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Original Research Article

COMPARATIVE PATHOLOGICAL STUDY OF EXPERIMENTAL INFECTION OF NEWCASTLE DISEASE VIRUS GENOTYPES VII IN CHICKENS AND DUCKS

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Abstract : Newcastle disease virus (NDV) is a highly contagious virus infects a wide range of avian species including chicken and ducks. The disease can be fatal for many types of them. It was believed that ducks and geese act as natural reservoirs of NDV because of their natural resistance. Recently, NDV isolates were pathogenic for ducks. Therefore, our study aimed to investigate the pathogenicity of highly pathogenic NDV genotype VII in white Ross commercial broiler chickens and Mallard ducks on the basis of clinical, histological observation and immunohistochemistry findings of virus antigen through studying the sequential pathological lesions of both species at 2nd, 5th and 10th dpi. Both species were inoculated via intranasal route by NDV genotype VII (accession number MT887290). Clinical lesions and mortalities were evident mostly in chickens rather than in ducks. Respiratory, digestive and nervous signs were clearly manifested in chickens. Ducks showed low level of mortalities and the nervous signs were the most obvious symptoms. Microscopic findings of chickens showed severe pancreatitis, pneumonia, enteritis as well as pericarditis and brain malacia. Similar findings were noticed in ducks but with lower degree of severity. Brain lesions in ducks included encephalitis and perivascular reaction mostly within the cerebellar tissue. Immunohistochemistry reaction against NDV antigen showed marked immunostaining within epithelial cells, purkinje cells and inflammatory cells mostly the macrophages. The immunohistochemistry reaction was correlated with the pathological findings in both species. In conclusion, the mortality and lesions of NDV in both species were correlated with systemic alterations associated with pancreatitis, pneumonia and encephalopathy. Moreover, the obtained data revealed possible susceptibility of ducks to NDV genotype VII, which might be need forthcoming viral vaccination strategy for ducks against NDV.

Key words: Newcastle disease; chicken; duck; virulent NDV-genotype VII challenge

Introduction

Newcastle disease (ND) is an acute and highly contagious disease caused by Newcastle disease virus (NDV), a member of the avian avulavirus 1 and formerly as avian paramyxomvirus -1 (APMV-1) (1) paramyxovirus serotype-1 (APMV-1) (1). NDV belongs to the genus *Orthoavulavirus* from the Paramyxoviridae family (2, 3). Viruses of Newcastle disease (ND) currently infected a wide range of avian species numbering about 27 orders of birds (4). Both domestic and wild species are affected with varying degrees of mortality and morbidity depending upon the host species and also the strain of virus (5).Among poultry species, chickens and turkeys are the most susceptible, while ducks and geese are the least susceptible (6, 7). Waterfowl are believed to act as a reservoir of a virulent ND viruses. However the epidemiology of these viruses in wild birds is unclear (8).

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Ducks are considered as the natural reservoir for all genotypes of NDV and typically experienced asymptomatic infection or little pathology with velogenic NDV strains lethal to chickens (9,10). Virulent strains have been isolated in commercial ducks with no obvious clinical signs recorded (11). However, recent outbreaks of high virulence genotype VII NDV strain showed high mortalities in ducks (12, 13), but the pathogenesis of genotype VII NDV in ducks has not been clearly clarified.

Clinical signs produced by the ND virus are influenced by numerous factors, including the species infected, the age and the production status or health of the host, especially in the presence of co-infections with other viruses, route of inoculations, and association with bacteria or parasites (12). In addition, the level of immunity to the virus, that may be passively derived maternal antibodies or actively induced by vaccination (14)? The susceptibility of ducks to infection with NDV decreased with age. Ducks infected with Newcastle via I/N and I/O routes exhibited clinical signs, which were primarily neurologic. The rate of virus isolation in tissues from infected ducks was generally low, even in those from dead birds, and it appeared to be unrelated to bird age and infection route. At necropsy most dead ducks did not have obvious postmortem lesions (12).

Our study aimed to investigate the main difference in pathogenicity of highly pathogenic NDV genotype VII in male white Ross commercial chickens and male Mallard ducks through clinical, pathological lesions observation and immunohistochemical localization of viral antigen through sequential examination of different organs.

Material and methods

Animals and experimental design

About forty five male white Ross commercial chickens and forty five male Mallard ducks at day one old were raised till 28-day-old. All groups were reared separately in bird-standard batteries in biohazard rooms and given water and commercial diets modified according to bird breeds and ages. The food and water intake were given ad-libitum and the groups were monitored clinically. Each raised Ross chickens and Mallard ducks were divided into two groups control group containing 15 birds and challenged group containing 30 bird. The challenged groups (chickens and ducks) infected via intranasal route with 0.1 ml of viral solution containing 10^6 EID_{50} of NDV isolated strain. The virus strain was isolated in The National Laboratory for Veterinary Control on Poultry Production, Animal Health Research Institute, Cairo, Egypt, under the accession number (*MT887290*). The isolated strain was tested for its pathogenicity index according that mentioned by OIE (15); Intracerebral pathogenicity index (ICPI) and Mean Death Time (MDT) were 1.83 and 48 h respectively.

After inoculation, birds from challenged chicken and duck groups were euthanized under Halothane inhalation anesthesia on the 2nd, 5th, 10th day post inoculation (dpi). The ethics committee of experimented animal from Kafrelsheikh University approved the experimental protocol on 2019/8.

Sampling

Necropsy inspection of dead and euthanized birds were carried out immediately and tissue samples from brain, eyelid, nostrils, trachea, spleen, thymus, bursa, proventriculus, gizzard, intestine, cecal tonsil, lung, heart, liver, pancreas, and kidney were collected and fixed in 10% neutral buffered formalin (NBF). Blood samples were collected from brachial and wing veins for haemagglutination inhibition (HI) test. The blood samples were allowed to coagulate and were centrifuged at 3500 rpm for 15 min to obtain serum, and the serum samples were kept in Eppendorf tubes at −20 °C until tested

Haemagglutination inhibition (HI) test

Haemagglutination inhibition (HI) test were carried out for NDV antibody titers in collected samples from 5 birds per each group on 2nd, 5th and 10th dpi according to OIE (15), using 4 HA units per 50 μl of antigen suspension and the serum was diluted using two folds serial dilution. The results were expressed as $\log_2$ of the reciprocal of the last dilution. Chicken sera don't need pretreatment before HI test performance. On the other hand, other species sera as ducks need to remove nonspecific hemagglutination agents. Sera from ducks were treated by adsorbing the collected duck sera by adding 0.025 ml of packed chicken RBCs to each 0.5 ml of collected duck antisera samples (15).

Histopathological study

For histopathological examination, the fixed tissues were routinely processed, sectioned (5-6 µm thick section) and stained with haematoxylin and eosin stain according to (16). Lesions score of examined H&E stained sections was performed as previously mentioned by Anis et al, (17) which graded the lesions as the following; normal tissues without any pathological alterations (-), mild lesions which represented with focal and slight degenerative changes (+), moderate lesions which associated with remarkable degenerative changes reach to necrosis level (++),severe degree accompanied with diffuse necrotic changes (+++).

Immunohistochemical reaction (IHC)

For the IHC, tissue sections were applied on Poly- L- Lysine coated slides which deparaffinized, and rehydrated as routine tissue technique. A heat induced antigen retrieval, blocking of non-specific protein binding and endogenous peroxide were performed. The tissue sections were then incubated with a polyclonal primary antibody (rabbit anti NDV Ig) for overnight which was obtained kindly from faculty of veterinary medicine Cairo University, Cairo, Egypt and was followed by incubation with horseradish peroxidase-conjugated goat polyclonal secondary antibody to rabbit Ig (SM802 EnVision™ FLEX/HRP). Color was developed with 3, 32 - Diaminobenzidine (DAB) substrate (DM827 EnVision™ FLEX DAB+ Chromogen) and counterstained with Mayer's hematoxylin and subsequently observed in the optical microscope, in a200x magnification (18, 19). Control negative samples were done by adding saline instead of the primary antibody which used as a reaction guide to avoid nonspecific immunostaining.

Statistical analysis

Data in the tables are presented as geometric mean of NDV (Log base 2) of HI antibody titers (± standard deviation) in all groups. The data were analyzed by SPSS 11.00 software for Windows (SPSS, Chicago, IL, USA) using T-test.

Results

Intracerebral pathogenicity index

The pathogenicity of the isolated virus was determined by high ICPI (1.83) (Table 1).

Clinical signs

The early stage of NDV inoculation within chickens showed respiratory and digestive manifestations. While the nervous signs started later on 5th dpi, and appeared as deviated head and neck and suboptimal response of perching reflex. On the other hand, the inoculated ducks showed clear nervous signs on the 4th dpi which was observed as incoordination, ataxia and reluctance to move.

Death occurred in both chicken and duck challenged groups. Two ducks were found dead on the 5th dpi. Chicken challenged group demonstrated 15 dead birds; 4 birds on the 5th dpi, then 6 on the 6th dpi, 3 on the 7th and 2 on the 10th dpi.

The deaths and clinical manifestation of both chickens and ducks inoculated groups were summarized in (Table 2).

Gross lesions

The macroscopic lesions of examined birds of both inoculated chickens and ducks were summarized in (Table 2) and Figure 1. No gross lesions were detected in control chicken and duck groups examined at different time points of the study. On the 2nd dpi, chickens showed congestion and petechial hemorrhages in the thymus. The gall bladder was distended with bile. Ducks showed petechial hemorrhages on the thymus and slight congestion of bursa. On the 5th dpi, chickens showed edema of the proventriculus tips, slight hemorrhages at the esophageal and junction. Hemorrhages within the different organs were also observed. On the 10th dpi, the lesions were in a diffuse manner in challenged chickens but with less degree in the inoculated ducks.

NDV specific antibody titers

Results of antibody titers against NDV were summarized in (Table 3). There was an increased antibody levels throughout the all experimental periods.

Table 1: ICPI calculation

Clinical signs	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day 8	Total	Score	ICPI
Normal	2	0	0	0	0	0	0	0	2×0	0	1.83
Sick	7	2	0	0	0	0	0	0	9×1	9	
Dead	1	8	10	10	10	10	10	10	69×2	138	
									Total	147	

Table 2: Summary of clinical signs and gross lesions in chickens and ducks inoculated with NDV MT887290 strain via the intranasal route

G		Day post infection (dpi)		
		2-4 dpi	5-7 dpi	8 up to 10dpi
Chicken	Clinical signs	• Signs observed on 2nd dpi were slightly decreased feed and water intake • On 3-4 dpi: • Off food, ruffled feathers • Closed eye , inflamed eyelid and watery eyes /+++ • Lethargy and recumbence, abnormal respiratory sound /+++ • Deviated head/++	• Signs severely progressed than 4th dpi • Mucoid nasal discharge /(+++) • Nervous signs/+ • Labored breathing, grasping (+++)	• As signs observed on 7th dpi.
		• No mortalities	• 4 died birds on 5th dpi per 30 birds (mortality rate 13.33 %) • 6 died birds on 6th dpi per 26 birds (mortality rate 23.07%) • 3 died birds on 7th dpi per 20 birds (mortality rate 15%)	• 2 bird died on 10th dpi per 17 birds • Mortality rate (11.76%)
		• Total mortality rate 15dead birds per 30 birds (50%)		
	postmortem	• Hemorrhage w congestion in thymus began on 2nd dpi/ ++ • Hemorrhage w congestion in proventriculus, gizzard, liver and intestine 5th dpi / +++ • Congested or hemorrhagic kidney and hemorrhages myocardium / 5th dpi ++to+++ • Necrotic areas in small intestine5th dpi • Congestion of brain blood vessels (5th dpi)/(+) • Atrophied spleen or w few white spots in spleen5th dpi-7th • Fibrinous pericarditis(5th dpi) , thickened air sac /+++ • Hemorrhage w congestion in lung 5th dpi/+++ • atrophied hemorrhagic cecal tonsils 5th dpi		
Duck	Clinical signs	• Signs observed on 3rd dpi: were slight decreased feed and water intake/+ • Ataxia and diarrhea on 4th dpi	• Signs progressed than 4th dpi • Recumbence and ataxia/+++ • labored breathing /+	• Signs decreased in severity
		• No mortalities	• 2 birds died on 5th dpi per 30 birds (mortality rate 6.0%):	• No mortalities
		• Total mortality rate 2dead birds per 30 birds (6%)		
	postmortem	• thymus w congestion and hemorrhage's 2nd dpi / ++to +++ • congestion of brain blood vessels 5th dpi / ++ • Hemorrhage w congestion in lung 5th dpi/++ • Hemorrhages on heart 5th dpi/++ • Friable kidney		

Dpi=day post infection; w= with; /S=Score or degree of lesions; (+= mild; ++= moderate; +++= severe).

Table 3: Geometric mean of NDV (Log base 2) of HI antibody titers (± standard deviation) in all groups of ducks and chickens

	Chickens		Ducks	
	Control(5/group)	challenged(5/group)	control(5/group)	challenged(5/group)
2nd dpi	0.5418±0.134	1.168 ±0.227	0.1806±0.164	0.602±0.212*
5th dpi	0.4214±0.164	2.107±0.309	ND	1.023±0.164*#
10th dpi	0.3612±0.134	2.709±0.212#	ND	1.264±0.251**#

No antibody titer detected (ND), * indicates significance differences in challenged chickens and ducks (P <0.05), ** indicates significance differences in challenged chickens and ducks (P <0.005), # Indicates significance differences of 2nd dpi compared with 5th and 10th dpi separately (P <0.05)

Table 4: Microscopic lesions of NDV in Both Chickens and Ducks

		Chickens			Ducks		
D.P.I		2nd D.P.I	5th D.P.I	10th D.P.I	2nd D.P.I	5th D.P.I	10th D.P.I
Brain	Cerebrum	++ PC, D, M, N, V	`++	+	-/+	+ D, N, V, C	+ .N, D, G, t P.C
	Cerebellum	++ P.L, N,.V	++	+	+ N	+ Neuro-denderitic D,N, V, P.L, H,G	+ N, V, P.L, H, C,G
	Pones	+	+	-/+	-	+ .D, N, V, C,	-/+
	Medulla oblingata	+	+	-/+	-	+ .D, N, V, C	-/+
Conjunctiva		-/+	+	-/+	-	-/+	+
Nostril		+++ H.F, I, I.B	++/+++	+ R	++	+ Focal M F, I	-/+ I
Trachea		+++	++	+ R	++ I	+	-/+ Focal F
Lung		+++ FIB BR-PN	+++ BR-PN	+++ INT PN, BR- PN ,	+ C, I,	++/+++ C, H, F, BR- PN	+ BR- PN
Gizzard		+ N. F	++ N.F, FIB. F	+	-	-	-
Proventriculus		+++ H.F, N.F	+++ N	+++	+ I	+ I	+ N, D, I
Intestine		+++ H.F, N.F	++/+++ N	+++	++ FIB	++ N, FIB	+ FIB
Liver		++/+ ++ C, N,I, I.B	+++	+/++ N, C,Focal I,	+ D, I,	+/++ V, N,I,C	-/+
Pancreas		+ N	+++ FocalN, I	++	-	-/+ One N. foci	-/+
Kidney		+++ GL.Np (C, IB, N, H.C)/ VA	+++ Diffuse N, Np,	++ INT NP, /, GL.HC, GL.N or GL.D /T. R	+ C, I	+	+
Heart	Pericardium	-/++	+++	+++	-	+ E, C	-/+
	Myocardium	+/++ C, D, Few I	+++	+	-	+ C, H, I	-/+
Spleen		+/++ L.D	+++ N, L.D	+++ L.D	+ L.D	+ L.D	-/+
Thymus		+++	++	+++ D	+ L.D	+ L.D	-/+
Bursa		++ L.D, N	++	+++ D	+ L.D	+ L.D	-/+
Cecal tonsil		++ H.F, N.F	+++ N	+++			

normal (-), mild(+),moderate (++), marked and sever degree (+++), infiltration (I), inflammation (F), hemorrhagic inflammation (H.F), necrotic inflammation (N.F), lymphocytic inflammation (L.F), degeneration (D), necrosis (N), hemorrhages (H), fibrin (FIB), congestion (C), lymphoid depletion (L.D), vacuolation (V), fatty vacuolation (F.V), neural vacuolation (N.V), gliosis (G), perkinje cell loss (P.L), malacia (M), inclusion bodies (I.B), perivascular cuffing (P.C), spongiosis (S), regeneration (R), pneumonia (PN), interstitial (INT), bronchi (BR.), vasculitis (VA), alveoli (ALV), emphysema (EMPH), tubular (T), glomerular (GL), nephritis (NP) hyper cellularity (H.C), edema (E)

Table 5: Immunolabeling for Newcastle Disease Viral Nucleoprotein Antigen by Immunohistochemistry in Both Chickens and Ducks

Organs	Chickens						Ducks					
	Control			Challenged			Control			Challenged		
	2nd dpi	5th dpi	10th dpi	2nd dpi	5th dpi	10th dpi	2nd dpi	5th dpi	10th dpi	2nd dpi	5th dpi	10th dpi
Trachea	-	-	-	++ M.	++++ M., L. P.	+++ M., S.M	-	-	-	+ E. cell	+++ M., L. P.	++ M.
Lung	-	-	-	++ A. E.	++++ A. E., F. cells	+++ A. E., F. cells	-	-	-	++ E. cell (A. Pb.),	++++ E. cell (A., Pb.), F. cell	++ F. cell
Brain	-	-	-	+ P. cells	+++ P.cell, G.cell	+++ S.cell, G.cell	-	-	-	+ P.cell, G.cell	+++ P.cell	++ G.cell
Intestine	-	-	-	++ E. cell (V, GL)	+++ E. cell (V), F. cell	++ E. cell (V)	-	-	-	+ E. cell (Gl.), F. cell	++ E. cell (V., GL)	++ E. cell (Gl.)

*No labeling (-); rare positive cells (+); frequent positive cells (++); abundant positive cells (+++); diffuse marked positive cells (++++). purkinji cells (P. cell), epithelial cells (E. cell), inflammatory cells (F. cell), granule cells (G. cells), astroglia (S. cells), alveoli (A.), lamina propria (L.P.), mucosa (M.), submucosa (S.M.) villi (V), gland (GL).

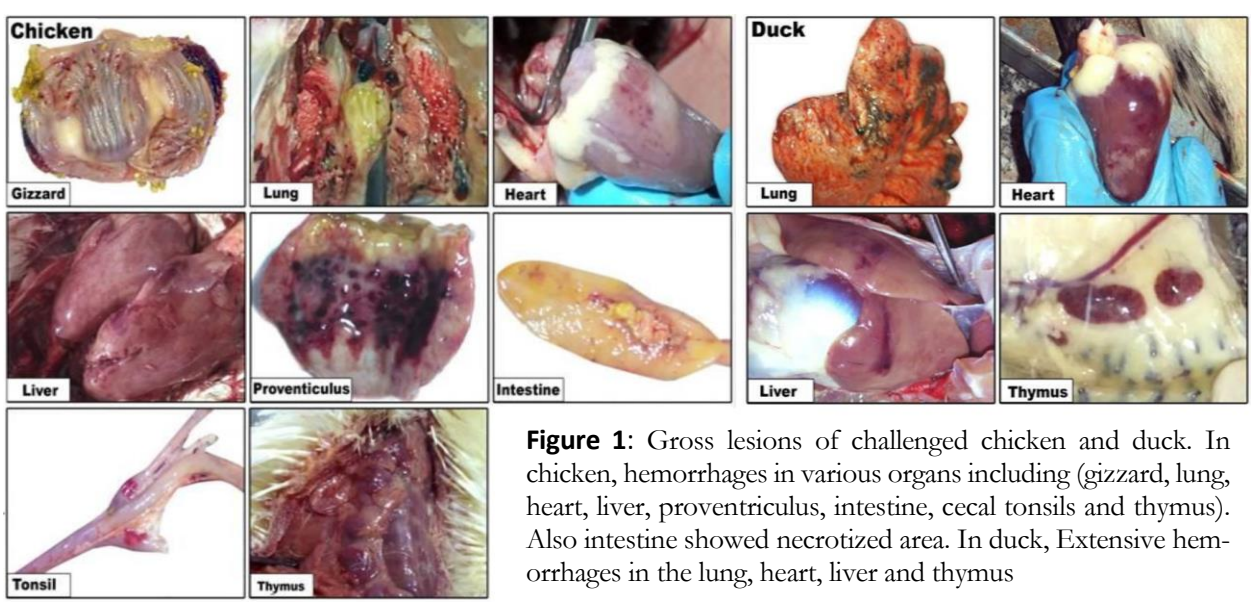

Figure 1: Gross lesions of challenged chicken and duck. In chicken, hemorrhages in various organs including (gizzard, lung, heart, liver, proventriculus, intestine, cecal tonsils and thymus). Also intestine showed necrotized area. In duck, Extensive hemorrhages in the lung, heart, liver and thymus

Histopathology and immunohistochemy

Histopathological and immunohistochemical observations by measuring the percentage of positive expressions within different organs in ducks and chickens inoculated with *NDV MT887290* via the intranasal route (I/N) are summarized in (Table 4 and 5) and illustrated in (Figures 2, 3, 4, 5, 6, 7, 8 and 9). The severity and distribution of the lesions differed depending on the bird species.

Intranasal-inoculated chicken group

On the 2nd dpi, nostrils showed severe rhinitis associated with hemorrhage and erosive lesions. Necrosis and desquamation of the epithelium and significant inflammatory cells infiltrations especially macrophages, heterophils, plasma cells and lymphocytes were detected. Trachea showed focal tracheitis represented by focal necrosis of covering epithelium as well as loss of cilia, congestion and inflammatory cells infiltration of lamina propria. Lung showed mild degree of pneumonia revealed as congestion, mononuclear cellular infiltration and thickening of inter-alveolar septa. Some examined birds showed bronchopneumonia accompanied with typical bronchitis consisted of thickened bronchial wall, epithelial covering necrosis and

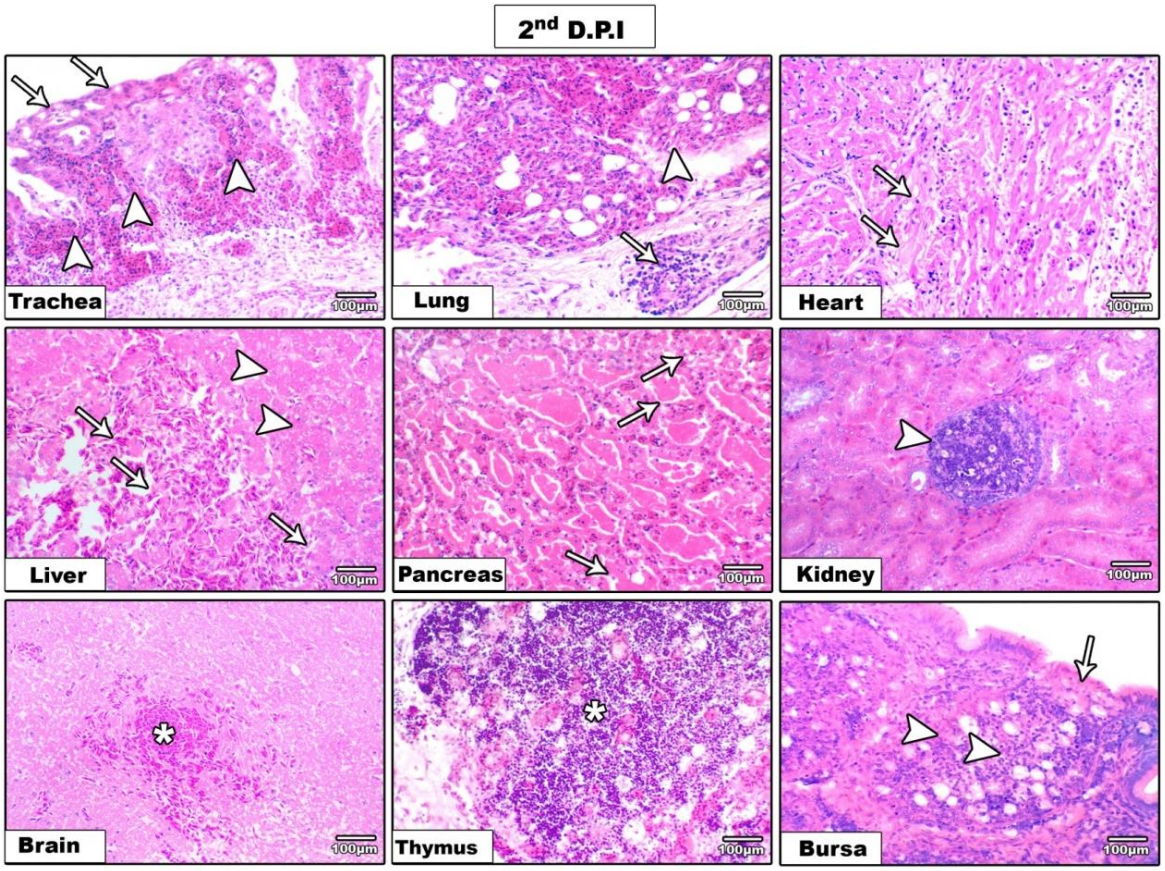

Figure 2: (2nd dpi) Systemic histopathological findings of chicken challenged with NDV-VII (2nd dpi). Trachea showing severe tracheitis associated with necrosis of covering epithelium(arrows) as well as congestions and hemorrhages in mucosa(arrowhead) Lung showing hemorrhagic pneumonia associated with mononuclear cells. infiltration(arrow) and edema (arrowheads). Heart showing marked myocarditis associated with myolsis (arrows). Liver showing severe degree of hepatitis revealed as hepatocytes degeneration(arrowheads) and necrosis (arrow). Pancreas showing severe pancreatic necrosis (arrows). Kidney showing interstitial nephritis associated with mononuclear cellular infiltration(arrowhead). Brain showing malacia associated with perivascular hemorrhages (asterisk). Thymus showing severe necrosis of thymic compartments. Bursa showing follicular lymphocytic depletion (arrows) and epithelial lining necrosis(arrowheads)

desquamation in the lumen, and inflammatory cells infiltration in lamina propria. The proventriculus revealed severe proventiculitis appeared as necrosis of covered epithelium necrosis with sloughing, congestion, hemorrhages and inflammatory cellular infiltration, which extended to gizzard to form focal areas of inflammation associated with sloughing epithelium. The Intestine showed mild enteritis mostly within the cecum associated with inflammatory cells infiltration and sloughed epithelial cells. The liver of examined birds showed moderate to severe degree of hepatitis associated with congestion, hemorrhage, inflammatory cells infiltration and necrosis. Pancreas showed pancreatitis associated with inflammatory cells infiltration with mild degree of necrosis as multiple necrotic foci of pancreatic acini within some examined birds. Heart showed myocarditis associated with congestion, mild mononuclear cellular infiltration, fatty vacuolation and myocardial degeneration characterized by eosinophilic cytoplasmic granules. Kidneys showed nephritis associated with vascular congestion, hemorrhages, inflammatory cells infiltration, and glomerular cells hyperplasia causing losses of bowman's spaces. Lymphoid organs showed varied degree of lymphoid depletion. The cecal tonsils and bursa showed moderate lymphocytic depletion, while the bursa showed cortical lymphoid depletion and marked cellular necrosis in medulla.

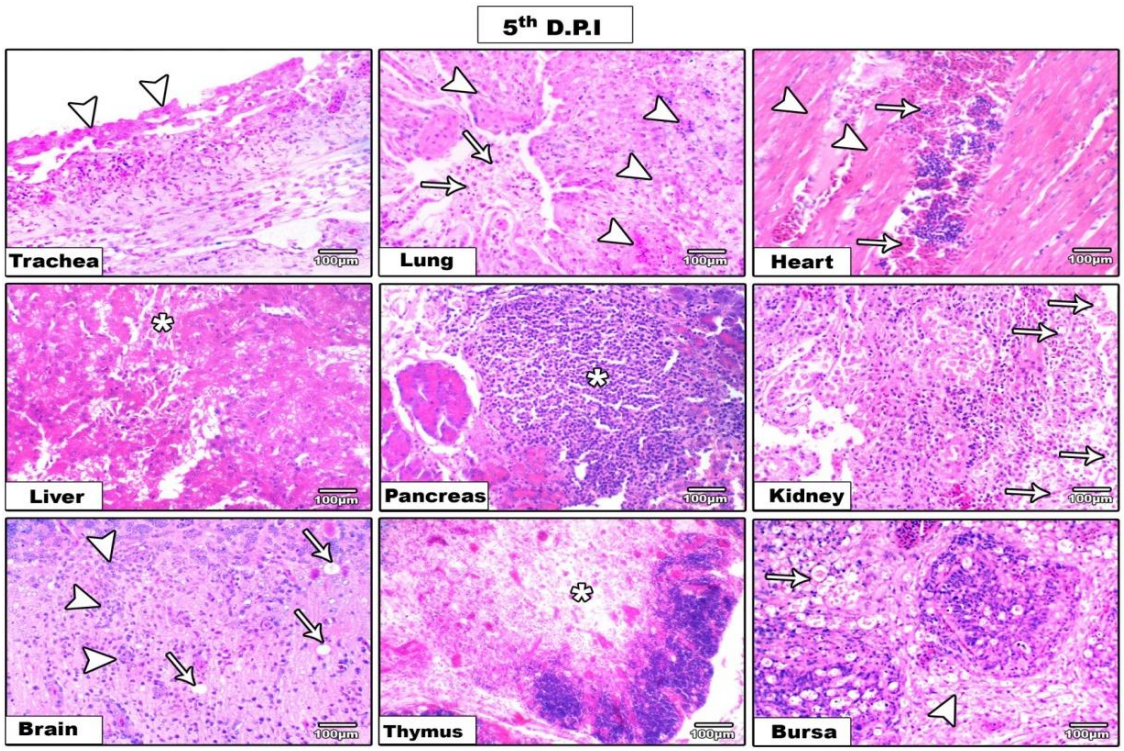

Figure 3: (5th dpi) Systemic histopathological findings of chicken challenged with NDV-VII (5th dpi). Trachea showing severe necrosis of covering epithelium (arrowhead). Lung showing severe fibrinous pneumonia revealed as consolidation with the absence of air capillaries (arrowheads) and fibrin deposition with mononuclear cellular infiltration in parabronchial lumen (arrow). Heart showing myocardial necrosis (arrowheads) accompanied with interstitial hemorrhage (arrow). Liver showing diffuse periportal hepatocytes necrosis (asterisk). Pancreas showing diffuse pancreatic necrosis (arrow), with diffuse inflammatory cells infiltration (asterisk). Kidney revealing the advanced degree of tubular necrosis (arrows). Brain showing neuronal necrosis with microgliosis (arrowhead) and spongiosis (arrows). Thymus showing severe lymphoid depletion in medulla than cortex with fibrin deposition in medulla (star). Bursa showing a severe degree of lymphoid depletion (arrows).

The spleen revealed very mild lymphoid depletion. Brain showed congestion and mild neuronal necrosis in cerebral cortex with obvious necrosis of purkinje cells in cerebellum.

On the 5th dpi, the lesions were similar to the previous sacrifice but with severe nature which appeared with hemorrhagic inflammation. Severe pneumonia, confluent pancreatic necrosis, malacia, perivascular cuffing and proliferative glomerulonephritis were noticed in this stage.

On the 10th dpi, the previous lesion was less severe than that observed on the 5th dpi. Hyperplastic and regenerative changes within the mucosal lining of the bronchi and intestine were detected.

Intranasal-inoculated duck group

On the 2nd dpi, nostrils revealed moderate degree of mucosal rhinitis. Trachea showed tracheitis characterized by partial losses of epithelial cilia, mononuclear cellular infiltration and epithelial cells degeneration. Lungs revealed mild pneumonia consisted of vascular congestion, thickening of interstitial alveolar septa and mononuclear cellular infiltration.

Proventriculus showed only mild mononuclear cells infiltration at the junction with esophagus. Intestine showed mild to moderate catarrhal enteritis. Liver showed hepatitis appeared as mild degree of hepatic sinusoids congestion, mononuclear inflammatory cells infiltration mostly lymphocytes and vacuolar degeneration of hepatocytes. Pancreatic tissue showed normal architectures in most examined ducks.

Heart of all examined birds revealed normal myocardial architecture. Kidney revealed only mild blood vessels congestion and lymphocytic infiltration. Lymphoid organs as spleen, and bursa had very mild depletion. The most affected portion within the brain tissue was the cerebellum

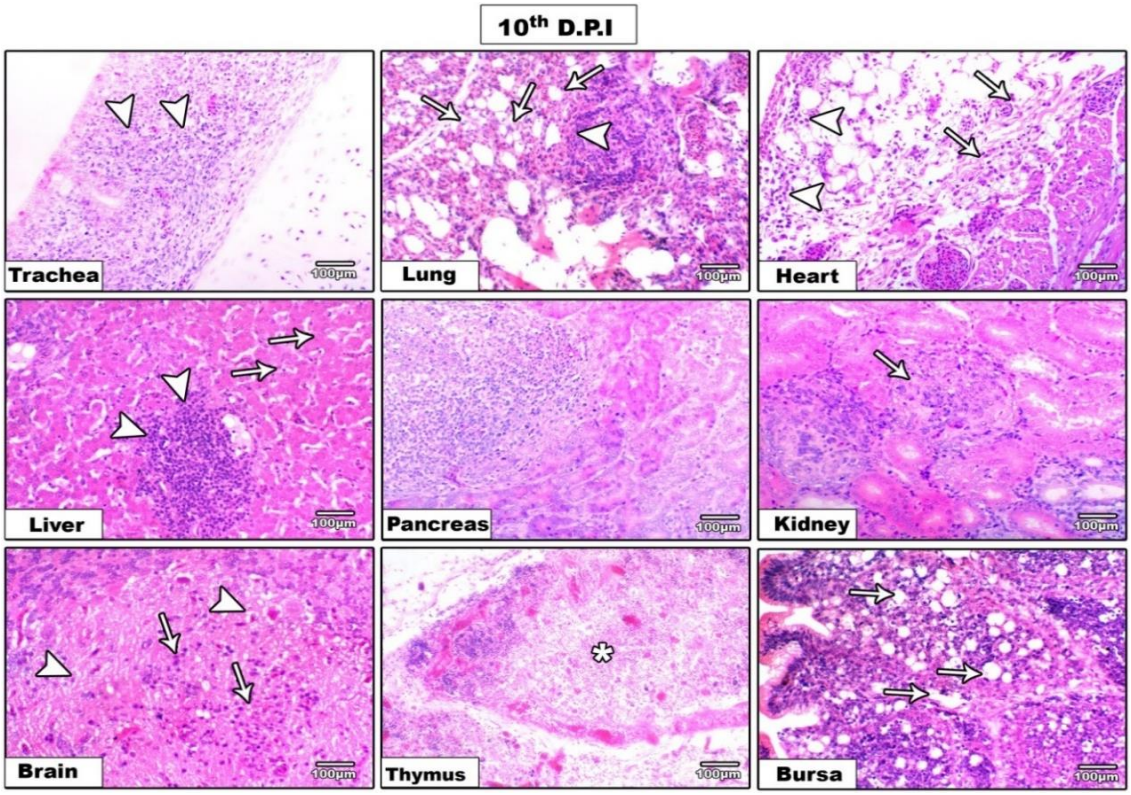

Figure 4: (10th dpi) Systemic histopathological findings of chicken challenged with NDV-VII (10th dpi). Trachea showing severe tracheitis associated with severe infiltration of mononuclear cells (arrowhead). Lung showing pneumonia associated with necrosis (arrowhead) and distortion of the alveoli (arrows). Heart showing pericarditis accompanied with inflammatory cells infiltration (arrowheads) and marked organization (arrow). Liver showing hepatocytes necrosis (arrows), and diffuse mononuclear cellular infiltration (stars). Pancreas showing diffuse pancreatic necrosis (arrow), with diffuse inflammatory cells infiltration (arrowheads). The kidney revealed proliferative glomerulonephritis (arrows). Brain showing malacia (arrowheads) and diffuse gliosis (arrows) Thymus showing severe lymphoid necrosis of the cortex and medulla with marked fibrin deposition (asterisk). Bursa showing marked lymphoid depletion (arrows)

which revealed segmental losses of purkinje cells, while the cerebral tissue showed very mild congestion. On the 5th dpi, mild conjunctivitis was observed. Nasal and tracheal epithelium revealed very mild focal inflammatory reaction. The lungs revealed severe hemorrhagic pneumonia / or bronchopneumonia. The intestinal mucosa showed moderate degree of enteritis with fibrinous exudate and necrosis of villi epithelial covering. Liver showed moderate congestion of hepatic sinusoid, mononuclear cellular infiltration, fatty vacuolation, and very mild necrosis of hepatocytes. Pancreas showed small two focal area of necrosis in the pancreatic acini. Heart revealed pericarditis consisted of edema and congestion of pericardium and myocarditis as myocardial congestion and hemorrhage with very few mononuclear infiltrations. Kidneys revealed lesions similar to the lesions on the 2nd dpi. Lymphoid organs; thymus, spleen and bursa showed very mild depletion. The most affected brain portions were cerebellum, pons and medulla which showed congestion, neural degeneration, vacuolation and necrosis. The cerebellum showed also gliosis, neurodendritic degeneration besides vacuolation and necrosis of perkinje cells.

On the 10th dpi, different examined tissues showed mild inflammation which was noticed as mononuclear inflammatory cells. Lymphoid organs revealed normal architecture to mild lymphoid depletion. However, spleen showed hyperplasia in some lymphoid follicles. Brain showed encephalitis revealed as cerebral neuronal degeneration, necrosis and gliosis. Meanwhile, the main lesion was in cerebellum and consisted of congestion, perivascular hemorrhage, and vacuolation.

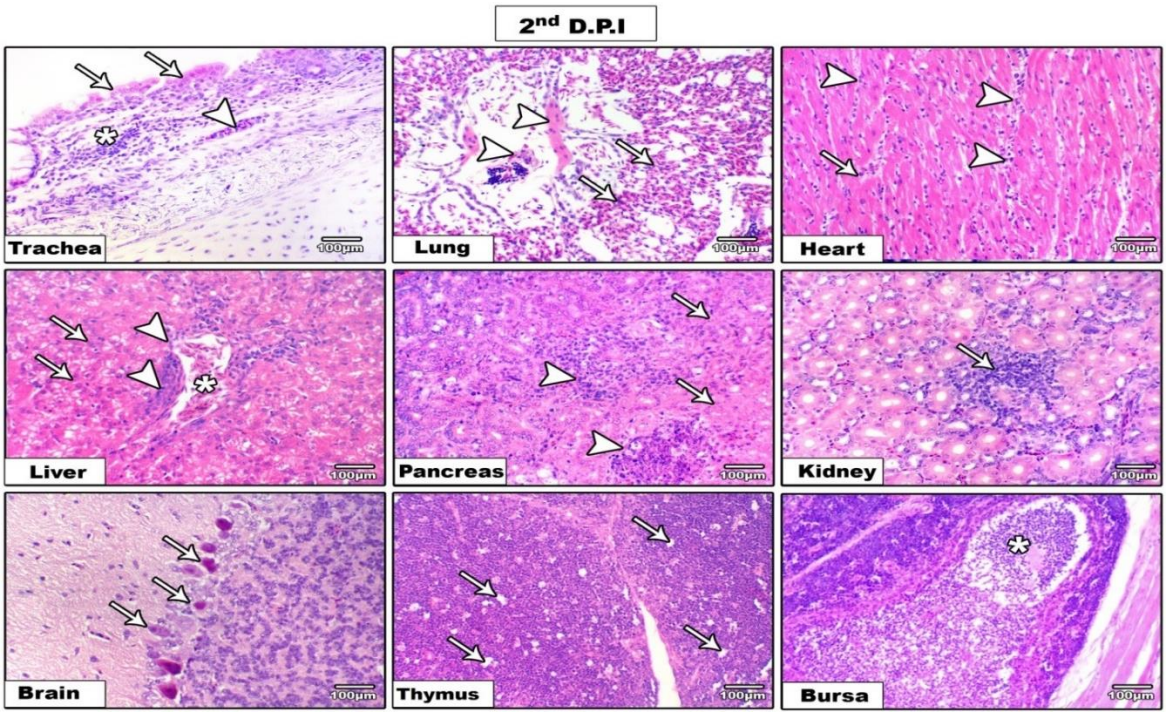

Figure 5: (2nd dpi) Systemic histopathological findings of duck challenged with NDV-VII (2nd dpi). Trachea showing necrosis of covering epithelium as well as loss of cilia (arrowhead) with vascular congestion(asterisk) and mononuclear cells infiltration.(arrows) in lamina propria. Lung showing congestion of blood capillaries (arrows) and parabronchial lumen fibrin deposition and cellular infiltration (arrowheads). Heart showing mild to moderate myocarditis associated with myocytes degeneration (arrows) and mild cellular infiltration (arrowheads). Liver showing congested portal vein (asterisk) with periportal cellular infiltration (arrows) and diffuse hepatocytes vacuolar degeneration (arrows). Pancreas showing pancreatitis with necrotic foci within the pancreatic acini (arrow) and marked inflammatory cells infiltration (arrowheads). Kidney showing tubulointerstitial nephritis (arrow indicates mononuclear cells infiltration). rain showing a severe degree of ischemic neuronal injury within the Purkinje cell layer (arrows). Thymus showing mild lymphoid depletion. Bursa showing follicular lymphocytic depletion (asterisk).

Immunohistochemistry

Regarding to immunolabellings for NDV N.P (nucleoprotein), the control negative samples of different organs didn't show any immunostaining of NDV antigen, however the other examined tested slides showed immunostaining of virus N.P antigen within the most of examined organs. The immunoreaction was well correlated with NDV lesions in both species, with marked immunostaining of epithelial lining of various organs including the upper respiratory epithelial covering and alveolar epithelial lining. Immunolabeling expression was also detected in the proventriculus and intestinal mucosa. Neuronal cells showed marked immunoreaction within the neuronal cells including their bodies and dendrites, purkinje cells and astroglia cells. Also, the virus was detected within the mononuclear inflammatory cells mostly macrophages. The virus was minimally detected in kidney, heart and liver. Immunostaining of NDV was noticed on the 2nd dpi the reaction, reached the peak of immunostaining on the 5th dpi then declined on the 10th dpi in duck. The labeling intensity and distribution of NDV were illustrated in (Table 5).

Discussion

Although Newcastle disease virus has pandemic nature, severe virulent strains were detected all over the world. Several epidemics affected the Asian, Middle East regions (20) and in South and West Africa within the last few years (21). Recently, the most NDV outbreaks in Egypt among poultry species caused by NDV-VII (22-26). Migratory birds have an important role in the transmission of the virus between the continents (8, 17). The NDV was isolated from different migratory bird including Tufted duck (8, 11). Some of

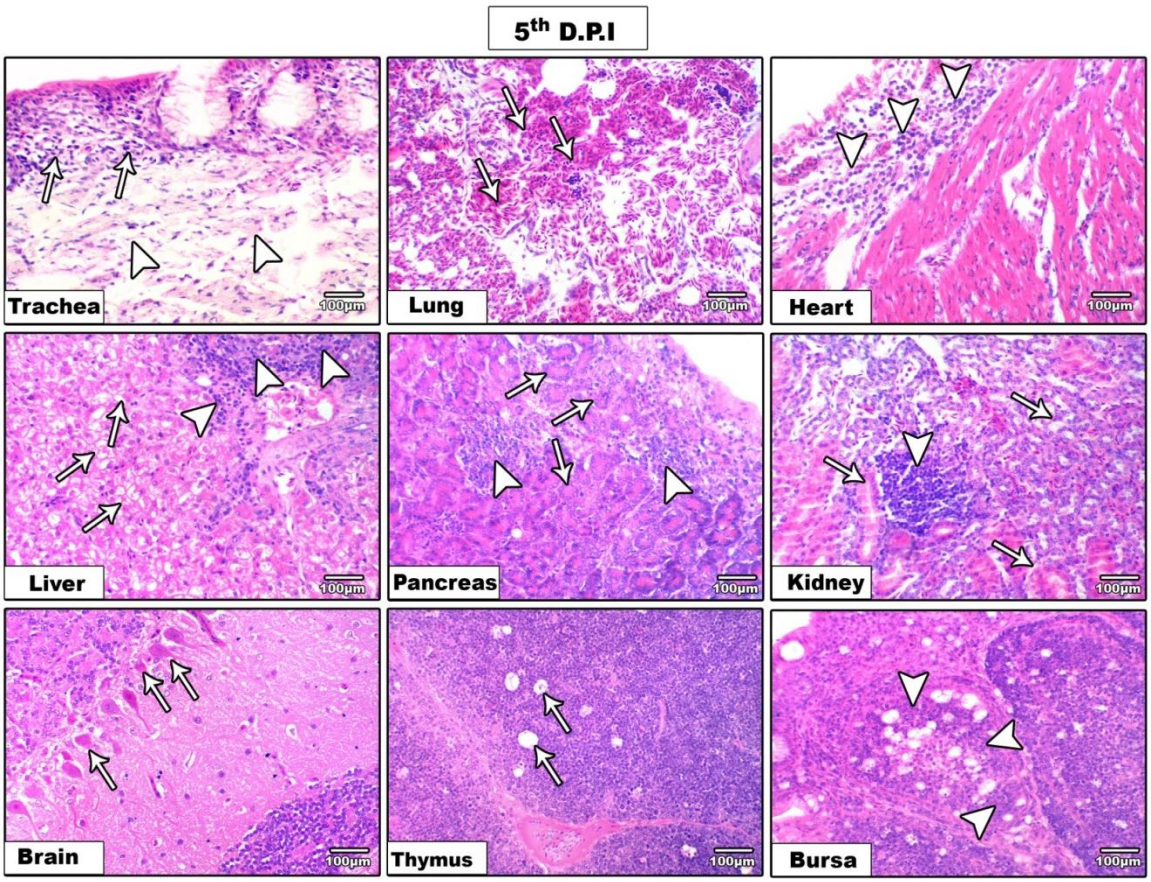

Figure 6: (5th dpi) Systemic histopathological findings of duck challenged with NDV-VII (5th dpi). Trachea showing tracheitis associated with both mononuclear cells infiltration (arrows) and edema in lamina propria (arrowheads). Lung showing hemorrhagic pneumonia (arrows indicate hemorrhages). Heart showing marked pericarditis (arrowheads indicate mononuclear cellular infiltration). Liver showing marked hepatitis associated with marked periportal mononuclear cellular infiltration (arrowheads) and hepatocytes fatty vacuolation and necrosis. Pancreas showing confluent necrosis pancreatic tissues (arrows) associated with diffuse inflammatory cells infiltration (arrowheads). Kidney showing severe tubular nephritis with losses of renal architectures due to severe tubular necrosis (arrows) and marked mononuclear cells (arrowheads). Brain cerebellum showing marked degree of Purkinje cells ischemic degeneration (arrows). Thymus showing lymphoid depletion (arrows). Bursa showing marked lymphoid depletion (arrowheads)

naturally circulating NDV strains with high virulence were associated with severe illness and mortalities within different duck species including;

Mallard, Gaoyou, Shaoxing, Jinding, Shanma, and Pekin ducks. The bird susceptibility to NDV varied among bird according to species and even breeds within the species, age, route of infection as well as the maternal immunity (12, 27). From different duck species, the most susceptible breed is the Mallard duck and the most resistant one is the Pekin duck (12).

NDV is characterized by multiple amino acids configuration especially the F protein cleavage site with different virulence. It is noteworthy that the high virulent strain of NDV had [112 R-R-Q- R-R 116] at the F site cleavage site (28). The virus is associated with respiratory, digestive, and nervous manifestations. The disease is associated with great mortalities within the affected flocks lower than 20% in mesogenic strains and reaches up to 100% mortality within Velogenic strains (15, 26).

In the current study, the isolated field strain *NDV MT887290* has a virulent arrangement of four basic amino acids [112 R-R-Q-R-R 116] motif at the cleavage site of F protein, and had an ICPI score greater than 1.83, which indicates a virulent NDV strain (29).

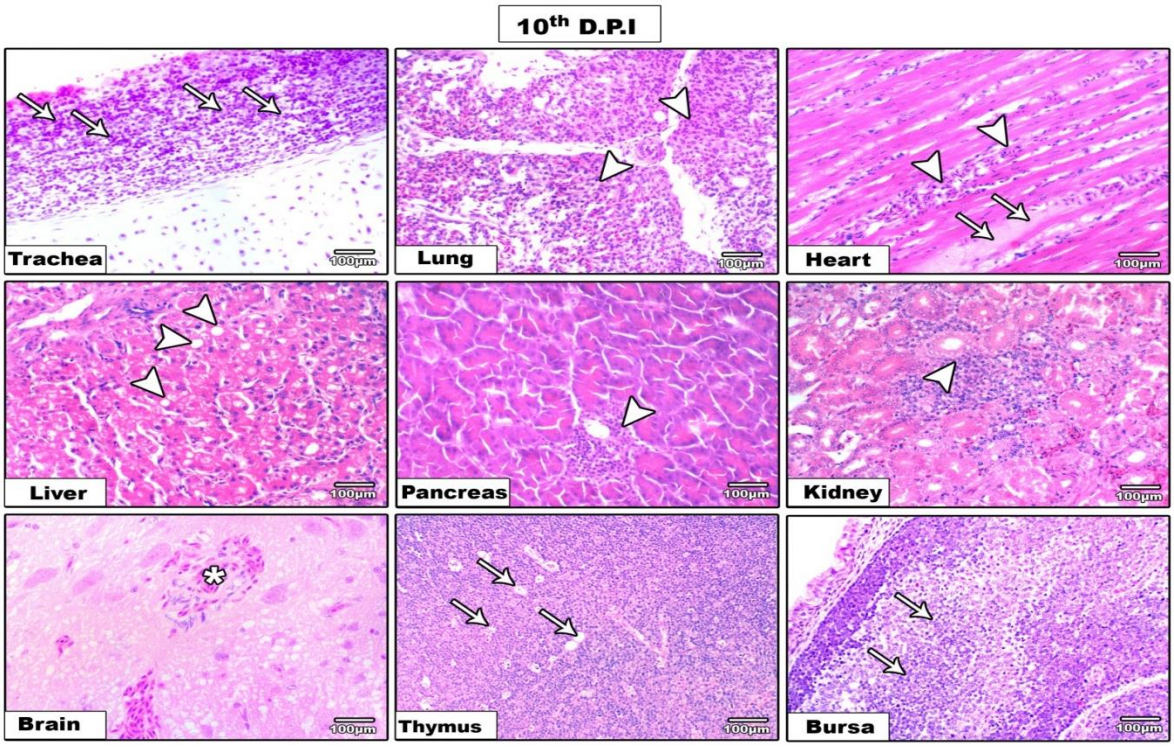

Figure 7: (10th dpi) Systemic histopathological findings of duck challenged with NDV-VII (10th dpi). Trachea showing tracheitis associated with mononuclear cellular infiltration in the mucosa (arrows). Lung showing severe pneumonia associated with consolidation of the alveolar spaces (arrowheads) indicate marked mononuclear inflammatory cells infiltration). Heart showing myocarditis (arrowheads) indicate inflammatory cells infiltration and arrow revealing myolysis). Liver showing fatty vacuolation (arrowheads). Pancreas showing focal pancreatitis accompanied with inflammatory cells infiltration (arrowheads). Kidney revealed interstitial nephritis (arrows) indicate interstitial mononuclear cellular infiltration). Brain showing cerebral neuronal malacia (asterisk). Thymus showing lymphoid depletion (arrows). Bursa showing epithelial lining necrosis (arrowheads) and lymphocytic depletion in germinal center (arrows)

The challenged chicks showed early reduced food intake, lethargy, watery greenish diarrhea and ruffled feathers nasal discharge (30-34). Nervous manifestation such as incoordination and torticollis were appeared later on 5th dpi. Similar observations were noticed by Miller & Koch and Ezema et al (31, 35). While the duck infected group showed clear nervous signs mainly ataxia on the 4th dpi consistent with findings observed by previous reports (12, 36).

NDV has a great affinity to multiple cells (pantropism) including epithelial cells (14). The moralities due to the virus are mostly associated with vascular hemorrhages of various organs. It was noteworthy, the virus has great affinity tropism to endothelial cells, this affinity was highlighted with severe vasculitis and systemic hemorrhages. The affinity to the endothelial cells is usually associated with increased pathogenicity of the viruses (37).

In the current study, two deaths on 5th dpi were recorded in challenged duck with NDV. This finding was consistent with Dai et al (12), who reported deaths of duck challenged with ND virus. This data showed recent higher pathogenicity of NDV-VII in ducks than previous data which showed absence of deaths between ducks infected with NDV (14, 38, 39).

Our sequential study of NDV on chickens and ducks demonstrated that the severe necrotic and degenerative changes within the nasal mucosa in the early days post infection, then systemic lesions including pneumonia, lymphoid depletion as well as pancreatitis and pericarditis. Higher mortalities within chickens than ducks may be associated with more severe pancreatitis. Although, severe pneumonia and malacia within the affected chickens may be also be considered as a death factor. Ducks showed similar lesions, the.

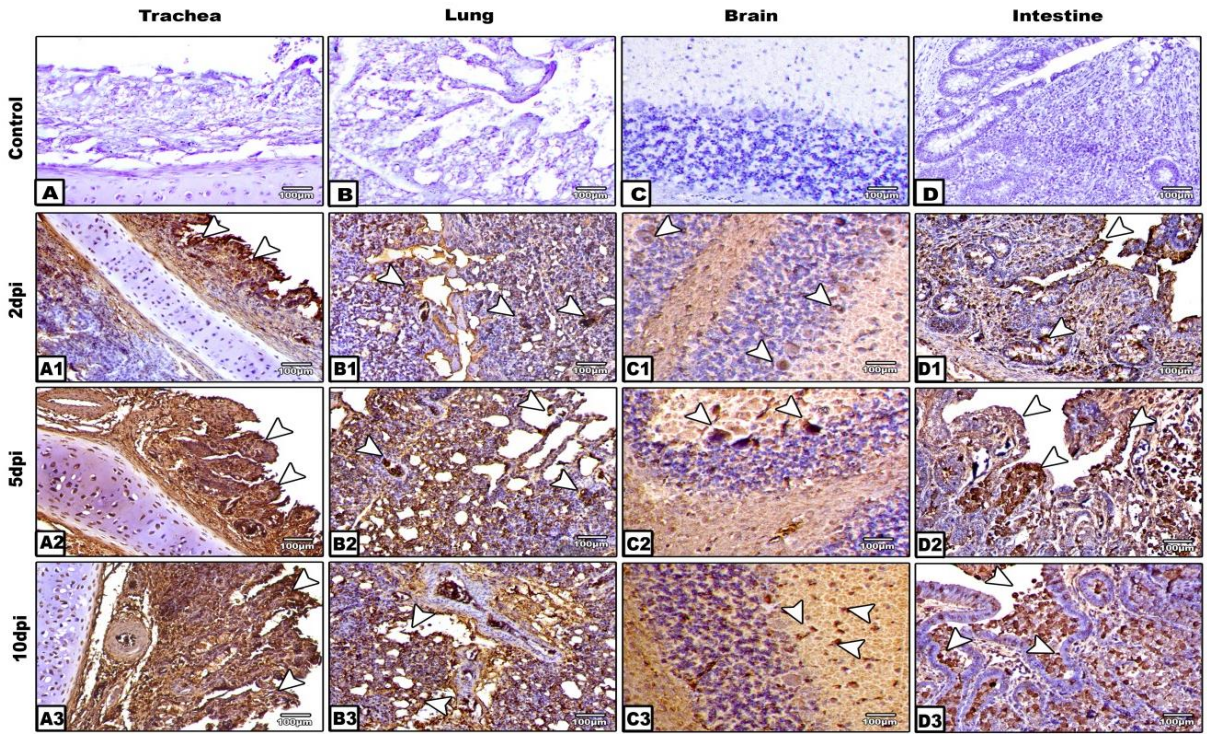

Figure 8: Systemic immuno-histopathological findings of chickens challenged with NDV-VII nucleoprotein (NP). Upper panel represents the control negative samples of tracheal (A), pulmonary (B), cerebellar (C) and intestinal (D) sections. On the 2nd dpi (A1 represents tracheal section, B1 represents pulmonary section, C1 represents cerebellar section and D1 represents intestinal section), NDV- NP immune-staining were detected on the tracheal epithelium, Purkinje cell layer, alveolar lining epithelium, within the intestinal lining epithelium and intestinal (arrowheads). On the 5th dpi (A2 represents tracheal section, B2 represents pulmonary section, C2 represents cerebellar section and D2 represents intestinal section), strong NDV- NP immune-staining were detected on the tracheal epithelium, inflammatory cells in mucosa and lamina propria, Purkinje cell layer and granular layer of the cerebellum, alveolar lining epithelium, inflammatory cells of the lung, within the intestinal epithelium and within the interstitial inflammatory cells of the intestinal mucosa (arrowheads). On the 10th dpi (A 3 represents tracheal section, B3 represents pulmonary section, C3 represents cerebellar section and D3 represents intestinal section), NDV- NP immuno-staining was detected on the epithelium lining of the trachea, astroglia cells, within granular layer of the cerebellum, alveolar lining epithelium and intestinal epithelium (arrowheads)

NDV in ducks was associated with similar epithelial cell necrosis, lymphoid depletion, pneumonia and even neuronal lesions. Meanwhile the pancreatitis was less than observed in chickens. Therefore, these findings may attribute to the higher mortalities in chickens (17, 26, 28, 40, 41).

In general, the immunolabeling reaction of NDV protein revealed stronger reaction in chickens than in ducks (17). The reaction appeared in various organs on 2nd dpi and increased until the 5th dpi and then the staining intensity was decreased on the 10th dpi, which may refer to viral elimination and recovery stage. In chicken brain, the immunolabeling reaction was detected in neurons, glia cells, astroglia and purkinje cells. While ducks showed expression of NDV within the cerebellar granular and purkinje layers in ducks.

NDV distribution and immunopositive reaction was similar to that noticed by previous studies (42, 43). A previous report (17) showed brain lesions only within the brain of chickens, while the infected duck showed absence of the brain immunolabeling when challenged with NDV-9a5b mutant type.

Immunopositive reaction in both chickens and ducks showed marked staining of NDV antigen lining epithelium and in lumen in both of the respiratory organ (trachea and lung). Trachea stronger reaction was detected in chickens than ducks in mucosa and mononuclear infiltrated cells. In lungs, the immunolabeling reaction was detected in epithelial lining of alveoli, parabronchi and mononuclear cells in interstitial tissue and

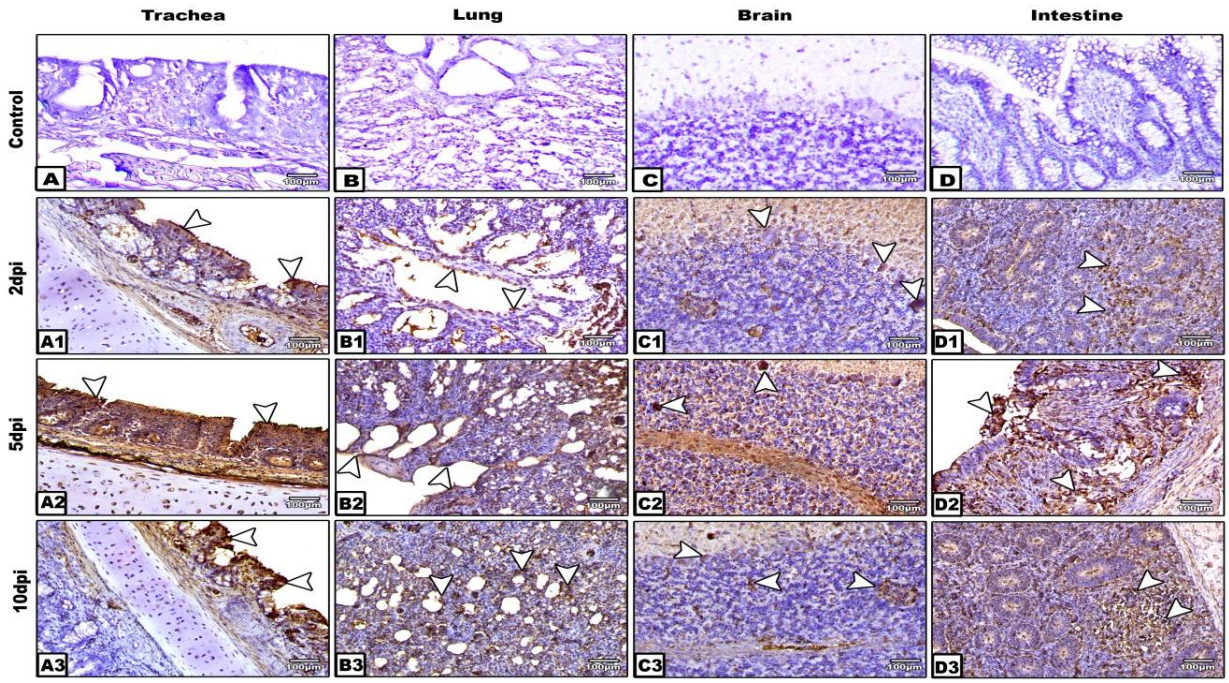

Figure 9: Systemic immuno-histopathological findings of ducks challenged with NDV-VII nucleoprotein (NP). Upper panel represents the control negative samples of tracheal (A), pulmonary (B), cerebellar (C) and intestinal (D) sections. On the 2nd dpi (A1 represents tracheal section, B1 represents pulmonary section, C1 represents cerebellar section and D1 represents intestinal section), NDV- NP immuno-staining was detected on the tracheal epithelium, Purkinje cell layer, parabronchial and alveolar lining epithelium and within the interstitial inflammatory cells of the intestinal mucosa (arrowheads). On the 5th dpi (A2 represents tracheal section, B2 represents pulmonary section, C2 represents cerebellar section and D2 represents intestinal section), strong NDV- NP immuno-staining was detected on the tracheal epithelium, inflammatory cells in mucosa and lamina propria, Purkinje cell layer and granular layer of the cerebellum, parabronchial and alveolar lining epithelium, within the intestinal epithelium, interstitial inflammatory cells of the intestinal mucosa and intestinal gland lining epithelium (arrowheads). On the 10th dpi (A3 represents tracheal section, B3 represents pulmonary section, C3 represents cerebellar section and D3 represents intestinal section), NDV- NP immuno-staining was detected on the necrotized epithelium lining of the trachea, necrotized Purkinje cell layer, in some granule cells within granular layer of the cerebellum, alveolar lining epithelium, within the intestinal epithelium and interstitial inflammatory cells of the intestinal mucosa (arrowheads)

in alveolar septa. These findings was consistent with mentioned by other reports (18, 44).

The intestinal epithelium showed immunolabelling reaction in the necrotized epithelium

and in the degenerative epithelium of villi and intestinal gland. The immunopositive reaction of liver, proventriculus and intestinal tissues of infected chickens which expressed within the cytoplasm and nuclei of the necrotic cells in addition to Kupffer cells and circulating monocytes in the liver (34, 44).

NDV in both challenged chickens and ducks showing a stronger lymphoid tissue tropism within mononuclear cells in both lymphocytes and macrophages, and also a positive staining lymphoid-associated tissue that found throughout the upper respiratory and gastrointestinal tracts (21, 43). The lymphoid cells within the bursa showed immunolabelling reaction to NDV (21).

Upon the histopathological and immunohistochemical findings, NDV genotype VII had a wide systemic distribution, especially targeting lymphoid organs and mucosa-associated lymphoid tissues in the respiratory and intestinal tracts, respiratory organs, intestine, and brain in both challenged duck and chicken group (29, 43, 44).

Maternal immunity against of NDV showed high level of maternal antibody in chickens than ducks. Previous data revealed absence of antibodies titer against NDV within ducks (45). Recently the antibody titer of NDV was recently detected

in the serum of duck, and gradually increased post infection. This finding was similar to our results, which may be referred to the circulation of NDV within the domestic water fowl (46).

Conclusion

Intranasal inoculation of NDV in both chicken and duck showed systemic distribution of the virus within the different tissues. In general the mortalities and detected lesions were more severe in chickens than in ducks. Pancreatitis, pericarditis and severe lymphoid depletion were mostly severe in chickens. The microscopic and immunohistochemical findings of NDV-challenged ducks revealed that increase the susceptibility to new circulating NDV genotype VII with possible deaths within the ducks. Therefore, we recommend the follow-up of NDV-VII in duck population and possible vaccination strategy should be rolled out.

The authors declare no conflict of interest.

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