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(Research Article)

PCR-based Detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in Philippine Broiler Poultry Flocks

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Abstract

Background: Two major bacterial poultry pathogens that cause chronic respiratory disease and infectious synovitis are *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS). To date, no nationwide MG and MS molecular detection data are available in the Philippines. This study aimed to detect MG and MS in broiler poultry flocks. This study aimed to detect the presence of MG and MS in Philippine broiler poultry flocks. **Methods:** From 2020 to 2023, 2,127 broiler flocks with clinical or subclinical respiratory signs were purposively sampled from 13 Philippine regions. Oropharyngeal swabs and selected tissues were screened by conventional PCR targeting the *mgc2* (cytadhesin protein gene) and *16S rRNA* genes of MG and MS, respectively. Positivity estimates with 95% confidence intervals were calculated, and differences were assessed using the chi-square test. **Results:** PCR amplicons yielded 302-bp (MG) and 211-bp (MS) products. Overall, MG and MS were detected in 13.31% (283/2,127) and 7.33%

(156/2,127) of flocks, respectively. Higher detection levels were observed in CAR, Regions IVA, VII, and III. Regional differences in MG and MS detection were statistically significant ($p < 0.001$). **Conclusion:** This study confirmed the molecular detection of MG and MS among clinically affected broiler poultry flocks in the Philippines. Findings reflect detection in sampled flocks and not a population-level prevalence due to purposive sampling. Given the limitations of this PCR-based design, future surveillance should consider rapid, field-adapted, and strain-discriminative approaches, including advanced AI- or machine learning-assisted LAMP assays, to improve pathogen differentiation and control.

Keywords

Mycoplasma gallisepticum, Mycoplasma synoviae, PCR, Philippine poultry

1. Introduction

Two major economically significant bacterial respiratory pathogens that cause chronic respiratory disease (CRD) and infectious synovitis are *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS), respectively. These organisms are known to cause major economic losses by reducing egg production, decreasing feed conversion efficiency, increasing embryo mortality, and increasing carcass condemnations [1,2,3].

Variable respiratory lesions, including airsacculitis, sinusitis, and tracheitis, can be seen with MG and MS infections. It has been reported that concurrent infections with bacterial *Escherichia coli* and *Avibacterium paragallinarum,* and viral infections with infectious bronchitis, are associated with severe clinical outcomes [1,4,5]. Although commonly reported to cause less severe respiratory conditions, infection with MS often progresses to synovitis. This results in joint swelling and clinical lameness, and, with its chronic progression, transmission between and within poultry farms becomes evident [3,6].

Poultry mycoplasma infection continues to be a challenging problem despite the availability of vaccines and efforts to improve farm biosecurity and management. Disease expressions may vary depending on surveillance, production systems, and geographical settings. Prevalence estimates ranging from 7% to 70% have been reported in continental Asia, Europe, and Africa. High prevalence levels are most observed in areas with intensive poultry farming [2,6,7]. Of particular importance in interpreting prevalence data are the intensity of sampling and the diagnostic procedures or tools used. A study conducted in Myanmar, which detected concurrent infections with MG and MS, has demonstrated that these pathogens can indeed co-circulate on poultry farms [2]. In contrast, lower infection prevalence was observed in poultry and wild birds in Belgium [7]. Such findings suggested that migratory birds may be responsible for transmissions of poultry Mycoplasma infections.

In the Philippines, there is limited information on poultry mycoplasma infections. Most studies used serologic rather than molecular methods. An early local study in the country on MG has confirmed the presence of chronic respiratory disease (CRD) using cultural and serologic methods [8]. A recent study involving broilers in South Luzon detected MG antibodies using hemagglutination-inhibition and enzyme-linked immunosorbent assay [9]. Obviously, no large-scale molecular-based detection data is available in the country.

In detecting MG and MS, the PCR method is considered the gold standard for obtaining reliable diagnostic and surveillance data [2,5,7,10]. This is understandable given its rapid, sensitive, and specific outcomes compared with traditional isolation and the common reliance on markers of immune responses. The *mgc2* gene, encoding a cytadhesin involved in attachment to host epithelial cells, is a well-established molecular target for MG detection, while the *16S rRNA* gene provides a reliable marker for MS identification [2,5,6].

With the continually expanding broiler poultry industry in the Philippines, which is a major contributor to national animal protein supply and commercial livestock production, and the growing focus on One Health initiatives, it is imperative that molecular data on Mycoplasma infections in the country be established. This study determined the molecular positivity detection of MG and MS across major poultry-producing regions, which constituted the first large-scale PCR-based screening of Mycoplasma infections

nationwide. This was done to improve diagnostic innovation beyond the usual levels for Philippine poultry flocks [11,12].

2. Materials and Methods

2.1 Ethical Consideration

All procedures for sample collection were approved by the Institutional Animal Care and Use Committee (IACUC) of each collaborating institution of the University of the Philippines, Los Baños - College of Veterinary Medicine (Protocol No. 2019-0027); the Cavite State University (with IACUC Exemption Certification); and the University of Eastern Philippines – College of Veterinary Medicine (Protocol No. 2020-001). Informed consent was obtained prior to sampling from poultry operators/owners. An official letter from the Director of the Bureau of Animal Industry, encouraging poultry farm operators/owners to participate in the study, was made available prior to the actual field sampling.

2.2 Sample Collection

A total of 2,127 poultry flocks from 13 Philippine regions (Fig. 1) sampled between 2020-2023 were included in this study. Each flock represented a farm unit with 25 sampled birds. Within each selected flock, birds were purposively sampled, prioritizing individuals exhibiting respiratory or other clinical signs consistent with MG/MS infection, with additional birds sampled from the same housing unit to achieve a minimum of 25 birds per flock. Overall, this corresponded to a total of 53,175 birds sampled across all flocks included in the study. Flock-level classification was based on pooled PCR results, with a flock considered positive if the pooled sample tested positive; individual bird infection status could not be determined due to pooling. No Mycoplasma vaccination was practiced in the broiler flocks included in this study. Sampling was conducted only with the consent and cooperation of poultry operators/owners.

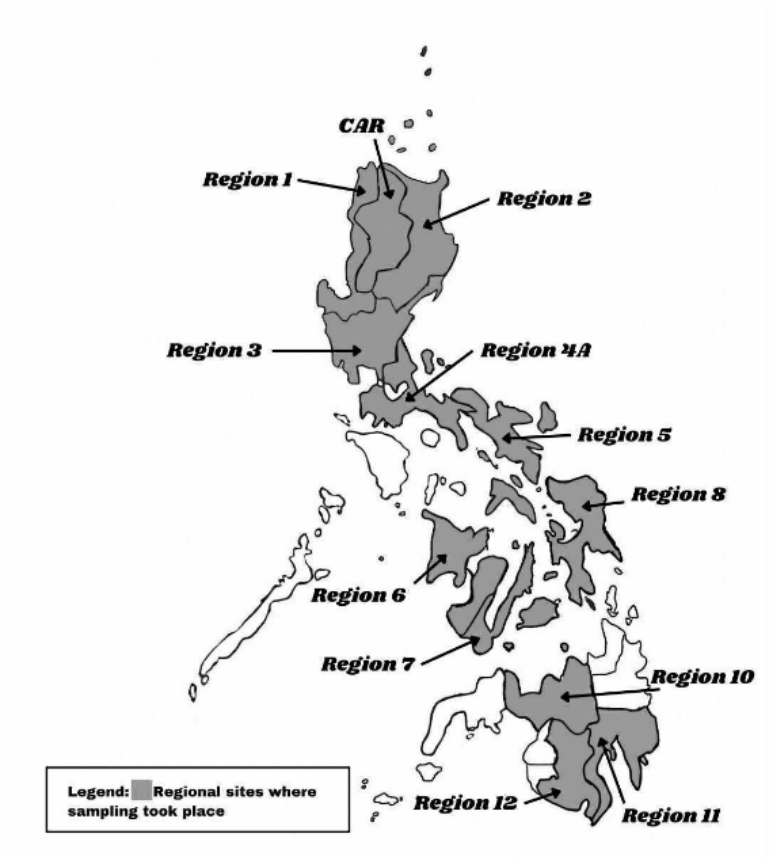

Figure 1. Map of the Philippines showing sampling sites from various poultry farms.

2.3 Sampling Methods

Oropharyngeal swabs were obtained using sterile cotton applicators following the standard procedures [10]. Each swab tip was placed in isotonic phosphate-buffered saline (PBS) containing penicillin (2,000 U/mL), streptomycin (2 mg/mL), gentamicin (50 µg/mL), and mycostatin (1,000 U/mL), transported at 4°C, and subsequently stored at −20 °C until DNA extraction. For each flock, tissue samples from the trachea, lungs, and air sacs were collected from selected birds suspected of MG/MS infection, pooled at the flock level, placed in airtight sterile containers, and stored at −20 °C until analysis.

2.4 DNA Extraction

Approximately 5–10 g of homogenized pooled tissue and swab samples were mixed with 30% normal saline solution containing penicillin (10,000 U/mL) and streptomycin (10 mg/mL). The mixture was centrifuged at 6,000 rpm for 10 min, and the resulting supernatant was stored at −80 °C. DNA extraction was performed using the QIAamp® DNA Mini Kit (Qiagen, West Sussex, UK) according to the manufacturer's protocol.

2.5 Polymerase Chain Reaction (PCR)

Conventional PCR assays were performed to amplify species-specific gene targets for MG (*mgc2*) and MS (*16S rRNA*) using the primer sets listed in Table 1. The *mgc2* gene encodes a cytadhesin protein involved in adhesion to the host respiratory epithelium and contains conserved regions that enable reliable species-specific detection of MG. In contrast, the *16S rRNA* gene region provides highly conserved sequences commonly used for bacterial species-level identification. These loci were selected according to the protocol for PCR detection of poultry Mycoplasma [2,15,16]. Positive control DNA was derived from commercially available live attenuated poultry vaccines used as positive controls for MG and MS PCR assays. A no-template control was included as a negative control in each run. The positive controls consistently produced amplicons of the expected sizes (302 bp for *mgc2* and 211 bp for *16S rRNA*), while the negative controls showed no amplification. [2,13,14,15].

PCR reactions were prepared in 25 µL total volume containing 2.5 µL 10× PCR buffer, 2 µL $MgCl_2$ (50 mM), 0.2 µL dNTPs (10 mM each), 0.1 µL of each primer (10 µM), 0.1 µL Taq DNA polymerase (5 U/µL), 19 µL nuclease-free water, and 1 µL of extracted DNA template. Amplification was performed using an ABI SimpliAmp™ Thermal Cycler (Applied Biosystems, Thermo Scientific) under the following thermocycling conditions: initial denaturation at 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s, 60 °C for 10 s, and 72 °C for 10 s, with a final extension step at 72 °C for 5 min.

2.6 Analysis of Data

The proportion of poultry flocks that tested positive for MG and MS by PCR was determined for each region and overall. The PCR-detected

Table 1. Primer sequences used for PCR detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae.*

Target species	Gene target	Primer name	Sequence (5′–3′)	Target Amplicon	References
M. gallisepticum	*mgc2*	mgc2-F	CGCAATTTGGTCCTA ATCCCCAAC	302 bp	[10, 13, 14]
		mgc2-R	TAAACCCACCTCCAG CTTTATTTC		
M. synoviae	16S rRNA	MSL1 (F)	GAGAAGCAAAATAGT GATATCA	211 bp	[10, 15]
		MSL2 (R)	CAGTCGTCTCCGAAG TTAACAA		

positivity rate for each pathogen was computed using the formula [10,16]:

$$\text{PCR Positivity Rate (\%)} = \frac{\text{Number of PCR-positive flocks}}{\text{Total number of flocks tested}} \times 100$$

Ninety-five percent confidence intervals (95% CI) for flock-level positivity estimates were calculated using Wilson's score method for binomial proportions [17]. All analyses treated the flock as the epidemiological unit. Results are presented in tables and figures to summarize spatial variation in MG and MS molecular detection across regions.

Differences in PCR-detected flock-level positivity between pathogens (MG vs. MS) and across regions were evaluated using the chi-square test of independence. Flocks were considered the unit of analysis. Statistical significance was set at $\alpha = 0.05$, and exact p-values are reported.

3. Results

3.1 PCR-Detection of Mycoplasma in Philippine Poultry

PCR-detected amplicons of MG and MS using gel-electrophoresis are presented in Fig. 2 and 3. The figure shows the distinct presence of the target

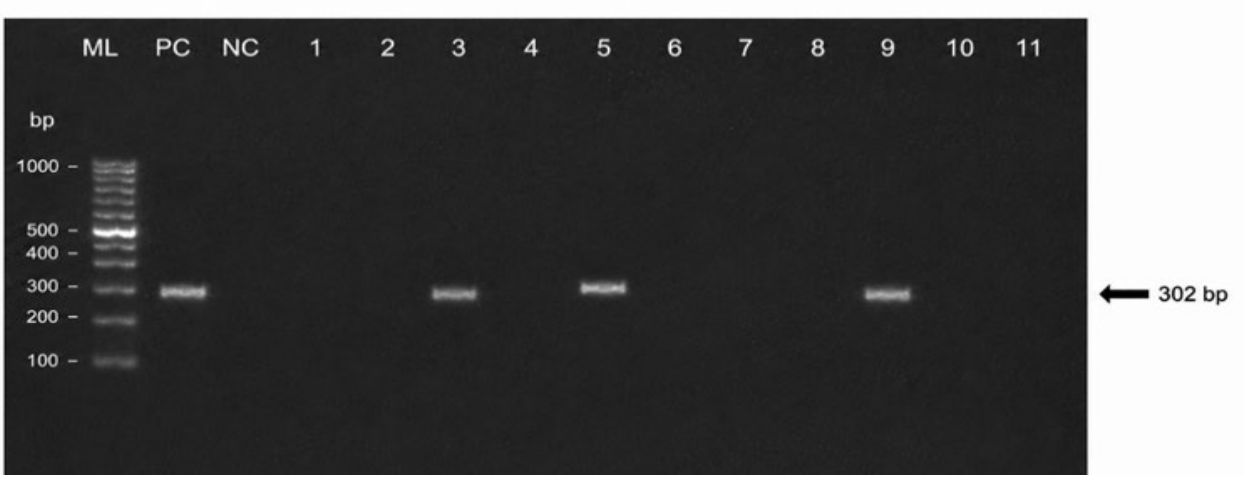

Figure 2. Electrophoretogram of PCR-amplified products of the mgc2 gene of *Mycoplasma gallisepticum* showing a 302 bp product; Lane ML: 100 bp DNA Ladder; lane PC: positive control (*M. gallisepticum* vaccine strain); lane NC: negative control; lanes 1-11: isolates from Luzon, Philippine commercial broiler farms.

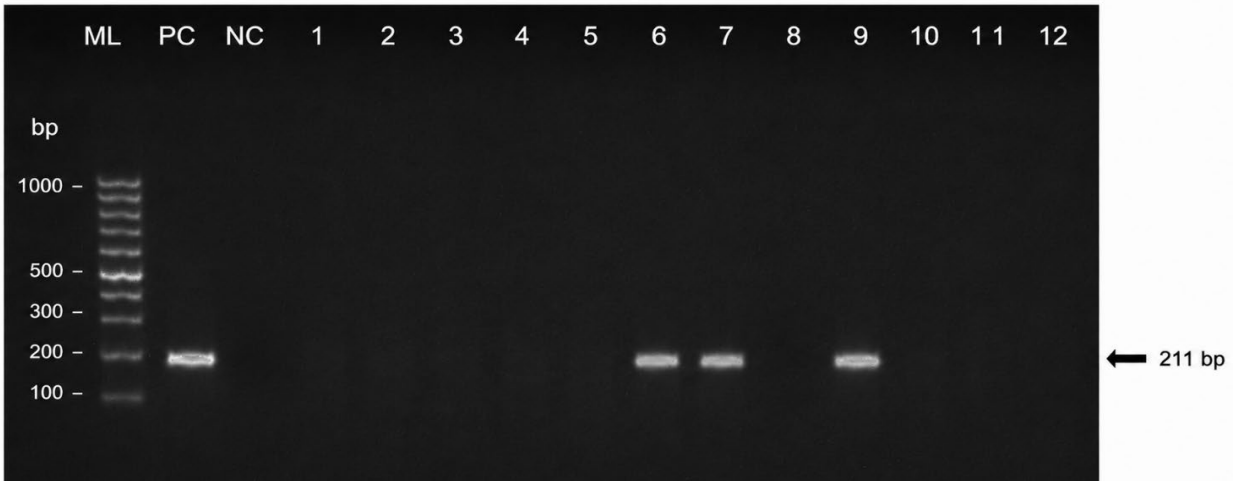

Figure 3. Electrophoretogram of PCR-amplified products of the 16S rRNA gene of *Mycoplasma synoviae* showing a 211 bp product; Lane ML: 100 bp DNA ladder; lane PC: positive control *(M. synoviae* vaccine strain); lane NC: negative control; lanes 1-12: isolates from Luzon, Philippine commercial broiler farms.

MG organisms using polymerase chain reaction (PCR) of the cytadhesin-encoding *mgc2 gene* (Fig. 2), which produced 302 bp products, and the *16S rRNA* genes of MS, producing 211 bp products (Fig. 3).

3.2 PCR-Based Detection of MG and MS in Philippine Broiler Poultry

Poultry broiler farms in 13 geographic regions demonstrated the presence of MG and MS in varying proportions from 2020 to 2023 (Table 2). Nationally, MG was detected in 13.31% of flocks (95% CI: 11.93–14.81), whereas MS was detected in 7.33% (95% CI: 6.30–8.52).

MG positivity varied across regions, ranging from 0% to 17.65%. Higher detection rates were observed in CAR (17.65%; 95% CI: 6.19–41.03), Region IVA (17.55%; 95% CI: 15.21–20.16), and Region VII (16.67%; 95% CI: 5.84–39.22). Intermediate levels were observed in Regions VI (14.29%; 95% CI: 7.95–24.34), Region X (14.29%; 95% CI: 2.57–51.31), Region XII (12.50%; 95% CI: 4.97–28.07), and Region II (12.12%; 95% CI: 4.82–27.33). Lower detection rates were observed in Region III (10.81%; 95% CI: 8.91–13.07), Region VIII (10.00%; 95% CI: 2.79–30.10), Region I (3.33%; 95% CI: 0.59–16.67), and Region XI (2.36%; 95% CI: 0.81–6.72), while no MG-positive flocks were detected in Region V. These regional differences were statistically significant based on Chi-square analysis ($\chi^2 = 36.39$, df = 11, $p < 0.001$).

MS detection ranged from 0% to 17.65% across regions. Higher MS detection rates were

Table 2. PCR-detected positivity rates of *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) among poultry flocks in the Philippines (2020–2023).

Region	Total flocks examined	MG Positive (n)	% MG Positive (95% CI)	MS Positive (n)	% MS Positive (95% CI)
CAR	17	3	17.65 (6.19–41.03)	3	17.65 (6.19–41.03)
Region IVA	906	159	17.55 (15.21–20.16)	94	10.38 (8.55–12.53)
Region VII	18	3	16.67 (5.84–39.22)	0	0.00 (0.00–17.59)
Region VI	70	10	14.29 (7.95–24.34)	1	1.43 (0.25–7.66)
Region X	7	1	14.29 (2.57–51.31)	0	0.00 (0.00–35.43)
Region XII	32	4	12.50 (4.97–28.07)	0	0.00 (0.00–10.72)
Region II	33	4	12.12 (4.82–27.33)	0	0.00 (0.00–10.43)
Region III	860	93	10.81 (8.91–13.07)	54	6.28 (4.84–8.10)
Region VIII	20	2	10.00 (2.79–30.10)	1	5.00 (0.89–23.61)
Region I	30	1	3.33 (0.59–16.67)	0	0.00 (0.00–11.35)
Region XI	127	3	2.36 (0.81–6.72)	3	2.36 (0.81–6.72)
Region V	7	0	0.00 (0.00–35.43)	0	0.00 (0.00–35.43)
TOTAL	**2,127**	**283**	**13.31 (11.93–14.81)**	**156**	**7.33 (6.30–8.52)**

observed in CAR (17.65%; 95% CI: 6.19–41.03) and Region IVA (10.38%; 95% CI: 8.55–12.53). Intermediate levels were noted in Region III (6.28%; 95% CI: 4.84–8.10) and Region VIII (5.00%; 95% CI: 0.89–23.61), whereas the remaining regions showed low to zero detection. Regional differences in MS detection were also statistically significant ($\chi^2 = 34.82$, df = 11, $p < 0.001$).

MS was detected in 7.33% (156/2,127) of flocks, whereas MG was detected in 13.31% (283/2,127). MS-positive flocks represented 55.12% of MG-positive flocks at the population level. MS detection occurred only in regions where MG was also identified.

4. Discussion

4.1 PCR Detection Targets

The existing MG and MS detection protocol uses the target genes *mgc2* for MG and *16S rRNA* for MS as PCR targets [10,13,14]. Extensively used in both conventional and advanced PCR protocols, the *mgc2* gene, a conserved region that encodes the cytadhesin protein, is essential for respiratory epithelial attachment [13,14]. High-throughput MS identification in diverse clinical samples targets the *16S rRNA* locus, a highly conserved ribosomal region that contains species-variable sequences, enabling accurate, high-throughput identification [15]. In this study, detected bands were interpreted based on the expected amplicon sizes, species-specific PCR targets, and appropriate positive and negative controls. Although minor differences in band migration may occur during gel electrophoresis, as observed in lane 5 of Figure 2, the positive bands remained within the expected target region. Sequencing of PCR-positive amplicons was not included in the present study as the primary objective was large-scale molecular surveillance rather than strain characterization. Species-specific, internationally validated PCR targets and appropriate positive and negative controls were used to ensure detection specificity. Future studies will incorporate sequencing and strain-level analyses.

While both targets are recommended [10] for confirming avian mycoplasmosis, it is noteworthy that neither gene could offer an absolute "*Differentiating Infected from Vaccinated Animals*" (DIVA) capability. In commercial broiler production in the Philippines, MG and MS vaccination is not routinely practiced; therefore, vaccine-derived detection is unlikely in the broiler flocks included in this study. However, in layer operations where vaccination may occur, conventional PCR assays lack DIVA capability and cannot distinguish between field and vaccine strains. Hence, vaccination history should be considered when interpreting PCR-positive results. Moreover, since this study focused on purposive sampling of birds exhibiting clinical signs of MG/MS, it can be inferred that the birds detected as positive may have been suffering from active MG/MS infections. Future studies should incorporate detailed production and health management data to strengthen epidemiological interpretation.

With advances in molecular diagnostics, LAMP-based platforms are increasingly being explored as rapid and field-adapted tools to support pathogen detection. Previous LAMP simulation and machine learning studies reported modified F1-scores ranging from 71.19% to 72.10%, with specificity reaching 85.45% at optimized reaction conditions [11,12]. These findings suggest that advanced AI- or machine learning-assisted LAMP approaches may complement conventional PCR by improving primer evaluation, amplification prediction, and pathogen differentiation. However, these methods should be further validated for MG and MS detection before routine diagnostic application.

4.2 Nationwide Detection of MG and MS

The current overall molecular detection of MG/MS (13.3% and 7.3%, respectively) has demonstrated the endemic presence of poultry mycoplasma infections in the Philippines. This finding is consistent with molecular positivity rates for MG/MS across Asia and Europe. Molecular surveys of MG/MS have reported positivity rates of 6-20% in Myanmar, 5-10% in Belgium, and as high as 25% in Egypt [2,6,7]. Also aligned with global trends is the predominance of MG over MS in Philippine poultry flocks. MG is considered to be the more infectious and economically important poultry respiratory pathogen [3].

Managing poultry respiratory mycoplasmas, particularly on dense, multi-aged rearing farms, may be cumbersome in Central Luzon and

Calabarzon regions, where co-circulation of MG and MS has been documented. Disease persistence and horizontal spread of infections are commonly observed in poultry operations with overlapping production cycles [7,10]. In the present study, MG was observed in all regions except in Region V (Bicol). Future studies should include larger clinical samples in this area, as a previous serologic study covering South Luzon and Bicol areas reported MG-seropositive flocks [9]. Aside from this, a far-improved diagnostic platform must be explored, such as the use of advanced, machine-learning-enhanced LAMP that can offer better detection accuracy than conventional PCR methods [11, 12]. More importantly, it is well to note that the currently observed detection rates reflect PCR positivity among clinically affected flocks and should not be interpreted as a true national prevalence due to purposive sampling.

In this study, region-specific positivity estimates with 95% confidence intervals were used to provide a clear representation of spatial variation in MG and MS detection [17]. Significant regional variation in MG and MS positivity was observed across regions in the Philippines (χ^2 test, $p < 0.001$), suggesting that pathogen distribution may not be random and may be influenced by region-specific factors. However, given the purposive sampling design, these results should be interpreted as representative only of the sampled flocks and not of the general poultry population.

The relatively high detection in the Cordillera Administrative Region (CAR) (17.65% for both MG and MS) may be attributed to its environmental condition. Findings from low-temperature, high-altitude zones in other Asian regions indicate that cold stress often exacerbates the expression of respiratory disease [19]. The lowest MG PCR positivity rates (2–3%) were observed in Regions XI and I, respectively, while the lowest MS positivity rate was observed in Region VII. These findings are consistent with the statistically significant regional variation identified in this study (χ^2 test, $p < 0.001$). These observations may be attributed to multifactorial poultry-raising management and geographic separation across the country.

The reported MG detection rates in neighboring Asian countries — Japan (14.7%), Malaysia (15.3%), and China (12.6%) [1, 2]—have aligned with the current overall Philippine MG PCR-positive rate of 13.3%. The co-detection of MG with MS in the same geographic regions has highlighted the potential for synergistic poultry respiratory disease outbreaks. Chronic colonization with MG and MS is known to increase antimicrobial resistance and exacerbations of tissue inflammatory lesions [10, 18,19].

Despite antimicrobial use to combat poultry mycoplasmosis, the persistence of MG and MS suggests the possibility of inconsistent/improper antimicrobial administration, or the emergence of resistant field strains of the organisms. These observations are likewise noted in other Asian poultry farms [18,19]. Considering this, an integrated surveillance approach is recommended, including strain identification and monitoring of resistance patterns. This strategy could help assess emerging trends and, thereafter, support targeted immunological and antimicrobial treatments. The risk of prolonged poultry mycoplasma infection could be mitigated by addressing the underlying resistance strains.

4.3. MG and MS Co-circulation Patterns

The observation that MS detection occurred only in regions where MG was also identified suggests overlapping circulation of these pathogens in broiler production systems. This pattern is consistent with their shared transmission ecology and recognized involvement in respiratory disease complexes [1,3,7]. In this study, MG was significantly higher than MS as indicated by non-overlapping 95% confidence intervals [17].

At the population level, MS-positive flocks represented 55.12% of MG-positive flocks in this study, indicating substantial epidemiological overlap between MG and MS in affected broiler populations. Such co-occurrence is biologically plausible because both organisms colonize the respiratory tract and share similar vertical and horizontal transmission pathways [2, 3]. Mycoplasma exposure has been associated with more severe respiratory compromise, higher incidence of airsacculitis, poorer growth performance, and greater susceptibility to secondary bacterial infections [3,6,10]. Although broilers have a relatively short production cycle,

these effects may still contribute to subclinical production losses and increased antimicrobial usage [18,19].

The regional co-distribution of MG and MS may also reflect gaps in biosecurity and source flock control, as both pathogens can be transmitted vertically and horizontally [3,10]. These findings support the need for integrated Mycoplasma monitoring rather than single-pathogen surveillance, particularly in production systems where vaccination against Mycoplasma is not routinely implemented in broilers [1,10].

From an epidemiological perspective, the co-circulation pattern observed in this study underscores the complexity of respiratory disease ecology in poultry and highlights the value of molecular surveillance strategies capable of detecting multiple pathogens simultaneously [10,11,12,20,21].

A limitation of this study is that summarized pathogen detection did not permit direct confirmation of flock-level MG/MS co-infection within the same pooled samples. Although the regional distribution suggests epidemiological overlap, precise quantification of concurrent infection would require paired pathogen testing within the same individual flocks. Future studies incorporating integrated molecular detection approaches, including advanced AI- or machine learning-assisted LAMP assays [11,12], may help clarify co-infection dynamics and their potential impact on production performance and disease severity.

5. Conclusions

This study provides nationwide molecular evidence of MG and MS positivity in Philippine broiler flocks. Because sampling was purposive and focused on clinically affected flocks, the results represent molecular detection rather than population-level prevalence. The predominance of MG highlights its comparatively greater pathogenic significance and potential economic impact on commercial production. The co-detection of MG and MS further underscores the ongoing risk of concurrent infections, which may exacerbate respiratory disease severity and compromise flock performance. Future investigations should incorporate antimicrobial resistance profiling and well-designed intervention trials, alongside the evaluation of newer PCR-based diagnostic platforms, including AI- or machine learning-assisted LAMP assays, to enable more precise pathogen discrimination and improved field-level surveillance.

Availability of Data and Materials

All data are presented in the manuscript.

Author Contributions

Conceptualization, G.A.C., M.C.R.D.C., R.D.D., and D.V.U.; Methodology, G.A.C., M.C.R.D.C, G.M.G., R.D.D., M.L.G.A., and D.V.U.; Investigation, G.A.C., M.C.R.D.C., G.M.G., M.L.G.A., P.L.D.R., E.J.T.S., E.J.A., L.A.D.L., Y.R.M.T., D.B.R.P., C.C.P., C.M.D.P., J.P.R., and D.V.U.; Writing – Original Draft, G.A.C.; Writing – Review & Editing, G.A.C., M.C.R.D.C., G.M.G., R.D.D. and D.V.U.; Funding Acquisition, G.A.C., M.C.R.D.C., R.D.D., and D.V.U.; Resources, G.A.C., M.C.R.D.C., R.D.D., and D.V.U.; Supervision, G.A.C., M.C.R.D.C., and D.V.U.; Project Administration, G.A.C., M.C.R.D.C., R.D.D., and D.V.U.

Ethics Approval and Consent to Participate

All procedures for sample collection were approved by the Institutional Animal Care and Use Committee (IACUC) of each collaborating institution of the University of the Philippines, Los Baños - College of Veterinary Medicine (Protocol No. 2019-0027); the Cavite State University (with IACUC Exemption Certification); and the University of Eastern Philippines – College of Veterinary Medicine (Protocol No. 2020-001). Informed consent was obtained prior to sampling from poultry operators/owners. An official letter from the Director of the Bureau of Animal Industry, encouraging poultry farm operators/owners, was made available prior to the actual field sampling.

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Conflict of Interest

The authors declare no conflict of interest.

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