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# Seroprevalence of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in broiler chickens from Haryana, India

**Jay Prakash Yadav[1,2], Kanisht Batra[3] and Yarvendra Singh[1*]**

[1]Department of Veterinary Public Health and Epidemiology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, India, [2]Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Rampura Phul, Bathinda, India, [3]Department of Animal Biotechnology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, India

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**Abstract**

*Mycoplasma gallisepticum (MG) and Mycoplasma synoviae (MS) are economically important respiratory pathogens of birds causing significant losses to poultry industry. The present study was aimed at serodetection of MG and MS antibodies in broiler chicken serum samples using standard plate agglutination test (SPAT). A total of 306 blood samples were collected from birds in the age group of 4-6 weeks slaughtered at local meat shops of five districts of Haryana, India i.e., Bhiwani (n=07); Fatehabad (n=49), Hisar (n=118), Jind (n=103) and Karnal (n=29). The SPAT was carried out using coloured antigens and standard sera of MG and MS (Soleil Biovac, Animal health, France). In SPAT, 21.57% (66/306; 95% CI: 17.33-26.51), 13.40% (41/306; 95% CI: 10.03-17.67) and 14.38% (44/306; 95% CI: 10.89-18.75) serum samples were found positive for MG, MS and mixed (both MG and MS) infections, respectively. The serodominance of MG and MS antibodies were reported from Hisar (30.51%) and Jind (19.42%) districts, respectively. As similar to MS serodominance, mixed infection of MG and MS were reported maximum from Jind (18.44%) district. The study reveals that MG and MS infection is prevalent in commercial broiler chicken flocks that poses risk to poultry farming in terms of production losses. It is also reported that enzyme linked immunosorbent assay (ELISA) based assays should be used along with SPAT for accurate serodetection of avian mycoplasmas infection in poultry flocks affected with respiratory tract infections.*

**Keywords:** Broiler chicken; *Mycoplasma gallisepticum*; *Mycoplasma synoviae*; Standard plate agglutination test

Commercial poultry farming in Haryana is very much prominent and the state is ranking among the top five states in poultry meat production. The main hubs of broiler chicken production in Haryana are Fatehabad, Hisar, Jind, Karnal, Kaithal, Panipat, Sirsa and Yamunanagar. The poultry industry in Haryana has seen significant growth, transitioning from backyard farming to commercial operations (Mahajan et al., 2023). However, one of the major challenges of poultry farming is the outbreaks of different infectious diseases caused by pathogenic avian mycoplasmas [*Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS)], avian pathogenic *Escherichia coli* (APEC), avian influenza virus (AIV), avian pneumovirus (APV), infectious bronchitis virus (IBV) and Newcastle disease virus (NDV), individually or concurrently (Roussan et al., 2008; Gowthaman et al., 2017; Yadav et al., 2023). MG and MS may lead to significant economic losses in

**Corresponding author: ysingh@indovax.com, Ph. 9416648148*

the poultry industry by causing 5-10% increase in embryo mortality, 10-20% reduction in productivity, as well as cost incurred in prevention and control of disease (Kleven, 1990; Nascimento et al., 2005). MG is responsible for causation of chronic respiratory disease (CRD) in chickens; however, MS are mainly involved in arthritis, air sacculitis, infectious synovitis, mild respiratory tract infection, with or without symptom of septicemia (Kleven, 2008; Sprygin et al., 2010; Yadav et al., 2021). Early detection of the disease is important to prevent further spread of infection into the flock. Different diagnostic techniques such as culture, polymerase chain reaction (PCR) based assays and serological tests are commonly used for laboratory detection of avian mycoplasmas. Culture of pathogen is considered to be gold standard test for detection of avian mycoplasmas. However, this technique is laborious, time consuming and faces over growth problem of other commensal avian *Mycoplasma* species such as *M. gallinarum*, *M. gallinaceum* and *Acholeplasma laidlawii* (Kleven, 2008; Sprygin et al., 2010; Yadav et al., 2025a). PCR based assays are commonly used for rapid and specific detection of avian mycoplasmas (Tomar et al., 2017a; Yadav et al., 2024). However, it requires sophisticated laboratory facilities, trained man power and cost involved in purchase of molecular biology reagents, which hinders its wide application in resource poor laboratories, mostly in developing countries (Ahmed et al., 2015; Yadav et al., 2021). Serological techniques such as serum plate agglutination test (SPAT), enzyme linked immunosorbent assay (ELISA) and haemagglutination inhibition (HI) are commonly used for detection of MG and MS antibodies (Sumitha et al., 2015, Tomar et al., 2017b, Rajkumar et al., 2018; Yadav et al., 2022). SPAT is fast, inexpensive, sensitive and can be used for detection of MG and MS antibodies in field conditions (Kleven, 1998; Seifi and Shirzad, 2012). Its sensitivity is reported to be superior as compare to ELISA and HI, as it detects acute infection by measuring IgM antibodies (Sumitha and Sukumar, 2017). Earlier studies also reported seroprevalence of MG and MS antibodies from broiler chickens of similar areas of Haryana, India (Tomar et al., 2017b; Yadav et al., 2021). However, Tomar et al. (2017b) used most of the samples from day old chicks and Yadav et al. (2021) used indirect ELISA (iELISA) and DOT-ELISA for serodetection of MG and MS antibodies. This study was designed to screen the serum samples from broiler chickens aging 4-6 weeks sampled from local meat shops located in different districts of Haryana using SPAT for serodetection of MG and MS antibodies, followed by comparing the result with earlier standardized iELISA and DOT-ELISA.

## Materials and methods

### *Sample collection and study areas*

A total of 306 blood samples were collected from broiler chickens aging 4-6 weeks slaughtered at local meat shops of five districts of Haryana, India *i.e.,* Bhiwani (n=07); Fatehabad (n=49), Hisar (n=118), Jind (n=103) and Karnal (n=29) for the detection of MG and MS antibodies (Table 1). For serum separation, the blood sample collected from each bird into 5 mL capacity tube (BD Vacutainer® SST II Advance) was kept at 4°C for 30-60 min, followed by centrifugation at 2500 × g for 10 min. The collected sera were used for detection of MG and MS antibodies using SPAT.

### *Serum plate agglutination test*

The SPAT was carried out using coloured antigens of MG and MS (Soleil Biovac, Animal health, France) as per earlier described protocol (Shadmanesh and Mokhtari, 2013). The test was performed by taking equal volume (25 µl) of MG/MS coloured antigen and test serum on a grease free glass slide, mixed it with the help of micropipette tips and rotated clockwise and counter-clockwise direction up to two minutes. The appearance of clear blue violet agglutinates (clumps) was considered positive, whereas no agglutination indicates sample negative for avian mycoplasmas. The MG and MS commercial test sera received from Soleil, BioVac Animal Health, France were used as positive and negative test control to perform the assay. Each sample was tested twice to prevent false positive/negative test results. Samples having inconclusive test result was tested again for confirmatory diagnosis.

### *Statistical analysis*

The data obtained using SPAT was statistically analyzed using Epitools Epidemiological Calculators (Rogan and Gladen, 1978). The test sensitivity and specificity were considered as 90% and 99%, respectively at 95% confidence interval (CI). The CI for apparent and true prevalence was calculated using Wilson and Blaker methods, respectively. The positive and negative predictive values and likelihood ratios (positive and negative) were calculated considering estimated true prevalence with an imperfect test of Epitools Epidemiological Calculators.

## Results and discussion

A total of 306 serum samples collected from five districts of Haryana, India were used for the serodetection of MG and MS antibodies using SPAT. The apparent (WilsonCL) and true (BlakerCL) seropositivity of avian mycoplasmas was found to be 49.34% (151/306; 95% CI: 43.79-54.92) and 54.32% (95% CI: 48.08-60.59), respectively. However, positive and negative predictive values were found to be 0.9907 and 0.8928, respectively. The positive and negative likelihood ratios were found to be 90 and 0.101, respectively. Among 151 avian mycoplasmas positive samples, highest seropositivity was found in Jind (61.16%), followed by Hisar (53.39%), Fatehabad (36.73%), Bhiwani (28.57%) and Karnal (17.24%) districts. The seropositivity of MG, MS and mixed (both MG and MS) infection were found to be 21.57% (66/306; 95% CI: 17.33-26.51), 13.40% (41/306; 95% CI:

10.03-17.67) and 14.38% (44/306; 95% CI: 10.89-18.75), respectively. The serodominance of MG and MS antibodies were reported from Hisar (30.51%) and Jind (19.42%) districts, respectively. As similar to MS serodominance, mixed infection of MG and MS were reported maximum from Jind (18.44%) district (Table 1; Fig 1). On screening the same serum samples using iELISA, 50.32% (95% CI; 44.76–55.89) and 61.76% (95% CI; 56.21–67.03) serum samples were found positive for MG and MS antibodies, respectively. However, in DOT-ELISA, 41.83% (95% CI; 36.44–47.42) and 53.92% (95% CI; 48.32–59.42) serum samples were found positive for MG and MS antibodies, respectively (Yadav et al., 2021). Statistical analysis for serodetection of MG and MS antibodies using SPAT is given in table 2. Diagnostic efficacy of SPAT in comparison to iELISA and DOT-ELISA are given in table 3.

In the present study, the SPAT was used for serodetection of MG and MS antibodies in broiler chickens slaughtered at local meat shops of five districts (Bhiwani, Fatehabad, Hisar, Jind and Karnal) of Haryana, India. Serological tests are generally preferred for early detection of *Mycoplasma* infection in poultry flocks (Ley, 2003; Xavier et al, 2011; Yadav et al., 2025b). The SPAT is a rapid, economical, sensitive and can be widely used as a primary screening test for flock monitoring and serodiagnosis of *Mycoplasma* infection (Kleven and Bradbury, 2008). It can detect seroconversion a few days

**Table 1:** Serodetection of *Mycoplasma gallisepticum and Mycoplasma synoviae* using serum plate agglutination test

| Location of poultry flocks | Number of sera tested | Samples positive for avian mycoplasmas | | | Total number of positive samples |
|---|---|---|---|---|---|
| | | *M. gallisepticum* (MG) | *M. synoviae* (MS) | Both MG & MS | |
| Hisar | 118 | 36 | 09 | 18 | 63 |
| Bhiwani | 07 | 02 | 00 | 00 | 02 |
| Jind | 103 | 24 | 20 | 19 | 63 |
| Fatehabad | 49 | 04 | 07 | 07 | 18 |
| Karnal | 29 | 00 | 05 | 00 | 05 |
| Total | 306 | 66/306 (21.57)* | 41/306 (13.40) | 44/306 (14.38) | 151/306 (49.34) |

*Value in parentheses indicates percentage

**Table 2:** Statistical analysis of serum plate agglutination test for serodetection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* infection in broiler chickens

| Variables | Avian mycoplasmas | | |
|---|---|---|---|
| | MG [% (95% CI)] | MS [% (95% CI)] | MG & MS [% (95% CI)] |
| Apparent prevalence | 21.57 (17.33-26.51) | 13.40 (10.03-17.67) | 14.38 (10.89-18.75) |
| True prevalence | 23.11 (18.35-28.67) | 13.93 (10.15-18.73) | 15.03 (11.11-19.95) |
| PPV | 0.9644 | 0.9358 | 0.9409 |
| NPV | 0.9705 | 0.9839 | 0.9824 |
| LR (+ve) | 90 | 90 | 90 |
| LR (-ve) | 0.101 | 0.101 | 0.101 |

MG- *M. gallisepticum*; MS- *M. synoviae;* PPV- positive predictive value (proportion of individuals with a positive test result who actually have the disease); NPV- negative predictive value (proportion of individuals with a negative test result who actually free from the disease); LR- Likelihood ratio (LR (+ve)- indicates how much more likely a positive test result in individual with the disease compared to those without it; LR (-ve)- indicates how much less likely a negative test result in individual with the disease compared to those without it. LR+ > 1 suggests the presence of disease is more likely with a positive result [sensitivity/(1-specificity]; LR- > 1 suggests the presence of disease is less likely with a negative result [(1-sensitivity)/specificity].

**Table 3:** Diagnostic efficacy of SPAT in comparison to iELISA and DOT-ELISA

| Variables | SPAT vs iELISA (reference test) | | SPAT vs DOT-ELISA (reference test) | |
|---|---|---|---|---|
| | MG (CI) | MS (CI) | MG (CI) | MS (CI) |
| Relative sensitivity (%) | 72.73 (64.97-79.58) | 44.97 (37.75-52.36) | 62.02 (53.05-70.41) | 46.67 (38.87-54.58) |
| Relative specificity (%) | 100 (97.60-100) | 100 (96.90-100) | 81.92 (75.45-87.29) | 94.33 (89.13-97.52) |
| Kappa value | 0.7260 | 0.3846 | 0.4474 | 0.3937 |

SPAT- serum plate agglutination test; iELISA- indirect enzyme linked immunosorbent assay; MG- M. gallisepticum; MS- M. synoviae; CI- confidence interval; Cohen's Kappa values between 0.10-0.20, 0.21-0.40, 0.41-0.60, 0.61-0.80 and 0.80-0.99 indicate slight agreement, fair agreement, moderate agreement, substantial agreement and near perfect agreement, respectively.

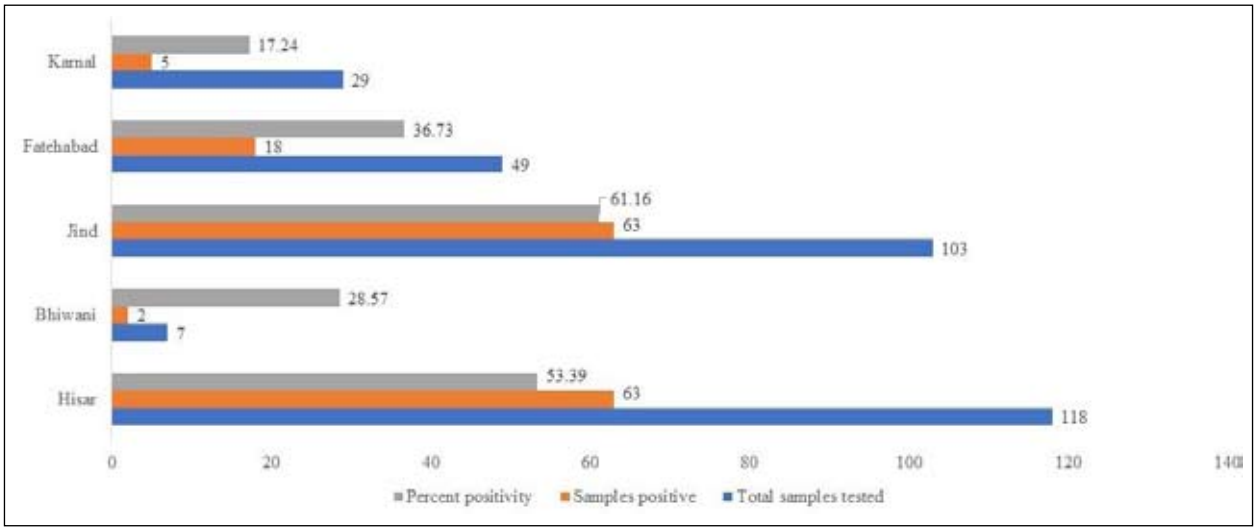


**Fig. 1:** Seropositivity of avian mycoplasmosis using serum plate agglutination test

earlier than HI and ELISA, as it measures IgM antibodies, which appear first after infection (Feberwee et al., 2005). It is considered more sensitive as compare to i-ELISA and hemagglutination test (Ley, 2008). Overall, the present study reported 35.94% (110/306) and 27.77% (85/306) seropositivity of MG and MS antibodies from different regions of Haryana. The findings of the present study are concurrent with earlier studies from India that reported 22.4% and 18.3-51.1% seropositivity of MG and MS antibodies in chicken using SPAT (Sumitha and Sukumar, 2017; Tomar et al., 2017b). Similarly, Aimeur et al. (2010) reported 43.3% and 26.7% seropositivity of MG and MS antibodies, respectively in chicken at Eastern Algeria. Xavier et al. (2011) reported 55.1% and 100% chicken farms were seropositive for MG and MS antibodies, respectively in the state of Entre Ríos in Argentina. Helleli et al. (2012) reported 69.9% and 66.3% seropositivity of MG and MS in chicken at Batna commercial farms in Algeria. Nouzha *et al*. (2013) reported 61.1% seropositivity of MG in chicken at Batna Governorate, Algeria. Shadmanesh and Mokhtari (2013) reported 85% seropositivity of MG in native hens of Eghlid, Iran. Silva et al. (2015) reported 4.3% and 7.1% seropositivity of MG and MS in chicken from State of Minas Gerais, Brazil. Shoaib et al. (2020) reported 45.9% and 40.4% seropositivity of MG and MS in chicken from the poultry farms located in the different regions of Rawalpindi, Pakistan. The differences in seropositivity of MG and MS in different studies may be due to difference in health status of birds, season of studies and different geographical areas. The present study reported variation in serodetection of MG and MS antibodies from different districts. It might be due to difference in managemental practices (biosecurity measures in the poultry flocks, degree of infection in the feed, water and litter) and the chicken rearing system (Xavier et al., 2011; Messa Júnior et al., 2017). The lack of effective biosecurity measures, such as the low use of footbaths and inadequate litter management, contributes to the spread of avian respiratory infections (Kadja et al., 2024). To prevent the entry of pathogen and maintaining poultry flocks free from avian mycoplasmas, it is required to purchase fertile eggs and chicks from *Mycoplasma* free source, followed by rearing the birds with adequate hygiene and biosecurity measures (Hong et al., 2004). The variation in seroprevalence of MG and MS in different districts may also be due to flock density, as it can exacerbate the spread of avian mycoplasmas, leading to increased morbidity and mortality rates. Trade practices, such as the use of mycoplasma-free breeding stock and strict biosecurity measures, are essential to prevent the transmission of the disease and maintain disease free flock (Malik et al., 2022). Additionally, the high rate of vaccination failures underscores the urgent need for improved vaccine quality and administration techniques.

The SPAT may sometimes give false positive reaction due to cross reactions between closely related *Mycoplasma* species (MG, MS, and *Mycoplasma imitans*), nonspecific reactions and use of inactivated vaccines (Osman et al., 2009; Luciano et al., 2011). To overcome these problems, the test sera had been inactivated by heating at 56°C for 30 min and nonspecific reactions tried to reduce by diluting the test sera (Xavier et al., 2011). None of the poultry flocks had been vaccinated against MG and MS. It is seen that a single test is not appropriate for accurate diagnosis of MG and MS in poultry farms (Feberwee et al., 2005). Considering these, we have also tested the same serum samples for detection of MG and MS antibodies using iELISA and DOT ELISA. In iELISA and DOT ELISA, higher seropositivity of MG and MS antibodies were detected as compared to SPAT. Higher seropositivity of MG and MS in ELISA based assays may be due to these techniques are more sensitive and specific as compared to conventional serological tests, such as SPAT (Wanasawaeng et al., 2015; Muhammad et al., 2018). Also, these tests can be used to screen a large number of sera with accuracy and having higher reliability (Miao et al., 2000). The present study has certain limitations. The sample sizes across districts are highly

unequal (ranges from 7 - 118 samples). Also, the samples were collected from slaughtered birds at local meat shops, which may not truly represent live commercial flocks. In future it is recommended to design a systematic sample collection strategy to reveals true prevalence of MG and MS in different regions of Haryana, India.

**Conclusions**

The present serological study reveals that MG and MS infection is prevalent in broiler chickens slaughtered at local meat shops of selected districts of Haryana, India. Among the targeted districts, the serodominance of MG and MS antibodies were reported from Hisar and Jind districts, respectively. To prevent the endemicity of infection, the broiler chicken flocks should be checked periodically to determine the status of *Mycoplasma* infection. The seropositive birds should be culled to prevent further spread of infection in the flock. Effort need be taken to educate the poultry farmers about sign and symptoms of MG and MS infections in birds and actions need to be taken for effective control through appropriate prophylactic or therapeutic measures. Vaccination should also be followed in breeding stock to reduce transmission of infection through egg.

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**Declaration of competing interest**

The authors have declared that there is no conflict of interest.

**Data availability statement**

All the data generated are included in the manuscript and tables.

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