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Article:

Clinical and Histopathological Investigation of Pigeon Orthoavulavirus in Sohag Governorate

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Abstract

Newcastle disease (ND) is a highly contagious and destructive viral disease that affects poultry and other birds worldwide. Pigeon paramyxovirus serotype 1 is the antigenic "pigeon variant" of the virus that causes ND in pigeons, also known as avian Orthoavulavirus. Central nervous system symptoms and occasionally high death rates are noted during infection. This study aimed to examine the columbiforms naturally infected with avian orthoavulavirus based on clinical signs, gross lesions, and histological changes. Some organs from affected pigeons were obtained for the study. They compromise the brain, spinal cord, liver, trachea, pancreas, proventriculus, duodenum, and heart. The histological examination of these organs revealed numerous histological alterations, including fibrosis and vascular alterations in certain areas, as well as degenerative, necrotic, and inflammatory alterations in all the tissues examined. Our study explains why it is necessary to investigate avian orthoavulavirus infection in pigeons in the Sohag governorate. It is an important step toward a deeper comprehension of the virus in columbiforms. Our study results showed that because the virus can replicate in many tissue organs, it causes lesions and major problems.

Keywords: Avian orthoavulavirus, Histopathology, Newcastle disease, Pigeon, Pigeon paramyxovirus.

Introduction

Newcastel disease is a highly contagious and destructive viral disease that affects poultry and other birds worldwide, recognized by the World Organization for Animal Health as significantly affecting international trade and involving approximately 240 bird species, including pigeons **[1–4]**. ND in pigeons (Columba Livia) is closely related to ND in poultry. This disease was first identified in 1980 in the Middle East and subsequently disseminated globally, becoming endemic among wild and domestic pigeons in numerous countries. In Egypt, pigeons showed the clinical signs of PPMV-1 in 1981 **[5–8]**.

Avian orthoavulavirus type 1 strains (AOAV-1) are the cause of ND, a significant avian illness that results in high mortality rates and financial losses. Over the past 30 years, several outbreaks in Columbiformes have been documented globally. These outbreaks have been attributed to a modified strain of pigeon paramyxovirus type 1 (PPMV-1) **[9]**. These viruses belong to class II genotype XXI **[10].**

There is a wide spectrum of clinical manifestations associated with AOAV-1 infections, ranging from mild (with little to no mortality) to systemic (with moderate to high mortality) **[11]**. ND is the name given to the latter form, which is brought on by virulent strains of AOAV-1.

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Pigeons can become infected with AOAV-1 at any age or during any season. Pigeons are thought to be natural reservoirs. The virulence of the particular isolate and the host's immunity determine the clinical symptoms in infected pigeons **[6, 8, 12–14]**. High rates of morbidity and mortality are caused by this virus in pigeons; it replicates in various tissues, primarily the brain, lung, kidney, trachea, spleen, liver, bursa of Fabricius, and pancreas **[15, 16].**

In recent years, AOAV-1 has become more common in pigeons, and asymptomatic pigeons have a major role in infection **[6]** The severity of the infection may be reduced by treating sick birds **[12]** AOAV-1 outbreaks in flocks pose a threat to chickens and other birds. The virus poses a significant threat due to changes in the AOAV-1 genome composition and transmission to chickens **[17, 18]**. Pigeons must be taken seriously as a potential source of NDV infection and disease for commercial poultry flocks **[19]** They may be sub-clinically infected, spreading the virus for a significant time without clinical signs **[20, 21]**.

As there have been few studies on the histopathology of orthoavulavirus in pigeons, the purpose of this study was to identify clinical signs, postmortem lesions of Avian Orthoavulavirus-1 infection in pigeons in Sohag Governorate, and histopathological lesions that play a preliminary role in the diagnosis of AOAV-1 infection **[22].**

Materials and Methods

Ethical considerations

The current study was approved by the Veterinary Medical Research Ethics Committee, Faculty of Veterinary Medicine, Sohag University, Sohag, Egypt, according to the OIE standards for the use of animals in research, with number Soh.un.vet/00078 R.

Source of samples

Samples were collected from 43 diseased or recently dead pigeons in various cities throughout Sohag Governorate. The samples were collected between July 4th, 2022, and March 2024, during different seasons. Standard procedures were followed during diagnostic necropsies. The brain, spinal cord, liver, trachea, pancreas, proventriculus, duodenum, and heart were collected for histopathological investigation.

Samples preparations

The brain, spinal cord, liver, trachea, pancreas, proventriculus, duodenum, and heart **Table. (1)** were fixed at 10% neutral buffered formalin and were dehydrated in a graded alcohol series, cleared with xylene, embedded in paraffin wax, sectioned at 4-5 um thickness, and stained with hematoxylin and eosin for histological examination with light microscopy **[23]**. Sections of stained tissue were examined under light microscopy (Olympus, Japan) and photographed with a digital camera (Olympus, Japan).

Table 1. Collected histopathological samples.

Histopathological samples	Brain	Liver	Gizzard	Heart	Trachea	Duodenum	Pancreas	Spinal cord
Number	17	18	5	12	10	9	11	4

Results

Clinical findings

Pigeons infected with avian orthoavulavirus were showing the following clinical signs: neck twisting, greenish diarrhea, and paresis or paralysis of wing and/or leg **Figure. 1**.

Gross lesions

Inspection of various organs in postmortem examination of infected pigeons **Figure. 2**shows congestion of brain blood vessels **(Figure. 2a)**, the content of gizzard is green due to anorexia **(Figure. 2b)**, hemorrhage is visible following the removal of gizzard content and the cuticle **(Figure. 2d)**.

Histopathological findings

In the brain, there were multifocal areas of neuronal necrosis and gliosis characterized by shrunken, eosinophilic, and pyknotic neurons **(Figure. 3a)**, neuronophagia **(Figure. 3b)**, neuropil vacuolation, congestion of blood vessels, and lymphoplasmacytic perivascular cuffs **(Figure. 3c)**. However, the main lesion in the spinal cord was demyelination of nerve fibers in the white matter, represented by extensive vacuolation and spongy appearance of spinal cord **(Figure. 3d)**.

In the liver, congestion of blood vessels in the portal area and perivascular mononuclear inflammatory cell infiltration **(Figure. 4a)**. There was vacuolar degeneration

of hepatocytes **(Figure. 4b)** as well as multifocal areas of mononuclear cell infiltration **(Figure. 4c)**. In the portal area, there was damage of endothelial lining of portal vein, and the surrounding hepatocytes showed necrotic changes characterized by acidophilic cytoplasmic and pyknotic nucleus **(Figure. 4d)**.

The proventriculus was the most affected organ in the alimentary tract, as it appeared with thickness in the mucosa due to profuse leucocytic cell infiltration and submucosal hemorrhage **(Figure. 5a)**. In addition, thickening of interlobular septa and severe necrosis of glandular tissue **(Figure. 5b)**. However, the duodenal mucosa showed degeneration, necrosis, and submucosal inflammatory cell infiltration **(Figure. 5c)**. In the trachea, there was necrosis, sloughing of respiratory epithelium, mononuclear cell infiltration, and lumen filled with necrotic material **(Figure. 5d)**.

In the heart, there was multifocal mononuclear myocarditis characterized by myocardial degeneration, fragmented muscles, intermuscular edema **(Figure. 6a)**, and congested blood vessels **(Figure. 6b).** The predominant lesion recorded in the pancreas was necrotizing pancreatitis, which characterized by necrosis of pancreatic acini, thickening of interlobular septa, and mononuclear cell infiltration **(Figure. 6c, d)**.

Figure 1. Clinical manifestations of pigeons infected with AOAV-1: Showing the twisted neck (neurological sign) (arrow) and greenish diarrhea (stars).

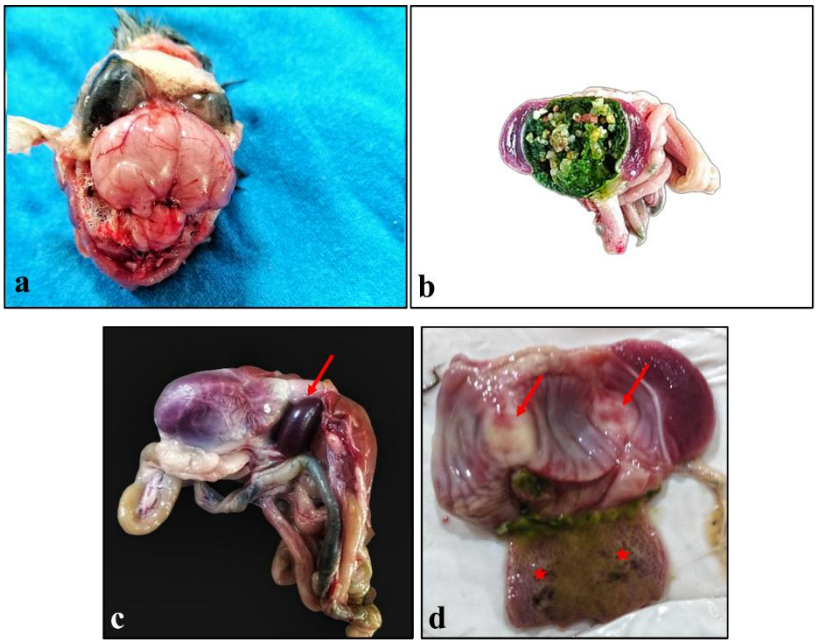

Figure 2. Gross lesions of pigeons infected with AOAV-1: a) Congestion of brain tissue. b) Greenish content of gizzard. c) Splenomegaly (arrow). d) Haemorrhage in gizzard after removing of cuticle (arrows) and in proventriculus (stars).

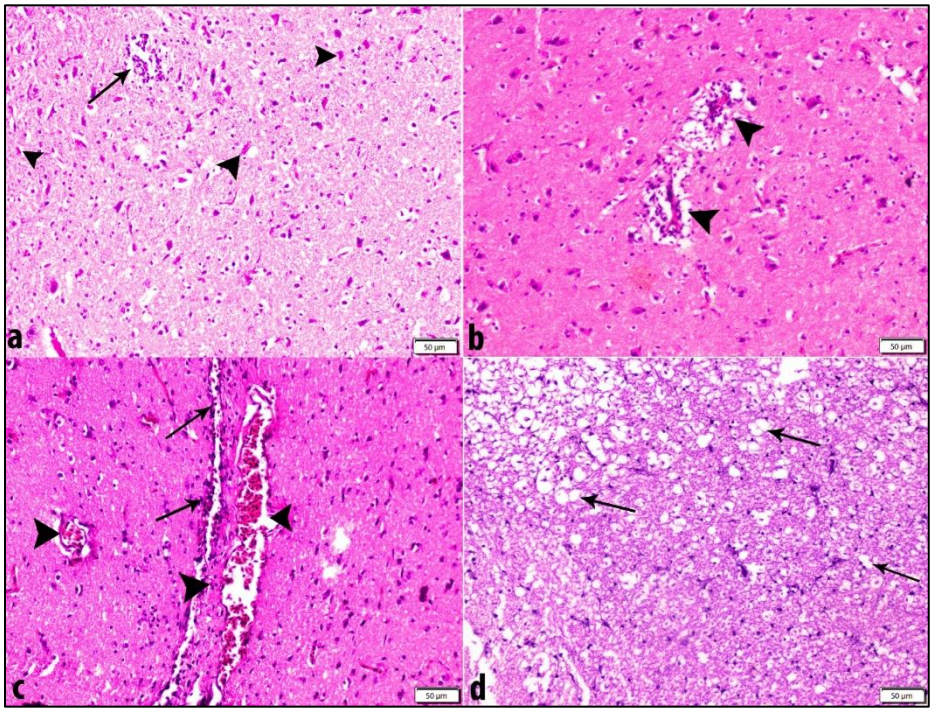

Figure 3. Histopathological sections of brain and Spinal Cord of pigeon infected with AOAV-1: a) Neuronal necrosis are characterized by shrunken, eosinophilic, and pyknotic neurons (arrow head) and focal gliosis (arrow) in the brain (HE, Bar = 50 µm). b) Brain tissue section showing necrotic neurons undergoing phagocytosis (neuronophagia) by many microglia cells (arrow head) (HE, Bar = 50 µm). c) Blood vessels from the brain presenting congestion (arrow heads) and perivascular cuffs are composed mainly of lymphocytes (arrow) (HE, Bar = 50 µm). d) Demyelination of nerve fibers in the white matter of spinal cord represented by extensive vacuolation and spongy appearance of spinal cord (arrows) (HE, Bar = 50 µm).

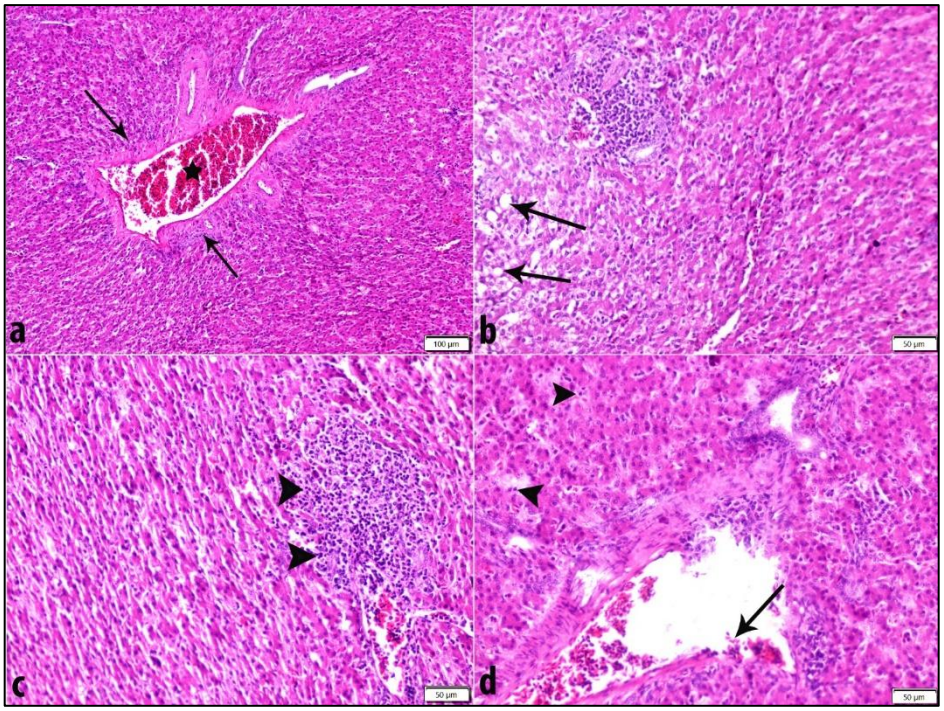

Figure 4. Histopathological sections of liver of pigeon infected with AOAV-1: a) Congestion of blood vessel in the portal area (star) and perivascular mononuclear inflammatory cells infiltration (arrow) (HE, Bar = 100 µm). b) Vacuolar degeneration of hepatocytes represented by cytoplasmic vacuoles and pyknotic nuclei (arrows) (HE, Bar = 50 µm). C) Focal area of mononuclear cellular infiltration (arrowheads) (HE, Bar = 50 µm). d) Damage of endothelial lining of portal vein with desquamated endothelium in the lumen (arrow), lymphocytic cells infiltration and necrosis of hepatocytes represented by acidophilic cytoplasm and pyknosis of nucleus (arrowheads) (HE, Bar = 50 µm).

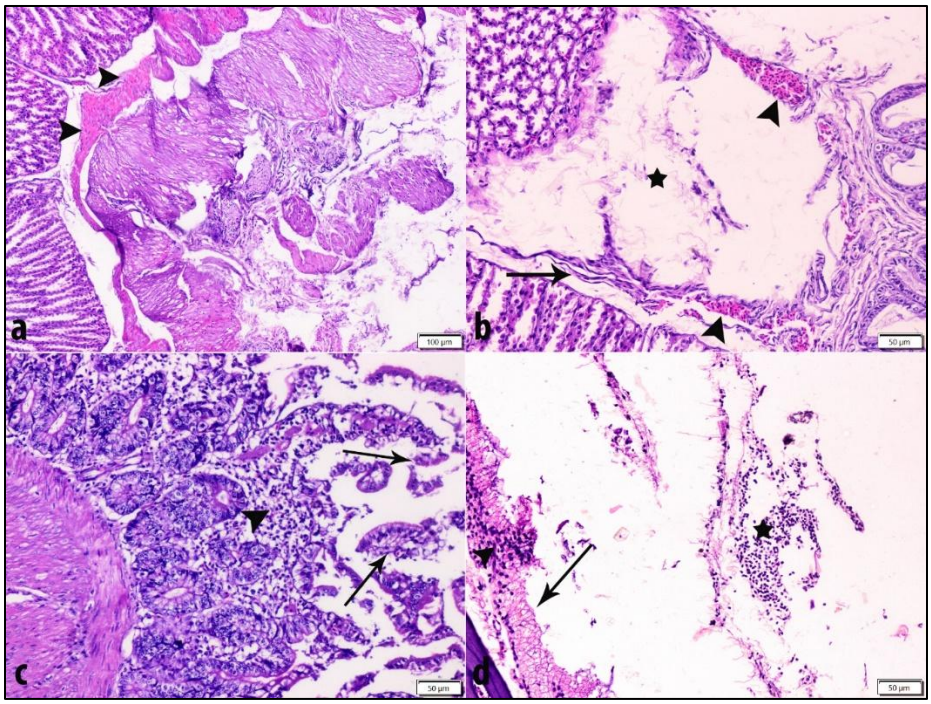

Figure 5. Histopathological sections of proventriculus, duodenum and trachea of pigeon infected with AOAV-1: a) Thickening of proventricular mucosa due to necrotic changes and profuse leucocytic cells infiltration in mucosa and submucosal hemorrhage (arrowheads) (HE, Bar = 100 µm). b) Severe proventricular gland lobule necrosis, sloughing (star) and thickening of inter-glandular C.T by edematous fluid (arrow), leucocytic cells infiltration and hemorrhage (arrowhead) (HE, Bar = 50 µm). C) Duodenal section showing degeneration and necrosis in the mucosa (arrows), leucocytic cells infiltration (arrowhead) and duodenal gland degeneration (HE, Bar = 50 µm). d) tracheal section showing severe necrosis, sloughing of lining epithelium (arrow), profuse inflammatory cells infiltration (arrowhead) and tracheal lumen contained necrotic materials and inflammatory cells (star) (HE, Bar = 50 µm).

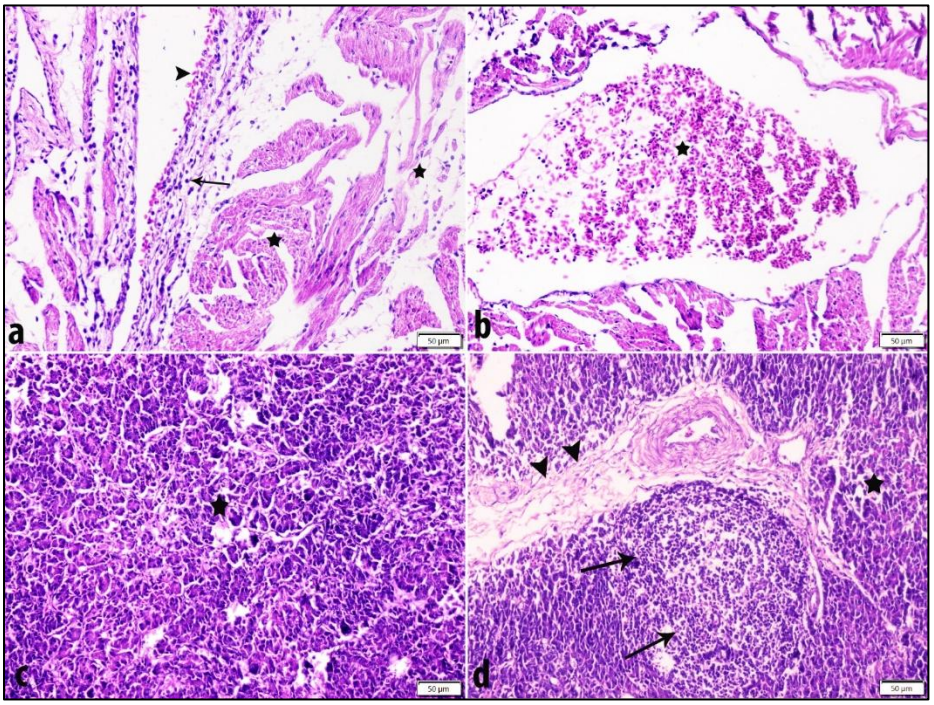

Figure 6. Histopathological sections of heart (a, b) and pancreas (c, d) of pigeon infected with AOAV-1: a) myocarditis are characterized by necrosis, intermuscular edema (star), mononuclear cells infiltration (arrow) and hemorrhage (arrowhead) (HE, Bar = 50 µm). b) Congestion of blood vessel with endothelial lining damage (star) (HE, Bar = 50 µm). C, D) necrotizing pancreatitis characterized by necrosis of pancreatic acini (star), thickening of interlobular septa (arrowhead) and mononuclear cells infiltration (arrow) (HE, Bar = 50 µm).

Discussion

In this study, pigeons exhibited neurological signs such as ataxia, head tremors, incoordination, and twisted neck, similar to those seen in the Columbiformes **[7, 24]**. The central nervous system accounted for the majority of the lesions observed, which strongly implies that the strain under investigation is neurotropic. Clinical manifestations and pathological lesions driven from elevated interleukin-6 (IL-6) levels indicate a rise in AOAV-1 infections in all infected tissues. IL-6 expression in AOAV-1-infected chickens and pigeons encourages tissue damage and inflammation **[25, 26]**.

The application of histopathology in combination with other diagnostic instruments as histochemical and molecular tools is a crucial to confirm the diagnosis of avian orthoavulavirus infection. A high level of virus expression is correlated with the severity of histopathological lesions. More pronounced histopathologic lesions were seen in the brain, spinal cord, and coelomic cavity organs, including liver, proventriculus, trachea, pancreas, duodenum, and heart, matched with those previously reported **[9, 27, 28]**.

Histopathologic examination of the central nervous system (CNS) showed lymphoplasmacytic perivascular cuff, neuronophagia, neuropil vacuolization, and neural necrosis and gliosis. The presence of CNS lesions in the majority of the pigeons examined in our study is explained by the significant amount of AOAV-1 detected in the brains of experimentally infected pigeons, which points to a high concentration of this viral strain in the central nervous system **[19]**. Necrotizing pancreatitis was typically seen as an acute alteration characterized by vacuolar degeneration and necrosis of virus-infected exocrine cells. The subacute stages of the pancreatic infection were characterized by interstitial expansion and inflammatory cells replacing the lost parenchyma over degeneration and necrosis **[24]**. Activated proteolytic enzymes and proinflammatory cytokines released into the bloodstream by acute pancreatitis may result in multi-organic failure and a systemic inflammatory response syndrome, which could kill the bird **[29]**. As reported for NDV in chickens **[30]**, AOAV-1 may be effectively eliminated by aerosolized respiratory secretion due to its ability to infect the respiratory epithelium in the trachea, bronchi, parabronchi, and abdominal air sacs. Inhalation may be a significant route of AOAV-1 infection for the same reason.

Conclusion

The prevalence of avian orthoavulavirus infection in pigeons has increased recently, and since pigeons are considered reservoirs, poultry is seriously at risk. The need to look into AOAV-1 infection in columbiforms in Sohag Governorate is explained by our study. It is a significant step towards a better understanding of the virus in its natural hosts. The findings showed that although the AOAV-1 infection causes gross and histological abnormalities in many tissues, the most severely affected organs are the brain and most of the celomic cavity organs. The findings demonstrated that the virus causes lesions and serious issues because it can replicate in many tissue organs.

Conflict of interest

The authors declare no conflict of interest regarding the publication of this article.

Authors' contribution

Each author received an equal share of the work. The final draft of the manuscript has been reviewed and approved by all authors.

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