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Table of Contents

Original Article

Gross and Microscopic Pathology of Pigeon Paramyxovirus Serotype 1 (PPMV-1) Infection in Racing Pigeons (*Columba livia domestica*) from Luzon, Philippines

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Abstract

Background: Pigeon paramyxovirus serotype 1 and other virulent NDVs are known to cause multi-systemic infection in birds. However, very little is known about its pathology in poultry species. Methods: In this study, the gross and histopathological changes in various organs of PPMV-1-infected racing pigeons were characterized and morphologically diagnosed. A total of fifteen organs, grouped into organ systems, were analyzed. Results: Clinical history revealed that all pigeons were exhibiting nervous and gastrointestinal signs such as torticollis and green-white diarrhea. Morbidity and mortality rates were spiking at 10 to 15% and 10%, respectively. Gross and in situ examination showed that the brain, heart, lungs, and gastrointestinal tract were mostly affected. The majority of the macroscopic lesions include hemorrhage and variable signs of necrosis and degeneration. Histologically, the brain, kidneys, lungs, and portion of the gastrointestinal tract had presented multiple lesions which include congestion, hemorrhage, and vasculitis. Diffuse lymphoid depletion and necrosis were also notable in all lymphatic organs and related tissues. Conclusion: The results of this investigation support the multi-systemic involvement of the infection which further validates the need for stronger preventive and control measures against PPMV- 1 and other avian paramyxoviruses not only for the domestic poultry but also for the other avian species.

Keywords

gross pathology, histopathology, multi-systemic infection, PPMV-1, racing pigeons, virulent NDV

1. Introduction

Pigeon paramyxovirus serotype 1 (PPMV- 1) belongs to the genus Avulavirus of the family Paramyxoviridae, under the order Mononegavirales [1]. PPMV-1 is termed genotype VI Newcastle disease virus (NDV) which is known to cause enteric and neurological disease in infected birds [2]. Since its first report in the 1970s, PPMV-1 has been detected in pigeon populations throughout the world. Its contagious potential has allowed itself to spread rapidly among different bird populations [3].

The hallmark feature of PPMV-1 infection is its association with a variety of systemic involvement. Infection with NDV has also been referred to as avian distemper because of its effect on the respiratory, circulatory, gastrointestinal, and nervous systems [4]. In pigeons, PPMV-1 typically produces

gastrointestinal and neurological symptoms that mimic signs similar to those seen with velogenic NDV infection in chickens [3, 5]. For these reasons, the pigeon and poultry industries could face economic problems due to high morbidity and mortality.

Unfortunately, despite the current threats in the pigeon industry, there is still a lack of sufficient knowledge regarding PPMV-1 infection in the Philippines. Currently, there has never been a study conducted regarding its clinical and pathological implications for pigeons. This study aimed to characterize the clinical signs and lesions caused by PPMV-1 in racing pigeons as a reference tool to recognize the disease using routine H&E staining technique. The practical approach to its early detection does not only guarantee prompt diagnosis, but it may also help pigeon raisers and fanciers recognize the disease for field case reporting.

2 Materials and Methods

2.1 Ethical approval

The conduct of this research has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the College of Veterinary Medicine, University of the Philippines Los Baños with assigned protocol number: 2018-0018.

2.2 Sampling protocols

Five animals solely infected with pigeon paramyxovirus serotype 1 (PPMV-1) from Luzon, Philippines were purposefully selected as study units. The samples were acquired from a One- loft racing facility where racing pigeons from Regions 1, 3, and 4A were housed at the time of submission. Specifically, pigeons were three to five months old, and of the Racing Homer breed. All pigeons were clinically ill at the time of study and were humanely euthanized by cervical dislocation. Six organ systems comprising the circulatory, gastrointestinal, hepato-biliary, lymphatic, nervous, respiratory, and urinary systems were collected and characterized. Prior to tissue sample collection, the carcasses were examined for gross characterization. Examination and sample collection were done within 1-2 hours, and all submitted samples were placed and fixed in 10% buffered neutral formalin for routine histopathological processing.

2.3 Detection of pathogen

All racing pigeons used in this study were confirmed to be solely infected by velogenic PPMV-1 through the use of multiplex reverse transcription polymerase chain reaction (mRT- PCR) and nested RT-PCR (nRT-PCR), as we previously reported [6]. Phylogenetic analysis and subgenotyping revealed that the pathogen belongs to the subgenotype VIb/2 NDV group. Other pathogens that produce similar clinical signs such as avian influenza Virus, infectious laryngotracheitis virus, and infectious bronchitis virus have been ruled out using the same laboratory techniques.

2.4 Gross and histopathological analyses

Post-mortem examination and tissue collection were methodically performed to ensure that all tissue samples were of superior quality. Gross characterization of the organs was done through necropsy. All formalin-fixed samples were embedded in paraffin and then sectioned for histological staining using hematoxylin and eosin (H and E). All tissue samples were clustered into pools according to their corresponding organ systems. Histopathological sections were read and analyzed following a systemic approach. Approximately five to ten microscopic fields per sample were evaluated in this study. Lesion assessment was performed descriptively according to the presence of cell degeneration, hemorrhage, congestion, and inflammatory cell infiltration; as previously described [7]. Morphological descriptions and diagnoses were made and verified by a veterinary pathologist. Lesions and other findings were photographed and documented using the EVOSTM XL Core Cell Imaging System (Thermo Fisher Scientific, Inc.) under different magnifications.

3. Results and Discussion

Clinical manifestations of the infection were mostly associated with the nervous and gastrointestinal systems. Particularly, erythematous conjunctivitis, periorbital edema, torticollis (Figure 1-A), and green-white diarrhea were apparent to all study units. All signs appear to have a strong correlation with the velogenic properties of the field strain isolate. The development of gastrointestinal disturbances and respiratory distress followed by neurologic disease is a classical feature

of velogenic NDV strains [8]. These clinical symptoms resulted in the morbidity and mortality rates observed within the flock. Correspondingly, gross lesions were most frequently observed in the gastrointestinal, nervous, and respiratory systems; whereas, samples from the brain, kidneys, lungs, and portion of the gastrointestinal tract presented the most number of microscopic lesions and were found to be the most affected organs.

3.1 Circulatory system

Grossly, pale myocardial infarction and engorgement of the cardiac blood vessels were noted in the heart (Figure 1-B).

On histopathology, congestion, myocardial degeneration, and necrosis (Figure 2-A) were the primary lesions observed in the samples. Severe infiltration of mononuclear cells was also found in virtually 60% of the organs. Most of the lesions found were in constant agreement with those previously described [7], which attributed cardiac lesions to the viremic phase of the disease. Since the virus can spread hematogenously, endothelial damage is highly plausible which compromises effective blood circulation. This, in return, lowers oxygen perfusion to the organ, leading to myocardial infarction [9]. In addition, a previous study also mentioned that the apoptotic mechanism by pressure overload, as induced by NDV infection, in the heart causes myocardial cell abnormalities [10]. Similarly, other significant findings such as

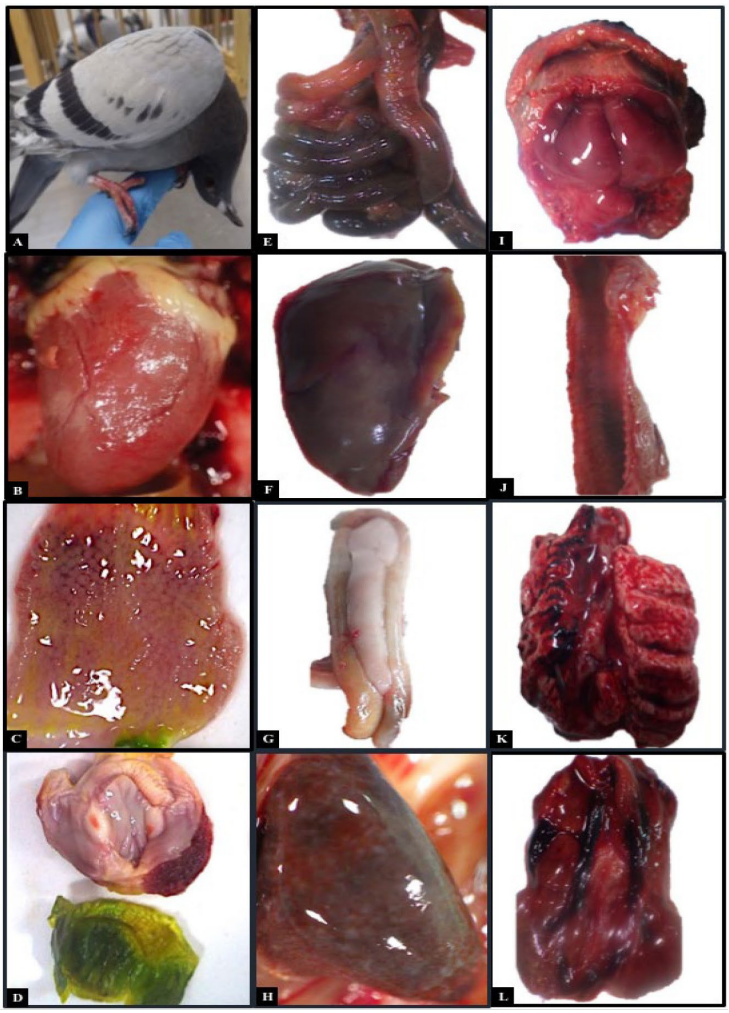

Fig. 1. Clinical sign and gross morphology (representative images). (A) torticollis of an affected bird. (B) pale infarction on the heart. (C) red-to-yellow discoloration of the proventriculus. (D) reddened mucosal surface of the ventriculus (koilin removed). (E) hemorrhagic serosal surface of the gastrointestinal tract. (F) liver with areas of congestion. (G) pale surface of the pancreas. (H) mottling on the surface of the spleen. (I) hemorrhagic and edematous brain. (J) congested tracheal lumen. (K) hemorrhagic lungs with black spots. (L) hemorrhagic kidneys with engorged renal blood vessels. Note: organs were removed and placed on a clean sheet; background was removed.

severe multifocal mononuclear cell infiltration and myocardial degeneration and necrosis were found to share similarities with documented reports [11, 12]. These findings supported the extensive vascular involvement of the organ which further exacerbates the negative impact of the viral infection to its host.

3.2 Gastrointestinal and hepato-biliary systems

One of the classical characteristics of velogenic NDV infection is its association with different digestive disturbances accompanied by neurologic signs. Based on medical history, all five pigeons were exhibiting clinical signs

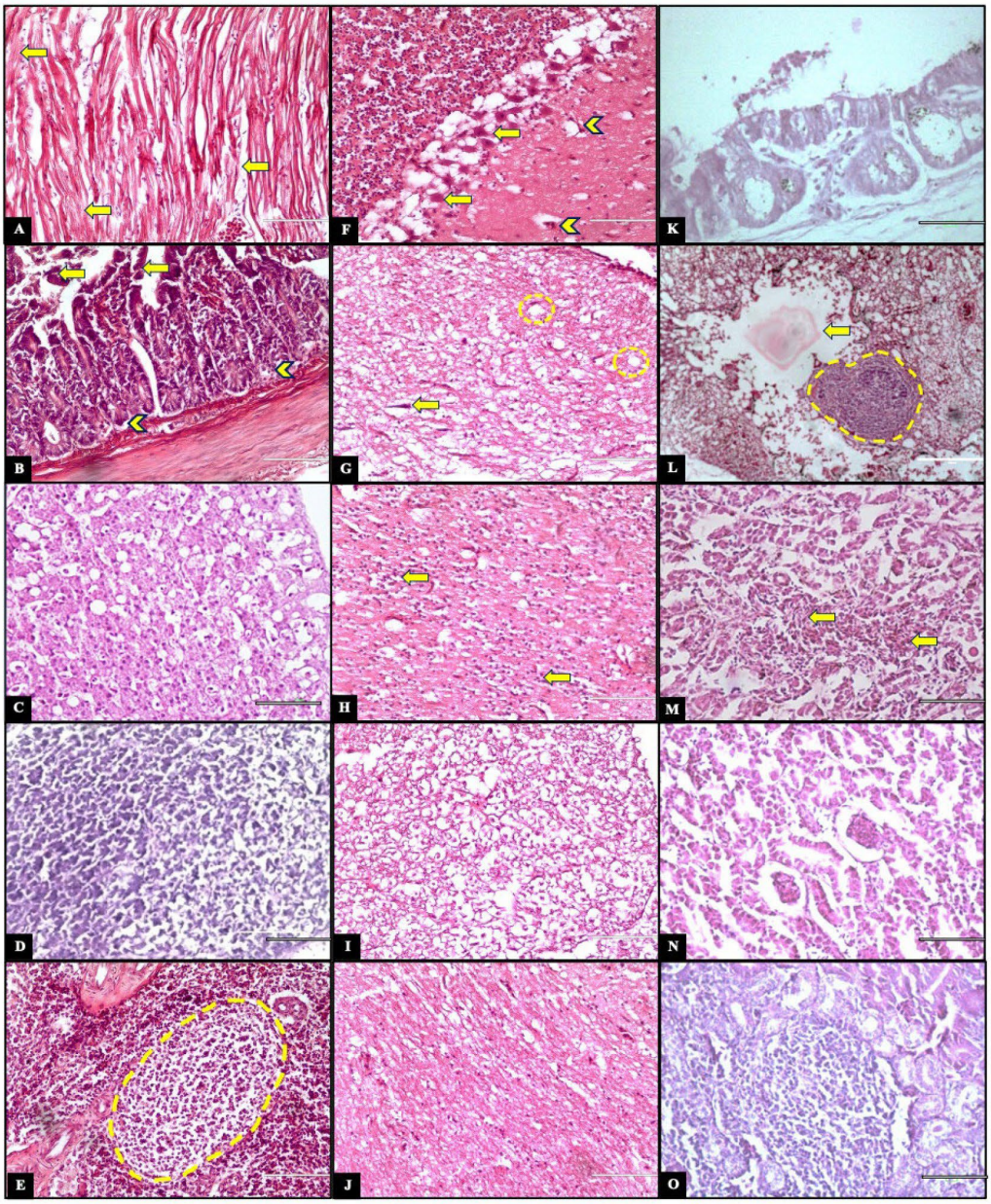

Fig. 2. Representative photos of the microscopic examination of tissues. (A) myocardial fiber disintegration (arrows) with lymphocytic infiltration. (B) sloughing off of intestinal mucosal epithelium (arrows) and necrosis of intestinal glands (arrowheads). (C) perihepatic fatty vacuolation. (D) necrosis of the pancreas. (E) depletion of lymphoid tissues (outlined). (F) degeneration of the cerebellar Purkinje cells (arrow) and presence of anoxic neurons (arrowheads). (G) status spongiosus in the brain (encircled) and anoxic neuron (arrow). (H) gliosis in the cerebrum (arrows). (I) degeneration and necrosis of the spinal cord. (J) gliosis and status spongiosus of the spinal cord. (K) tracheal hemorrhage. (L) mucoid exudate (arrow) in the parabronchus with nodular lymphoid hyperplasia (outlined). (M) infiltration of erythrocytes admixed with lymphoplasmacytic cells (arrows) in the renal parenchyma. (N) necrosis of the renal tubular glands. (O) nodular lymphocytic abscess in the kidney. H and E stain. 400x magnification. Bar=100 μm.

related to the gastrointestinal tract, with green- white diarrhea being the most notable sign. This type of diarrhea can be attributed to the presence of unmetabolized bile acids in the feces. Physiologically, impairment of the entero- hepatic function may yield excessive bile acid concentration in the colon, which, in turn, would activate certain receptors for colonic motility stimulation and fluid secretion [13]. However, this cascade of events is still not established in NDV infection. Nevertheless, the presence of green-white diarrhea is highly suggestive of NDV or PPMV-1 infection as supported by the experimental inoculation of velogenic NDV in naive birds [14], which later produced similar clinical manifestations noted in this study.

Most gastrointestinal organs exhibited moderate to severe macroscopic lesions during inspection. On gross examination, the luminal surface of the crop and esophagus appeared red and hyperemic. The samples were also observed to be extremely thin-walled. Most proventriculus and ventriculus showed dark discoloration of mucosal surfaces (Figure 1-C, D). Moreover, the esophago-proventricular junction and distal proventriculus both presented mucosal erosion covered by yellow- to green-colored membranes. Microscopically, the esophagus displayed severe congestion with submucosal lymphocytic infiltration at the level of the esophageal-crop junction. The proventriculus presented a denuded mucosal surface with marked degenerative changes in the majority of the proventricular tubular glands. Likewise, hyperemic submucosal layers with mild mucosal hemorrhage were seen in the ventriculus. The majority of the results obtained in these analyses share high similarities with other reported studies in the field [7, 11, 14]. The presence of circulatory disturbances such as hyperemia, congestion, and hemorrhage in the gastrointestinal organs has been attributed to the vascular endothelial damage caused by the virus during active infection. In addition, moderate to marked degeneration of the glandular epithelium of the organs noted in this study also coincides with other reports [12, 15]; however, it is noteworthy that the findings of the former were obtained from a guinea fowl infected with NDV.

Meanwhile, at the level of the small intestine, the serosal surface of the organs showed moderate dark discoloration with segmental areas of severe hyperemia and congestion (Figure 1-E). Microscopically, the lesions found were mostly confined to the mucosal surfaces and submucosal layers of the organs; with marked epithelial mucosal cell necrosis and lymphocytic infiltration being the most common lesions. In addition, the presence of lymphoid depletion most notably in the ileum was observed. Supportive evidence of extensive mucosal damage can be seen in the entire gastrointestinal tract (Figure 2-B). These findings were also described in several studies about virulent strains of avian paramyxoviruses [11, 14], indicating that diffuse lymphoplasmacytic infiltration to the gastrointestinal mucosa could be a common finding of the viral infection.

Most of the microscopic observations in this study follow the same results obtained from a previous study [7]. The widespread sloughing off of the mucosal epithelium is largely affected by the ability of the virus to replicate within the intestinal lymphoid tissues, which subsequently causes blood endothelial damage leading to vasculitis, hemorrhage, and edema [16]. In the process of virus replication, enhanced pathogenesis is observed when the host immune response is already overwhelmed by the number of virus particles [17]. Although the process has not been established in PPMV- 1 yet, excessive pro-inflammatory responses by the gut-associated lymphoid tissues (GALT) and individual enterocytes, as induced by the virus during infection, can also explain the rapid destruction and denudation of the intestinal tract [18].

The gross appearance of the liver showed the presence of multifocal areas of moderate to severe congestion with dark red discoloration at the visceral surface (Figure 1-F). Histologic sections of the tissue samples showed zones of circulatory disturbances such as congestion and hemorrhage, along with multifocal lymphocytic nodules, hepatocyte necrosis, and mononuclear cell infiltration; presenting similar gross observations in previous studies

[5, 14]. Correspondingly, our histopathologic findings also agree with reported cases of NDV infection [11, 15], citing the presence of necrosis in the organ with lymphoplasmacytic infiltrates traversing through the hepatic parenchyma. Additionally, the presence of fatty vacuolar degeneration (Figure 2-C) within the hepatic parenchyma observed in this study is also supported by previous studies [5, 19].

The appearance of hepatic steatosis indicates an abnormality in lipid metabolism, with the autophagic signaling related to the apoptotic pathways being implicated as one of the molecular mechanisms for its occurrence [20].

Parallel to the findings in the liver, the pancreas appeared grossly as pale organs with inconsistent pigmented patterns (Figure 1-G), a finding that had been reported in a previous study. In retrospect, a study on NDV infection also observed the presence of white foci in the pancreas of NDV-infected birds which was later revealed to be caused by the histologic derangements in the organ [12]. In this study, microscopic examination of the organ revealed extensive abscess formation and multifocal areas of congestion and necrosis (Figure 2-D). The exact mechanism in this regard has not been established yet, however, a similar report implicated that it may be strongly related to the affinity of the velogenic strain to the digestive tract [12], causing an extensive degeneration in the organ with concurrent depletion and necrosis of the acinar cells. Although these findings were not strictly observed in all tissue samples, as with those described by [21], it is still noteworthy that disparity among the studies can be an indication of variability in the disease progression among isolates.

3.3 Lymphatic system

All submitted spleen samples were mildly enlarged. Numerous multifocal white pinpoint markings on the spleen surface were also highly noticeable (Figure 1-H). Microscopic analysis of the organ showed diffuse congestion with marked lymphoid depletion (Figure 2-E), along with hemosiderin deposition and moderate hemorrhage and congestion. Our observations in both gross and microscopic examinations share high similarities with a previous study [11] which demonstrated splenic enlargement and mottling in experimentally infected birds. While the previous report showed how experimental infection affects birds, the enlargement of the organ in this study can be linked to its initial response to viral infection. During viremia, endothelial damage will cause abnormal perfusion of blood, to which the spleen will react by clearing the vascular system. This, in return, would make the spleen overactive [22]. On the other hand, significant microscopic changes in the spleen can be explained by the forced mobilization of lymphocytes at a faster rate caused by virulent NDV strains. Consequently, the loss of compact lymphoid tissue follicles in infected birds can be anticipated, as previously described [23].

Meanwhile, histologic evaluation of the thymus showed the presence of congestion and mononuclear cell infiltration on its parenchyma. Despite showing distinct lobules, the tissue samples showed the development of cystic structures lined by epithelial cells which contain mucoid substances. These findings are similar to those reported [24], but the exact mechanism remains poorly elucidated. In addition, necrosis and degeneration of lymphoid tissues were observed in the organ which can be attributed to the apoptotic mechanism during NDV infection [11]. The results of this study conform to those previously demonstrated cases [25], suggesting that apoptosis in the lymphoid organs could be a consequence of viral replication and increased interferon (IFN) stimulation.

3.4 Nervous system

Most brain samples presented moderate to severe hyperemia of the cerebral surface. Vascular engorgement was also evident in all the samples, with one brain showing severe hemorrhagic and necrotic surfaces (Figure 1-I). In situ inspection revealed the presence of brain edema in all birds. Microscopic examination of the organs revealed the presence of severe lymphoplasmacytic non-suppurative encephalitis with neuronal degeneration, vasculitis, gliosis, and status spongiosus. Specifically, the cerebellum consistently showed Purkinje cell degeneration and diffuse status spongiosus (Figure 2-F, G). In the cerebrum, focal areas of congestion and vasculitis, neuronal degeneration, and gliosis (Figure 2-H) were markedly observed. Likewise, large areas of hemorrhage with extensive loss of

tissue architecture were observed in the spinal cord. Variable signs of cell injury such as neuronal and Wallerian degeneration, status spongiosus, and gliosis were also found in 100% of the samples. In 1990 [19], a study demonstrated the lethal effects of PPMV-1 infection on the brain which yielded similar results obtained in this study. The formation of status spongiosus (Figure 2-G, J) has been linked to the degenerative response of the organs to the infection, indicating disturbance in the glial cell function [26]. Correspondingly, the lesions in the brain and spinal cord have severely affected the motor function of the organs which led to the development of the neurological signs observed. Furthermore, the presence of extensive malacia in this study suggests a high tropism of PPMV-1 to infect the nervous tissues [12].

3.5 Respiratory system

Among the different respiratory organs, the lungs were observed to be the most affected. Velogenic strains of NDV can cause mild to severe respiratory distress to an infected animal. In this study, only mild respiratory disturbance was observed among all pigeons; and, the reason for this can be attributed to the rapid clearing of the virus, as supported by a previous study [27]. Grossly, severe hemorrhage and edema of the organ were noted in all tissue samples (Figure 1-K). Microscopic analysis indicated the presence of severe pneumonia, heterophilic and lymphocytic; with intralesional hemorrhage, edema, and anthracosis (Figure 2-L). In addition, hyperplastic nodular lymphoid tissues and carbon deposition were also observed in the samples. Based on the findings, all pigeons had suffered from severe hemorrhagic pneumonia with mononuclear cell infiltration, nodular lymphoid hyperplasia, and anthracosis. Similarly, on histopathology, tracheal tissue samples showed hemorrhages with moderate to severe sloughed-off epithelium (Figure 2-K). These histopathological changes in both the trachea and lungs coincide with the ones previously observed [7]. Impairment of the respiratory tract can be attributed to the initial attachment of the virus to the respiratory epithelial cells during natural infection. Upon attachment to the host cell receptors, it induces the production of specific immunoglobulin A by the regional immunity system [7]. The progression of these events may have caused mucosal congestion and exudation; thereby, affecting normal respiratory function.

3.6 Urinary system

Grossly, all kidney samples presented moderate dark red discoloration (Figure 1-L). In situ, the organs appeared congested with visible inconsistent polygonal surface patterns. On histopathology, tissue sections showed focal areas of congestion with diffuse necrosis of the renal tubular epithelial cells (Figure 2-M). The renal parenchyma also presented moderate degenerating glomeruli with increased Bowman's space (Figure 2-N) and multiple renal abscesses composed of lymphocytes (Figure 2-O). Considering these, all tissue samples were morphologically diagnosed with interstitial nephritis with focal areas of congestion, diffuse tubular epithelial necrosis, and lymphocytic abscess. In 2015, similar observations were documented [12], wherein infected doves had developed renal tubular epithelial cell necrosis and moderate lymphocytic interstitial nephritis even without overt urinary signs. The current findings obtained were also in line with a previous report [7], demonstrating the presence of hemorrhagic nephritis in field cases of NDV. The histologic changes observed in the kidneys can be associated with the hematogenous spread of the virus from the respiratory and gastrointestinal tract. NDV distribution to the kidneys due to secondary viremia is already established in previous reports [7, 12]; although, the direct renal tropism of the virus in feral pigeons has been proposed [20]. The formation of renal abscesses and presence of the lymphocytic infiltrates within the renal tubular interstitium can be associated with the unwarranted response by the immune system to the invading virus.

4. Conclusions

The observations made in this investigation have proven the multi-systemic involvement of PPMV-1 in pigeons from Luzon, Philippines. Quantification using a standard gauge system could be implemented for a standardized lesion grading. Mean death time

and intracerebral pathogenicity index tests may be performed to establish the pathogenicity and virulence of the current isolate. Immunohistochemistry can be done as well to visualize and detect the presence and distribution of viral nucleoproteins in the target organs. With these tests, tissue tropism of the virus can also be elucidated. Together, these findings may help in understanding the pathogenesis of the disease, which may aid in the formulation of diagnostic, preventive, and control procedures against PPMV-1 in the country.

Author Contributions

Conceptualization, D.V.U. and C.B.M.; Methodology, D.V.U., C.B.M., and M.G.L.; Investigation, C.B.M. and M.G.L.; Writing – Original Draft, C.B.M.; Writing – Review and Editing, D.V.U. and C.B.M.; Funding Acquisition, D.V.U. and C.B.M.; Resources, D.V.U. and C.B.M.; Supervision, D.V.U. and M.G.L.

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Conflict of Interest

The authors declare no conflict of interest.

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