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(Case Report)

Co-infection of Avian Reovirus, *Mycoplasma synoviae,* and Marek's Disease in a Gamefowl (*Gallus gallus domesticus* L.) Flock in Ibaan-San Jose, Batangas, Philippines

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Abstract

Background: Avian reovirus (ARV) affects nearly all domestic poultry species and commonly associated with severe viral arthritis. This predisposes the birds to other viral and bacterial infections such as Marek's disease (MD), and *Mycoplasma synoviae* (MS). Approximately 25% of gamefowls with swollen shanks, exudation on the keel bone, and nasal discharge were reported by the farm. Hence, this paper aims to describe the clinical presentation of a gamefowl flock infected with ARV, MD, and *M. synoviae*. **Methods:** Blood and organ samples were collected from ten (10) morbid birds for serology, histopathology and qPCR to confirm the diagnosis. **Results:** Necropsy findings showed lesions in the tarsometatarsal, keel, and tracheal regions. Serum samples tested positive for ARV and MS antibodies using ELISA. Real-time RT-PCR detected ARV RNA, while PCR detected MDV DNA in the pooled tissue samples. Follow-up necropsy, conventional PCR, and phylogenetic analysis were also conducted post-vaccination. Phylogenetic analysis classified the MD-positive samples as *Gallid alphaherpesvirus* type 2 and suggested a possible association with VV+ strains. **Conclusion:** To the best of the authors' knowledge, this is the first documented case of ARV with co-infection with MS and MD in gamefowls in the Philippines.

Keywords

Avian reovirus, Marek's disease, *Mycoplasma synoviae*, Gamefowls

1. Introduction

Avian reovirus (ARV), a virus from the family Reoviridae, subfamily Spinareovirinae, and genus Orthoreovirus, affects nearly all domestic poultry species [1]. This virus is associated with malabsorption syndrome, runting-stunting syndrome, hepatitis, myocarditis, immunosuppression, and severe arthritis – the most common clinical sign [2]. The associated

arthritis and tenosynovitis can negatively affect the performance parameters and marketability of the birds due to reduced feed conversion and weight gain. Additionally, Reovirus infections can increase the susceptibility of birds to other bacterial or viral infections [3].

Marek's disease (MD), another major viral threat to poultry worldwide, typically manifests as a neoplastic disease in visceral organs, neurological disorders leading to paralysis, and immunosuppression [4]. The disease is caused by Gallid alphaherpesvirus-2 (Serotype 1/MDV 1), and serotype 1 strains are considered pathogenic [5].

Furthermore, *Mycoplasma synoviae* (MS), a 300–500 nm bacterium lacking a cell wall, is a significant pathogen causing synovitis, airsacculitis, and abnormalities in eggshell and laying patterns in chickens [6]. This bacterial disease can interact with other pathogens, further amplifying its pathogenic effects in chickens [7].

Infections with these pathogens pose significant losses to poultry producers. Studies have reported the occurrence of ARV in backyard chickens in Bangladesh [8], Africa [9], Brazil [10], and Canada [11]. However, the detection of ARV, MS, and MD in the Philippines has not been documented in any published literature. Hence, this paper aims to describe the clinical presentation of a gamefowl flock infected with ARV, MD, and MS.

2. Case Presentation

2.1. History

The farm of interest is a brooding and growing gamefowl farm located in Ibaan-San Jose, Batangas. The facility has a total of 11 open-range pens, and each pen can accommodate up to 200 birds depending on size. The birds are of mixed strains (Sweater, Roundhead, Hatch, Kelso, and Albany), and 30% of the parent stock is imported from Tennessee, USA. The vaccines given are shown in Table 1.

Table 1. Vaccination history of the gamefowl flock.

Age (days)	Vaccine (Type)	Strain	Route
1	*Eimeria* spp. (Live)	*E. acervulina, E. mivati, E. maxima, E. tenella*	Mouthdrop
7	Avian Encephalitis + Fowlpox (Live)	AE virus strain Calnek Live Fowl Pox virus strain Gibbs	Wingweb
	Newcastle Disease (ND) (Live)	LaSota	Intranasal
21	Infectious Bronchitis (IB) (Live) ND (live)	Ma5 Clone 30	Intranasal
31	Avian Encephalitis + Fowlpox (Live)	AE virus strain Calnek Live Fowl Pox virus strain Gibbs	Wingweb
41	Inactivated Coryza (Inactivated)	Serotypes A, B, and C	Intramuscular (IM)
51	Infectious Laryngotracheitis (live)	TCO	Intranasal
61	*Pasteurella multocida* (live)	X-73 (Type 1)	Wingweb
71	Inactivated Coryza (Inactivated)	Serotypes A, B, and C	Intramuscular (IM)
84	IB + Gumboro Disease + ND (Inactivated)	IB M41 Gumboro D78 ND Clone 30	Intramuscular (IM)
120	*Pasteurella multocida* (live)	X-73 (Type 1)	Wingweb
150	IB (Live) ND (Live)	IB ma5 ND Clone 30	Intranasal

On March 22, 2024, the management reported that approximately 25% of birds exhibited swollen shanks, exudation on the keel bone, and nasal discharge. The clinical onset was at around 3.5 to 5 months of age and leg lameness was more apparent in 4-month-old birds. Given these clinical signs, the farm veterinarian initially suspected MS as the causative agent. Hence, Tilmicosin + Florfenicol + Aspirin (Nutribiz Corp.) was administered via drinking water for 7 days to assess treatment response. Unfortunately, the response was slow and only around 40% of the symptomatic birds recovered. Hence, on June 6, 2024, 8-month-old birds (n=30) from Batch 5 were physically examined for further testing. Blood (n=30) and organ samples (n=10) were collected for serology, histopathology, and qPCR.

2.2 Necropsy

Ten (10) morbid birds were humanely euthanized to assess the external and internal lesions. Externally, the hock joints appeared inflamed, with swelling extending downward through the tarsometatarsal region (Fig. 1A). Despite these external signs, gross examination of the joint capsule revealed no significant gross lesions. In contrast, the sternal region of the bird showed poor feathering and erythema (Fig. 1B). Beneath the skin, there was visible accumulation of yellow seromucous exudate around the keel bone and surrounding muscles (Fig. 1C). Mucosal hyperemia was also seen in the tracheal mucosa (Fig. 1D).

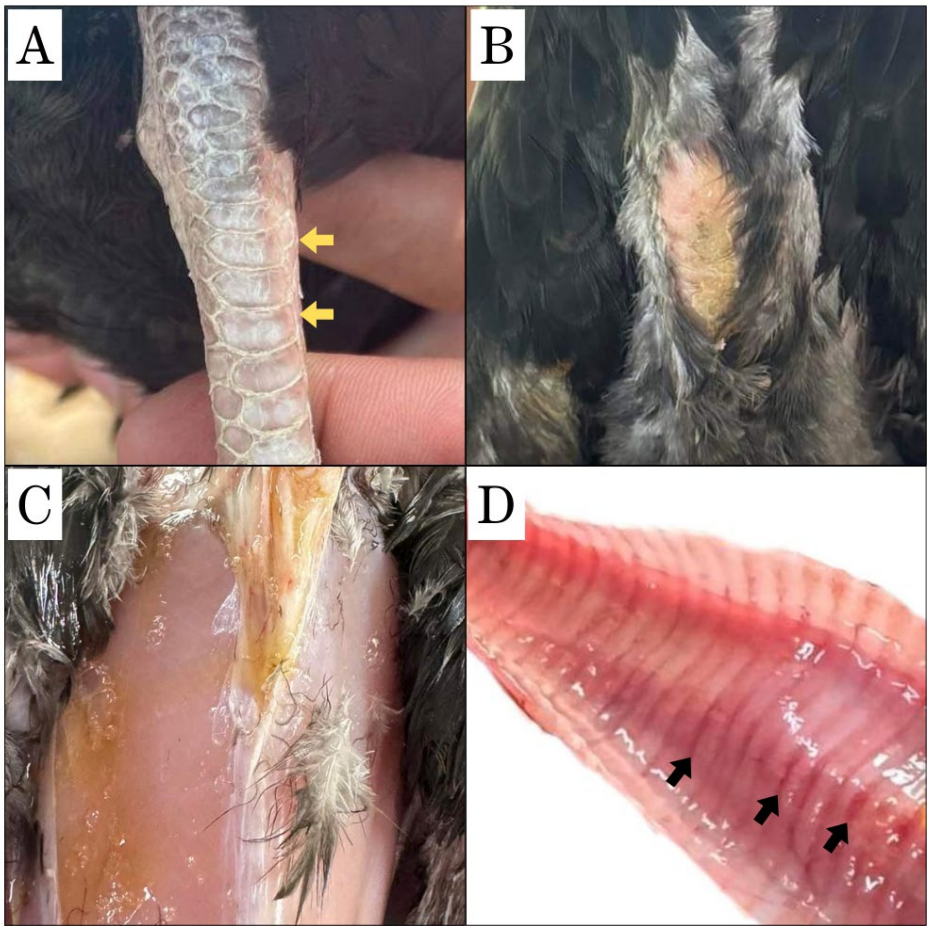

Figure 1. Necropsy findings in a gamefowl flock. (A) Swollen and erythematous tarsometatarsal region (yellow arrows). (B) and (C) Sternal bursitis marked by accumulation of yellowish exudate in the keel region. (D) Mucosal hyperemia in the tracheal mucosa (black arrows).

2.3. Histopathology

The liver, lung, spleen, bone marrow and tracheal samples were fixed using 10% buffered formalin, sectioned and stained using the standard hematoxylin-eosin stain (Fig. 2). Histopathological examination of the liver revealed moderate to severe, multifocal infiltration of inflammatory cells was observed in the perivascular regions of the periportal vessels and hepatic sinusoids. The portal vessels and sinusoidal capillaries also showed mild to moderate congestion. The lungs revealed severe, multifocal to diffuse infiltration of heterogeneous lymphoid cells within the interstitial areas surrounding the air capillaries. Additionally, there was moderate to severe congestion of the pulmonary blood vessels. The spleen showed mild, diffuse lymphoid depletion in the central areas of the splenic parenchyma, which appeared as slightly pale zones upon sub gross examination. In the bone marrow, there was a moderate, diffuse reduction in overall cellular density, with moderate to severe replacement of hematopoietic elements by adipocytes, indicative of marrow suppression or atrophy. The tracheal mucosa exhibited moderate to severe, multifocal to diffuse infiltration of inflammatory cells within the lamina propria. The normal epithelial and mucosal glandular architecture was disrupted and replaced by an irregular arrangement of lymphoid cells, interspersed with disorganized goblet cell-like structures.

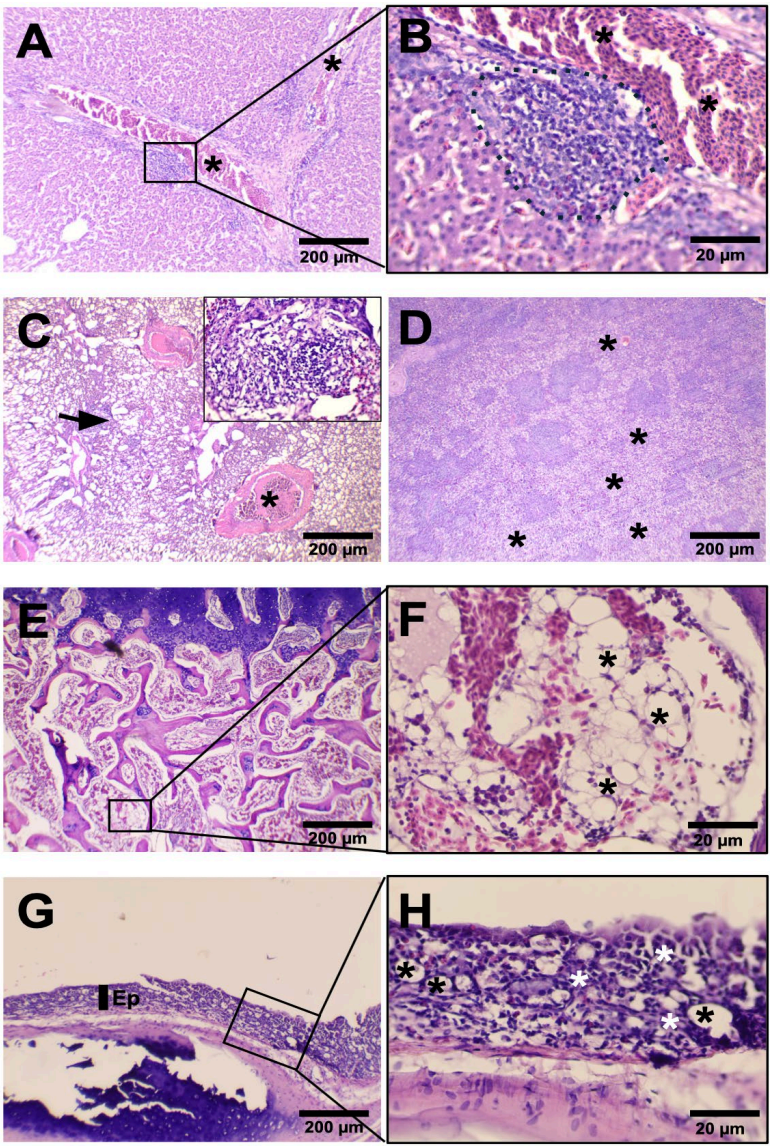

Figure 2. Histopathological images of lesions in the liver, lungs, spleen, bone marrow and trachea. (A) low and (B) high magnification images of the liver showing focal infiltration of inflammatory cells (demarcated with broken lines) in the perivascular area and moderate congestion in the portal vessel (asterisks). (C) low magnification images of the lung showing focal infiltration of heterogenous lymphoid cells in the interstitium of air capillaries (arrow and inset) and severe congestion within pulmonary vessels (asterisk). (D) low magnification image of the spleen showing pale areas of the parenchyma (asterisks), with mild depletion of lymphoid cells. (E) low and (F) high magnification images of the bone marrow show adipocyte replacement of hematopoietic cells (asterisk). (G) low and (H) high magnification images of the tracheal mucosa showing severe infiltration of inflammatory cells in the lamina propria (white asterisk) and disorganized goblet cells (black asterisk); Ep: epithelium.

2.4. Serology

Thirty (30) serum samples were sent to the Precisione International Research and Diagnostic Laboratory Inc. in Marilao, Bulacan for antibody titer assessment against ARV and MS using ELISA. Results are shown in Table 2. All samples tested positive for ARV antibodies, and the mean titer exceeds the expected range for vaccinated birds (7,000-20,000). The coefficient of variation (CV) for ARV is low, which suggests that the antibody titer is relatively uniform across all samples. Five (5) out of 30 samples also tested positive for MS antibodies, with a CV (%) of 187. Since the birds were unvaccinated for these diseases, the results show clear evidence of exposure.

Table 2. Antibody titer results for ARV and MS using ELISA.

Disease Agent	Mean Titer	Min-Max Titer	ELISA standard (Biochek)	CV (%)	Remarks
ARV	27790	11845-37000	live vaccine- 2 000 - 6 000 3 - 5 wks post vaccination; inact. vaccine 7 000 - 20 000 5 - 8 wks post vaccination; Suspect titer >6,000	23	Positive (No ARV vaccination in the farm)
MS	359	1-3247	live MS-H (eye drop)- 500 - 3 000 6 -12 wks post vaccination 30- 70% Pos; Suspect titer > 5000 and > 90% Pos	187	Positive (No MS vaccination in the farm)

2.5 Qualitative Polymerase Chain Reaction (qPCR) and sequencing

Liver, spleen, cecal tonsil, feather, and bone samples from each bird were pooled (n=5) via Flinders Technology Associates cards (Qiagen) and were sent to the ANICON Veterinary Diagnostics and Services Laboratory in Höltinghausen, Germany for REO and MD PCR screening and sequencing. The samples tested positive for both ARV and MD (Table 3). All CT values below 30 were processed for sequencing.

2.6. Consensus sequence alignment and BLAST Search

Nucleotide sequence analyses were conducted using MEGA 11 (Molecular Evolutionary Genetics Analysis) software (Philadelphia, PA, USA). The quality of all newly obtained gene sequences was assessed by visual inspection of chromatograms. Forward and reverse sequences were aligned to generate consensus sequences. Sequence similarity searches were performed using the Basic Local Alignment Search Tool (BLAST) against the NCBI GenBank database to identify the most closely related sequences. Representative nucleotide sequences generated from each organ in this study were deposited in GenBank (Accession Numbers PX833342 – PX833346).

2.7. Phylogenetic analysis

Analysis of aligned multiple *Meq* gene sequences was also done using MEGA 11 software. Alignments were visually inspected and trimmed to ensure comparable sequence lengths prior to phylogenetic analysis. A phylogenetic tree was reconstructed for the *Meq* gene sequences, with *Gallid gammaherpesvirus* 2 used as the outgroup. The best-fit nucleotide substitution model was selected based on the lowest corrected Akaike Information Criterion (AICc) value.

The Kimura 2-parameter (K2P) model was identified as the optimal substitution model for the *Meq* gene, with no rate heterogeneity among sites supported (AICc = 6369.160). A maximum-likelihood tree was constructed with 1,000 bootstrap replicates, and branches with bootstrap support below 70% were collapsed to improve the reliability of the tree.

2.8. Treatment and Follow-up

On June 23, 2024, live MS and killed ARV emergency vaccines were given to apparently healthy birds. Three hundred (300) out of 500 birds were culled from Batch 5 to prevent further disease transmission. The vaccination program of the younger batches was also adjusted accordingly to include MS and ARV.

The post-vaccination status of the birds was assessed on August 26, 2024. Following vaccination, 318 out of 354 cockerels (90%) from the succeeding batches no longer showed signs of leg lameness. Necropsy was once again conducted in four morbid birds. Splenomegaly was observed in all birds with the spleen size comparable to that of the heart. In addition, corneal opacity and iris discoloration was also observed in a 6-month-old bird. Tissue samples from the tendon, spleen, heart, liver, lungs, and feathers, along with oropharyngeal and cloacal swabs, were screened for ARV, MS, and MD using real time PCR. The feather samples tested positive for MD.

3. Discussion

The gamefowl industry is an economically important poultry sector in the Philippines, where birds are raised for entertainment, specifically cockfighting. Despite its economic importance, epidemiological data on key avian pathogens affecting gamefowls such as ARV, MDV, and MS, remain limited and underreported.

To the authors' knowledge this is the first documented case of ARV, MS, and MD detection in gamefowls in the Philippines. Diagnosing avian diseases is often challenging since many pathogens present similar clinical signs. To optimize the use of resources, the testing procedures focused on the pathogens most likely to cause the infection based on the clinical signs, necropsy findings, and vaccination history. This list included ARV, MD, and MS. *Mycoplasma gallisepticum* (MG) and Newcastle disease (ND) were initially considered in the differential diagnoses. MG is known to cause chronic respiratory disease in chickens and the tracheal lesions observed could also be attributed to this pathogen [12]. However, tenosynovitis, the chief complaint for this case, is more commonly associated with MS infections in chickens. Hence, MG was excluded from the diagnostic testing. Similarly, ND, which is linked to leg paralysis and respiratory signs [13], was ruled out due to the vaccination history of the birds against this disease.

Avian Reovirus infections are usually asymptomatic. Tenosynovitis/arthritis is the predominant clinical manifestation among all genotypic clusters of ARV [3]. In clinical cases, this is characterized by swelling and redness of the hock joint [13-15]. Several ARV strains previously reported in Asia include ARV LY383 China 2016, HeN130728, LN160607-1, GX150816, SD150806, JS170705-1, A15-48/Wild bird/ Korea 2015, A18-205/Wild bird/Korea2018, ARV2IR018, and ARV1IR019. These strains have been identified from China, Korea, and Iran [3]. Further virus isolation and propagation are recommended prior to sequencing.

Similar to ARV, most MS infections are subclinical. Symptomatic cases primarily affect the synovial structures and upper respiratory tract [6,16]. The clinical signs in this case are consistent with the typical ARV and MS symptoms. Experimental infection of chickens with both ARV and MS showed that the tibiotarsal-metatarsal joint is the main predilection site of dual-infection [17]. As a result, erythema is often observed in the synovial membrane and tendon sheaths.

Sternal bursitis and tracheitis are primarily associated with MS [18], while most reported ARV infections are focused on arthritic lesions and stunted growth [9,13-14]. In this particular case, aside from bacterial infection, repeated contact of the keel region with the ground may have induced trauma, which could also be linked with the apparent leg lameness. Leg lameness can also be induced by the enlargement of peripheral nerves due to MD. Typical MD lesions are lymphoproliferative and neoplastic. The most consistent gross lesion involves the enlargement of the sciatic nerve [5,19]. However, the

histopathological findings for this case only revealed mild to moderate congestion of the visceral organs and presence of inflammatory cells in bone sections (Fig. 2). Additionally, it is possible that the enlargement of the sciatic nerve was not observed due its bilateral nature and the lack of comparison with the sciatic nerve of normal chickens.

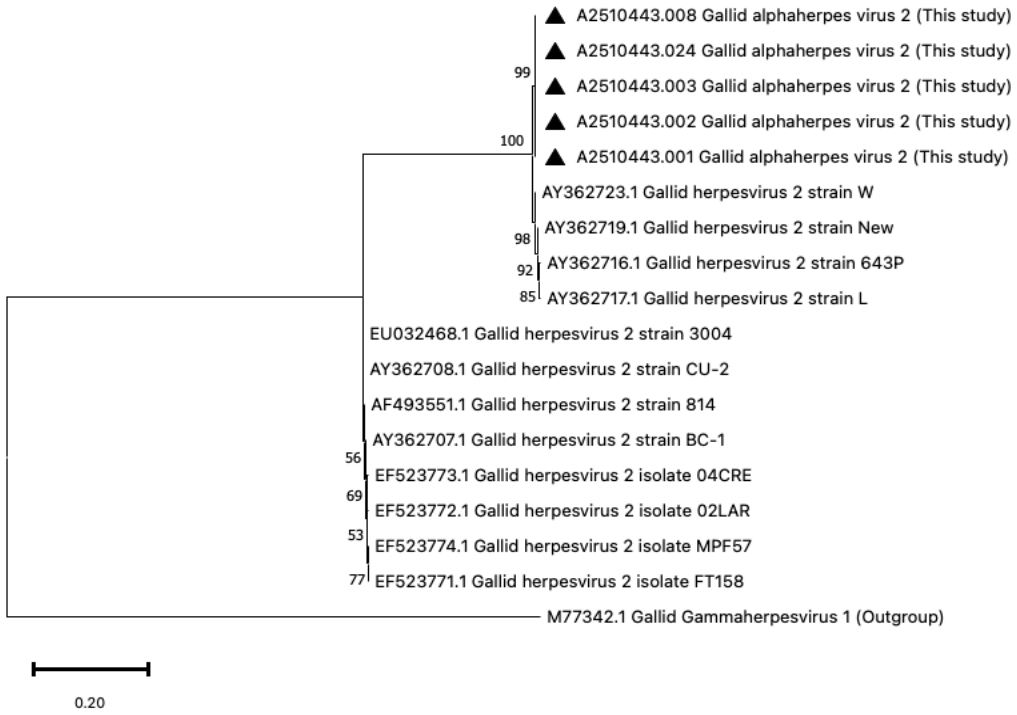

Figure 3. Phylogenetic reconstruction of *Gallid alphaherpes virus 2* in gamefowls from Ibaan-San Jose Batangas based on *Meq* gene.

The positive PCR result for MD in the follow-up suggests that the ocular discoloration represents lymphoid infiltration of MDV in the iris [12]. REO detection via qPCR was not sequenced due to high CT value. Factors such as timing of sample collection, low viral load, or viral shedding variability may explained the results [20-22].

The qPCR results indicated that some samples had a low viral load, rendering further sequencing inadvisable, whereas all MD-positive samples with higher viral loads were used for subsequent analysis to determine the specific MDV strain. Sequencing analysis confirmed that all analyzed samples showed 99.88% homology to *Gallid alphaherpesvirus* type 2 isolated from the feather follicle of chickens in India (GenBank accession number: MK639178.1). Phylogenetic analysis showed that the identified sequences clustered closely with VV+ strains (Fig. 3), with a strong bootstrap support of 100, suggesting possible association with VV+ strains. Unlike ARV and MS, which are both transmitted horizontally and vertically [14,6], MDV is only limited to horizontal transmission [12]. MDV can survive in poultry dust and feather dander for extended periods [23]. Hence, even without vertical transmission, MDV can still proliferate in the farm. Given that this is a brooding to growing farm with an open-range set-up, contaminated sources can easily transfer from the growing to the brooding area and vice-versa. The occurrence and transmission of ARV and MS are influenced by farm setup and production practices. ARV is primarily spread through vertical and fecal–oral transmission, whereas MS is transmitted vertically and through the respiratory route. Factors such as housing conditions and biosecurity measures can affect both horizontal and vertical transmission, contributing to the persistence and broader transmission of these viruses within poultry operations [6,14].

Free-range birds may be predisposed to co-infections due to lack of physical barriers and risk

of contact with wild birds [24]. Immunosuppression induced by stress and exposure to other pathogens also increases the likelihood of co-infections in a gamefowl flock [4,24]. Therefore, regular monitoring and surveillance should be conducted using sensitive diagnostic tests, such as qPCR. If possible, the testing procedures should also include all possible differentials.

Culling of infected birds is recommended since there is no well-established treatment for ARV, MDV, and MS [12]. Vaccination should be implemented at the breeding, brooding, and growing sections of the farm to prevent horizontal and vertical transmission. Improved biosecurity practices such as addition of footbaths can help mitigate the spread of infectious diseases.

For MD vaccination, considerations should be made regarding its potential side effects. All MD vaccines consist of live viruses administered to 18-day-old embryos or post-hatch [23]. It is important to note that vaccinated flocks can still be infected by virulent MD. Marek's Disease Virus transmission to other chickens occurs via the feather follicle epithelium [25]. As such, post-vaccination infections typically occur in immunosuppressed birds. Birds may develop the clinical signs of the virulent form which includes ocular changes [12]. Ocular lesions may significantly affect the performance and economic value of gamefowls. Hence, possible adverse effects should always be relayed to the client prior to vaccine administration. Additionally, it is recommended to formulate a vaccination program for gamefowls to minimize the financial losses caused by field challenges. Proper vaccine handling and administration is also crucial in preventing vaccination failure [25]. The implementation of immunoprophylaxis and strict biosecurity measures among poultry farms in the Philippines is essential in effective controlling the introduction and transmission dynamics of ARV, MDV, and MS infections. These strategies can also effectively reduce and limit virus transmission within poultry farms and limit the spread of infections within and between poultry operations.

4. Conclusion

This paper reports and confirms the presence of MD, MS, and ARV in a gamefowl flock in the Philippines. Moreover, immunosuppressed birds can be predisposed to secondary bacterial infections. The clinical signs of these pathogens may overlap, further complicating the diagnosis and treatment.

This underscores the need for an integrated approach that combines tailored immunoprophylaxis programs and strict biosecurity implementation among farms in the Philippines to limit the transmission dynamic of MS, MD, and ARV. The addition of sensitive, specific, and cost-effective diagnostic tests into surveillance and control programs is essential to enable an effective and sustainable evidence-based interventions against emerging avian diseases.

Availability of Data and Materials

All data are available in this study.

Author Contributions

Conceptualization, S.I.C., and M.L.P.P; Methodology, S.I.C., F.P.D.V., M.J.C.A., and M.L.P.P.; Resources, S.I.C.; Writing - Original Draft, M.L.P.P.; Data Analysis, S.R.E; Writing - Review & Editing, S.I.C and F.P.D.V.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Brugère-Picoux, J., & Vaillancourt, J.-P. (Eds.). (2015). Manual of poultry diseases. AFAS.

[2] Farnoushi, Y., Heller, D., & Lublin, A. (2022). Development of a wide-range real-time RT-PCR assay for detection of Avian reovirus (ARV). *Journal of Virological Methods, 310*, 114613 – 114613. https: // doi.org / 10. 1016/j. jviromet.2022.114613.

[3] Rafique, S., Rashid, F., Wei, Y., Zeng, T., Xie, L., & Xie, Z. (2024). Avian orthoreoviruses: A systematic review of their distribution, dissemination patterns, and genotypic clustering. *Viruses, 16*(7), 1056–1056. https://doi.org/10.3390/v16071056.

[4] Prabhu, M., Gopal, S., Gopal, K., Kumar, V., Rajeswar, J. J., Malmarugan, S., & Anne, N. S. (2024). Differential detection and molecular characterization of multiple avian oncogenic viruses in backyard poultry of Tamil Nadu, India. *The Indian Journal of Animal Sciences, 94(1)*, 11–16. https://doi.org/10.56093/ijans.v94.i1.134611.

[5] Mescolini, G., Lupini, C., Antonietta Di Francesco, Davidson, I., Felice, V., Bellinati, L., Mattia Cecchinato, & Catelli, E. (2022). Marek's disease in genetically susceptible Cochin chickens in Italy: A case report. *Directory of Open Access Journals, 58*(1), 117–124. https://doi.org/10.12834/vetit.2120. 12221.1.

[6] Kleven, S. H., & Ferguson-Noel, N. (2008). *Mycoplasma synoviae* Infection. In Y. M. Saif (Ed.), Diseases of Poultry. Blackwell Publishing.

[7] Han, S., Wang, Y., Chang, W., Wang, L., Fang, J., Han, J., Hou, X., Qi, X., & Wang, J. (2024). Evaluation of the protective efficacy of six major immunogenic proteins of *Mycoplasma synoviae*. *Frontiers in Veterinary Science, 10*. https://doi.org/10.3389/fvets.2023.1334638.

[8] Islam, M., Sabuj, A., Haque, Z., Pondit, A., Hossain, M., & Saha, S. (2020). Seroprevalence and risk factors of avian reovirus in backyard chickens in different areas of Mymensingh district in Bangladesh. *Journal of Advanced Veterinary and Animal Research, 7*(3), 546. https:// doi.org/ 10.5455/ javar.2020.g452.

[9] Pinto, S.C., Aleixo, J., Kleidy Camela, Chilundo, A.G., & Bila, C.G. (2022). Seroprevalence of infectious bronchitis virus and avian reovirus in free backyard chickens. *Onderstepoort Journal of Veterinary Research, 89*(1). https://doi.org/10.4102/ojvr. v89i1.2042.

[10] Brochu, N.M., Guerin, M.T., Varga, C., Lillie, B.N., Brash, M.L., & Susta, L. (2019). A two-year prospective study of small poultry flocks in Ontario, Canada, part 1: Prevalence of viral and bacterial pathogens. *Journal of Veterinary Diagnostic Investigation, 31*(3), 327–335. https://doi.org/10.1177/104063871 9843577.

[11] Lunge, V.R., De Carli, S., Fonseca, A.S.K., & Ikuta, N. (2023). Avian reoviruses in poultry farms from Brazil. *Avian Diseases, 66*(4). https://doi.org/10.1637/aviandiseases-d-22-99998.

[12] Boulianne, M.C. (2012). Avian disease manual. American Association of Avian Pathologists, Inc.

[13] Dimitrov, K. (2023). Newcastle Disease in Poultry. MSD Veterinary Manual. https://www.msdvetmanual.com/poultry/newcastle - disease -and -other-paramyxovirus infections/newcastle-disease-in-poultry.

[14] Egana-Labrin, S., & Broadbent, A.J. (2023). Avian reovirus: A furious and fast evolving pathogen. *Journal of Medical Microbiology,* 72(10). https://doi.org/10.1099/jmm.0.0017 61.

[15] Shabbir, M.Z., Yu, H., Lighty, M.E., Dunn, P.A., Wallner-Pendleton, E.A., & Lu, H. (2024). Diagnostic investigation of avian reovirus field variants circulating in broiler chickens in Pennsylvania of United States between 2017-2022. *Avian Pathology, 1–8*. https://doi.org/10.1080/03079457.2024.23428 89.

[16] El-Gazzar, M. (2020). *Mycoplasma synoviae* Infection in Poultry. Merck Veterinary Manual. https://www.merckvetmanual.com/ poultry/mycoplasmosis/mycoplasma-synoviae-infection-in-poultry#Clinical-Findings-and-Lesions_v8986588.

[17] Bradbury, J.M., & Garuti, A. (1978). Dual infection with *Mycoplasma synoviae* and a tenosynovitis-inducing reovirus in chickens. *Avian Pathology, 7*(3), 407–419. https://doi.org/10.1080/03079457808418294

[18] Crespo, R. (2023, March). Breast Blisters in Poultry. Merck Veterinary Manual; Merck Veterinary Manual. https:// www. merckvet manual.com/ poultry/ miscellaneous - condi tions-of-poultry/breast-blisters-in-poultry.

[19] Abreu, D.L.C., Santos, F.F., José, D.S., Tortelly, R., Nascimento, E.R., & Pereira, V.L.A. (2016). Pathological aspects of a subclinical Marek's Disease case in free-range chickens. *Brazilian Journal of Poultry Science, 18*, 197–200. https://doi.org/10.1590/ 18069061-2015-0001.

[20] Mouliou, D. S., & Gourgoulianis, K. I. (2021). False-positive and false-negative COVID-19 cases: Respiratory prevention and management strategies, vaccination, and further perspectives. *Expert Review of Respiratory Medicine*, 1–10. https://doi.org/10.1080/17476348.2021.19173 89.

[21] Huang, L., Xie, Z., Xie, L., Deng, X., Xie, Z., Luo, S., Huang, J., Zeng, T., & Feng, J. (2015). A duplex real-time PCR assay for the detection and quantification of avian reovirus and *Mycoplasma synoviae*. *Virology Journal*, *12*(1). https://doi.org/10.1186/s129 85-015-0255-y.

[22] Wu, S., Ding, T., Shao, H., Qian, K., Ye, J., & Qin, A. (2023). A quadruplex real-time PCR assay combined with a conventional PCR for the differential detection of Marek's disease virus vaccines and field strains. *Frontiers in Veterinary Science*, *10*. https: // doi.org/ 10. 3389/fvets.2023.1161441.

[23] Bull, J.J., & Antia, R. (2022). Which "imperfect vaccines" encourage the evolution of higher virulence?. *Evolution, Medicine, and Public Health, 10*(1), 202–213. https://doi.org/10.1093/emph/eoac015.

[24] McMullin, P. (2022). Infectious diseases in free-range compared to conventional poultry production. *Avian Pathology, 51*(5), 424–434. https://doi.org/10.1080/03079457.2022.20864 48.

[25] Miles, A. M. (2008). Marek's Disease. In Brugère-PicouxJ. & J.-P. Vaillancourt (Eds.), Manual of Poultry Diseases. AFAS.