

# Prevalence of mycoplasma infection in poultry (*Gallus gallus domesticus*) and evaluation of some diagnostic techniques

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## ABSTRACT

Investigation for most feasible, easy, and cheapest laboratory methods for diagnosis of mycoplasma infection is a worldwide need. The present study aimed to estimate the prevalence of *Mycoplasma* infection (*Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS)) in poultries and evaluating three serological techniques, serum plate agglutination (SPA) technique, enzyme-linked immunosorbent assay (ELISA), and Hemagglutination inhibition (HI) technique with polymerase chain reaction (PCR) technique done in previous work. Serum samples (276) were collected from chickens with respiratory and articular diseases (202 and 74 respectively). A high detecting rate for MG (31.8%) and MS (19.5%) was observed using the SPA technique in comparison with other techniques. The sensitivity and specificity of the three techniques according to the PCR technique varied, 100% specificity recorded using ELISA and HI techniques. The current study concluded that the SPA technique is a more appropriate technique for detecting MG and MS. ELISA and HI techniques are highly specific. Although, a certain level of false-positive results can be expected in any test.

**Keywords:** Mycoplasma, poultry, SPA, ELISA, Hemagglutination Inhibition.

## Introduction

*Mycoplasma* spp. are associated with different diseases [1-3]. The most important poultry mycoplasmosis is caused by *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) [4]. The diseases caused by these microorganisms include chronic respiratory disease of chicken caused by MG and infectious synovitis of chicken caused by MS [5]. Symptoms of respiratory infection include respiratory rates, coughing, and nasal discharges. Infection with MS, on the other hand, causes acute or chronic disease of chickens and turkeys constitutes primarily an infection of synovial membranes of joints and tendons sheath, also, it causes airsacculitis [5].

The techniques used in the detection of mycoplasma infection are very important since clinical diagnosis (signs and pathological lesions) cannot confirm the source of infection. Prevention of the spread of infection and decreasing the economic losses in the poultry products are an attractive vision especially in the finding of the most reliable and rapid diagnostic detection of mycoplasma infections. Three approaches in the diagnosis of mycoplasma infection were submitted, they are, isolation and identification of the microorganisms via culture method, detecting of its DNA, and identification of specific antibodies in the serum [4, 6, 7]. The serological tests which are commonly used for the diagnosis of mycoplasma include serum plate agglutination (SPA), enzyme-linked immunosorbent assay (ELISA), and hemagglutination inhibition (HI) techniques [8-16].

The present study aimed to estimate the rate of mycoplasma infection in chickens with respiratory and articular diseases and evaluation of some serological diagnostic methods.

## Material and Methods

### Samples collection

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Blood samples were collected from 276 infected chickens suffering from respiratory disease including (cough, sneeze, nasal discharge, and inflammation of eyes) and/or joint infection (lameness and swelling of joints).

### Serological tests

**Serum plate agglutination (SPA) test:** Fresh sera were tested against MG and MS antigens (Charles River-USA), following the manufacturer's instructions. Briefly, thirty µl of serum was mixed with thirty µl of antigen and then incubated at room temperature (25 °C) for 1-2 min before the result was read. Negative and positive sera were included in each test.

**ELISA test:** It was applied by using 2 kits (Biocheck company-Holland); one for diagnosis of MS and the other for MG.

**Hemagglutination inhibition (HI) test:** The test was done in the Microtiter plate using kits provided by Charles River-USA. The kit contained two types of antigens for the bacteria MG and MS, and the test was done according to [17].

### Evaluation of the test techniques

The results of the 3 serological tests were evaluated according to the results of PCR for the same samples which were recorded in the previous paper [18].

### Statistical analysis

- Positive agreement =

$$\frac{\text{No. of samples gave positive results in the first test}}{\text{No. of samples gave positive results in the second test}} \times 100$$

- Negative agreement =

$$\frac{\text{No. of samples gave negative results in the first test}}{\text{No. of the samples gave negative results in the second test}} \times 100$$

- Sensitivity =  $\frac{\text{True positive}}{\text{True positive} + \text{false negative}} \times 100$

- Specificity =  $\frac{\text{True negative}}{\text{True negative} + \text{false positive}} \times 100$

- Positive predictive values =  $\frac{\text{True positive}}{\text{True positive} + \text{false positive}} \times 100$

- Negative predictive values =  $\frac{\text{True negative}}{\text{True negative} + \text{false negative}} \times 100$

## Results

In the current study, chickens suffering from the two types of diseases were studied.

**First group:** Chickens with respiratory signs including cough, sneeze, nasal discharge, and inflammation of eyes.

**Second group:** Chickens with lameness and joint swellings.

### Serological tests

**Serum plate agglutination test (SPA):** The results showed that out of 276 sera samples, a total of 142 (51.4%) gave positive anti-MG and anti-MS. Infection with MG [88(31.8%)] was more than MS infection [54 (19.5%)]. In addition, respiratory MG infection [84(41.5%)] was more than MS infection [27(13.3%)]. While MS infection [27(36.4%)] was more than MG infection [4(5.4%)] in chickens with joint diseases (Table 1).

**Table (1): Serum plate agglutination test for chickens suffering from respiratory and articular sings.**

| Type of samples      | No of samples | Positive samples for MG antibody |       | Positive samples for MS antibody |       | Total |       |
|----------------------|---------------|----------------------------------|-------|----------------------------------|-------|-------|-------|
|                      |               | No                               | %     | No                               | %     | No    | %     |
| Respiratory diseases | 202           | 84                               | 41.5% | 27                               | 13.3  | 111   | 54.9% |
| Articular diseases   | 74            | 4                                | 5.4%  | 27                               | 36.4% | 29    | 39.1% |
| Total                | 276           | 88                               | 31.8% | 54                               | 19.5% | 142   | 51.4% |

When comparing the SPA test and PCR for MG & MS, the results of sensitivity and specificity were respectively 80.9% and 76.9% for MG and 91.7% and 90.4% for MS (Tables 2&3).

**Table (2): Comparison between PCR and serum plate agglutination (SPA) test for MG.**

|               | Result of SPA test |    |                |     | Total |
|---------------|--------------------|----|----------------|-----|-------|
|               | Positive           |    | Negative       |     |       |
| Result of PCR | True positive      | 34 | False-positive | 54  | 88    |
|               | False-negative     | 8  | True negative  | 180 | 188   |
| Total         |                    | 42 |                | 234 | 276   |

Sensitivity = 80.9%, Specificity = 76.9%

**Table (3): Comparison between PCR and serum plate agglutination (SPA) test for MS.**

|               | Result of SPA test |    |                |     | Total |
|---------------|--------------------|----|----------------|-----|-------|
|               | Positive           |    | Negative       |     |       |
| Result of PCR | True positive      | 31 | False-positive | 23  | 54    |
|               | False-negative     | 3  | True negative  | 219 | 222   |
| Total         |                    | 34 |                | 242 | 276   |

Sensitivity = 91.7%, Specificity = 90.4%

**Results of ELISA test:** ELISA test detected antibodies against MG in 43 (21.1%) from the total of 202 chickens with respiratory signs, in contrast, anti-MS was detected in only 9 (4.4%) of the same chickens. In cases of chickens with articular signs, ELISA test detected antibodies against MS in 17 (22.9%) of the total 74 chickens, while, it detected antibodies against MG in only 5 (6.7%) from the same chickens. This means that the ELISA technique detected antibodies against MG in 48 (15.7%) chickens with respiratory or articular signs and detected antibodies against MS in 26 (9.4%) chickens (Table 4).

**Table (4): ELISA test results for anti-MG and anti-MS in chickens with respiratory and articular signs.**

| Type of disease   | No. of samples | Positive samples for MG antibody |       | Positive samples for MS antibody |       | Total |       |
|-------------------|----------------|----------------------------------|-------|----------------------------------|-------|-------|-------|
|                   |                | No                               | %     | No                               | %     | No    | %     |
| Respiratory signs | 202            | 43                               | 21.2% | 9                                | 4.4%  | 52    | 25.7% |
| Articular signs   | 74             | 5                                | 6.7%  | 17                               | 22.9% | 22    | 29.7% |
| Total             | 276            | 48                               | 15.7% | 26                               | 9.4%  | 74    | 26.8% |

When comparing ELISA test results with PCR, the sensitivity and specificity of the ELISA test was 73.8% and 92.7% for MG and 76.4% and 100% for MS (tables 5 & 6).

**Table (5): Sensitivity and specificity of ELISA test according to PCR in the diagnosis of MG.**

| Result of PCR  | Result of plate agglutination test |                |               |                | Total |
|----------------|------------------------------------|----------------|---------------|----------------|-------|
|                | Positive                           |                | Negative      |                |       |
|                | True positive                      | False positive | True negative | False negative |       |
| True positive  | 31                                 | 17             | 48            |                |       |
| False negative | 11                                 | 217            | 228           |                |       |
| Total          | 42                                 | 234            | 276           |                |       |

sensitivity= 73.8%, specificity= 92.7%

**Table (6): Sensitivity and specificity of ELISA test according to PCR in the diagnosis of MS.**

| Result of PCR  | Result of Serum plate agglutination test |                |               |                | Total |
|----------------|--|----------------|---------------|----------------|-------|
|                | Positive                                 |                | Negative      |                |       |
|                | True positive                            | False positive | True negative | False negative |       |
| True positive  | 26                                       | 0              | 26            |                |       |
| False negative | 8  | 232            | 240           |                |       |
| Total          | 34                                       | 232            | 276           |                |       |

sensitivity= 76.4%, specificity= 100%

- Positive agreement between ELISA and Serum plate agglutination test for MG and MS was 54.5% and 48.1% respectively.
- Total agreement between ELISA and Serum plate agglutination test for MG and MS was 49.4% and 25.3% respectively.

## Results of Hemagglutination inhibition test (HI)

HI test for MG gave positive results at a rate of 12.3%, while the test for MS gave positive results at a rate of 9.7% (Table 7).

**Table (7): Results of Hemagglutination inhibition test.**

| Type of samples   | No of samples | Positive samples for MG antibody |       | Positive samples for MS antibody |       | Total |       |
|-------------------|---------------|----------------------------------|-------|----------------------------------|-------|-------|-------|
|                   |               | No                               | %     | No                               | %     | No    | %     |
| Respiratory signs | 202           | 34                               | 16.8% | 6                                | 2.9%  | 40    | 19.8% |
| Articular signs   | 74            | 0                                | 0%    | 21                               | 28.3% | 21    | 28.3% |
| Total             | 276           | 34                               | 12.3% | 27                               | 9.7%  | 61    | 22.1% |

When comparing HI test results with PCR, the sensitivity and specificity of the HI test were respectively 80.9% and 100% for MG and 79.4% and 100% for MS (tables 8 & 9).

**Table (8): Sensitivity and specificity of HI test according to PCR in the diagnosis of MG.**

| Result of PCR  | Result of the HI test |                |               |                | Total |
|----------------|-----------------------|----------------|---------------|----------------|-------|
|                | Positive              |                | Negative      |                |       |
|                | True positive         | False positive | True negative | False negative |       |
| True positive  | 34                    | 0              | 34            |                |       |
| False negative | 8                     | 234            | 242           |                |       |
| Total          | 42                    | 234            | 276           |                |       |

sensitivity= 80.9%, specificity= 100%

**Table (9): Sensitivity and specificity of HI test according to PCR in the diagnosis of MS.**

| Result of PCR  | Result of Serum plate agglutination test |                |               |                | Total |
|----------------|--|----------------|---------------|----------------|-------|
|                | Positive                                 |                | Negative      |                |       |
|                | True positive                            | False positive | True negative | False negative |       |
| True positive  | 27                                       | 0              | 27            |                |       |
| False negative | 7  | 242            | 249           |                |       |
| Total          | 34                                       | 242            | 276           |                |       |

sensitivity= 79.4%, specificity= 100%

- Positive agreement between ELISA and HI test for MG and MS: 70.8% and 96.2%, respectively.
- Total agreement between ELISA and HI test for MG and MS: 70.8% and 50.9%, respectively.
- Positive agreement between Serum plate agglutination test and HI test for MG and MS: 38.6% and 50%, respectively.
- Total agreement between HI and Serum plate agglutination test for MG and MS: 27.8% and 33.3%, respectively.

## Discussion

Various techniques can be applied in the diagnosis of mycoplasma. Serological tests have been used to detect

antibodies against the pathogen, including SPA, HI, and ELISA tests. Also, tests have been used to detect the mycoplasma to find either the organisms by culture and isolation or their DNA using PCR procedures<sup>[19]</sup>. In the current study, the serological tests were performed according to the results of the PCR technique. The results of the serological test revealed that the results of the ELISA test were similar to those of HI, since, both of them can detect IgG antibodies<sup>[20]</sup>. The results also showed that the highest positive results were recorded by the SPA test in comparison with ELISA and HI tests due to its ability in the detection of IgM. Therefore, the SPA test considered the best serological method and it is easy, cheap, and highly sensitive<sup>[20]</sup>. Even though, false-positive results can be expected in certain levels in any test<sup>[21]</sup>. So, because of the variety of false-positive results between several serologic tests, it is not advisable to depend completely on one technique<sup>[22]</sup>. The high number of false-positive results in several tests may be due to factors such as serum of avian which recently infected with a heterologous *Mycoplasma* spp., heat inactivation lack, age of the avian and applying inactivated vaccines<sup>[23]</sup>. The World Organization for Animal Health (OIE) recommends the use of serological techniques for avian mycoplasmosis only as screening tools in the diagnosis of flocks, not of individual birds<sup>[4, 22]</sup>.

## Conclusion

Respiratory *M. gallisepticum* (MG) infection is more frequent than *M. synoviae* (MS) infection in chickens. While, the MS infection is more frequent than MG infection in chickens with joint diseases. The most appropriate technique for detecting (MG) and (MS) is SPA. ELISA and HI techniques are highly specific. Although, certain level of false positive results can be expected in any test.

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