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Pathogenesis of Isolated Newcastle Disease Virus Genotype VII .1.1 in Turkey Poults in Egypt

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Abstract

Recurrent infection with Newcastle disease virus in flocks that have received vaccinations and high economic losses in Egypt in the last few years urged us to study the diversity and genetic changes in isolated NDV from chickens and its pathogenesis in other species such as turkey poults. Fifteen positive NDVs were isolated from chicken flocks suffering from a respiratory infection. Sequencing of three isolates out from the 15 NDV positive isolates (20%) revealed that NDV was genotype VII.1.1. When compared to other previously isolated worldwide and Egyptian strains, the three isolates' amino acid sequences show (99.1-99.8 %) identity with genotype VII.1.1.Thirty four weeks old Black Burzi turkey poults, separated into two groups: control (n=15) and infected (n=15), were used to study the pathogenesis of the isolated NDV genotype VII.1.1. Each afflicted bird was given an inoculation with 0.1 mL of 106EID50 of NDV genotype VII.1.1(ND /chicken /Egypt / Dakahlia /31 /2020) at 4 weeks of age via an ocular route. Proventriculus, conjunctiva, lung, spleen, trachea, and caecal tonsil samples were collected from both groups at (6, 12, 24, 48 hours, and 5 days after infection) and tested for the presence of NDV using Quantitative Reverse transcription-polymerase Chain Reaction (QRT-PCR). The virus was found in the challenged birds' spleen as soon as 12 hours after infection, followed by the lungs and trachea. After 2 and 5 days after NDV infection, histologically significant lesions were found, particularly in lymphoid organs. It is concluded that the presence of NDV in Egyptian flocks of chickens could induce major disease in commercial turkeys, necessitating the development of novel vaccinations based on the circulating NDV genotype VII.1.1 in Egypt to protect domestic poultry from recurrent infection.

KEYWORDS
Newcastle disease virus, Turkey poults, virus shedding, RT-PCR, Histological changes

INTRODUCTION

Newcastle disease (ND) virus is extremely contagious and fatal (Afonso *et al.*, 2016), affects a minimum of 250 kinds of domestic and wild birds of various ages (Smietanka *et al.*, 2014). A virulent virus of the avian paramyxovirus serotype I (APMV-1) of the Avulavirus genus pertaining to the family paramyxoviridae and the order Mononegavirales is the disease's causal agent (Li *et al.*, 2019). The most isolated virulent viruses with great losses among bird species were genotype VII (Hegazy *et al.*, 2021). Turkey rearing is believed to be quickly developing in Egypt to the point where it now ranks second to the chicken sector. Newcastle disease had its first appearance in Turkey species in 1948 (Abdoshah *et al.*, 2012). Thereafter several outbreaks in the same species have been reported (Capua *et al.*, 2002). In 1999, the number of Newcastle disease outbreaks in turkeys increased dramatically. Turkeys and fowl have similar sensitivity; however, turkeys' clinical symptoms are less severe. A turkey outbreak with substantial morbidity and mortality has recently been reported (Gowthaman *et al.*, 2013). Shedding of NDV in the digestive and respiratory systems of turkeys has already been optimized using reverse transcription-polymerase chain reaction (Quantitative RT-PCR) (Boroomand *et al.*, 2016), because it is regarded as a more sensitive technique in comparison to additional confirmation test procedures in the field of ND laboratory diagnosis (Krzysztof *et al.*, 2006). Therefore, the current work employed Quantitative RT-PCR and histological alterations to elucidate the pathogenesis of NDV genotype VII.1.1 in turkey poults. Serum samples were also taken at various intervals after infection to determine the seroconversion rate.

MATERIALS AND METHODS

Fifteen positive NDV samples were isolated from chicken flocks that suffered from respiratory signs as in (Table 1) and identified by Quantitative RT-PCR as shown in Figure 1. The challenged virus was isolated from trachea, lung, liver, spleen, and brain in a group of of broiler chicken flock during an outbreak that occurred in 2020 in Egypt using commercial embryonated chicken eggs (ECEs), 9 day old via the allantoic sac. The dangerous genotype VII.1.1 strain of the Newcastle disease virus is

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Table 1. Descriptive data of examined flocks with RT-PCR results and sequencing.

Flock number	Locality	Breed	Age/day	Total number	Mortality% till submission day	Vaccination against NDV	RT-PCR	
1	Damietta	Cobb	30	4500	3	+	+	
2	Farskour	Cobb	25	4500	2	+	+	
3	Farskour	Ross	35	6000	2	+	+	MZ668300
4	Farskour	Hisex	250	2000	1.5	-	+	
5	Karm and Razok	Arbor Acres	31	10000	4	+	+	
6	Karm and Razok	Ross	20	5000	2	-	+	
7	Meet Abo-Ghalib	Arbor Acres	35	6000	16.6	-	+	
8	EL-Manzla	Saso native breed	42	20000	8.5	+	+	
9	EL-Manzla	Saso native breed	45	1500	1	+	+	
10	EL-Manzla	Arbor Acres	25	3000	2	+	+	
11	EL-Manzla	Arbor Acres	35	16000	8	+	+	
12	EL-Manzla	Ross	35	8000	3	+	+	
13	Karm and Razok	Arbor Acres	25	6000	3	+	+	
14	Gamassa	Arbor Acres	32	18000	6	+	+	MZ668301
15	Gamassa	Ross	31	3000	2	+	+	MZ668302

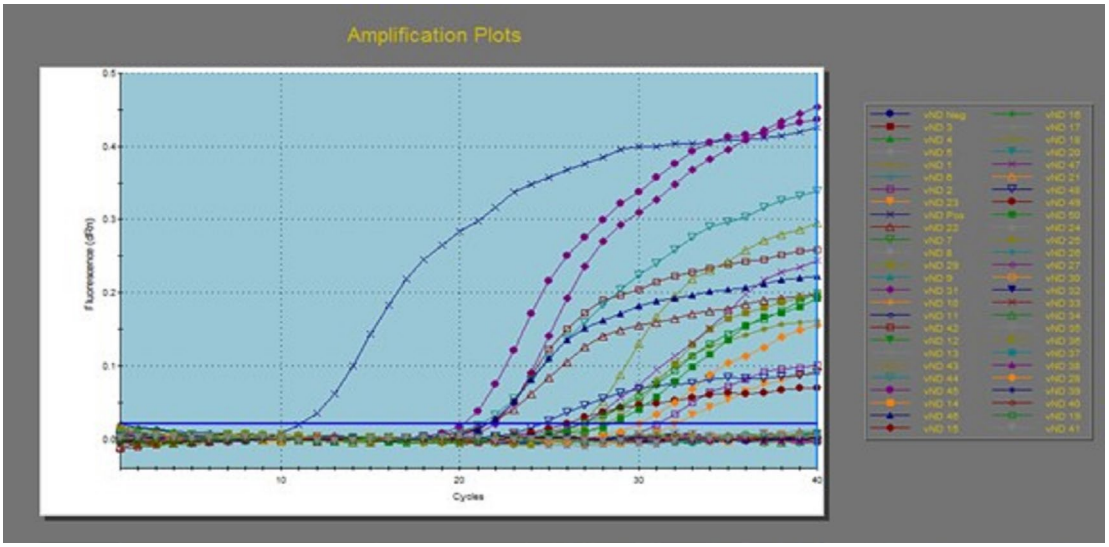

Figure 1. The amplification curve of 15 positive NDVs was isolated and identified by QRT-PCR and found to be NDV genotype VII.1.1 is the main genotype.

known as ND /chicken /Egypt /Dakahlia /31 /2020 and has the accession number MZ668300. The virus spread and titrated in accordance with OIE (1996). The extracted allantoic fluid was administered via the ocular route after being titrated in ECE and adjusted to contain approximately 106 embryo infective dose 50 (EID50)/0.1 ml.

Experimental birds

Thirty-four weeks old commercial black bruzi turkey poults were obtained from Dakahlia province. The birds were grown using a floor-based method in the animal house, the experimental facility at the Faculty of Veterinary Medicine, Zagazig University,. Under permission number ZU-IACUC/2F/119/2021, Zagazig University's institutional animal care and use committee (ZU-IACUC) approved the study. The turkey poults were divided into two groups of 15 birds each. The initial group was challenged with 0.1 ml of 10^6 EID_{50} of (ND /chicken /Egypt /Dakahlia /31 /2020) via the ocular route. Organs (N=6/bird) from each bird were harvested at 6,12,24,48 h, and 5 days post-inoculation. The organs were divided and put to use for both RNA extraction using the QIAamp Viral RNA purification kit manufacturer's instructions. The primer sequences a probe-based (Wise *et al*., 2004). PCR reaction was done and the specific amplified PCR product was detected by agarose gel electrophoresis according to (Sambrook and Russel,(2001) and histopathological examination according to (Suvarna and Layton,(2013). The second group (the control group) received only PBS via the ocular route and had been used to determine the fundamental level of humoral immune responses. Serum samples from 3 birds were harvested at the same time of tissue collection and used for evaluation of antibodies seroconversion throughout the experiment OIE (2012).

Statistical analysis

One-way ANOVA (PROC ANOVA; SAS Institute Inc., 2012) was run with the level of significance set at $\alpha = 0.05$

RESULTS

Detection of vNDV genotype VII from natural outbreaks in poultry flocks

The investigated flocks suffered from high morbidity and mortality which ranged from(1.5-16.6%),and severe respiratory symptoms (dyspnea and tracheal rales).The gross lesions were sinusitis, severe tracheitis, pneumonia, air vasculitis, and petechial hemorrhages on the proventriculus. The fifteen positive NDV genotype VII.1.1 were identified by Quantitative RT-PCR and by choosing 3 isolates that show more severe respiratory signs and mortalities for sequencing it was found to be NDV genotype VII.1.1 with cleavage site 112RRQKRF117 that is consistent velogenic NDV viruses with accession numbers MZ668300, MZ668301, and MZ668302.

Experimental infection of vNDV genotype VII in turkey poults

The results of the experimental infection showed no abnormal clinical signs in the control group (PBS-inoculated) and 6 h post-infection in the challenged group. While 12 hours post-infection, 3 birds showed signs of conjunctivitis and depression. After 24 h post-infection another 3 birds had severe respiratory signs. On the other hand, after 48 h the turkey poults showed loss of appetite, weakness, severe depression with bilateral conjunctivitis, and oculo-nasal discharge. After 5 days post-infection conjunctivitis, dyspnea, incoordination, paralysis, and head shaking with the uncontrolled movement of the head were recorded with Greenish diarrhea.

Virus detection shedding and Serological examination

NDV virus was subjected to Quantitative RT-PCR from experimentally infected tissues (trachea, lung, conjunctival membrane, proventriculus, caecal tonsils, and spleen). After 6 h post-infection,no virus was detected in all organs, after 12 and 24 h the virus was detected in the lung, spleen, and trachea,while after 48 h and 5 days PI (Table 2),the virus was detected in all investigated tissues. By sequencing, the detected virus found that belongs to NDV genotype VII .1.1 (Figure 2). The virus, on the other hand, was not found in any organ samples taken from non-infected control birds. At the time of challenge the immunity of turkey poults was zero and there is no maternal immunity. The results of the haemagglutination test revealed a significant seroconversion in the infected group 2 and 5 days PI ($P < 0.001$) as shown (Figure 3).

Figure 2. Phylogenetic tree of the shedding NDV virus genotype VIId indicated by a black triangle and compared with other related reference strains of NDV.

Histopathological findings after challenge with virulent NDV genotype VII.1.1 in different organs at different times

Trachea, lung, and spleen specimens are the most prevalent organs showing histopathological lesions early 12 h PI and the severity of lesions increased post 24, 48 h, and 5 days PI as shown in (Figures 4-7).

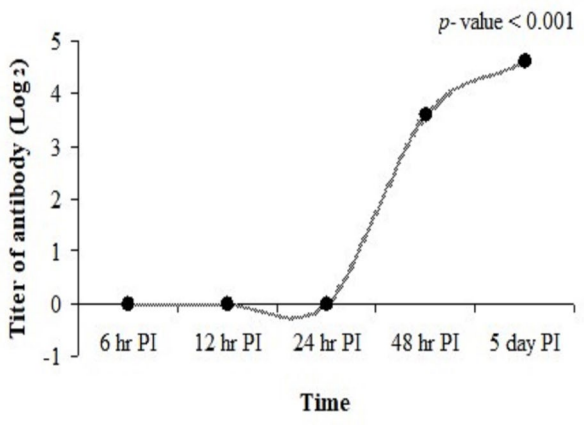

Figure 3. Pathogenesis of NDV genotype VII.1.1 and seroconversion post-Newcastle challenge at 4 weeks old age.

DISCUSSION

One of the most prevalent infectious and viral diseases in chicken is Newcastle disease, causing substantial financial losses in poultry industry each year (Miller and Koch, 2013; Megahed *et al.*,2018). The ability to recognize and identify viruses quickly is critical for developing an effective disease control strategy. As a result, molecular approaches (QRT-PCR) can be used for quick and confirmed detection of NDV from any ND epidemic (Dharmayanti *et al.*, 2014; Manual, 2008). Fifteen positive NDV chicken flocks suffering from respiratory infection were identified by QRT-PCR. Sequencing of 3 isolates out from the 15 NDV isolates (20%) revealed that NDV genotype VII.1.1 is the main genotype with a cleavage site that carries motif 112RRQKRF117 that corresponds to velogenic NDV viruses. Also, the current study sheds light on the direct detection of NDV in various organs at various times in experimentally infected turkey poults as stated by (Kant *et al.*,(1997) who found that direct QRT-PCR detection from organ samples carrying virulent NDV was possible. According to (Gohm *et al.*, (2000), quick NDV detection can be performed by using QRT-PCR immediately on samples from infected birds without first isolation of the virus. Turkey poults in research in this study showed moderate respiratory discomfort with conjunctivitis or nasal discharge, especially after 2 days PI, and became more obvious at 5 days after exposure which is consistent with (Piacenti *et al.*,(2006) and (Gowthaman *et al.*,(2013). As opposed to (Wakamatsu *et al.*, (2006) who discovered that commercial turkeys with velogenic

Table 2. Pathogenies of Newcastle disease virus in experimentally infected turkey poults by RT-PCR.

Time post challenge	Sample type	RT-PCR	Titer (EID50/ml)
6 hours	Proventriculus	-	-
	Conjunctiva	-	-
	Lung	-	-
	Spleen	-	-
	Trachea	-	-
	Caecal Tonsil	-	-
12 hour	Proventriculus	-	-
	Conjunctiva	-	-
	Lung	+	7.652×10^{1}
	Spleen	+	1.829×10^{2}
	Trachea	+	1.151×10^{2}
	Caecal Tonsil	-	-
24 hour	Proventriculus	-	-
	Conjunctiva	-	-
	Lung	+	6.138×10^{2}
	Spleen	+	1.082×10^{3}
	Trachea	+	2.790×10^{2}
	Caecal Tonsil	-	-
48 hour	Proventriculus	+	4.919×10^{2}
	Conjunctiva	+	3.915×10^{2}
	Lung	+	9.433×10^{3}
	Spleen	+	1.306×10^{4}
	Trachea	+	7.405×10^{3}
	Caecal Tonsil	+	9.042×10^{2}
5 days	Proventriculus	+	4.377×10^{3}
	Conjunctiva	+	2.534×10^{3}
	Lung	+	4.928×10^{4}
	Spleen	+	1.665×10^{5}
	Trachea	+	4.004×10^{4}
	Caecal Tonsil	+	8.614×10^{2}

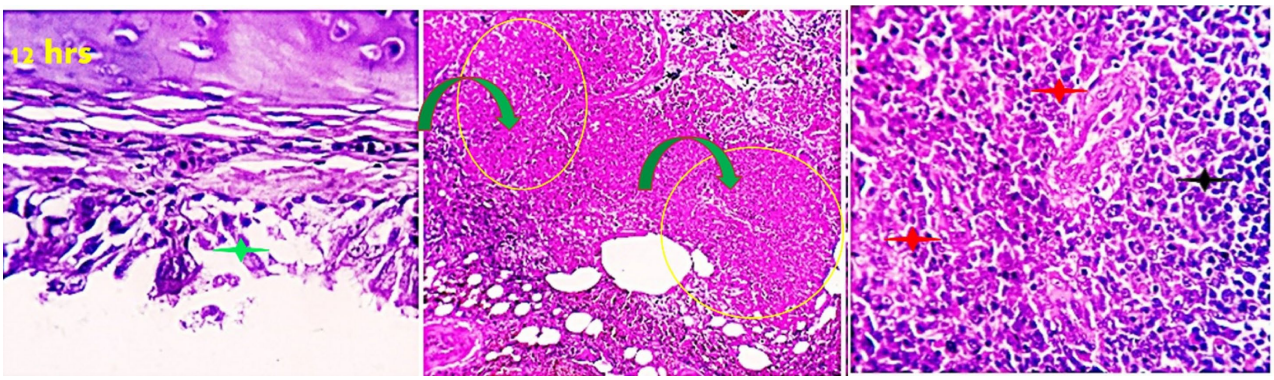

Figure 4. Photomicrographs from different organs (12-hour PI) showing submucosal round cells infiltration (green star). The pulmonary tissue reveals, moderate vascular dilatation and multifocal replacement of the pulmonary tissue by granulomatous nodular structures with central caseated necrosis and peripheral foamy cellular aggregations (curved green arrows and yellow circles). The spleen shows expanded white pulp marginal zones, focal reticuloendotheliosis, and red pulp small lymphocytic infiltration with remarkable splenic cords (black and red stars) H&E X 100.

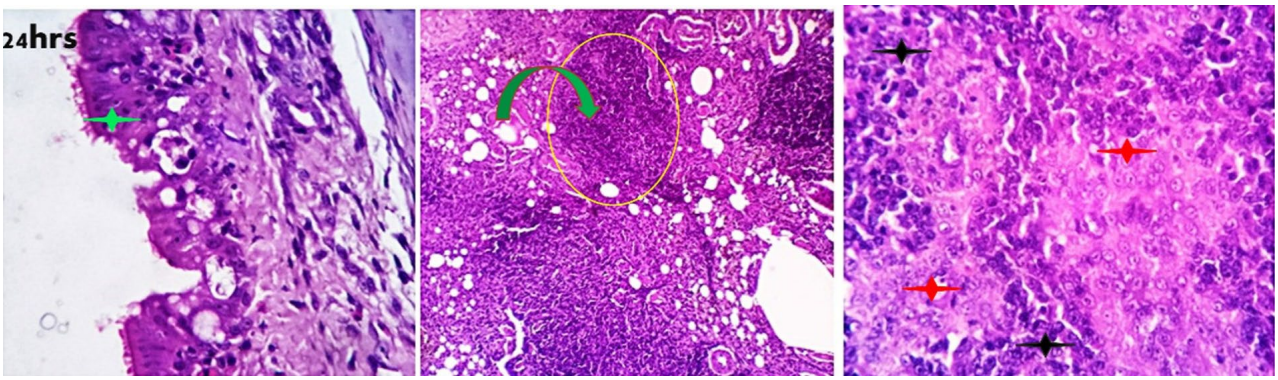

Figure 5. (24-hour PI) showing tracheal focal epithelial erosion, and an increase in goblet cell number (green star). The lungs demonstrate perivascular and interstitial infiltration and aggregation of lymphocytes beside perivascular and interalveolar edema (green curved arrows and yellow circles). The spleen reveals moderate white pulp lymphocytic proliferations (black arrows). The red pulp showed marked histiocytic proliferation (reticuloendotheliosis) (red arrows) H&E X 100.

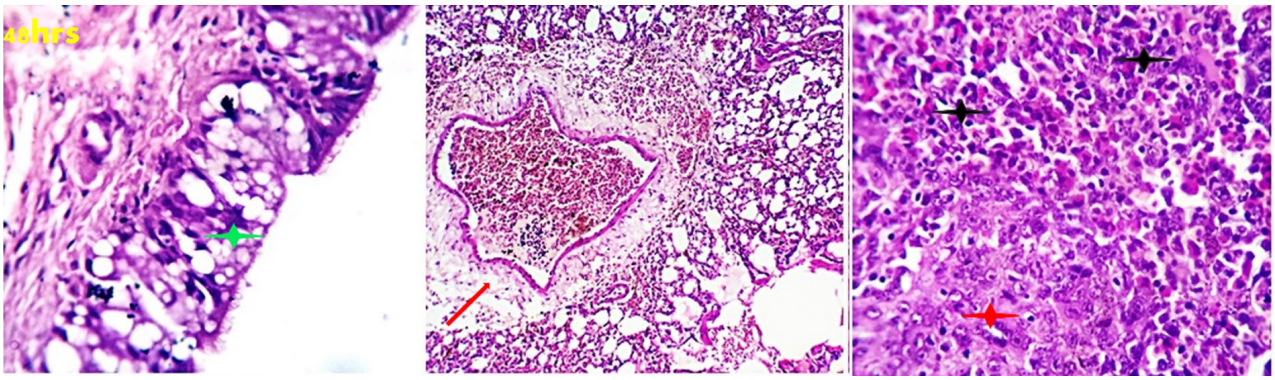

Figure 6. (48-hour PI) showing tracheal mucosal and glandular epithelial goblet cells hyper-reactivity (marked increase in number) (green stars). The lungs demonstrate characteristic perivascular inflammatory edema and hemorrhages (red arrow), focal per-bronchiolar and peri-alveolar aggregations of acute inflammatory cells with a predominance of lymphocytes and histiocytes (green curved arrows and yellow circles). The spleen shows marked with pulp lymphocytic proliferation involving the germinal, mantle, and marginal zoned (black stars) and the red pulp showed characteristic histiocytosis (red stars) H&E X 100.

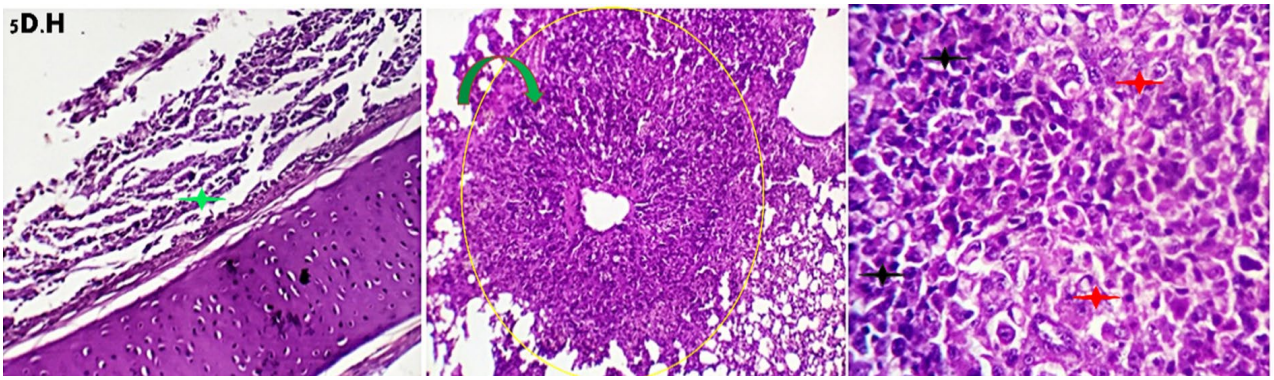

Figure 7. (5 days PI) showing total bronchial mucosal ulceration due to necrosis of most of the mucosal cells with exposure of the underlying submucosa which appeared edematous and infiltrated by mononuclear cells (green stars). The lungs show marked broncho-alveolar lymphocytic pneumonia (curved green arrow), bronchiolar epithelial hyperplasia (green circle, black stars), perivascular and interstitial edema, marked hyperemia, and focal hemorrhages (red arrow). The spleen shows moderate to marked lymphoid and histiocytoid proliferative reactions in the white and red pulps respectively (black and red arrows) H&E X 100.

NDV infection at the age of 4 weeks, clinical indications emerged at 6 days PI with no mortality. The general indicators reported in the current study such as diarrhea and central nervous system problems (head tremor, incoordination, and muscle paralysis) are the most prominent signs. These clinical symptoms have been formerly observed by (Piacenti *et al*., (2006 ; and Diel *et al*., (2012). Moreover, the current study showed that spleen had a high virus detection rate of $1.829x10^2$, $1.08x10^3$, $1.306x10^4$, and $1.665x10^5$ at 12, 24, 48 h, and 5 days PI, respectively. Similar results (Haque *et al*., 2010) reported that the virus isolation rate is greater in the spleen (100%) because the spleen is a lymphoid organ that is responsible for blood filtering during viremia, NDV invasion from the blood poses a threat to the spleen making it one of the best sources of the virus among organs taken during the collection of samples from experimental cases. Most researchers came up with the same conclusions (Majid and Peter, 2006; Krzysztof *et al*., 2006). The virus was also detected in the lung with the concentration of $7.652x10^1$, $6.138x10^2$, $9.433x10^3$, and $4.928x10^4$ at 12, 24, 48 h and 5 days respectively, while in the trachea the virus titer was $1.151x10^2$, $2.790x10^2$, $7.405x10^3$, $4.004x10^4$ at 12, 24, 48 h and 5 days respectively, this because NDV is generally propagated via ingestion and aerosol, which allow the virus to shed quickly through the trachea. This is generally in line with the results of earlier studies (Haque *et al*., (2010) that found NDV tropism may influence the increased detection or isolation rate of NDV from tracheal swabs. Results of the haemagglutination test revealed that the seroconversion was 3.6 $\log_2$ and 4.6 $\log_2$ in the infected group post 2 and 5 days PI respectively these results supported with findings of (Seal *et al*., (2005). Furthermore, in research by (Piacenti *et al*., (2006), ten days after the initial inoculation, commercial turkeys with velogenic NDV infection began to seroconvert. These results imply the rapid and comparable changes in serum antibodies following NDV infection in turkeys. After 6 days, antibodies are visible in the blood of chickens with NDV infection, peaking after 21–28 days (Miller and Koch, 2013). Histologically, at 48 h PI, the severity increased with tracheal mucosal and glandular epithelial goblet cells showed hyper-reactivity associated with submucosal edema and lymphocytic infiltration. The lungs demonstrate characteristic perivascular inflammatory edema and hemorrhages, focal peribronchiolar and perialveolar aggregations of acute inflammatory cells with a predominance of lymphocytes and histiocytes but 5 days PI lesions become more severe which shows total bronchial mucosal ulceration due to necrosis of most of the mucosal cells with exposure of the underlying submucosa which appeared edematous and infiltrated by mononuclear cells. The lungs show marked broncho-alveolar lymphocytic pneumonia, bronchiolar epithelial hyperplasia, perivascular and interstitial edema, marked hyperemia, and focal hemorrhages. The spleen showed marked histopathological lesions post 12 and 24 hours and more severe lesions were observed after 2 and 5 days PI. Similar results were observed by (Saravi *et al*., (2021) who observed microscopic lesions of spleen 3-day PI, such as numerous macrophages in the white pulp of the spleen along with lymphocytes and plasma cells. On day 5 PI of NDV, the white pulp was infiltrated with macrophages, in association with necrosis of some lymphocytes.

CONCLUSION

It is concluded that The NDV found in Egyptian chicken flocks has the potential to induce serious sickness in commercial turkeys which need new vaccine preparation from the circulating NDV genotype VII.1.1 in Egypt to protect domestic poultry from recurrent infection.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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