Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Experimental Infection of Japanese Quails (*Coturnix coturnix japonica*) with Avian Orthoavula Virus-1: Pathogenicity and Transmissibility of Velogenic Genotype VII.1.1

Khaled El Sayed Abdelaal[1], Shimaa M.G. Mansour[1*], Mohamed El-Bakry Ismaiel[1] and Ahmed A.H. Ali[1]

[1] *Department of Virology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Sharkia 44511, Egypt*.

Abstract

*Avian orthoavula virus*1, formerly known as Newcastle disease virus (NDV) is able to infect a wide variety of bird species resulting in various clinical signs and consequences. The easiness of transmission has endorsed the virus to spread globally showing variable levels of virulence based on host species and virus strain. The pathogenicity and transmissibility of field Egyptian chicken-origin NDV genotype VII 1.1 to induce the disease in 35-day-old Japanese quails were investigated. Twenty-five percent of quails inoculated oculonasally with 10^6EID_{50} showed conjunctivitis and depression, whereas 11% of cases exhibited greenish diarrhea. Neurologic signs consisting of incoordination and tremors were recorded in 37.5% of infected quails at day 8 post-infection (dpi). Congested meningeal blood vessels and hemorrhagic cecal tonsils were seen in both NDV-infected and contact quails. Microscopically, the NDV-infected quails showed brain edema, lymphocytic encephalitis and pulmonary congestion. Using hemagglutination inhibition (HI) assay, the anti-NDV antibodies for NDV-infected and LaSota-vaccinated at 7 dpi were relatively similar. The antibody titers reached 6±0.8 $\log_2$ in the NDV-infected birds and 5.25±0.5 and 4±0.8 $\log_2$ in LaSota-vaccinated and contact quails, respectively at 21 dpi. Additionally, the infected quails shed virus via the oropharynx and cloaca. Virus titers of approximately $10^{2.8}$ and $10^{5.2}$ EID_{50}/mL were determined in the oropharyngeal and cloacal swabs on day 5 post-infection, respectively. These results demonstrate that Japanese quails can indeed be susceptible to NDV genotype VII 1.1 and might be a source of infection to additional birds.

Keywords Newcastle disease virus; Histopathology; HI; Shedding; qRRT-PCR; Egypt.

Introduction

The *avian orthoavulavirus* 1, also known as Newcastle disease virus (NDV), is an enveloped, negative-sense, single-stranded RNA virus, which belongs to the subfamily *Avulavirinae*, family *Paramyxoviridae* under the order *Mononegavirales* [1]. The viral genome is approximately 15.200 base pairs long and encodes six distinct proteins; haemagglutinin–neuraminidase (HN), fusion (F) protein, matrix (M) protein, nucleocapsid protein (NP), large RNA polymerase (L) and phosphoprotein (P). The antigenicity and pathogenicity of NDV are influenced by the HN and F surface glycoproteins [2]. Two additional proteins (V and W) could potentially be encoded by phosphoprotein mRNA editing [3]. The HN and F proteins denote the crucial targets of the immune responses against NDV [4]. Based on complete-genome and F gene sequences, all NDV strains are categorized into a single serotype encompasses two classes of NDVs: class 1 and 2. A single genotype was present in class 1 (1.I) comprises non-virulent NDV strains that are generally asymptomatic in aquatic wild birds. However, there are 21 genotypes (2.I-2.XXI) within class 2 NDV [5]. In line with their dissemination, NDV strains with genotypes II, VI, and VII are the predominant NDV genotypes in Egypt and other North African nations [6]. Accordingly, NDV genotype VII was first recognized in 2011 in Egypt [7].

Newcastle disease (ND), a viral highly contagious notifiable disease, has a major clinical impact and causes large financial losses for the poultry industry globally [8]. It is included in the OIE list A and classified as the second-utmost endemic disease in several nations [9]. Numerous

*Corresponding authors: Shimaa M.G. Mansour, E-mail: shimaagalal@gmail.com, Tel.: 01111221024
(Received 12 November 2024, accepted 02 February 2025)
DOI: 10.21608/EJVS.2025.334876.2490

domestic and wild birds are affected by the ND, which has widely varied pathogenicity spanning from asymptomatic disease to peracute disease (with up to 100% mortality). Amongst poultry; chickens, turkeys and other gallinaceous species are frequently affected [10, 11], whereas pigeons and geese are only reasonably susceptible [12-14]. Conversely, ducks, while susceptible, are highly resistant to NDV infection [15, 11]. In Egypt, quails were brought to commercial poultry industry primarily for food consumption. They were regarded as NDV carriers and/or vulnerable hosts [16-18]. In Assiut Province, the viscerotropic velogenic ND was isolated from quails [19]. Prior research revealed that quails experimentally infected with pigeon paramyxovirus-1 resulted in mild infection and 5% mortality in quails; however, contact pigeons exhibited greenish diarrhea besides neurological manifestations (25%) followed by deaths with a percentage of 20% [20]. The Japanese quails were also vulnerable to infection with NDV of genotype VII, where the virus resulted in 33% and 100% mortality in quails and chickens, respectively. The virus induced a classic ND picture, which was more severe in chickens than in quails [21]. Quails infected with NDV genotypes VI and VII showed mild to severe neurological symptoms, with corresponding mortality rates of 46% and 33%, respectively [22]. Nevertheless, another study recorded a decreased pathogenicity caused by NDV VIId with no mortality in quails [23].

It is worth mentioning that the pathogenicity of NDV genotype VII in quails and their role in NDV transmission to domestic birds remain questionable. Along with the view of the accumulative curiosity in quail farming by several Egyptian farmers, imperative information regarding the vulnerability of the Japanese quails to NDV infection and immunization approaches to prevent and control the NDV infection should be kept in mind. The pathogenicity and transmissibility of field Egyptian NDV genotype VII 1.1 formerly isolated from chickens to induce the disease in 35-day-old Japanese quails were investigated. A systematic approach was taken into consideration in order to ascertain the clinical picture, histopathological findings, along with the level and magnitude of NDV replication as determined by the detection of virus in tissues and mucosal secretions. Further, contact transmission trails with naïve quails were used to assess transmission potential.

Material and Methods

Newcastle disease virus and vaccine

Field strain of virulent Newcastle disease virus sub-genotype VII 1.1 strain designated as "NDV/Chicken/Egypt/ALEX/ZU-NM99/2019" and published in GenBank under the accession number OP219680 was kindly supplied by Dr. Amal Eid, Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt. The virus was propagated and titrated in 10-days old embryonated chicken eggs (ECEs). LaSota NDV vaccine strain (CEVAC NEW L LaSota Vaccine) was used.

Propagation and titration of NDV in Embryonated Chicken Eggs:

Ten day old ECEs were used for propagation and titration of NDV. The supernatant (200 µL) of the NDV/Chicken/Egypt/ALEX/ZU-NM99/2019 strain was inoculated into the allantoic cavity of ECEs (n=10) following the standard inoculation procedures described elsewhere [24]. Additionally, five fertile eggs were kept without inoculation as a control negative. The allantoic fluids were collected and examined by rapid hemagglutination test with 10% (v/v) washed chicken red blood cells (RBCs). The harvest was titrated by inoculation in 10-day-old ECEs to determine median embryo infective dose 50 (EID_{50}) according to Reed and Munch [25] and used with a dose of 10^6 EID_{50}/0.1 ml (100 µL/bird; 50 µL using eye drops and 50 µL by the nasal route) at 35 days of age.

Experimental quails

A total of 80 unvaccinated Japanese quails (*Coturnix coturnix japonica*), were obtained from a commercial farm in Dakahlia Province, Egypt at 21 days of age. Upon arrival, serum samples were collected from random quails (n=10), and examined with LaSota NDV strain to verify the seronegativity of the quails for NDV infection by hemagglutination inhibition (HI) assay [9]. Additionally, oropharyngeal and cloacal swabs were collected from quails and tested by quantitative real-time RT–PCR (qRRT-PCR) [26]. The quails were raised in disinfected cages in the experimental animal households at Faculty of Veterinary Medicine, Zagazig University under controlled environmental circumstances. They were acclimatized to the rooms for 14 days before NDV infection at 35 days of age.

Pathogenicity and transmissibility of NDV in quails

Before inoculation of quails, three birds were randomly selected, euthanized and tissue samples were collected. To determine whether NDV genotype VII 1.1 could cause infection in quails under experimental circumstances, a group of 20 healthy quails (n=10 per cage), serologically negative for NDV, were inoculated oculonasally with 100 µL virus fluid containing 10^6 EID_{50}. Ten quails (5 per cage) were added as sentinels into the quails' cage to detect transmissibility. The sentinel quails had direct contact with the excreta of the inoculated quails and shared a communal source of drinking water. Another group of quails (n=20; 10 per cage) were vaccinated with LaSota NDV vaccine (CEVAC NEW L LaSota Vaccine) according to manufacturer's recommendation. Additionally, twenty-five sham-

inoculated quails were kept in separate accommodation to the other birds to serve as a control negative group, and was inoculated with sterile phosphate-buffered saline (PBS; pH =7.4). The quails were checked two or three times daily for a total of 21 days and the clinical signs were documented. Oropharyngeal and cloacal swabs were collected from four quails (n=2 per cage) at 0, 3, 5, 7 and 10 days post-infection (dpi), placed in 1.0 ml of PBS with antibiotics for subsequent measurement of viral shedding. The experimental studies were permitted by the Research Ethical Committee for Animal Studies, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Pathology

Quails were euthanized at different time points, necropsied and internal organs were sampled for macroscopic, microscopic examination and qRRT-PCR. For histopathological examination, part of selected organs (brain, lungs, liver, proventriculus and intestine) were preserved in 10% buffered formalin, processed and embedded in paraffin. Paraffin blocks were sectioned in duplicate at 5µm and routinely stained by haematoxylin and eosin [27]. Subsequently, multiparametric multiorgan semiquantitative lesion scoring was carried out according to Gibson-Corley et al. [28]. Lesion score system was estimated as the followings: (0 = no noticeable histopathological modifications, 1 = rarely minimal or focal, 2 = multifocal, 3 = patchy or diffuse). Another part was stored for subsequent analysis with qRRT-PCR.

Detection of NDV in swabs and tissues

The RNA was extracted following the instructions of QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA). The Quantitative real-time RT–PCR (qRRT-PCR) was performed using Qiagen One Step RT-PCR Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The PCR was performed in 25 µL in sterile 0.2-mL PCR tubes as previously described using a specific primer set and probe amplifying and identifying a highly preserved sequence within NDV M gene viral RNAs [26]. The RT-PCR thermal profile included one cycle of 50^{o}C for 30 min followed by another cycle of 95^{o}C for 15 min. The PCR cycling profile consisted of 40 cycles of (i) 94^{o}C for 15 s, (ii) 52^{o}C for 30 s, and (ii) 72^{o}C for 10 s. The cycle thresholds (Ct) values were determined after calculating the standard curve. The EID_{50} of virulent NDV genotype VII 1.1 strain from the samples were determined based on the Ct values, using a standard curve derived from standard RNA concentrations of the reference virus. The detection limit of each qRRT-PCR runs was determined using the standard curve. The results were expressed as EID_{50}/mL equivalents.

Hemagglutination inhibition assay

Serum samples were collected from quails at 0, 7, 14 and 21 dpi from four birds per group (2 per cage) randomly selected for blood sampling. The HI assay was done using 4 HA units of LaSota NDV vaccine strain (CEVAC NEW L LaSota Vaccine) and 1% RBCs. The serum samples were subjected to heat inactivated at 56°C for 30 min. The serum was first mixed with equal parts of diluent and then subjected to a series of two fold dilution [9]. The reciprocal of the highest dilution demonstrating hemagglutination inhibition was documented. Titers were expressed as $\log_2$ geometric mean titers (GMT).

Statistical analysis

The collected data was analysed with GraphPad Prism version 8.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com). The geometric mean of NDV (log base 2) and of HI antibody titers (± standard deviation) was quantitatively shown in each group. Two-way ANOVA with Tukey's multiple range test was accomplished to assess the significant variations in antibody response over time and among different groups. The mean $\log_{10}$ virus titers in swabs by qRRT-PCR were compared using one-way ANOVA with Tukey's multiple range test. A p-value < 0.05 was deemed to have statistical significance.

Results

Clinical signs and gross lesions

The ND clinical signs and postmortem lesions were documented after the virus infection and throughout the duration of the experiment (Table 1). The sham-inoculated negative control group seemed normal during the entire experiment. In NDV-infected quails, five quails (25%) showed nasal discharges, conjunctivitis and slight depression at 3 dpi, along with decrease in feed intake. Greenish diarrhea was seen at 5 dpi in 11% of infected quails. At 8 dpi, neurologic signs consisting of incoordination and tremors were seen in 35.7% of infected quails. At 14 dpi, no clinical abnormalities were recorded in NDV-infected quails. No clinical signs were observed in contact quails In LaSota-vaccinated group, mild lacrimal and nasal discharges were seen at 2 dpi. No mortalities were recorded throughout the experiment in all groups. Upon necropsy in NDV-infected quails, at 5 dpi, mild congestion in trachea (Fig. 2A) and congested and oedematous lung (Fig. 2B) were seen. At 10 dpi, the gross examination revealed congested meningeal blood vessels (Fig. 2C) and enlarged spleen with hemorrhage. Hemorrhagic cecal tonsils (Fig. 2D) with a few pinpoint hemorrhages on the tips of the periventricular gland were recorded. In contact quails, the trachea (Fig. 2E) and lung (Fig. 2F) appeared normal with no, detectable lesions. Few petechial hemorrhages in cecal tonsils (Fig. 2H) and congested meningeal blood vessels (Fig. 2G) were seen in contact quails at 14 and 21 dpi, respectively.

However, the sham-inoculated control showed no lesions (Figs. 2I, J, K, L).

Histopathologic findings

The highly virulent NDV infections virtually cause severe lesions in several organs. Thus, in order to assess the pathological alterations, histopathological scoring was carried out in NDV-infected and LaSota-vaccinated quails. Microscopic examination of tissues from sham-inoculated and LaSota-vaccinated quails showed a normal histologic structure of the trachea, lungs, proventriculus, intestine, liver and brain along with the period of experiment (Fig. 3A, D, G, J). However, in NDV-infected birds, there were variables microscopic lesions in examined tissues and dpi (Table 2). The brain showed vascular and apoptotic changes; congested meningeal and cerebral blood vessels (Fig.3B), perivascular edema and hemorrhages (Fig. 3C) with nonsuppurative encephalitis. The examined section of pulmonary tissues revealed presence of exudates within lumen of bronchioles (Figure 3E), congested pulmonary blood vessels and perivascular edema (Fig.3F). Further, inflammatory cells admixed with desquamated epithelium were seen within some air vesicles. The proventriculus exposed mucous secretions adhered to mucosal folds (Fig. 3H); necrotic tips of some proventricular glands with lymphocytic aggregates, and dilated interstitial blood vessels (Fig. 3I). The intestine exhibited necrotic, detached epithelial lining villi and destructed intestinal crypts beside mild infiltration of macrophages and lymphocytes within the lamina propria (Fig. 3K and 3L). Liver showed congestion of hepatic blood vessels beside vacuolated large number of hepatocytes in addition to multifocal areas of hepatic necrosis replaced by lymphocytes aggregates (Data not shown). A semi-quantitative lesion scoring system for the histopathological changes of all scrutinized tissues among NDV-infected and LaSota-vaccinated quails was summarized in Table 2.

Detection of anti-NDV antibodies

No significant anti-NDV antibody titers were found in any sampled quail prior to infection or vaccination using HI assay. The sham-inoculated negative control groups didn't develop positive antibody to NDV throughout the experimental period (21 days). Quails infected with NDV genotype VII 1.1 strain showed positive in antibody titers against NDV at 14 dpi. There was an increase in NDV-antibody levels during the experimental period in both NDV-infected and LaSota-vaccinated quails as compared to the sham-inoculated control negative birds with a significant difference in antibody titers seen within the NDV-infected group. Interestingly, the contact quails expressed anti-NDV in their sera (Fig. 4).

Virus excretion and transmission kinetics

The NDV shedding was measured by titrating the quantity of viruses recovered from swabs taken from both the oropharynx and cloaca on 3, 5, 7 and 10 dpi using qRRT-PCR. Across all time points, the cloacal swabs yielded a higher NDV titer than the oropharyngeal ones (Fig. 5). In NDV-infected quails, the virus titers ranging from 2.8±0.5 to 5.2±0.3 Log_{10}/mL were determined in the collected swabs. A significant decrease in the titer of NDV recovered from oropharyngeal and cloacal swabs was documented in the infected groups at 7 and 10 dpi. No virus shedding was recorded in swabs collected from contact quails. Additionally, swabs of sham-inoculated negative control quails were negative. The virus load in lungs, cecal tonsil, and brain tissues was measured by qRRT-PCR. Systemic infection through 10 dpi was confirmed in the infected quails. The virus titers ranged from 2.9±0.4 to 4.6±0.6 log_{10}/g tissue. Furthermore, the virus was detected in the brains of the sentinel quails at 21 dpi.

Discussion

Newcastle disease is an extremely contagious devastating disease of birds. It is regarded as a major infectious challenge hindering the expansion of the poultry industry worldwide, particularly in endemic countries. Numerous ND outbreaks have been recorded within Egyptian poultry flocks in recent years, resulting in disastrous financial losses due to high mortality rates, implementing containment strategies, and imposing trade limitations. The Japanese quail (*Coturnix coturnix japonica*) is believed to be a significant carrier of NDV [16] and could have a critical impact on transmitting the virus among poultry species kept in close proximity to or with quails [29, 30]. The current study exhibits the capability of chicken-origin genotype VII 1.1 NDV to replicate in experimentally infected quails and to be transmitted to contact quails. The pathogenicity of NDV in quails is thought to be influenced by the virus strain, its infectious dose and infection's route [31]. The recommended infectious dose of NDV is at least $10^4 EID_{50}$ as mentioned by WOAH [9]. Many authors used up to 10^8 EID_{50} for infection of quails with NDV [23, 30]. Here, the quails were inoculated oculonasally with a virus dose in between (10^6 EID_{50} per bird) to ascertain the clinical picture, level and magnitude of NDV replication in tissues and mucosal secretions. No mortality was reported in infected quails. Similarly, neither morbidity nor mortality was recorded in 17-week-old Japanese quail's inoculated oculonasally with a virulent NDV strain [16]. Conversely, another study recorded 46% and 33% mortality in quails infected with Pigeon NDV genotype VI strain and NDVGHB-328 genotype VII 1.1 strain, respectively [22]. However, the virus caused 100% mortality in diseased chickens [21, 32]. Japanese quails experimentally infected oculonasally with velogenic NDV strains showed slight (13%) [29] to modest (25-28%) mortality [30] were

recorded. These findings suggest that quails are relatively resistant to the NDV infection compared to other avian species. In northern India, a spontaneous outbreak of ND in 22-week-old Japanese quails induced 30% morbidity and 20% mortality, with clinical nervous manifestation [33]. Remarkably, a previous study conducted in Brazil on Japanese quails infected with low dose of $10^{3.5}$ ELD_{50} NDV strain IBS 002 demonstrated clinical manifestations, presence of virus in tissue specimens and the development of anti-NDV antibodies [34].

In the present study, the infected quails displayed a representative clinical picture for NDV infection. The birds exhibited depression, conjunctivitis, greenish diarrhoea besides nervous signs consisting of incoordination and tremors were seen in 35.7% of infected quails at 8 dpi. The NDV genotype VI and VII caused serious nervous signs like depression, ataxia, head tremors, torticollis and paralysis in quails at 3 dpi and 4 dpi, respectively [22]. Comparable clinical symptoms were recorded in former studies [31, 35, 30, 21]. However, despite the fact that the virus is known to induce viremia, a number of authors have stated that quails infected with NDV did not display any symptoms of ND. They have reported that quails were generally resistant and perhaps played a limited epidemiological role in the spread of the NDV [36, 16]. Comparable results were recorded recently by Ali et al. [23] in quails inoculated with a high infectious dose (10^7 EID_{50}) of NDV genotype VII 1.1 (NDV/Chicken/Egypt/1/2015) inducing mild clinical manifestations and no microscopic findings noticed, indicating that NDVs have distinctive biological properties [37]. The aforementioned disparity could be ascribed to the quails' age, virus strain and route of infection.

Upon necropsy in NDV-infected lungs appeared congested and oedematous, congested meningeal blood vessels, hemorrhagic cecal tonsils. Comparable gross lesions were thoroughly documented [38, 33]. Despite not being the main distinctive gross lesions in NDV-infected birds in this study, proventricular haemorrhages have been recorded [18, 39]. Additionally, histopathological changes in quails infected with highly virulent NDV compared to LaSota-vaccinated quails were investigated. NDV-infected quails showed several lesions, including brain oedema, lymphocytic encephalitis, pulmonary congestion, necrosis in the proventriculus and intestine, and liver damage. It seems that the infected quails with 10^6 EID_{50} exhibited distinct clinical presentation and microscopic lesions without inducing deaths. In contrast, vaccinated quails exhibited normal tissue structures throughout the experiment. These findings are consistent with the previous lesions detected in NDV-infected quails and chickens [11, 35, 30].

Although the genotype VII 1.1 is currently the predominant NDV genotype in Egypt [40, 16], the NDV genotype II is still utilized in the manufacturing of commercial NDV vaccines [41]. Here, the HI assay on a representative serum samples prior to starting the experiment revealed that the quails were serologically negative to NDV. The HI antibody titers showed noticeable variations among the NDV-infected, LaSota-vaccinated in comparison to sham-inoculated control group. The antibody titers against the NDV genotype VII and II increased after 14 dpi and 21 dpi, indicating a fruitful immune response following the viral replication in quails' tissues. The NDV can naturally or experimentally infect quails, resulting in alterations in HI titers and damage to various organs [18]. In consistency, recent study reported the elevated HI antibody titers in non-vaccinated quails, following infection with NDV genotype VI or VII 1.1 [22].

In several countries, genotype VII demonstrated obvious virulence and broad tissue tropism. Furthermore, it was profoundly shed from oral and cloacal excretions and had a high capacity for infection and reproduction, signifying its rapid spread throughout geographical zones [42]. In the present study, the neurological signs (8 dpi) consistent with gross and microscopical findings in the brain, which were primarily noticed at 10 dpi and progressed in intensity by 14 dpi. Nervous envelopment at later phases of infection (after 5 dpi) is frequently seen with ND [11, 35, 30]. Accompanied by the pathologic findings, the qRRT-PCR results verified the existence of NDV in tissues and swabs from infected quails.

In NDV-infected quails, the virus titers in oropharyngeal swabs reached $10^{4.2}$ EID_{50}/mL on 3dpi, then decrease by 5 dpi. However, in cloacal swabs the titers began to increase post-infection, reaching $10^{5.2}$ EID50/ml at 5 dpi before declining dramatically by 7 and 10 dpi. These findings are in consistency with the results reported by Mohamed and Hafez [29] who noticed that the titers were increased from 1 to 5 dpi before declining by 7 dpi. Meanwhile, the shedding of NDV in challenged birds reached its highest point at 6 dpi and then significantly decreased by 9 dpi, signifying a petite period of viral reproduction [22]. There was evidence of inflammation in the peripheral nerve tissue in addition to the widespread involvement of the central nervous system (brain). The outcomes from experimental NDV infection conducted in this study in quails were parallel to those discovered in the previous investigation concerning systemic viral replication. The virus attained a titer of $10^{4.6}$ EID_{50}/g in the brains of NDV-infected quails. Additionally, the virus was detected the intestine and brain of the sentinel quails at 21 dpi. This was represented by the low level of virus reproduction, as seen by the minimal virus titers in oropharyngeal and cloacal

secretions. Correspondingly, no NDV-related mortality/signs were seen in contact quails, with only few petechial hemorrhages in cecal tonsils and congested meningeal blood vessels were seen at 14 and 21 dpi, respectively. As well, the NDV-infected quails didn't have a significant systemic infection when compared to chickens infected with lower or higher infectious doses of virus [43, 44]. The low mortality rates combined with sporadic nervous envelopment and distinct patterns of virus shedding are common findings in birds like cormorants [45], and ducks [46] with partial resistance, when exposed to highly virulent NDV strains.

Conclusion

In conclusion, the results of present study revealed that NDV genotype VII 1.1 have the ability to reproduce and shed from infected quails, raising the possibility of transmission to additional bird species. The reasonably reduced vulnerability of quails to NDV infection could be a consequence of the decreased reproduction capabilities of the NDV in infected quails' tissues. These findings are sponsored by no or low titers of virus shedding in contact and infected quails, respectively. Understanding the molecular and biological characteristics of NDVs could be valuable for emphasizing how virulent ND viruses spread to poultry and the mechanisms underlying their pathogenicity and virulence. Further studies, predominantly continued monitoring of quails is necessary.

Acknowledgments

The authors would like to thank Department of Virology, Faculty of Veterinary Medicine, Zagazig University, Egypt and Dr. Mohamed Salah, Department of Pathology, Faculty of Veterinary Medicine, Zagazig University, for his help in reading the histopathology.

Funding statement

This study didn't receive any funding support

Declaration of Conflict of Interest

All authors declare that they have no conflicts of interest.

TABLE 1. Summary of clinical signs and postmortem lesions in different groups of quails

Group	2-4 dpi	5-7 dpi	8-10 dpi	11-14 dpi	15-21 dpi
NDV-infected (VII 1.1.)	No signs detected at 2 dpi Signs appeared at 3dpi Depression, + Conjunctivitis, ++ decrease in feed intake, ++	Depression,++ greenish diarrhea at 5 dpi, ++	Nervous signs; incoordination, tremors at 8 dpi, +	Signs decreased in severity No clinical abnormalities were recorded at 14-21 dpi.	
	Hemorrhages in trachea at 3 dpi, +	Congested and oedematous lungs at 5 dpi, ++ Mild congestion in meningeal blood vessels at 7 dpi, +	At 10 dpi, congested meningeal blood vessels, ++ hemorrhagic cecal tonsils, + enlarged spleen, +	At 14 dpi, mild congestion in meningeal blood vessels, + hemorrhagic cecal tonsils, +++	No lesions were seen at 21 dpi
Contact quails	No clinical signs were observed in contact quails				
	N/A	No lesions were seen at 7 dpi	No lesions were seen at 7 dpi	Few petechial hemorrhages in cecal tonsils at 14 dpi	Congested meningeal blood vessels at 21 dpi, ++
LaSota-vaccinated	Mild lacrimal and nasal discharges at 2dpi, +	Depression, + Mild lacrimal and nasal discharges, +	No clinical abnormalities were recorded		
	No lesions were seen at 3 dpi	Hemorrhages in trachea at 5 dpi, +	No lesions were seen		
Sham-inoculated	The sham-inoculated negative control group appeared normal throughout the experiment (No clinical findings were seen)				

dpi: days post-infection; + mild; ++ moderate; +++ severe; N/A not applicable

TABLE 2. Lesions score of the severity extent in NDV-infected and LaSota-vaccinated quails

Organ	Lesions	LaSota-vaccinated	NDV-Infected (Genotype VII 1.1) dpi					
			3	5	7	10	14	21
Brain	Degenerated neurons	0	0	0	1	3	2	1
	Encephalomalacia	0	0	0	0	2	1	0
	Congested vasculatures	0	0	0	2	2	2	2
	Perivascular oedema	0	0	0	2	3	2	1
	Hemorrhages	0	0	0	1	1	1	0
Lung	Bronchiolitis	0	1	2	2	2	2	1
	Intraluminal air spaces exudation	0	1	2	1	1	1	1
	Congested vasculatures	0	1	3	2	2	2	1
	Oedema	1	1	2	2	2	2	2
Proventriculus	Mucous secretion	0	0	1	1	2	1	1
	Necrotic tips of glands	0	0	1	2	2	1	1
	Lymphocytic aggregates	1	1	2	2	2	2	2
	Dilated interstitial vasculature	0	0	1	1	2	1	1
	Oedema between glandular tissue	1	0	2	2	2	2	1
Intestine	Destructed epithelium and crypts	0	0	1	2	3	2	1
	Lymphocytic infiltrates	1	0	1	3	3	3	2
Liver	Congested hepatic vasculatures	1	0	0	2	3	2	2
	Vacuolated hepatocytes	0	0	0	2	2	2	1
	Focal necrotic area	0	0	0	1	2	1	1
	Focal areas of lymphocytic aggregates	1	0	1	2	3	2	2

Lesions score system was as follows: (0 = no detectable histopathological lesion, 1 = rarely minimal or focal, 2 = multifocal, 3 = patchy or diffuse) as a semi-quantitative method.

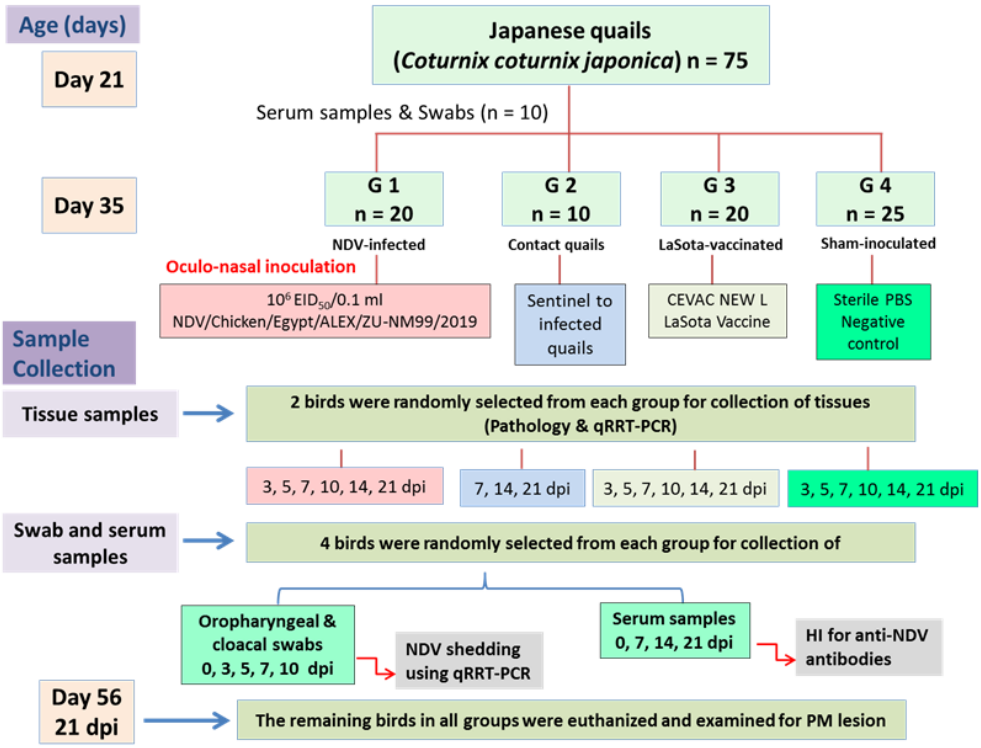

Fig. 1. Schematic illustration of the experimental design for studying the pathogenicity and transmissibility of NDV genotype VII.1.1 in quails

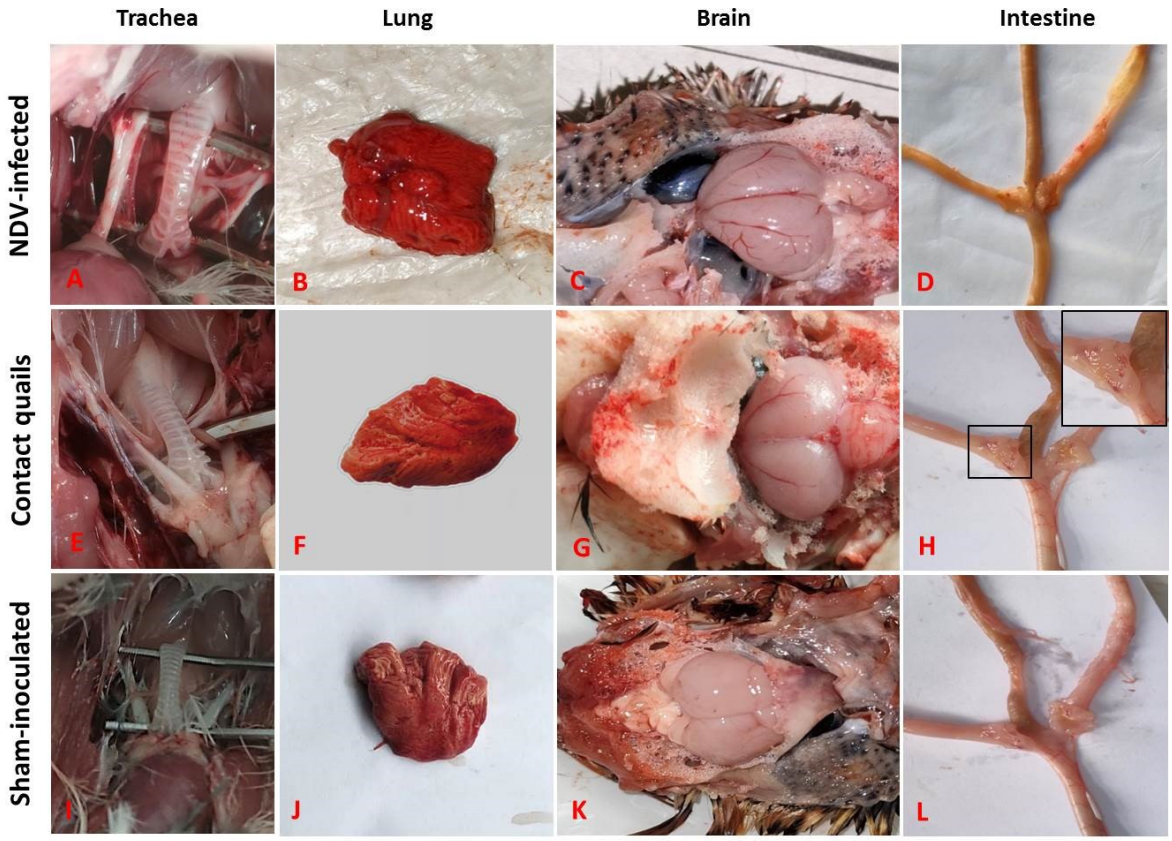

Fig. 2. Postmortem findings in NDV-infected quails, contact and sham-inoculated quails. NDV-infected quails: (A) Trachea showing mild congestion at 3 dpi **(B)** Lung showing oedema and congestion at 5 dpi. **(C)** Brain showing congested meningeal blood vessels at 10 dpi. **(D)** Intestine showing hemorrhagic cecal tonsils at 10 dpi. **Contact quails:** Normal Trachea **(E)** and Lung **(F)** with no lesions. **(G)** Brain showing congested meningeal blood vessels at 21 dpi**. (H)** Intestine showing mild hemorrhages in cecal tonsils at 14 dpi. **Sham-inoculated control quails: (I-L)** Normal appearance of tissues with no lesions.

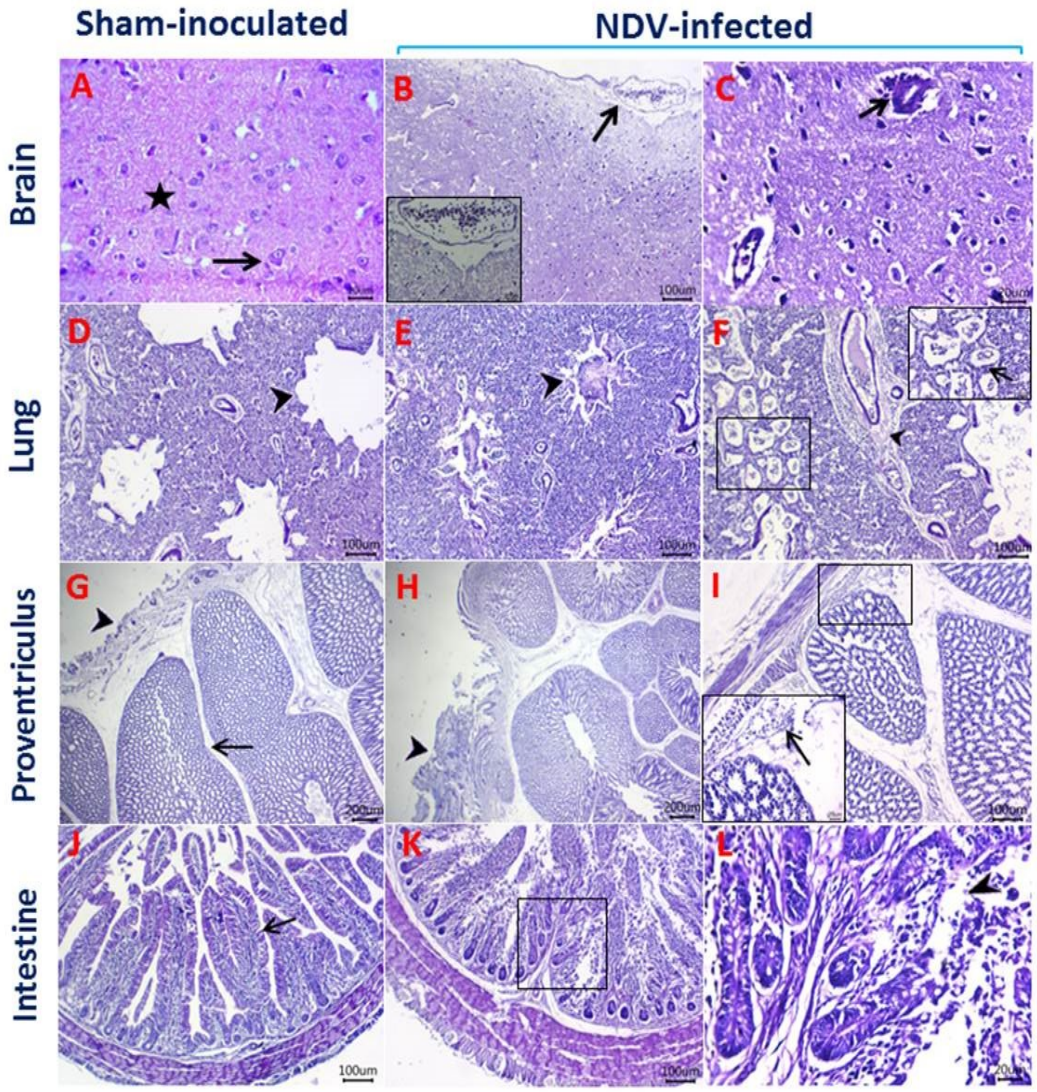

Fig. 3. Histopathologic findings of sham-inoculated and NDV-infected quails. Hematoxylin and eosin stain.

(A) Brain of sham-inoculated control quails showing normal morphology of neurons (arrow), neuropil (asterisk) and supporting cells. *Bar=100 µM*. **(B)** Brain of NDV-infected quails at 10 dpi showing congested meningeal blood vessels (arrow). *Bar=100 µM*, Square *Bar=20 µM*, and **(C)** Perivascular edema and hemorrhages (arrow) *Bar=100 µM*. **(D)** The pulmonary tissue of control quails appears with normal histomorphological structures of bronchi, tertiary bronchioles (arrowhead), air vesicles and stromal structures of pulmonary tissue. *Bar=100 µM* **(E)** Exudates within lumen of bronchioles(arrowhead)*Bar=100 µM*. **(F)** Congested pulmonary blood vessels and perivascular edema (arrowhead) Bar=100 µM, Square *Bar=20 µM*. **(G)** Normal histological structures of mucosal epithelium **(**arrowhead), lamina propria with loose connective tissue, proventricular glands (arrow), and muscular layer in control quails. *Bar=200 µM*. **(H)** Proventriculus showing mucous secretions adhered to mucosal folds (arrow head)*Bar=100 µM*, and **(I)** dilated interstitial blood vessels (arrowhead) *Bar=100 µM*, Square Bar = 20 µM. **(J)** Normal villous epithelium (arrow), crypts, lamina propria, submucosa and musculosa in control quails *Bar=100 µM*. **(K, L)** Intestine showing necrotic, detached epithelial lining villi (arrowhead) and destructed intestinal crypts beside mild infiltration of lymphocytes and macrophages within the lamina propria. **(K)** *Bar=100 µM*, **(L)** *Bar=20 µM*.

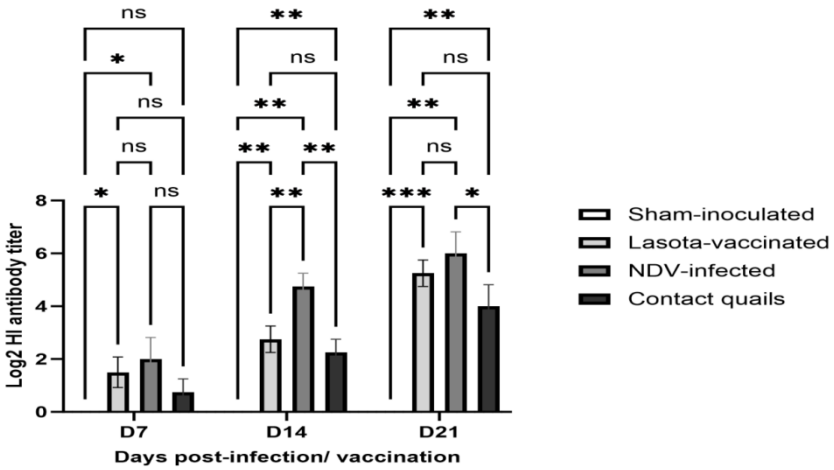

Fig. 4. Hemagglutination inhibition (HI) antibody titers in sham-inoculated, LaSota-vaccinated, NDV genotype VII 1.1 infected and contact quails. The birds were infected with NDV genotype VII 1.1 at 35 days old via oculonasal route. In Lasota-vaccinated group, the quails were vaccinated with LaSota NDV vaccine (CEVAC NEW L LaSota Vaccine). The mean value of Log2 HI antibody titer in sera collected from four birds ± standard deviation. Tukey`s multiple comparison two-way ANOVA was used to compare values against each other, considering the row matching factor. * for $p \leq 0.05$, ** for $p \leq 0.01$, and *** for $p \leq 0.001$.

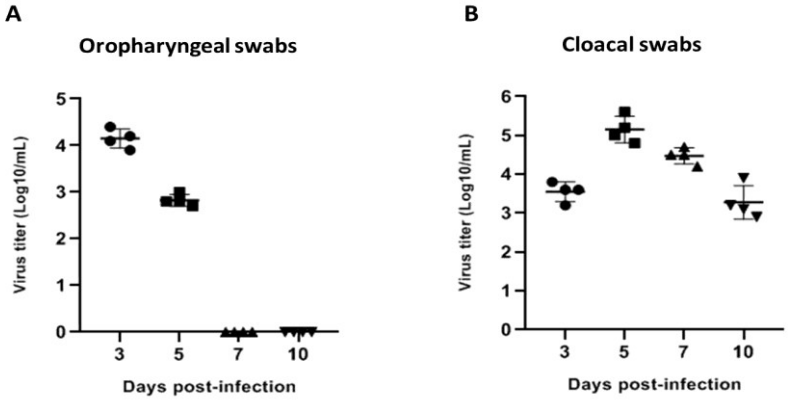

Fig. 5. Virulent NDV shedding in oropharyngeal (A) and cloacal swabs (B) collected from infected quails at 3, 5, 7 and 10 dpi. The virus titer (Log 10 EID_{50}/mL) was determined by qRRT-PCR in swabs collected from four birds. The birds were infected with NDV genotype VII 1.1 at 35 days old via oculonasal route.

References

1. ICTV. Virus Taxonomy: 2023 Release, EC 55, Jena, Germany, August 2023.
2. de Leeuw, O.S., Koch, G., Hartog, L., Ravenshorst, N. and Peeters, B.P.H. Virulence of Newcastle disease virus is determined by the cleavage site of the fusion protein and by both the stem region and globular head of the haemagglutinin neuraminidase protein. *Journal of General Virology*, **86**, 1759-1769 (2005).
3. Czegledi A., Ujvari D., Somogyi E., Wehmann E., Werner O. and Lomniczi B. Third genome size category of avian paramyxovirus serotype 1 (Newcastle disease virus) and evolutionary implications. *Virus Research,* **120**, 36-48 (2006).
4. Seal, B.S., King, D.J. and Sellers, H.S. The avian response to Newcastle disease virus. *Developmental & Comparative Immunology,* **24**, 257-268 (2000).
5. Dimitrov, K.M., Abolnik, C., Afonso, C.L., Albina, E., Bahl, J., Berg, M., Briand, F.X., Brown, I.H., Choi, K.S., Chvala, I., Diel, D.G., Durr, P.A., Ferreira, H.L., Fusaro, A., Gil, P., Goujgoulova, G.V., Grund, C., Hicks, J.T., Joannis, T.M., Torchetti, M.K., Kolosov, S., Lambrecht, B., Lewis, N.S., Liu, H., Liu, H., McCullough, S., Miller, P.J., Monne, I., Muller, C.P., Munir, M., Reischak, D., Sabra, M., Samal, S.K., Servan de Almeida, R., Shittu, I., Snoeck, C.J., Suarez, D.L., Van Borm, S., Wang, Z. and Wong, F.Y.K. Updated unified phylogenetic classification system and revised nomenclature for Newcastle disease virus. *Infection, Genetics and Evolution,* **74**, 103917 (2019).
6. Mansour, S.M., ElBakrey, R.M., Mohamed, F.F., Hamouda, E.E., Abdallah, M.S., Elbestawy, A.R., and Eid, A.A. Avian paramyxovirus type 1 in Egypt: epidemiology, evolutionary perspective, and vaccine approach. *Frontiers in Veterinary Science*, **8**, 647462 (2021).

7. Radwan, M.M., Darwish, S.F., El-Sabagh, I.M., El-Sanousi, A.A. and Shalaby, M.A. Isolation and molecular characterization of Newcastle disease virus genotypes II and VIId in Egypt between 2011 and 2012. *Virus Genes*, **47**, 311-316 (2013).

8. Alexander, D.J. and Senne, D.A. "Newcastle Disease, Other Avian Paramyxoviruses, and Pneumovirus Infections," In: Saif, Y.M., Fadly, A.M., Glisson, J.R., McDougald, L.R., Nolan, L.K. and Swayne, D.E. Eds., *Diseases of Poultry, Blackwell Publishing, Ames, ,* pp. 75-100(2008).

9. WOAH-World Organization for Animal Health. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Chapter 3.3.14.) (2018). Available online at: https://www.woah.org/app/uploads/2021/03/3-03-14-newcastle-dis.pdf (accessed May 14, 2020).

10. Aldous, E., Manvell, R., Cox, W., Ceeraz, V., Shell, W., Alexander, D.J. and Brown, I.H. Outbreak of Newcastle disease in pheasants (Phasianus colchicus) in south-east England in July 2005. *Veterinary Record,* **160**, 482-484 (2007).

11. Cattoli, G., Susta, L., Terregino, C. and Brown, C. Newcastle disease: a review of field recognition and current methods of laboratory detection. *Journal of Veterinary Diagnostic Investigation,* **23**, 637-656 (2011).

12. Erickson, G., Brugh, M. and Beard, C. Viscerotropic velogenic Newcastle disease in pigeons: clinical disease and immunization. *Avian Diseases,* **24**, 257-267 (1980).

13. Wan, H., Chen, L., Wu, L. and Liu, X. Newcastle disease in geese: natural occurrence and experimental infection. *Avian Pathology*, **33**, 216-221 (2004).

14. Wakamatsu, N., King, D., Kapczynski, D., Seal, B. and Brown, C. Experimental pathogenesis for chickens, turkeys, and pigeons of exotic Newcastle disease virus from an outbreak in California during 2002-2003. *Veterinary Pathology*, **43**, 925-933 (2006).

15. Nishizawa, M., Paulillo, A., Nakaghi, L., Nunes, A., Campioni, J. and Doretto Júnior, L. Newcastle disease in white Pekin ducks: response to experimental vaccination and challenge. *Brazilian Journal of Poultry Science*, **9**, 123-125 (2007).

16. Lima, F.S., Santin, E., Paulillo, A.C., Doretto Jr, L., De Moraes, V.R.M. and Schocken, R.P. Japanese quail (Coturnix coturnix japonica) as Newcastle disease virus carrier. *International Journal of Poultry Science***, 3**, 483-484 (2004).

17. Czirják, G.Á., Köbölkuti, L.B., Cadar, D., Ungvári, A., Niculae, M. and Bolf, A.P. An outbreak of the Newcastle disease in Japanese quail (Coturnix coturnix japonica). *Bull USAMV-CN.,* **64**, 1–2 (2007).

18. El-Tarabili, M.M., El-Shahiedy, M.S.., Hammouda, M.S.., Fetaih, H.A., Abdel-Wahab S.A. and Ramzy, N.M. Natural and experimental infections of quails (Couturnix couturnix japonica) with Newcastle disease virus. *Suez Canal Veterinary Medical Journal*, **16**, 67-80 (2009).

19. El-Zanaty, K. and Abd-El-Motelib, T.Y. Viscerotropic velogenic Newcastle disease in quails (Coturnix coturnix*). Assiut Veterinary Medical Journal*, **29**, 264-75 (1993).

20. Aly, B.A.A. Studies on pigeon paramyxovirus in quails (Master thesis). *Department of Virology, Faculty of Veterinary Medicine, Benha University, Benha, Egypt* (2014).

21. Abd El Aziem, A., Abd-Ellatieff, H., Elbestawy, A., Belih, S., El-Hamid, A and Abou-Rawash, A.R. Susceptibility of Japanese quail and chickens to infection with Newcastle disease virus genotype VIId. *Damanhour Journal of Veterinary Sciences*, **3**, 27-31 (2020).

22. Abd-Ellatieff, H.A., Abd El Aziem, A.N., Elbestawy, A.R., Goda, W.M., Belih, S.S., Ellakany, H.F., Abd El-Hamid, H.S., Yanai, T., AbouRawash, A.A. and El-Habashi, N. Efficacy of Vaccination against Infection with Velogenic Newcastle Disease Virus Genotypes VI and VII 1.1 Strains in Japanese Quails. *Journal of Comparative Pathology,* **186**, 35-50 (2021).

23. Ali, A.A.H., Abdallah, F., Abdelaziz, A., Madbouly, Y. and Kotb, G. Pathogenesis of different genotypes of Egyptian virulent Newcastle disease virus (NDV) previously isolated from chickens in Japanese quails (Coturnix coturnix japonica), Egypt. *Journal of Animal Health and Production*, **9**(s1), 90-96 (2020).

24. McGinnes, L.W., Pantua, H., Reitter, J. and Morrison, T.G. Newcastle disease virus: propagation, quantification, and storage. *Current Protocols in Microbiology*, **1**, 15F-2 **(**2006).

25. Reed, L. and Muench, H. A simple method of estimating fifty percent endpoints. *American Journal of Hygiene* **27**, 493-497 (1938).

26. Wise, M.G., Suarez, D.L., Seal, B.S., Pedersen, J.C., Senne, D.A., King, D.J., Kapczynski, D.R. and Spackman, E. Development of a real-time reverse-transcription PCR for detection of Newcastle disease virus RNA in clinical samples. *Journal of Clinical Microbiology,* **42**, 329-338 (2004).

27. Suvarna, K.S., Layton, C. and Bancroft, J.D. *Bancroft's theory and practice of histological techniques*. 8th edition, Amsterdam: E-Book, Elsevier Health Sciences, (2018).

28. Gibson-Corley, K.N., Olivier, A.K. and Meyerholz, D.K. Principles for valid histopathologic scoring in research. *Veterinary Pathology*, **50**, 1007-1015 (2013).

29. Mohamed, M.A. and Hafez, M.S.A. The susceptibility of Japanese quails to the infection with chicken originated Newcastle disease virus. *Journal of Advanced Veterinary Research,* **6**, 37-43 (2016).

30. Susta, L., Segovia, D., Olivier, T.L., Dimitrov, K.M., Shittu, I., Marcano, V. and Miller, P.J. Newcastle disease virus infection in quail. *Veterinary Pathology*, **55**, 682-692 (2018).

31. Oladele, SB., Enoch, I., Lawal, S. and Ibu, O. Clinico-pathological features of Newcastle disease in Japanese quails (Coturnix coturnix japonica) infected with Newcastle disease virus Kudu 113 strain. *International Journal of Poultry Science*, **7,** 165-168(2008).

32. Amer, S.A., Maatouq, A.M., Ahmed, H.M. and Hassan, E. R. Evaluation for Efficacy of Commercially Available Vaccines Against Challenge with Newcastle Disease Virus Genotype VII in Broilers. *Egypt. J. Vet. Sci.,* **51** (1), 35-41(2020).

33. Gowthaman, V., Singh, SD., Barathidasan, R., Ayanur, A. and Dhama, K. Natural outbreak of Newcastle disease in turkeys and Japanese quails housed along with chicken in a multi-species poultry farm in northern India. *Advances in Animal and Veterinary Sciences,* **1**, 17-20(2013).

34. Mazlan, L.F., Bachek, N.F., Mahamud, S.N.A., Idris, L.H., Wei, T.S., Omar, A.R. and Noor, M.H.M.: The positive expression of genotype VII Newcastle disease virus (Malaysian isolate) in Japanese quails (Coturnix coturnix japonica). *Veterinary World*, **10**(5), 542-548(2017).

35. Ecco, R., Susta, L., Afonso, C.L., Miller, P.J. and Brown, C. Neurological lesions in chickens experimentally infected with virulent Newcastle disease virus isolates. *Avian Pathology,* **40**, 145-152(2011).

36. Higgins, D. and Wong, F. Newcastle disease in a flock of Japanese quails. *Veterinary Record*, **83**, 437-440(1968).

37. Paldurai, A., Kim, S., Nayak, B., Xiao, S., Shive, H., Collins, P. and Sama, S. Evaluation of the contributions of the individual viral genes to Newcastle disease virus virulence and pathogenesis. *J. Virol.,* **88**(15),8579–8596(2014)..

38. Ellakany, H., Abd El-Hamid, H., Nasef, S., Nasr, S., Abdel Aziz, M., Gado, A. and Zedan, R. Evaluation of the protection of commercial live and inactivated NDV vaccines against Newcastle virus genotype VIId circulating in the field. *Damanhour J. Vet. Sci.,* **1**, 17–20(2019).

39. Sharawi, S., El-Habbaa, A., Heba, M. and Khodeir, M. Experimental infection of quail byNDV and its 47.

immune response to vaccination. *Benha Veterinary Medical Journal*, **29**, 218, e224 (2015).

40. Abdel-Glil, M.Y., Mor, S.K., Sharafeldin, T.A., Porter, R.E. and Goyal, S.M. Detection and characterization of Newcastle disease virus in formalin-fixed, paraffin embedded tissues from commercial broilers in Egypt. *Avian Diseases*, **58**, 118-123(2014).

41. Martinez, J., Chou, W., Berghman, L. and Carey, J. Evaluation of the effect of live La Sota Newcastle disease virus vaccine as primary immunization on immune development in broilers. *Poultry Science*, **97**, 455-462 (2018).

42. El-Morshidy, Y., Abdo, W., Elmahallawy, E.K., Abd El-Dayem, G.A., El-Sawak, A., El-Habashi, N., Mosad, S.M., Lokman, M.S., Albrakati, A. and Abou Asa, S. Pathogenesis of Velogenic Genotype VII.1.1 Newcastle Disease Virus Isolated from Chicken in Egypt via Different Inoculation Routes: Molecular, Histopathological, and Immunohistochemical Study. *Animals (Basel)*. **11**(12), 3567 (2021).

43. Susta, L., Miller, P., Afonso, C. and Brown, C. Clinicopathological characterization in poultry of three strains of Newcastle disease virus isolated from recent outbreaks. *Veterinary Pathology*, **48**, 349-360(2011).

44. Sedeik, M.E., Elbestawy, A.R., El-Shall, N.A., Abd El-Hack, M.E., Saadeldin, I.M. and Swelum, A.A. Comparative efficacy of commercial inactivated Newcastle disease virus vaccines against Newcastle disease virus genotype VII in broiler chickens. *Poultry Science,* **98**, 2000-2007 (2019).

45. Heckert, R.A., Collins, M.S., Manvell, R J., Strong, I., Pearson, J.E. and Alexander, D.J. Comparison of Newcastle disease viruses isolated from cormorants in Canada and the USA in 1975, 1990 and 1992. *Canadian Journal of Veterinary Research*, **60**, 50-54 **(**1996).

46. Lee, E.K., Jeon, W.J., Kwon, J.H., Yang, C.B. and Choi, K.S. Molecular epidemiological investigation of Newcastle disease virus from domestic ducks in Korea. *Veterinary Microbiology,* **134**, 241-248 (2009).

العدوى التجريبية للسمان الياباني (Coturnix coturnix japonica) بفيروس أورثوافولا الطيور-1: مسببات الأمراض وقابلية انتقال النمط الجيني VII 1.1

خالد السيد عبدالعال، شيماء محمد جلال منصور*، محمد البكرى عبدالرحيم اسماعيل، احمد عبدالسميع حسن على

قسم الفيرولوجيا، كلية الطب البيطرى، جامعة الزقازيق، الزقازيق، محافظة الشرقية، مصر.

الملخص

تعتبر فيروسات أورثوافولا الطيور1، المعروفة سابقًا باسم فيروس مرض نيوكاسل قادرة على إصابة مجموعة كبيرة ومتنوعة من أنواع الطيور بعلامات ونتائج سريرية مختلفة. وينتشر الفيروس عالميًا بمستويات متفاوتة من الضراوة اعتمادًا على نوع العائل وسلالة الفيروس. تم التحقق من قدرة فيروس نيوكاسل من النوع الجينى VII 1.1 المعزول سابقا من الدجاج المصري على إحداث المرض في السمان الياباني البالغ من العمر 35 يومًا. أظهر 25٪ من السمان المحقون بالفيروس عن طريق الأنف والعينين بـ 10^6EID_{50} التهاب غشاء العين، بينما أظهرت 11٪ من الحالات إسهالًا مخضرًا. لقد ظهرت أعراض عصبية مثل عدم التوازن والرعشة في 37.5٪ من السمان المصابة في اليوم الثامن بعد الإصابة. لوحظ احتقان الأوعية الدموية السحائية ونزيف فى الـ cecal tonsils في كل من السمان المصاب بفيروس نيوكاسل والمخالط. وبالفحص المجهري، أظهر السمان المصاب بالفيروس التهاب دماغي لمفاوي واحتقان رئوي. وباستخدام اختبار تثبيط التلزن الدموى (HI)، كانت الأجسام المضادة لفيروس النيوكاسل فى السمان المصاب بالفيروس والمحصن بلقاح اللا سوتا عند 7 أيام بعد أخذ اللقاح متشابهة نسبيًا. ووصلت مستويات الأجسام المضادة إلى log2 0.8±6 في الطيور المصابة بفيروس نيوكاسل و0.5±5.25 log2 و0.8±4 log2 في السمان المحصن بلقاح اللا سوتا والمخالط، على التوالي عند 21 يومًا بعد أخذ اللقاح. بالإضافة إلى ذلك، أظهرت الدراسة قدرة السمان المصاب على خروج الفيروس من الفم والأمعاء. تم تحديد عيارية الفيروس بحوالي $10^{2.8}$ and $10^{5.2}$ EID50/mL في مسحات الفم والأمعاء في اليوم الخامس بعد الإصابة على التوالي. توضح هذه النتائج أن السمان الياباني يمكن أن يكون عرضة للإصابة بالنمط الجيني VII 1.1 لفيروس النيوكاسل وقد يكون مصدرًا للعدوى لطيور أخرى.

الكلمات الدالة: فيروس مرض النيوكاسل، علم الأمراض النسيجي، اختبار تثبيط التلزن الدموى، خروج الفيروس، مصر.