

SEROLOGICAL INVESTIGATION OF MYCOPLASMA GALLISEPTICUM IN BROILER FARMS OF TABANAN REGENCY USING ELISA**Investigasi Serologis *Mycoplasma gallisepticum* Pada Peternakan Ayam Broiler di Kabupaten Tabanan Menggunakan ELISA**

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How to cite: Utami YM, Sudipa PH, Besung INK, Suarjana IGK, Mahatmi H, Lefira, Indrayudha PKP, Siahaan GYA, Merling ICS. 2025. Serological investigation of *Mycoplasma gallisepticum* in broiler farms of Tabanan Regency using ELISA. *Bul. Vet. Udayana*. 17(3): 949-956. DOI: <https://doi.org/10.24843/bulvet.2025.v17.i03.p41>

Abstract

Mycoplasma gallisepticum, the primary causative agent of Chronic Respiratory Disease (CRD), poses significant economic losses to the poultry industry through respiratory distress, decreased productivity, and elevated mortality in broilers. This study evaluated *Mycoplasma gallisepticum* antibody titers in broiler farms across Tabanan Regency, Bali, using the Enzyme-Linked Immunosorbent Assay (ELISA). Fifty serum samples were collected from five clinically healthy broiler farms (no observed respiratory signs) and analyzed with the IDEXX MG/MS Antibody Test ELISA kit. All unvaccinated broilers exhibited low detectable antibody titers, classified as non-reactive (<843). These results contrast with prior reports of high *M. gallisepticum* prevalence in Bali based on pathological findings. Potential explanations for this discrepancy include variations in chicken age, farm management, biosecurity protocols, sampling timing, and diagnostic methodologies. Our findings provide updated epidemiological data on *Mycoplasma gallisepticum* in Tabanan and highlight the necessity of regular serological surveillance to guide disease prevention strategies.

Keywords: Serology, ELISA, *Mycoplasma gallisepticum*, Chronic Respiratory Disease

Abstrak

Mycoplasma gallisepticum, agen penyebab utama Chronic Respiratory Disease (CRD), menimbulkan kerugian ekonomi signifikan pada industri perunggasan melalui gangguan pernapasan, penurunan produktivitas, dan peningkatan mortalitas pada ayam broiler. Penelitian ini mengevaluasi titer antibodi *Mycoplasma gallisepticum* di peternakan broiler di Kabupaten Tabanan, Bali, menggunakan metode Enzyme-Linked Immunosorbent Assay (ELISA). Sebanyak 50 sampel serum dikumpulkan dari lima peternakan broiler yang secara klinis sehat

(tanpa gejala pernapasan) dan dianalisis dengan kit uji ELISA IDEXX MG/MS Antibody Test. Semua ayam broiler yang tidak divaksinasi menunjukkan titer antibodi rendah yang terdeteksi, diklasifikasikan sebagai non-reaktif (<843). Hasil ini bertolak belakang dengan laporan sebelumnya yang mendokumentasikan prevalensi tinggi infeksi *Mycoplasma gallisepticum* di peternakan unggas Bali berdasarkan temuan patologis. Perbedaan ini dapat dijelaskan oleh variasi umur ayam, manajemen peternakan, protokol biosekuriti, waktu pengambilan sampel, dan perbedaan metode diagnostik. Temuan kami memberikan data epidemiologi terbaru tentang *M. gallisepticum* di Tabanan serta menegaskan pentingnya surveilans serologis rutin untuk mendukung strategi pencegahan dan pengendalian penyakit.

Kata Kunci: Serologi, ELISA, *Mycoplasma gallisepticum*, Chronic Respiratory Disease

INTRODUCTION

According to data from the Central Statistics Agency of Indonesia (2024), the Directorate General of Animal Husbandry and Animal Health reported that the population of broiler chickens in Bali Province was 68.720.589 in 2021, increasing to 72.373.629 in 2022. This indicates positive growth in the poultry industry in Bali, which is successfully meeting the demand for animal-based food. Poultry, particularly broiler chickens, plays a vital role in the economy and food security of Tabanan Regency. The production of healthy poultry contributes to the well-being of farmers and ensures the availability of poultry products for the local population.

One of the health challenges in the poultry industry is Chronic Respiratory Disease (CRD) caused by the bacterium *Mycoplasma gallisepticum* (MG), which affects the respiratory system of poultry (Annisa et al., 2024). Infection of MG can cause substantial economic losses due to high morbidity and mortality rates, reduced egg production, decreased hatchability, and decreased feed efficiency, ultimately hindering the weight gain of poultry (Karthik et al., 2018; Bharathi, Ravikumar, & Jothilakshmi, 2022).

Diagnosing MG infection in poultry farms based solely on clinical symptoms is challenging (Ali, Rahman, & Sultana, 2015). One approach to detecting MG infections is through serological testing (Widianingrum et al., 2022). Commonly used serological tests include Serum Plate Agglutination (SPA), Enzyme-Linked Immunosorbent Assay (ELISA), and Hemagglutination Inhibition (HI) tests (WOAH, 2021). ELISA is a rapid serological test used to detect and measure antibody titers, offering good sensitivity and specificity (Elyazee et al., 2020).

The poultry industry in Bali Province is distributed across all regencies and cities, with Tabanan Regency hosting the largest poultry population, accounting for 51.79%. As a result, Tabanan has become the primary poultry production centre in the province (Kurniawan et al., 2013) (Rastana et al., 2023). Previous reports on MG infection in broiler chickens in Bali have indicated a 43% prevalence based on anatomical pathology changes, with Tabanan experiencing a notably higher prevalence of 83.33% (Prasetyo, Rudyanto, & Berata, 2014). However, the available data is outdated, and no recent information on the seroprevalence of MG in Tabanan is available. Therefore, this study is essential to fill the data gap and offer an updated epidemiological perspective on MG infection in the region. Seroprevalence studies aim to assess the spread of MG in a region through antibody detection in poultry serum. Seroprevalence data serves as a basis for decision-making in disease prevention and control in poultry farms.

RESEARCH METHODS

Approval of Ethical Commission

Before conducting the study, ethical approval was granted by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Udayana University, under the reference number B/44/UN14.2.9/PT.01.04/2024.

Study period and location

Sampling was carried out in August-September 2024. Serum samples were processed at the Bacteriology and Mycology laboratory, Faculty of Veterinary Medicine, Udayana University.

Samples

This study evaluated 50 chicken bloods sample from Tabanan regency (n=50). Blood samples from chickens were collected via the brachial vein using a 3 mL syringe and left at room temperature ($\pm 25^{\circ}\text{C}$) for one to two hours until serum separation. The serum was then transferred into serum tubes, placed in a cooler box with ice packs, and transported to the laboratory (Diyantoro, Wibawan, & Pribadi, 2017). The serum was subsequently heated in a water bath at 56°C for 30 minutes prior to ELISA testing (WOAH, 2021). In this study, serum samples were randomly collected, with data on the poultry houses shown in Table 1. Serum samples were processed at the Microbiology Laboratory, Faculty of Veterinary Medicine, Udayana University.

Serological test

The serological test was conducted according to the ID.vet (ID Screen *M. gallisepticum* Indirect) test kit manufacturer's guidelines for the Enzyme-Linked Immunosorbent Assay (ELISA) to detect MG. Serum samples, diluted in the provided solvent, were applied to ELISA plates pre-coated with MG antigen. The plates were incubated, washed, and then treated with conjugated antibodies. After further incubation, the plates were washed again, and a substrate was added. Once the reaction occurred, a stop solution was applied to halt the reaction. The ELISA plates were then read using an ELISA reader, and the optical density (OD) values of the negative control, positive control, and samples were recorded and interpreted as per the manufacturer's instructions. Absorbance was measured at 450 nm, and samples were classified as reactive (S/P ratio > 0.5 ; Titter > 843) or non-reactive (S/P ratio < 0.5 ; titter < 843) based on the ELISA threshold.

Data Analysis

Statistical data, such as the mean, standard error (SE), maximum, and minimum values, were processed using IBM SPSS Statistics Data Editor. Prevalence was calculated using the following formula:

$$\text{Prevalensi (\%)} = \frac{\text{Sampel Positif}}{\text{total sampel}} \times 100$$

RESULTS AND DISCUSSION

Results

The OD readings and data analysis indicated that all poultry houses exhibited low S/P values. The serological test results showed that all samples had titers below 843 (non-reactive), with the highest titer observed in Farm C (750.064) and the lowest in Farm B (6.718) (Table 2). The titer calculations revealed that the average antibody titers across all chickens were relatively low. The seroprevalence of MG infection was found to be 0% (Table 3).

Discussion

Mycoplasma gallisepticum is the most virulent species in the *Mycoplasma* genus of the *Mycoplasmataceae* family, leading to substantial economic losses (Prabhu et al., 2021). *Mycoplasma* can be easily transmitted through human clothing and footwear, with transovarial and aerosol routes serving as primary modes of transmission (Greenacre & Morishita, 2021). Infected broiler chickens exhibit clinical signs such as ruffled feathers, reduced feed and water intake, depression, sneezing, coughing, diarrhoea, 3-20% mortality, severe respiratory symptoms (nasal and ocular discharge, laboured breathing), and unilateral or bilateral infraorbital sinus swelling (Al-baqir et al., 2023; WOA, 2021). Postmortem lesions include fibrinous perihepatitis, fibrinous airsacculitis, and caseous exudates in the infraorbital sinus (Bharathi et al., 2018). Diagnosis of MG infection can be performed serologically through Rapid Serum Agglutination (RSA), Hemagglutination Inhibition (HI), and Enzyme-Linked Immunosorbent Assay (ELISA), or molecularly by Polymerase Chain Reaction (PCR) (Amorim et al., 2024; Abdelrahman et al., 2021). The differential diagnosis of MG infection includes Newcastle Disease, Infectious Bronchitis, Infectious Laryngotracheitis, Avian Metapneumovirus and Colibacillosis, requiring the identification of specific agents through serological procedures to distinguish MG from other pathogenic microorganisms in chickens (Swayne et al., 2020; Habte et al., 2022).

This study aimed to investigate the seroprevalence of MG infection in broiler farms in Tabanan Regency using the ELISA serological test. A total of 50 serum samples were collected from 5 broiler farms (Table 1). ELISA was selected due to its high accuracy in detecting MG in poultry populations with low prevalence or low antibody levels. Indirect ELISA was used to assess the seroprevalence of MG in broilers that were not vaccinated with MG vaccine, which should ideally be zero (Bari & Shareef, 2023), but the results showed that all the sample collected samples tested (n=50) had low antibody titers (<843). The titer values were obtained from the optical density (OD) readings using an ELISA reader. By determining the OD values, the S/P ratio and titer values could be calculated. The OD readings indicated that all cages exhibited low S/P values (Table 2). The titer detected in unvaccinated chickens is the maternal antibody titer and provided relatively protection against the challenge (Leigh, Collier, Branton, & Evans, 2019). The highest average titer was observed in cage C, with a titer of 242.025 ± 235.850 . The serological testing results indicated that all samples had a titer <843 (non-reactive), with the highest titer in farm C (750.064) and the lowest in farm B (6.718) (Table 2). Titer calculations revealed that the average titer across all chickens was relatively low. The seroprevalence of MG infection was found to be 0% (Table 3). This result contrasts with previous studies, where anatomical changes detected in Bali Province showed 43% seroprevalence (Widianingrum et al., 2022). Other studies using pathological anatomy diagnosis reported suspected CRD infections at 83.33% in Tabanan, 23.33% in Bangli, 100% in Karangasem, and 30% in Jembrana (Prasetyo et al., 2014). The differences between these findings may be due to variations in sample size, chicken age and breed, management systems, farm sanitation, and agro-ecological location (Shiferaw et al., 2022). Variations in diagnostic methods also contribute to differing results, as ELISA detects active infection, whereas pathological anatomy detects latent or long-term effects. Negative antibody titers measured by ELISA do not necessarily mean that the poultry are free of MG, as this may be attributed to antigen virulence (Manalu, Tarigan, & Hutagaol, 2021). Low antigen virulence may fail to trigger apoptosis or an inflammatory response, which can facilitate persistent infections as a bacterial defence mechanism (Abdelsalam et al., 2020; Hoelzle, Ade, & Hoelzle, 2020; Tseng et al., 2013).

The low prevalence and titers observed in broiler chickens in Tabanan Regency may be influenced by factors such as chicken type and age. MG is more commonly found in layer farms (Diyantoro et al., 2017). Older chickens, high stocking densities, and poor farm hygiene contribute to higher infection rates (Hayati et al., 2015). In non-vaccinated broilers, which have a shorter lifespan, the average titer found in all samples was less than 834 (Table 2). Additionally, sample collection timing may have played a role, as the samples were collected during the summer, whereas MG infections typically peak in the rainy and colder seasons (Ali et al., 2015). The low prevalence and titers in Tabanan may thus result from various risk factors, including sample timing, chicken type, and age.

CONCLUSION AND RECOMMENDATIONS

Conclusion

This study found that all broiler chickens in Tabanan Regency, which had not been vaccinated against *M. gallisepticum*, exhibited detectable antibody titers at low levels and were classified as non-reactive based on ELISA testing. The absence of high antibody titers suggests no active infection in the tested population. Differences compared to previous studies may be influenced by factors such as diagnostic methods, farm management practices, biosecurity measures, chicken age, and sample collection timing. These findings highlight the importance of routine serological monitoring to assess poultry health status and support effective disease prevention strategies in the region.

Recommendations

Further research is recommended with an increased number of chicken samples, taking into consideration factors such as breed, age, housing conditions, clinical symptoms, and the timing of sample collection.

ACKNOWLEDGMENT

The authors would like to acknowledge Laboratory of Veterinary Bacteriology and Mycology, Veterinary Medicine of Udayana University for providing research facilities.

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Table

Table 1. Blood sample data from poultry farms in Tabanan Regency.

Farm	Chicken type	Age stage	Clinical symptoms*)	MG Vaccination	Number of serum samples
A	Broiler	Finisher	None	No	10
B	Broiler	Finisher	None	No	10
C	Broiler	Finisher	None	No	10
D	Broiler	Finisher	None	No	10
E	Broiler	Finisher	None	No	10

Total	50
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*) Clinical symptoms in the respiratory system related to *M. gallisepticum* infection.

Table 2. Descriptive Data of Serum Antibody Titters Against *M. gallisepticum*.

Samples	OD±SD	S/P Value±SD	Result	Minimum Titter	Maximum Titter
Positive Control ^{*)}	0,036	-	Positive	-	-
Negative Control ^{*)}	0,441	-	Negative	-	-
Farm A	0.040±0.030	0.010±0.074	Negative	18.256	240.494
Farm B	0.040±0.040	0.009±0.087	Negative	6.718	324.983
Farm C	0.064±0.074	0.070±0.183	Negative	44.568	750.064
Farm D	0.007±0.009	0.071±0.021	Negative	64.457	175.167
Farm E	0.010±0.008	0.063±0.021	Negative	59.55	175.167

*) Control form the ID.vet (ID Screen *M. gallisepticum* Indirect) test kit.

Table 3. Seroprevalence, Antibody titters of *M. gallisepticum*, and the coefficient of variation (CV) percentage in the evaluated broiler farm.

Farm	S/P Value						Titter Mean ± SD	All Titters >843	CV %
	Reactive			Non-Reactive					
	No	%	Total Prevalence	No	%	Total Prevalence			
A	0	0		10	100		122.199±72.759	-	59.54
B	0	0		10	100		115.870±113.86	-	98.27
C	0	0	0%	10	100	100%	242.025±235.85	-	97.45
D	0	0		10	100		141.997±39.361	-	27.72
E	0	0		10	100		127.884±38.865	-	30.39