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Pathology

Pathological and Epidemiological Study on Newcastle Disease in Broiler Chicken Farms in Egypt between 2021-2022.

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ABSTRACT

Newcastle disease virus (NDV) is an economic threat to the poultry industry worldwide. In this study, we aimed to investigate the incidence of NDV in the vaccinated Egyptian poultry flocks through histopathological and molecular investigation. One hundred and nine broiler chicken flocks were examined during the period from January 2021 to December 2022from seven different Egyptian governorates (El-Beheira, El-Fayoum, El-Gizza, El-Gharbia, El-Menofia, El-Qalyubia, and El-Sharqia). History, clinical signs, and gross examination of the affected flocks were recorded. Brain, nostril, trachea, lung, heart, proventriculus, duodenum, liver, pancreas, thymus, spleen, bursa, cecal tonsil, and kidney were collected for histopathological examination and only the tracheal tissues for Real-time polymerase chain reaction (RT-PCR). The most observed gross lesion in the examined samples was the hemorrhage in the proventriculus. Histopathologically, cerebral spongiosis, brain vasculitis, rhinitis, tracheitis, pneumonia, pericarditis, hemorrhage in the proventriculus, enteritis, perihepatitis, necrotizing pancreatitis, lymphoid depletion of the immune organs, and proliferation of lymphoid tissue in kidney. Furthermore, lymphoid proliferation was detected in the lung, proventriculus, and liver. From the examined samples, only forty- three samples were found to be positive by RT-PCR, which represented 39.5% of the total samples. From our results, we can conclude that NDV circulates in the vaccinated broiler flocks in Egypt. Therefore, reconsidering the immune status, vaccination programs, and surveillance of newly emerged strains are essential to reduce the incidence of ND in Egypt.

Keywords: NDV, Broiler, Egypt, Gross pathology, Histopathological, RT-PCR

INTRODUCTION

Newcastle disease is a serious viral poultry disease caused by an enveloped virus from the *Paramyxoviridae* family, subfamily Avulavirinae and genus Avulavirus (Yang et al., 2021). NDV is a negative sense non segmented single-stranded RNA virus (Shaw & Palese, 2013). This disease is considered the third most crucial poultry disease (Abd Elfatah et al., 2021). The first known ND outbreak occurred in Java, Indonesia, in 1926 (Kraneveld, 1926). Doyle established the term “Newcastle disease” after an attack in

Newcastle-upon-Tyne, England (Doyle, 1927, 1935). NDV was initially identified in Egypt in 1948 (Daubney & Mansy, 1948). Since then, Egypt has been regarded as an endemic country for ND (Nabila et al., 2014). NDV has three strains: velogenic (viscerotropic and neurotropic), mesogenic, and lentogenic. The velogenic strain is the most dangerous strain, with a high mortality rate of up to 100% and severe respiratory and neurological symptoms (Moura et al., 2016). Gasping, sneezing, coughing, and rales are all respiratory symptoms. Tremors, paralyzed wings and legs, twisted necks, and circling are all nervous symptoms. Other common symptoms include greenish diarrhea, depression, and inappetence, which can result in a partial or complete decrease in egg production and an increase in abnormally malformed eggs (Alexander et al., 2004). Mesogenic strains had mortality up to 10% and caused coughing and a drop in egg production and quality. Lentogenic strains had low mortality and few symptoms (Alexander, 2000). Grossly velogenic strains showed hemorrhage and necrosis in the proventriculus, cecal tonsils, and enlarged and mottled spleen with pinpoint focal areas of necrosis (Mostaree et al., 2021). Mesogenic strains have minimal gross lesions with severe morbidity observed in birds due to concurrent viral and secondary bacterial infections (Nakamura et al., 1994).

The histopathological findings of velogenic strains were congestion, edema, and extensive cellular infiltration of lymphocytes and macrophages in the mucosa of the upper respiratory tract. Furthermore, rhinitis, tracheitis, and interstitial pneumonia were also observed (Etriwati et al., 2017). Hemorrhage, necrosis, and ulceration of the digestive tracts' lamina propria and submucosa. Necrosis in the proventriculus, degeneration, and necrosis in the gizzard glandular epithelium, and proliferation of macrophages in the intestines' lamina propria were the main lesions observed in the organ of the digestive system (Mariappan et al., 2018). Pancreas showed necrotizing pancreatitis (El-Bahrawy et al., 2015; Mariappan et al., 2018). Depletion of lymphoid tissue was the prominent finding in lymphoid organs, including the thymus, spleen, bursa, intestinal lymphoid aggregates like Peyer's patches, and cecal tonsils (Mariappan et al., 2018). Histologically, mesogenic strains produced non-suppurative encephalitis, myocarditis, pancreatic and splenic necrosis. In the other hand, lentogenic strains rarely produce infection in adult chickens. In field outbreaks, some lentogenic isolates resulted in non-suppurative tracheitis in conjunction with *E. coli* (Hooper et al., 1999).

DNA sequencing and phylogenetic analysis of the F gene's entire or partial nucleotide sequences were used to demonstrate the genetic diversity amongst NDV strains (Abdisa & Tagesu, 2017). For the virus particles to be infectious, the host proteases must cleave the F protein from its inactive precursor (F0) to its active forms (F1 and F2) (Scheid and Choppin, 1974; Ganar et al., 2014). The cleavage specificity is determined by the amino acid sequence present at the cleavage site and varies with the strain type (Glickman et al., 1988). Some papers worked on amplifying the F gene of NDV from field isolates in Egypt (Radwan *et al*., 2013; Selim *et al*., 2018; Abozaid and Abdel-Moneim, 2022). Isolation and characterization of NDV from some outbreaks in Egypt governorates like Behera, Giza, and Fayoum during the period from 2011 to 2012 were done, and the results recorded the presence of velogenic isolates of genotype VII sub-genotype d NDV that closely related to the Middle East isolates (Radwan et al., 2013).

This study was initiated to determine the gross and histopathological features of ND, which attacks Egyptian chickens, concerning their demographical data (age and type of breeds) and the kind of given vaccination.

MATERIAL AND METHODS

1-Specimen collection

Three birds were collected from each examined vaccinated poultry flock from 7 Egyptian governorates, as recorded in Table 1. Samples were collected in the period from January 2021 to December 2022.

The collected samples (brain, nostril, trachea, lung, thymus, heart, proventriculus, duodenum, jejunum, cecal tonsil, pancreas, spleen, liver, kidney, and bursa) were fixed in 10% neutral buffered formalin for histopathological examination. From each sample we examined five different microscopic fields and take the average of the histopathological score. Parts from tracheal tissues were pooled and preserved at −80°C for RRT PCR.

Table 1. Distribution, number of flocks, age, and mortality among poultry flocks from seven governorates

Governorate	No. of flocks	Age (days)	Mortality rate
El -Beheira	62	18-41	4-25%
El -Fayoum	4	28-32	2-10%
El -Gizza	2	22-30	1-2%
El- Gharbia	3	21-28	1-5%
EL-Menofia	32	22-29	4-15%
El-Qalyubia	4	28-32	4-8%
El- Sharqia	2	26-28	10-12%

2. Histopathological examination

Formalin-fixed tissues were trimmed, washed, dehydrated in ascending grades of ethyl alcohol, cleared in methyl benzoate, and embedded in paraffin wax after the routine follow-up steps were completed. Sections at 3µm were obtained and then stained by hematoxylin and eosin (H&E) stain for light microscopical investigation according to Bancroft et al. (2013).

3. Molecular Identification of NDV

3.1. Viral RNA Extraction

Tracheal samples were homogenized in a sterile mortar and pestle with saline and antibiotics. The homogenate was

centrifuged after three cycles of freezing and thawing. The supernatants were collected and kept at -80°C. RNA was extracted from the supernatant of the homogenate using extraction Kit RNeasy ® Mini Kit (cat.nos.74104 and 74106) (QIAGEN, Germany) according to the manufacturer's instructions.

3.2. RNA amplification

The 101 bp F gene fragment was amplified from the isolated RNA using a previously established specialized primer set Forward Primer F+4839 - 5'TCCGGAGGATACAAGGGTCT-3', Reverse PrimerF−4939 - 5'AGCTGTTGCAACCCCAAG -3' and ProbeF+4894 - 5′FAMAAGCGTTTCTGTCTCCTTCCTC CA-TAMRA-3'(Selim et al., 2018). The commercial kit WizPure™ qRT-PCR Master (PROBE) (QIAGEN, Germany) was used for RRT-PCR. According to the manufacturer's instructions, this kit is an optimized, ready-to-use solution for one-step quantitative RT-PCR assays using concentration.

Using a real-time thermocycler (Tianlong, China), real-time PCR was conducted. Reverse transcription was done at 52°C for 20 min for one cycle, and initial denaturation at 95°C for 5 min for another cycle was followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing at 60°C for 1 min.

RESULTS

3.1. Clinical Signs

Some birds showed respiratory manifestations such as coughing, gasping, sneezing, and rales. Other birds showed nervous signs like tremors, paralyzed wings and legs, circling, and twisted necks, with general symptoms such as greenish diarrhea and inappetence.

3.2. Gross Lesion

Gross lesions are summarized in Table 2, and the most common gross lesions in the examined vaccinated chickens were congestion in the blood capillaries of the brain, copious mucus in the nostril, congestion and increased mucus secretion in the trachea, congestion, and pneumonia in the lung, the heart was enlarged, diffuse hemorrhage in between and on the tips of glands of the proventriculus (Fig. 1a), the button-shaped hemorrhagic ulcer was detected in the duodenum (Fig. 1b), pancreas had mottling appearance due to multifocal distribution of necrosis and hemorrhage, liver appeared with some necrotic areas, the thymus was congested, the spleen was severely enlarged and congested (Fig. 1c), with some whitish nodules that give the surface a mottling appearance, bursa revealed edema with pinpoint hemorrhage, kidney showed diffuse enlargement with whitish discoloration on its surface (Fig. 1d), and edema with some pinpoint hemorrhage was detected in cecal tonsils.

Table 2. Gross lesions of NDV in different organs from infected chickens.

Organs	Necrosis	Hemorrhage	Congestion	Edema	Enlargement
Brain	-	-	+	-	-
Nostril	-	-	+ +	-	-
Trachea	-	+	+	-	-
lung	-	+ +	+ +	+	+ +
Heart	-	+	+ +	-	+
Proventriculus	-	+ + +	-	-	-

duodenum	+	+ +	-	-	-
Liver	+	-	+ + +	-	+ +
Pancreas	+ + +	+	+	-	-
Thymus	-	-	+ + +	-	-
Spleen	+ + +	-	+ + + +	-	+ +
Bursa	+	+ +	-	+ +	+ +
Cecal tonsil	+	+ +	-	-	-
Kidney	+ +	-	+ +	-	+ + +

-Non observed lesion, + slight, + +Mild, + + +Moderate and ++++severe

3.3. ***Histopathology***

Histopathological findings are summarized in Table3.Microscopical examination revealed vasculitis with perivascular edema (Fig. 2a) and cerebral spongiosis (Fig. 2b) in the brain. Nostril showed severe lymphocytic rhinitis with some hemorrhages, deciliation, degeneration and necrosis of mucosa, and congestion in the submucosa (Fig. 2c). The trachea showed tracheitis with mild degeneration and necrosis in the mucosa, loss of cilia, diffuse heavy leukocytic cell infiltration and hemorrhage, and congestion in the blood vessels (Fig. 2d). Lung showed edema and congestion in the blood capillaries around parabronchus, mild leukocytic cell infiltration and atelectasis in alveoli**,** interstitial pneumonia, which is characterized by moderate inflammatory cell infiltration, hemorrhage, proliferation of periparabronchial lymphoid tissues, and proliferation of syncytial giant cell (Fig. 2e, f). Heart revealed pericarditis with thickness of the pericardium (Fig. 3a), as well as myocardial degeneration with focal areas of edema and hemorrhages between the myofibril (Fig. 3b). The proventriculus showed thickness in mucosa due to fibrin deposition, edema, moderate inflammatory cell infiltration, and focal area of lymphocytic aggregation. The proventriculus glands showed degeneration and/or necrosis with hemorrhages in between (Fig. 3c). The duodenum showed degeneration and necrosis in the mucosal epithelium with severe inflammatory cell infiltration, hemorrhages in the tip of intestinal villi, degeneration and necrosis in duodenal glands, and moderate inflammatory cell between duodenal glands (Fig. 3d). The liver showed perihepatitis with thickness in hepatic capsule due to fibrin deposition and severe inflammatory cell infiltration, dilatation of hepatic sinusoid, and proliferation of the lymphoid tissues (Fig. 3e).

Necrotizing pancreatitis with slight hemorrhage and infiltration of inflammatory cells were observed in the pancreas (Fig. 3f). Severe congestion in the cortex and medulla with severe lymphoid depletion was found in the thymus (Fig. 4a). Spleen showed severe necrosis and hemorrhage in the white pulp**,** lymphoid depletion in the germinal center with edema and rarification (Fig. 4b). Bursitis with severe inflammatory cell infiltration, hemorrhage with Heterophilic cell infiltration in the cortex and lymphoid depletion with numerous apoptotic bodies in medulla were the main lesions observed in bursa (Fig. 4c). Cecal tonsils showed lymphoid depletion, hemorrhage in the tip of villi necrosis and desquamation of the villi (Fig. 4d). In the kidney, proliferation of lymphoid tissues in the interstitial tissues (Fig. 4e), degeneration in renal tubules and hypercellularity in glomerular tuft were recorded (Fig. 4f).

Table 3. Histopathological lesions of NDV in different organs from infected chickens.

Organs	Necrosis	Hemorrhage	Congestion	Inflammatory cell infiltration	Edema	Lymphoid depletion
Brain	-	-	+	+	+	-
Nostril	+++ +	++ +	++ +	++ +	-	-
Trachea	+ +	+ + +	+ +	+ + + +	+ +	-
lung	-	+ +	+	+ + +	+ +	-
Heart	+	+ +	+ +	+ +	+ +	-
Proventriculus	+ +	+ +	-	+ + +	-	-
duodenum	+ +	+ + +	-	+ + +	-	-
Liver	+	+ + +	+ +	+ + +	+	-
Pancreas	+ + +	+	+	+ + +	-	-
Thymus	+ + + +	+ + + +	+ + +	+ + +	-	+ + +
Spleen	+ + +	+ + +	+ +	+ +	+ +	+ + + +
Bursa	+ +	+ +	+	+ + +	+	+ + + +
Cecal tonsil	+ +	+	-	+	-	+ +
Kidney	+ + +	+ + +	+ +	+ + +	+ +	-

-Non observed lesion, + slight, + +Mild, + + +Moderate and + + + +severe

3.4. Real-Time RT-PCR result

The results of the PCR showed that 43 samples from 109 farms (39.5%) were positive for NDV, as shown in Table 4.

Table 4. Prevalence of NDV according to RT-PCR:

Test	**Positive NDV**		**Negative NDV**	
	No.	**%**	**No.**	**%**
PCR of NDV (Tested Poultry farm= 109)	43	39.5	66	60.5

The incidence of infection was 18 to 41 days, and the peak of infection occurred between 20–and 30 days. The infection rate increased as age increased, as shown in Table 5. Avian 48 had the highest infection rate, while Cobb 500 had the lowest infection rate, as recorded in Table 6. The vaccination programs used in the flocks of chickens are described in Table 7. The farms that vaccinated vector vaccine at one day old and then gave live attenuated vaccine at 7th day then poster dose with inactivated vaccine, this program provides higher protection level against NDV than the program that did not use vector vaccine at one day old. The prevalence of NDV within the Egyptian governorates is explained in Table 8. El-Beheira had the most samples, whereas El-Menofia had the most significant infection rate.

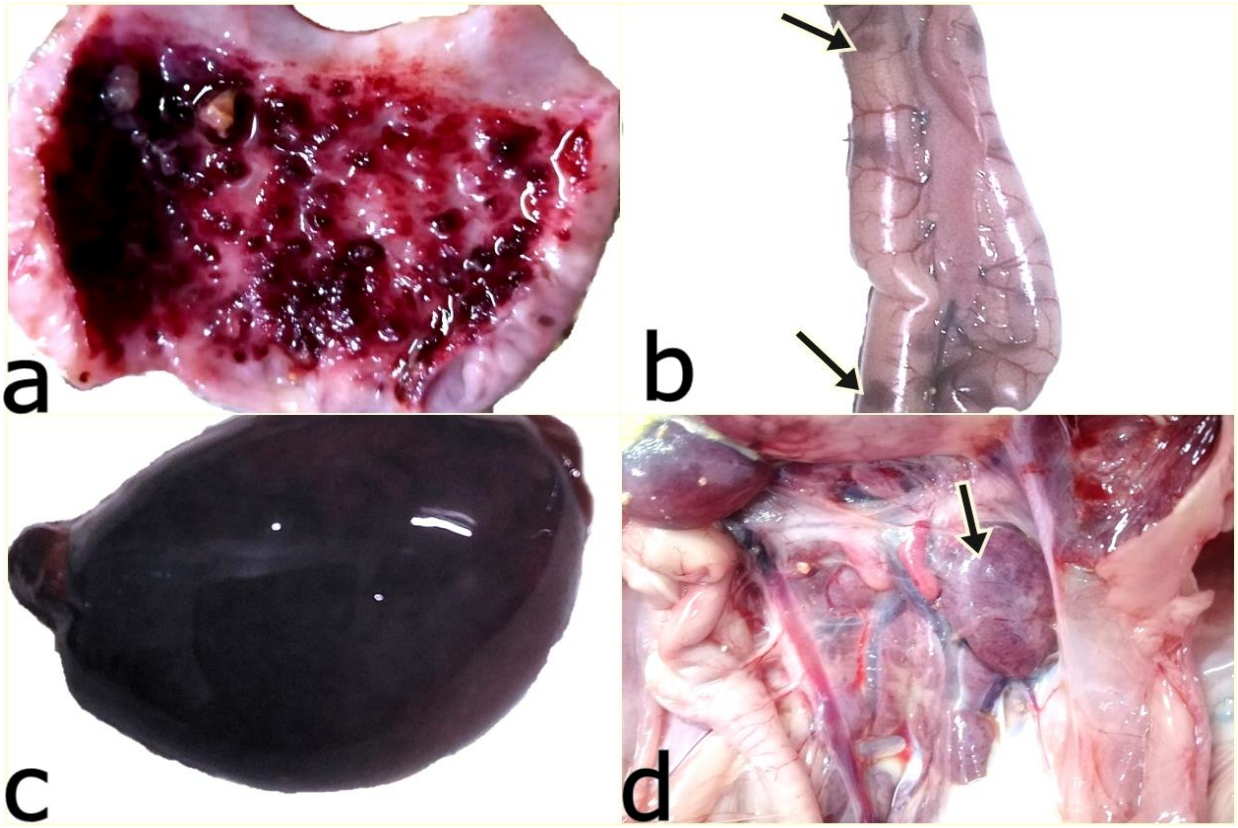

Fig. 1. a) Proventriculus, chicken: Shown diffuse hemorrhage in between and on the tips of glands of proventriculus. **b) Duodenum**; chicken: Shown button-shaped hemorrhagic ulcer (arrows).**c) Spleen,** chicken: severe congestion with splenomegaly. **d) Kidney** chicken: Shown enlargement with whitish discoloration on its surface (arrow).

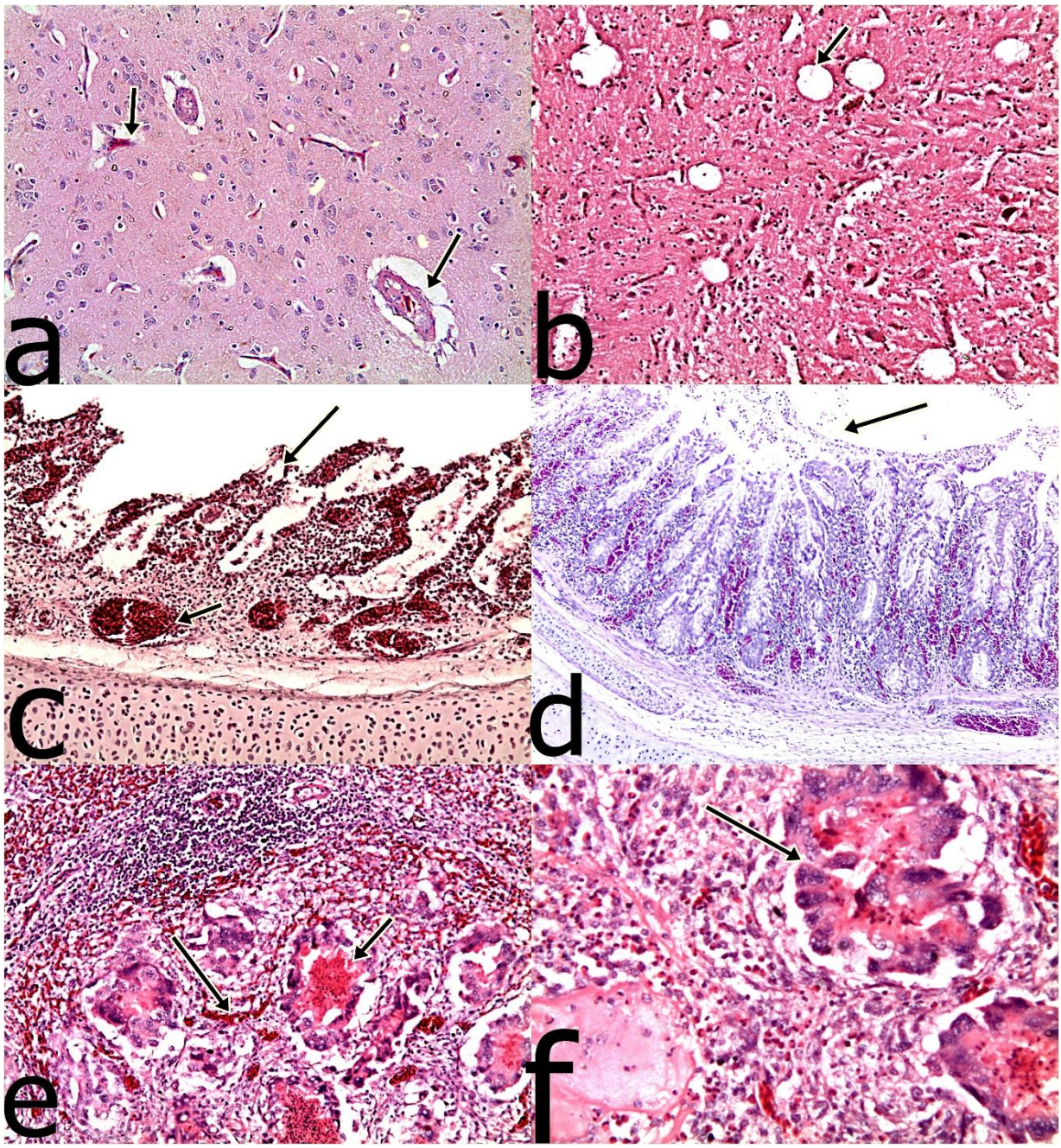

Fig. 2. Chicken naturally infected with NDV. (a) Brain. Showing vasculitis with perivascular edema (arrows). **(b) Brain**, chicken**.** Cerebral spongiosis (arrow). **(c) Nostril,** chicken. Showing severe lymphocytic rhinitis with degeneration and necrosis of the mucosal epithelial cells (long arrow) and congestion in the submucosa (short arrow). **(d) Trachea**, chicken. Showing tracheitis with mild degeneration of mucosa and loss of cilia (arrow), diffuse heavy leukocytic cell infiltration, hemorrhage, and congestion in blood vessels. **(e) Lung**, chicken. Interstitial pneumonia is characterized by moderate inflammatory cell infiltration and hemorrhage (long arrow) and proliferation of syncytial giant cells (short arrow). **(f) Lung**, chicken. High magnification from **(e)** showing syncytial giant cell (arrow). H&E stain, a, b, c, and d X10; c and f X20.

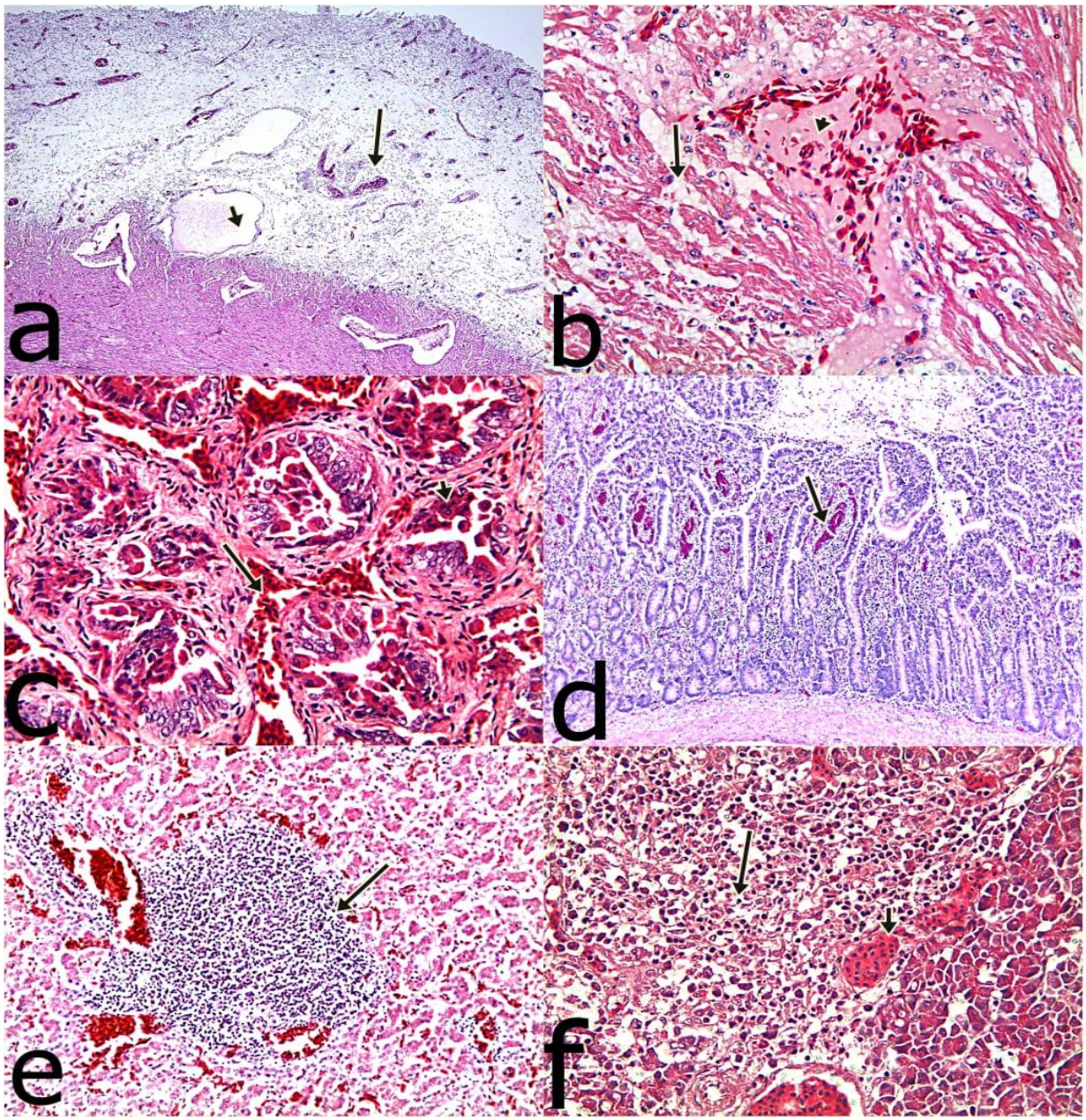

Fig. 3. Chicken naturally infected with NDV. (a)Heart, chicken**.** Pericarditis with thickness in the pericardium due to edema (short arrow), moderate inflammatory cell infiltration, and congestion in blood vessels(long arrow). **(b) Heart**, chicken**.** Degeneration and edema in myofibril (long arrow), focal area of edema, and hemorrhage between myofibril (short arrow). **(c) Proventriculus**, chicken**.** Showing degeneration and necrosis in glands (short arrow), hemorrhage between glands (long arrow). **(d) Duodenum,** chicken**.** Showing degeneration and necrosis in the mucosal epithelium with severe inflammatory cell infiltration and hemorrhage in the tip of intestinal villi (arrow). **(e) Liver**, chicken**.** Proliferation of the lymphoid tissue (arrow). **(f) Pancreas,** chicken**.** Necrotizing pancreatitis (long arrow), with slight hemorrhage (short arrow). H&E stain, a and d X4; e X10; b, c and f X20.

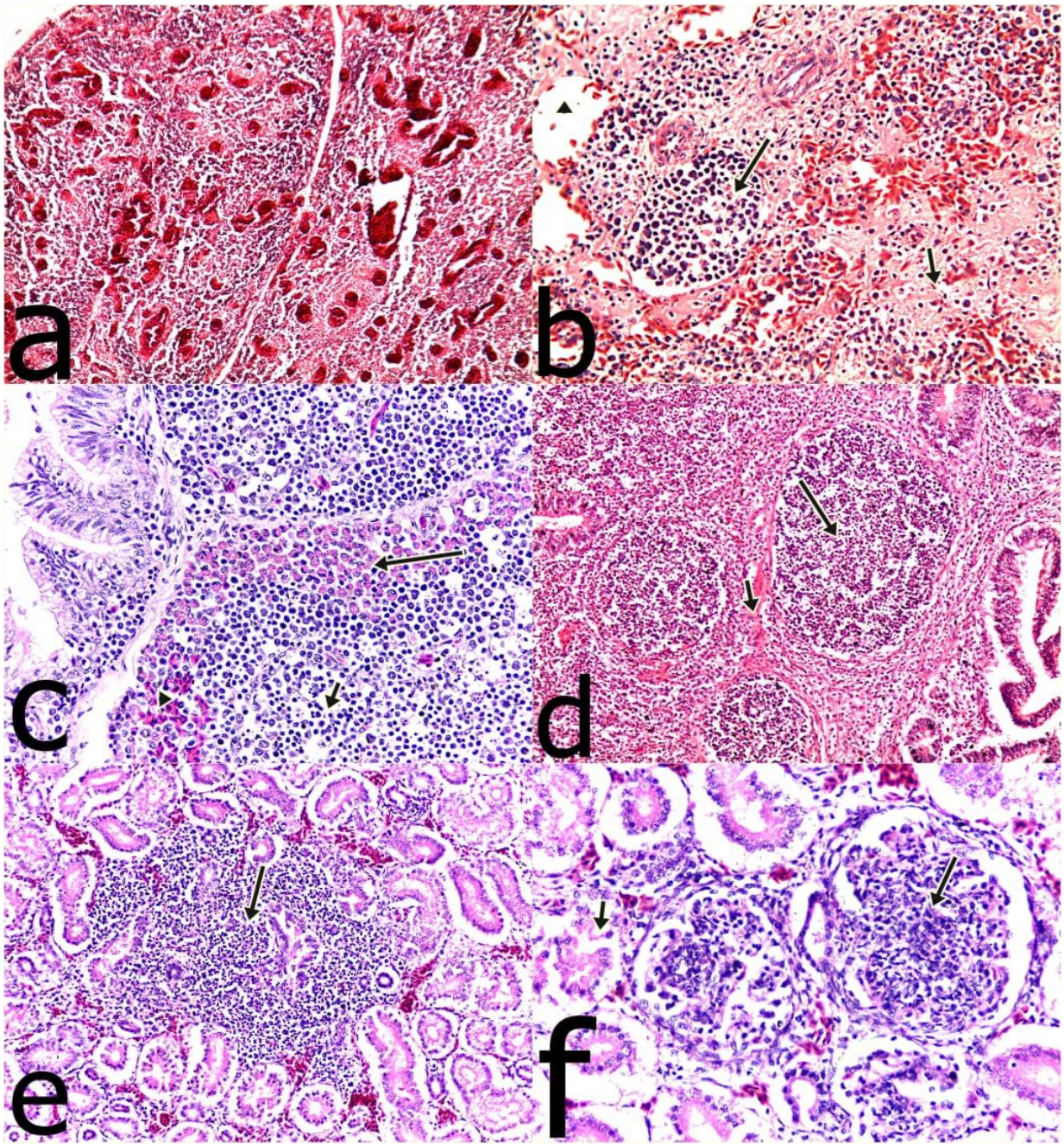

Fig. 4. Chicken naturally infected with NDV. (a) Thymus, chicken**.** Severe congestion in cortex and medulla with lymphoid depletion. **(b) Spleen**, chicken**.** Lymphoid depletion in the germinal center (long arrow), edema in the spleen (green arrow), rarification, and lymphoid depletion (short arrow). **(c) Bursa,** chicken**.** Lymphoid depletion with numerous apoptotic bodies in the medulla (short arrow), hemorrhage (green arrow), and heterophilic cell infiltration in the cortex (long arrow). **(d) Cecal tonsil**, chicken**.** Lymphoid depletion (long arrow) in cecal tonsils associated with interstitial fibrosis (short arrow). (**e) Kidney,** chicken**.** Degeneration in renal tubules with proliferation of the lymphoid tissues (arrow). **(f) Kidney,** chicken**.** Hypercellularity in glomerular tuft (long arrow), degeneration, and necrosis in renal tubules (short arrow).H&E stain, a, d, e, and f X10; b X20; c X40.

4. Results of Real-time Polymerase Chain Reaction (RT-PCR):

4.1. Table 5. Prevalence of NDV according to age of poultry.

Age group	Positive NDV		Negative NDV	
	No.	%	No.	%
10-20 days (No. = 14)	5	35.7	9	64.3
20-30 days (No. = 51)	22	43	29	57
>30 days (No. =44)	16	36.4	28	63.6
Total (No. = 109)	43	39.5	66	60.5

4.2. Table 6. Prevalence of NDV according to type of breed of broiler

Type of breed of broiler	Positive NDV		Negative NDV	
	No.	%	No.	%
Arbo (No.7)	3	43	4	57
Avian 48 (No.10)	7	70	3	30
Cobb 500 (No.19)	4	21	15	79
Indian River (IR) (No.30)	14	64.7	16	53.3
Ross 308 (No.43)	15	35	28	65
Total (No.109)	43	39.5	66	60.5

4.3. Table 7. Explains the vaccination programs against NDV in Egyptian farms.

Vaccination programs	Positive NDV		Negative NDV	
	No.	%	No.	%
Vector vaccine at one day old. Live attenuated vaccine on the 7th day. Inactivated at 14th day vaccine (No. 28)	8	28.6	20	71.4
Live attenuated vaccine on 7th day Inactivated on 14th day of vaccine (No. 81)	35	43	46	57
Total (No. 109)	43	39.5	66	60.5

4.4. Table 8. Prevalence of NDV according to Governorates.

Governorates	Positive NDV		Negative NDV	
	No.	%	No.	%
El -Beheira (No. 62)	23	37	39	63
El -Fayoum (No. 4)	1	25	3	75
El -Gizza (No. 2)	0	0	2	100
El- Gharbia (No. 3)	1	33,3	2	66.7
EL-Menofia (No. 32)	15	47	17	53
El - Qalyubia (No. 4)	1	25	3	75
El- Sherqia (No. 2)	2	100	0	0
Total (No. 109)	43	39.5	66	60.5

DISCUSSION

Newcastle disease is considered one of the most critical and devastating diseases due to its worldwide distribution and severe economic losses in domestic poultry (Baksh et al., 2021).In Egypt, the continuous spread of NDV has caused severe financial losses in the poultry industry since the first isolation of the velogenic NDV genotype VIId in 2011 and up until now, despite adopting several vaccination programs (Abd El-Hamid et al., 2020). This study was carried out to investigate the gross, histopathological, and epidemiological features of ND, which attacks broiler chickens in Egypt under field conditions. In the current study, one hundred and nine broiler chicken flocks were investigated from January 2021 to December 2022 from seven different Egyptian governorates for NDV infection. The flocks under examination were of varying ages, and their capacities ranged from 1,000 to 80000 birds. The mortality rates in the examined herds were varied between (1-25%). The infected chicken suffered from respiratory symptoms like sneezing, coughing, swollen sinuses with conjunctivitis, and diarrhea of various colors. Some flocks experienced gasping with enlarged sinuses. Other nervous symptoms included muscle tremors, wing drooping, neck and head twisting, and circling were also observed. From our reading in the previous studies (Kommers et al., 2002; Oladele et al., 2005; Abdisa and Tagesu, 2017; Abd El-Hamid et al., 2020; El-Morshidy et al., 2021), we did not detect any remarkable changes in the clinical signs of NDV under field conditions in this study.

The most critical gross features recorded in this study were congestion in the brain, copious mucus in the nostril, congestion and increased mucus secretion in the trachea, pneumonic lung, and enlarged heart was enlarged. In addition, the proventriculus revealed diffuse hemorrhage in between and on the tips of glands, and a button-shaped hemorrhagic ulcer was detected in the duodenum. The pancreas had a mottling appearance due to the multifocal distribution of necrosis and hemorrhage, and the liver showed hepatomegaly with some necrotic area. Furthermore, the thymus was congested, the spleen was severely enlarged with a mottling appearance, the bursa revealed edema with pinpoint hemorrhage, and the kidney showed diffuse enlargement with whitish discoloration on its surface. These results agreed with (Ellakany et al., 2019; Ogali et al., 2020). This result indicated that NDV had a severe effect on visceral organs, and the most frequent lesions

observed in proventriculus indicated that NDV circulated within the Egyptian flocks of velogenic viscerotropic nature. The current gross pathological features have not recorded any remarkable changes with those reported in previous studies (Kommers et al., 2002; Piacenti et al., 2006; Pansota et al., 2013; Brar et al., 2017; Fawzy et al., 2021).

Histopathological examination revealed cerebral spongiosis and vasculitis in the brain; this result was agreed with (Moura et al., 2016; El-Morshidy et al., 2021). Neurological disorders are brought on by NDV infection and replication in chickens because they damage the neurons (Angeliya et al., 2022). The respiratory tract is one of the primary entry points for NDV. The sialic acid on the host cell serves as a receptor for the attachment of the virus to the epithelial cell of the respiratory tract (Wen et al., 2016). NDV affects severely in the respiratory tract as the nostril shows necrosis of mucosa with severe lymphocytic cell infiltration (Anis et al., 2013). A severe inflammatory reaction was observed in the trachea, represented by diffuse heavy leukocytic cell infiltration with edema and hemorrhage. These findings were agreed with (Khader et al., 2020).In the current study, the lung showed the most characteristic lesion observed within the respiratory tract as it showed interstitial pneumonia with proliferation formation of syncytial giant cells (Mousa et al., 2020; Mousa et al., 2021; Gopal, 2023). The lesion observed in the lung may be due to circulatory disturbance caused by viremia and secondary bacterial infection (Lopez & Martinson, 2017). The lesion observed in the cardiovascular system was detected mainly in the heart, which showed pericarditis and degeneration in the myocardium, which was in harmony with (El-Morshidy et al., 2021; Mousa et al., 2021). Pericarditis may occur due to the compaination of Escherichia coli with ND infection, leading to fibrinous pericarditis, perihepatitis, and even fibrinous purulent lesion (Cattoli et al., 2011). NDV reaches the heart through blood (viremia) and is distributed within the heart tissue, and this is identified by an immuno-positive response to the blood vessel endothelial cells in the heart and other organs. The distribution of NDV in the heart is consistent with a previous report by (Bwala et al., 2012), who noted that chicken infected with a velogenic viscerotropic isolate displayed an accumulation of macrophage cells at the myocardium and immuno-positive reaction to NDV is identified at the myocardium and mononuclear cells. NDV can replicate inside the upper respiratory and digestive tracts' mucosal epithelial cells. The replication of NDV within the intestinal lymphoid follicle can lead to hemorrhage and edema in internal organs due to blood vessel damage(Chekwube Paul et al., 2014).The distribution of NDV in internal organs was detected by Nakamura et al. (2008) and Bwala et al. (2012), who recorded that the NDV antigen field isolate was immuno-positive in the proventriculus, duodenum, pancreas, and bursa of Fabricius. The proventriculus was the most affected organ in the digestive tract as it appeared with thickness in mucosa with a focal area of lymphocytic aggregation beside hemorrhage in the tip of the glandular epithelium, degeneration in the gland with hemorrhage and inflammatory cell infiltration between glands (Mousa et al., 2021). The intestinal tract showed degeneration and necrosis in the mucosal epithelium with severe inflammatory cell infiltration, hemorrhage in intestinal villi, and degeneration of the duodenal gland (Rehman et al., 2018; Chowdhary et al., 2020). The liver showed perihepatitis, hepatic sinusoid dilatation, and lymphoid

tissue proliferation (Anis et al., 2013; El-Morshidy et al., 2021). During their early development (up to 3–4 months of age), chickens produce a lot of lymphoid infiltrates in various tissues, partially due to local immunologic activation (Olah & Vervelde, 2008). In this study, lymphoid proliferation was found in multiple tissues such as (the lung, liver, proventriculus, and kidney). The pancreas showed necrotizing pancreatitis, considered one of the most characteristic lesions recorded with NDV infection (El-Bahrawy et al., 2015; Mousa et al., 2020). Our respiratory and digestive system findings indicated that NDV is of a velogenic viscerotropic nature. Lymphoid depletion and hemorrhage were all observed in the thymus, spleen, bursa, and cecal tonsil (El-Morshidy et al., 2021). It's possible that the virus multiplied and spread throughout lymphocytes, causing the observed lymphoid depletion and a significant decline in the birds' immune capacities (Mousa et al., 2019). Severe lymphoid depletion and hemorrhage were observed in thymus (Kabiraj et al., 2020). The main lesions observed in the spleen were severe necrosis, lymphoid depletion, rarification, and hemorrhage in the white pulp(El-Morshidy et al., 2021). Bursa was from the organ with characteristic lesions, which showed bursitis, hemorrhage with heterophilic cell infiltration in the cortex, and lymphoid depletion with numerous apoptotic bodies in the medulla (Anis et al., 2013; Chowdhary et al., 2020). The kidney in this study represents the effect of NDV in the urinary organs, which is observed with the proliferation of the lymphoid tissues in the interstitial tissue of the kidney with degeneration in renal tubules in addition to hypercellularity in glomerular tuft (El-Bahrawy et al., 2017; Kabiraj et al., 2020). As the virus enters through the respiratory system and is subsequently transported to the kidney by blood circulation, kidney nephritis develops. Once an infection occurs, the virus spreads through the bloodstream to the kidney and bone marrow, creating secondary viremia (Wen et al., 2016). The distribution of NDV in the kidney was detected in previous studies by (Nakamura *et al*., 2008; Etriwati *et al*., 2017), who discovered an immuno-positive response to NDV in kidney tubular epithelial cells. The current gross and pathological lesions observed in nervous, respiratory, cardiovascular, digestive, immune, and urinary organs indicate the pantropic nature of NDV, which agrees with (Mousa et al., 2021). According to the literature we reviewed from other studies, as mentioned above, we did not detect any remarkable changes in the histopathological features of NDV under field conditions in this study. Therefore, we believe that the pathological alteration of NDV in broiler chickens is somewhat stable.

PCR results showed that 43 samples from 109 broiler flocks were positive for NDV, representing about 39.5% of total samples. The present study recorded the broiler chicken's age at infection. The result recorded that with the increased age of birds, the percent of infection increased also, and the peak of infection was 20-30 days; this agreed with (Spalatin et al., 1976; East et al., 2006).This may be due to an increased chance of acquiring the infection with more contact with sources of infection during life and also may be due to increased stress on the bird with increasing gross weight due to a defect in vaccination. This may also be due to the natural degradation of the maternal-derived antibody (MDA) between 2 and 3 weeks of age (Hamal et al., 2006). The current study indicates the incidence of NDV in various broiler breeds. Avian 48 had a high infection rate among other broiler breeds. This may be due to the low innate

immunity produced by breeders. The Cobb500 breed gives the highest protection rate. Although the Ross 308 breed of broilers had the most significant expansion among Egyptian broiler flocks, the Cobb500 outperforms the Ross breed regarding average daily weight increases, growth performance, and immune protection against diseases (Mayahi et al., 2016; El-Tahawy et al., 2017; Pascalau et al., 2017; Gholami et al., 2020). This may have been caused by the different genotypes of the Cobb breed's strains compared to Ross breeds under repeated exposure to the endemic Newcastle virus in the area, the vaccine strains used in breeders' vaccination, and the schedule and application method used (Ezzulddin et al., 2022). Although the comprehensive immunization program used by the Egyptian farms, as all of the obtained samples were vaccinated against NDV, ND is still extensively prevalent, possibly due to inadequate farm management techniques. In endemic nations of Asia, Africa, and Central America, virulent NDV persists after strict vaccination regularly (Czegléd et al., 2006; Aldous et al., 2007; Gowthaman et al., 2019). Furthermore, it implies that while immunization may prevent the active disease from manifesting and safeguard the flock in the event of an epidemic, it might not wholly stop virus shedding. The investigated farms in this study used the vector vaccine at one day old and then gave live attenuated vaccine and then poster dose with inactivated vaccine; this program provides a higher protection level against NDV than the program that did not use the vector vaccine from the first day. This may be due to the high titer of antibodies produced early by the vector vaccine. The present study indicates that El-Menofia had the highest infection rate within other governorates, with a percentage of 47%, while El-Beheira had the highest number of samples. This may be due to poor management and inadequate vaccination programs as most of the flocks in EL-Menofia were of low capacity and farms located near each other. Most samples collected from El-Beheira were from large flocks with high capacity and good management systems. In the current study, the most characteristic pathological changes were cerebral spongiosis, lymphocytic rhinitis, tracheitis, interstitial pneumonia, pericarditis, hemorrhage in the proventriculus, perihepatitis, necrotizing pancreatitis, lymphoid depletion, and hemorrhage in immune organs, and proliferation of lymphoid tissue in some organs as lung, proventriculus, liver, and kidney. These changes were not as remarkable as the previous studies recorded in this study. It’s for sure that the observed pathological changes were closely related to age, type of breed, programs, and type of vaccination used in the examined farms.

CONCLUSION

The current study determined the distribution of NDV in Egyptian farms, which was supported by macroscopic, histopathological, and molecular analyses. The main histopathological lesions were severe lymphocytic rhinitis, interstitial pneumonia with proliferation of syncytial giant cells, pericarditis, perihepatitis, proliferation of heterophilic and lymphoid cells in some organs, necrotizing pancreatitis, and severe lymphoid depletion and hemorrhage in immune organs. Poultry of different ages and breeds were much more susceptible to natural infection with NDV. The Ross 308 had the most incredible spread between the Egyptian farms. The Avian 48 had the least protection against NDV, whereas the Cobb 500 generated high levels of security. The

most prevalent infection rate was in the EL-Menofia governorate.

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