive cases	Percentage %
Samples of trachea	**107**	**44**	**41.1%**
Samples of air sacs	**95**	**21**	**22.1%**
Total	**202**	**65**	**32.2%**

Detection of *Mycoplasma gallisepticum:* Mycoplasmas from broilers produced positive results when cultured on PPLO broth supplemented with 20 milliliters of horse serum, 25 milligrams of thallous acetate, and 2000 units of penicillin for a period of two days. The mycoplasmas that were isolated displayed colony shape similar to a "fried egg" on PPLO agar (Figure 1) (1). Found to be members of the *Mycoplasma gallisepticum* 48

(64.8%) in tracheal and 28(42.44%) air sac samples, Therefore the total of positive respiratory (tracheal and air sac) by used PCR technique that 76 (54.7%) (Table 2) .These result agreement with (27) .

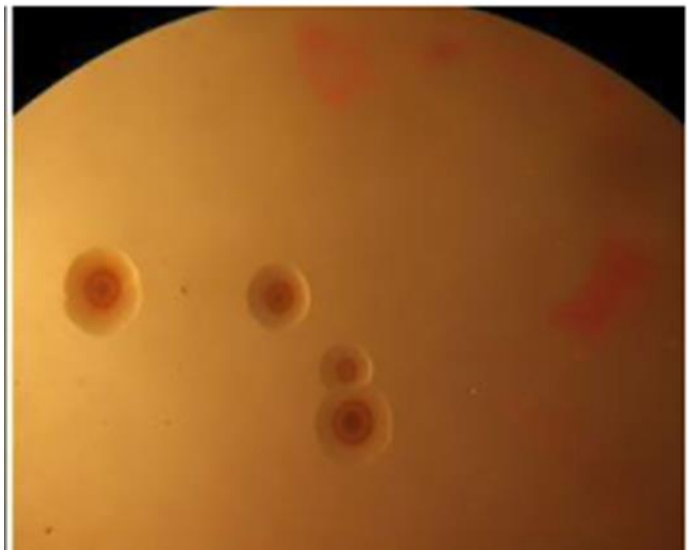

Figure 1. Fried egg colonies of *Mycoplasma gallisepticum* on PPLO agar 25X

Table 2. Detection of *Mycoplasma gallisepticum* ratio from trachea and air sac samples by used PCR technique

The type of samples	Number of the samples	Number of the positive cases	Percentage %
Samples of the trachea	74	48	64.8%
Samples of the air sacs	65	28	42.4%
Total	139	76	54.7%

Molecular detection for *Mycoplasma gallisepticum:* Mycoplasma colonies growing on PPLOs were positive for 16s protein, and the sequences of *Mycoplasma gallisepticum* (Figure 2).

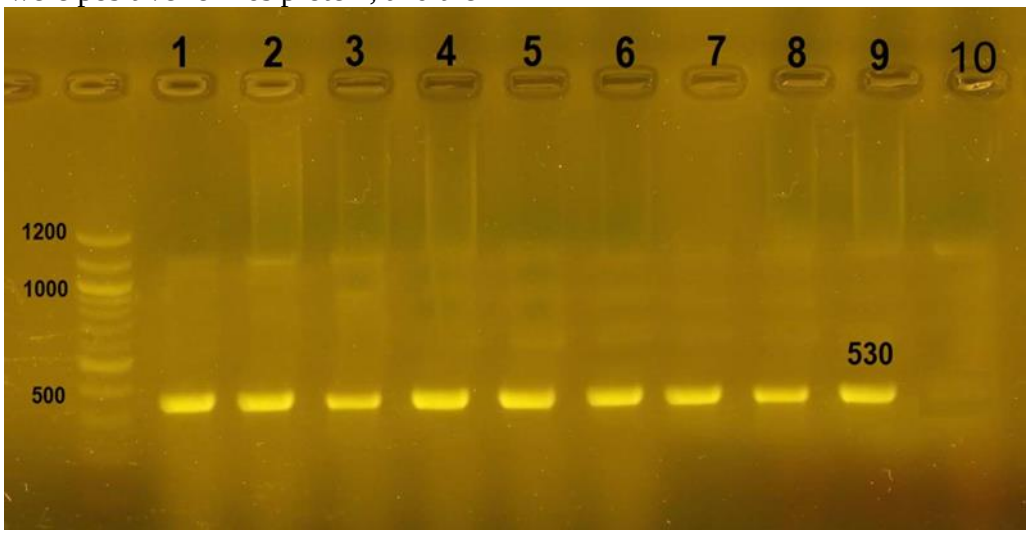

Figure 2. PCR operations are displayed by electrophoresis on a %1.5 agarose gel with ethidium bromide staining. M: Wells 1-9 positive samples of the DNA marker types and quantity of samples number of positive cases Mycoplasma using a 530 bp band size

Clinical and gross manifestation

The study recorded many clinical signs on the examined broiler chicken samples, which were represented by the presence of respiratory signs such as coughing, sneezing, and in addition to the presence of nasal secretions. The results were concurred with the researchers (3, 32). Also the study recorded found pathological changes represented by the occurrence and congestion in the trachea and air sacs (Figures 3 & 4), which is concurred with the results of the researchers (31).

Fig. 3. Congested of the trachea

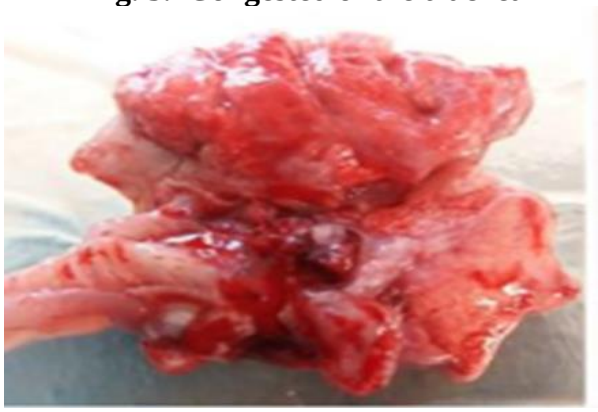

Fig. 4. Congested of the air sacs

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