Original Research

Isolation and molecular characterization of velogenic Newcastle disease virus genotype VII.1.1 from commercial broilers and wild birds in Matrouh governorate, Egypt

Ahmed R. Elbestawy[1*], Ahmed R. Elnagar[2*], Adel H. Saad[3], and Eman Khalifa[4]

1. Department of Bird and Rabbit Diseases, Faculty of Veterinary Medicine, Damanhour University, Elbeheira 22511, Egypt.
2. Department of Poultry and Fish Diseases, Faculty of Veterinary Medicine, Matrouh University, Fuka 51744, Matrouh, Egypt.
3. Department of Nutrition & Clinical Nutrition, Faculty of Veterinary Medicine, Matrouh University, Fuka 51744, Matrouh, Egypt.
4. Department of Microbiology, Faculty of Veterinary Medicine, Matrouh University, Fuka 51744, Matrouh, Egypt.

Correspondence*
Ahmed R. Elbestawy, Department of Bird and Rabbit Diseases, Faculty of Veterinary Medicine, Damanhour University, Elbeheira 22511, Egypt. ahmed.elbestawy@vetmed.dmu.edu.eg

Ahmed R. Elnagar, Department of Poultry and Fish Diseases, Faculty of Veterinary Medicine, Matrouh University, Fuka 51744, Matrouh, Egypt. ahmedelnagar@mau.edu.eg

Received: 14/11/2023
Accepted: 20/12/2023

ABSTRACT

Velogenic Newcastle disease virus (vNDV) genotype VII.1.1 continues to cause severe economic losses in chicken flocks since its first detection in 2011. In this study, pooled samples from 36 diseased commercial broiler chicken flocks (vaccinated against ND) which were suspected to be infected with vNDV and 15 pooled cloacal swabs from 6 wild bird species in Matrouh governorate were tested for vNDV genotype VII.1.1. The results showed that 5 broiler samples (5/36 = 13.8%) and 2 cloacal swabs from wild marbled ducks (2/15 = 13.3%) were positive by real-time RT-PCR and 6/7 isolates had mixed viral infections. F gene partial sequence analysis of 2 vNDV isolates (broiler origin, with a GenBank accession No. OR778089) and (wild marbled duck origin, with a GenBank accession No. OR778090), revealed 97.9-100% similarity with previous Egyptian isolates of vNDV-VII.1.1. Furthermore, the intracerebral pathogenicity index score of an isolate of duck origin (no. 44) was 1.82. In conclusion, wild marbled ducks could be an important source of transmission of vNDV-VII.1.1 to commercial broiler flocks. Furthermore, the isolation of vNDV-VII.1.1 from vaccinated commercial broiler flocks necessitates the use of more genetically and antigenically matched vaccines for effective control of this devastating viral infection.

Keywords: vNDV-VII.1.1; Fusion Protein; Matrouh

INTRODUCTION

Velogenic Newcastle disease virus (vNDV) or avian orthoavulavirus-1 (AOAV-1) genotype VII.1.1 is one of the most economically important diseases, causing high mortality and morbidity in susceptible broiler chickens in many countries ***(Miller & Koch, 2020)***. Recent classification of AOAV-1 depending on complete F gene sequence fusion divided it into two classes, class I (non-virulent) viruses, which have 3 genotypes, and class II (virulent and non-virulent) viruses, in which 20 genotypes (I to XXI) have recently been identified. This classification merged all sub-genotypes of AOAV-1 or vNDV genotype VII viruses into VII.1.1, VII.1.2, and VII.2 ***(Dimitrov et al., 2019)***.

In Egypt, ND was first identified in 1947 in the governorate of Kafr Elsheikh (***Daubney & Mansy, 1948)***. Currently, at least 4 NDV genotypes of class II are circulating in Egypt including II, VI.2 (PPMV-1), VII.1.1, and XXI.2.2 (former VIi). Several co-infections of NDV with infectious bronchitis and avian influenza (AI) viruses, especially low pathogenic AI-H9N2, have also been reported in Egypt ***(Radwan et al., 2013; Rohaim et al., 2016; Moharam et al., 2019; Shehata et al., 2019: Mansour et al., 2021; Naguib et al., 2022; Elbahnasawy et al., 2023 & Yehia et al., 2023)***.

Several records clarified the economic impact of vNDV-VII.1.1 in the Egyptian poultry industry and detected this virus in various outbreaks in both vaccinated and unvaccinated commercial poultry and wild birds since 2011 ***(Abdel-Glil et al., 2014; Awad et al., 2015; Saad et al., 2017; El Naggar et al., 2018; Abd El-Hamid et al., 2020; Abd Elfatah et al., 2021 & Abozaid & Abdel-Moneim, 2022)***. In addition, ***Amer et al. (2018)*** and ***Moharam et al. (2019)*** recorded that vNDV-VII.1.1 circulating in Egypt has the ability to be transmitted over long distances between neighboring farms and mentioned several pathogens as co-infections. It is well known that the F protein cleavage site, which determines the virulence of NDV strains, is commonly displayed as a dibasic amino acid sequence motif at the C-terminus of the F2 protein and a leucine amino acid at the N-terminus of F1 protein (i.e. 112GRQGRL117) in avirulent or lentogenic strains and as a polybasic motif with phenylalanine amino acid at the N-terminus of F1 protein (i.e. 112RRQKRF117) in virulent (mesogenic and velogenic) strains ***(Zhao & Peeters, 2003; Samal, 2008; Miller & Koch, 2020)***.

Previously, the circulation of AOAV-1 or vNDV-VII.1.1 in birds in the governorate of Matrouh had not been studied in detail. Therefore, this work aimed to make a survey and molecularly characterize vNDV-VII.1.1 strains from commercial broiler chickens and wild birds.

MATERIALS AND METHODS

Sample collection: The investigated 36 broiler chicken flocks were aged 23-46 days-old, vaccinated against NDV with double live and single inactivated vaccines, and suffered from increased mortality (0.5-6.25% in the last 3 days before sampling), as well as respiratory and enteric signs. Pooled tracheal swabs from live diseased, and tissue samples from lung, kidney, and spleen of freshly dead chickens (*n* = 5/each flock), as well as 15 cloacal swabs from 6 wild bird species (pooled from 5 birds/each swab) were collected during January 2021- January 2023, according to ***WOAH (2021)*** **(Table 1)**. The total number of the commercial broiler flocks ranged from 6000 to 16000 birds, while the total number of the 6 wild bird species were 75 including 15 wild pet or fig birds (orioles), 5 wild Japanese quails (*Coturnix japonica*), 45 wild marbled ducks [*Marmaronetta angustirostris* (n = 35)] and wild northern shoveler ducks (n = 10), 5 wild falcons *(Falco tinnunculus)*, and 5 wild European turtle doves.

Table (1): History of suspect NDV samples (2021-2023)

Sample Number	Year	Locality	Type of birds	Total Number	Age (days)	Mortality % (last 3 days before sampling)	History of Vaccination for NDV
1	2021	Marsa Matrouh	Commercial broiler chickens	6000	41	3 %	Hitchner vaccine at day 7 + killed (genotype II based) vaccine at day 8 + Clone 30 vaccine at day 18
2		El-Negela		6000	34	0.6 %	
3		El-Negela		6000	28	0.7 %	
4		Marsa Matrouh		10000	32	0.5 %	
5		Marsa Matrouh		10000	32	0.5 %	
6		El-Dabaa		12000	38	6.25 %	
7		El-Negela		6000	36	0.7 %	
8		El-Negela		6000	36	0.7 %	
9		Marsa Matrouh		7000	26	0.7 %	
10		Marsa Matrouh		6000	46	4.5 %	
11		Marsa Matrouh		7000	30	4.3 %	
12		Marsa Matrouh		12000	31	1.25 %	
13		El-Negela		6000	41	0.7 %	
14		Marsa Matrouh		6000	30	2.5%	
15		Marsa Matrouh		10000	40	5.4 %	
16		Marsa Matrouh		10000	40	5.4 %	
17		Marsa Matrouh		6000	26	1.5 %	
18		Marsa Matrouh		11000	38	0.9 %	
19		Marsa matrouh		6000	27	2.8 %	
20	2022	El-Dabaa		10000	25	0.9 %	
21		Marsa Matrouh		6500	26	0.5 %	
22		El-Negela		6000	41	1.8 %	
23		El-Alamin		10000	33	1.2 %	
24		Marsa Matrouh		6000	38	1.2 %	
25		El-Negela		14000	23	0.8 %	
26		Marsa Matrouh		6000	31	0.7 %	
27		Marsa Matrouh		16000	28	2.8 %	
28		Marsa Matrouh		8000	30	3 %	
29		Marsa Matrouh		12000	23	0.5 %	
30		Marsa Matrouh		7000	33	4.2 %	
31		Marsa Matrouh		6000	26	2 %	
32		Marsa Matrouh		7000	35	4.2 %	
33		Marsa Matrouh		6000	30	1.5 %	
34		El-Daba		10000	38	0.6 %	
35		Marsa Matrouh		10000	30	0.6 %	
36		Marsa Matrouh		10000	30	0.6 %	
37	2022	Migrating from Europe and captured in Matrouh governorate	Wild pet birds or Fig birds (Orioles)				Non - vaccinated
38							
39							
40			Wild Japanese quails (*Coturnix Japonica*)				
41			Wild marbled ducks (*Marmaronetta angustirostris*) Wild ducks (Northern shoveler)				
42							
43							
44							
45	2023						
46							
47							
48							
49							
50			Wild falcons (*Falco tinnunculus*)				
51			Wild European turtle doves				

Virus isolation and propagation:

Tissue homogenates and/or pooled swabs were inoculated via the allantoic sac of 10-day-old specific pathogen-free embryonated chicken eggs (SPF-ECE) (Kom Oshem, SPF Farm, El Fayoum) at 3 eggs/sample ***(WOAH, 2021)***. The harvested allantoic fluid of each sample was tested for haemagglutination (HA) activity by slide, and plate HA test after each passage. Confirmation of a negative HA test (no haemagglutinating agent) was considered after the 3rd passage ***(Terregino and Capua, 2009)***.

Real time polymerase chain reaction (rRT-PCR) detection:

All samples with positive HA titres were tested for velogenic NDV using primers and probe targeting the F gene (Metabion, Germany) according to ***Moharam et al. (2019)***. All the positive vNDV-VII.1.1 samples were also tested for other respiratory viruses using rRT-PCR (DT*lite* 4, DNA Technology, Moscow, Russia) according to the manufacturer's instructions. The used oligonucleotide primers and probes (Metabion, Germany) were used for AI ***(Hoffmann et al.,2016),*** for infectious bronchitis virus (IBV) ***(Acevedo et al.,2013)***, adenovirus ***(Günes et al.,2012)***. Furthermore, the positive vNDV samples were tested for infectious bursal disease virus (IBDV) ***(Tomás et al., 2012)***. For viral RNA extraction, 200 µl of bursal homogenate supernatant from each pooled sample was mixed with 1 ml of GENEzol™ Reagent (Geneaid, New Taipei City, Taiwan).

Extraction of viral RNA:

Total viral RNAs were extracted from all HA-positive allantoic fluids using GeneAll® EXGENE™ Viral RNA/DNA extraction kits (GeneAll Biotechnology Co., LTD, Seoul, South Korea) according to the manufacturer's instructions. A one-step rRT-PCR master mix kit (SMOBIO TaqMan-Rox, Beijing, China) was also used according to the manufacturer's protocol.

Partial sequence analysis of F-gene of vNDV:

Partial F gene sequencing (aa 1-149) was performed for 2 vNDV-VII.1.1 positive field isolates, one from commercial broilers (No. 20) and the other from wild marbled ducks, *Marmaronetta angustirostris* (No. 44), using primer sets designed by ***Selim et al. (2018)***. Purification using QIAquick Gel Extraction Kit (Qiagen, Germany) and one-step rRT-PCR reaction were performed according to the manufacturer's instructions. Big Dye Terminator V3.1 cycle sequencing kit (PerkinElmer, CA, USA) and Applied Biosystems 3130 Genetic Analyser (ABI, USA) were used for sequence analysis.

Bioinformatic and phylogenetic analysis:

Comparative sequence of the F gene of 2 selected vNDV field isolates with previously published NDV reference strains available in the public database (BLASTn, NCBI, USA) (http://www.ncbi.nlm.nih.gov/BLAST), using Bioedit software version 7.2.4®. Nucleotide similarity and divergence were performed in the MegAlign programme of the Laser Gene package (DNASTAR Inc., Madison, WI, USA). Molecular evolutionary genetics analysis using MEGA11 (version 1®) was applied to construct a phylogenetic tree for the F gene nucleotide and amino acid sequence alignments of both isolates ***(Kumar et al., 2018)***. Algorithms were applied to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach and the Tamura-Nei model ***(Tamura et al., 2013)***.

Pathogenicity evaluation of one selected vNDV isolate:

The intracerebral pathogenicity index (ICPI) was performed on 10 one-day-old SPF chicks to evaluate the pathogenicity of vNDV-VII.1.1 isolate no. 44. Fresh pure infective allantoic fluid, with a HA titre of 9 log2 was diluted 1/10 in sterile isotonic saline and 50 µl of the diluted virus was injected intracerebrally. The chicks were daily for 8 days and at each observation, they were scored: 0 if normal, 1 if sick, and 2 if dead. ICPI was calculated as the mean score per bird per observation over the 8-day period. Any score of ≥ 0.7 was considered virulent ***WOAH (2021)***.

RESULTS

Virus isolation, HA, and rRT-PCR

Embryos from inoculated eggs were died between 2 and 4 days after inoculation in 7 samples. Those 7 out of 36 (19.4%) samples, 5 from broilers (5/36 = 13.8%) and 2 from wild marbled ducks (2/15 = 13.3%), showed HA positive titres ranged from 4 log2. All the 7 haemagglutinating allantoic fluids were positive for vNDV-VII.1 by rRT-PCR. Six out of these 7 vNDV isolates were mixed viral infections with one or more of the tested viruses **(Table 2).**

Table (2): Positive samples for vNDV and other mixed infections

Sample Number	Slide HA activity after egg inoculation	Plate HA titer (log_2)	rRT-PCR results					
			vND	H5	H9	IB	Adeno	IBD
14	+	4	+	-	+	-	-	-
17	+	4	+	-	-	+	-	-
20	+	4	+	-	-	-	+	-
21	+	4	+	-	-	-	-	+
28	+	4	+	-	+	+	-	-
43	+	4	+	+	+	-	+	+
44	+	4	+	-	-	-	-	-

- The red colour indicates to positive results, the green colour indicates negative results

The annual migration routes of wild marbled ducks from their breeding grounds in Europe and Asia (autumn migration, from 15 August to 1 November) to their wintering grounds in Africa, including Egypt, to escape the cold weather, and then the spring or return migration to their breeding grounds along the same routes from 15 March to 25 May are shown in **Figure 1**.

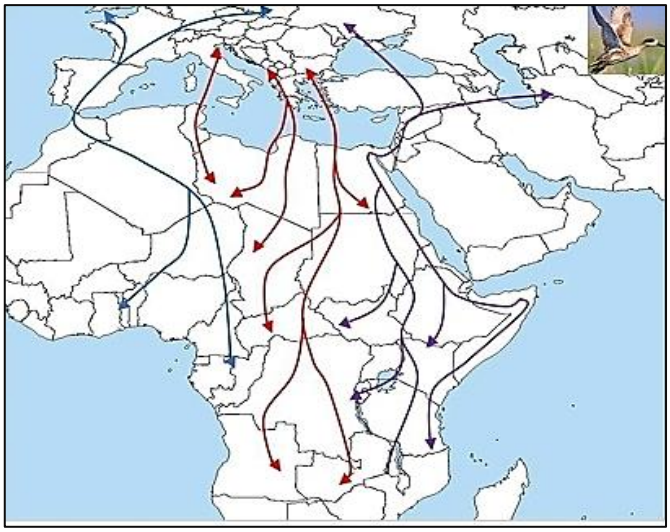

Figure 1: The annual migratory journey of wild marbled ducks. Red lines indicate the central migration route from western and central Europe to the Mediterranean Sea, across the Sahara to central and southern Africa, while, purple lines indicate the eastern migration route from eastern Europe and Asia through the Middle East down to eastern and southern Africa. Blue lines indicate the western migration route through Italy and Spain, across the Straits of Gibraltar, over the Atlas Mountains and the Western Sahara into West and Central Africa.

Partial sequence analysis of fusion protein, molecular pathotyping and phylogenetic tree of some vNDV isolates:

The partial-length F protein aa sequences obtained from 2 vNDV field isolates in 2022 (No. 20 and 44) were submitted to GenBank under accession numbers OR778089 and OR778090, respectively. Their BLASTN analysis showed 97.9-100% similarity with previous Egyptian isolates of vNDV-VII.1.1 for the same part of the sequenced F protein. Both isolates were identical (100%) to the F gene sequence of AOAV-1 chicken isolates NDV/chicken/Egypt/Dakahlia27/2016 (KY075890), NDV/Ch/Egypt/BeheiraB36/2017 (MK984236), NDV/Ch/Egypt/BeheiraR78/2018 (MK984238), NDV/Egypt/Giza10/2020 (OM243951). 96.5-98.6% to genotype VII.1.1 of old Chinese and Japanese isolates from 1998-2009 (AB853927, EF589133 and KC542905), 92.4-93.1% to Chinese genotype VII.1.2 (AY028995, DQ227246, GQ338309) and Congolese variant genotype VII.2 strains (MW363931 and MW363929). 88.9-89.6% to genotype VI (EU293914 and AY562989), 85.5-86.2% to genotype V (AY562986 AY562987), 88.9% to genotype IV (Herts/33, AY741404), 88.9% to genotype III (Mukteswar, JF950509), 82.7% to LaSota (JF950510) and clone-30 (Y18898), 82% to Avinew (KM056356) and 81.3% to B1 (AF309418) of genotype II and 82% for both V4 (AF217084) PHY-LMV42 (DQ097394) and 77.9% to Ulster (NC075404) of genotype I **(Figures 2 and 3)**.

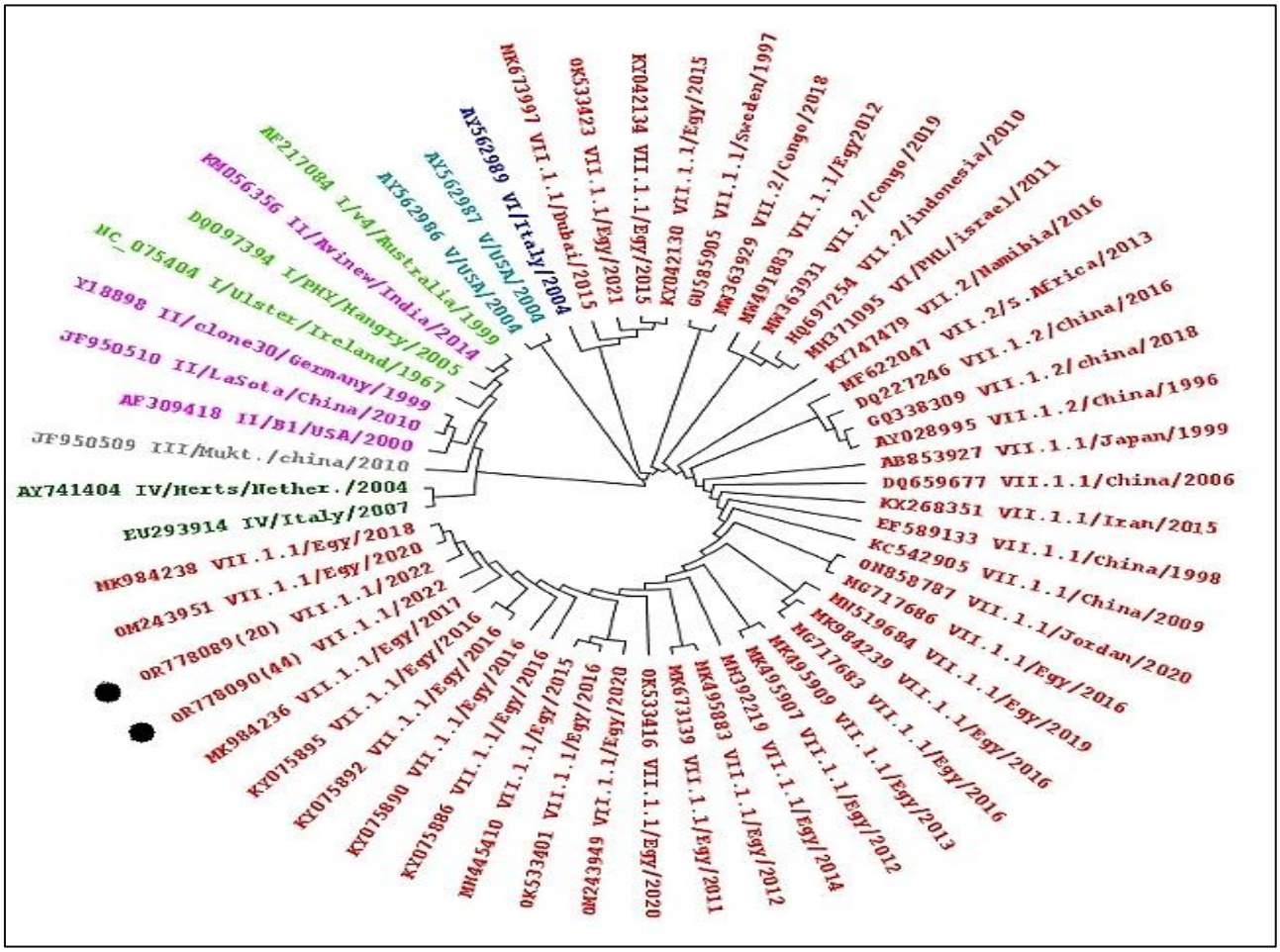

Figure 2: Phylogenetic analysis of the 2 obtained vNDV-VII.1.1 field isolates (no. 20: OR778089 and 44: OR778090) (black dots) and other NDV isolates resembling genotypes I-VII.

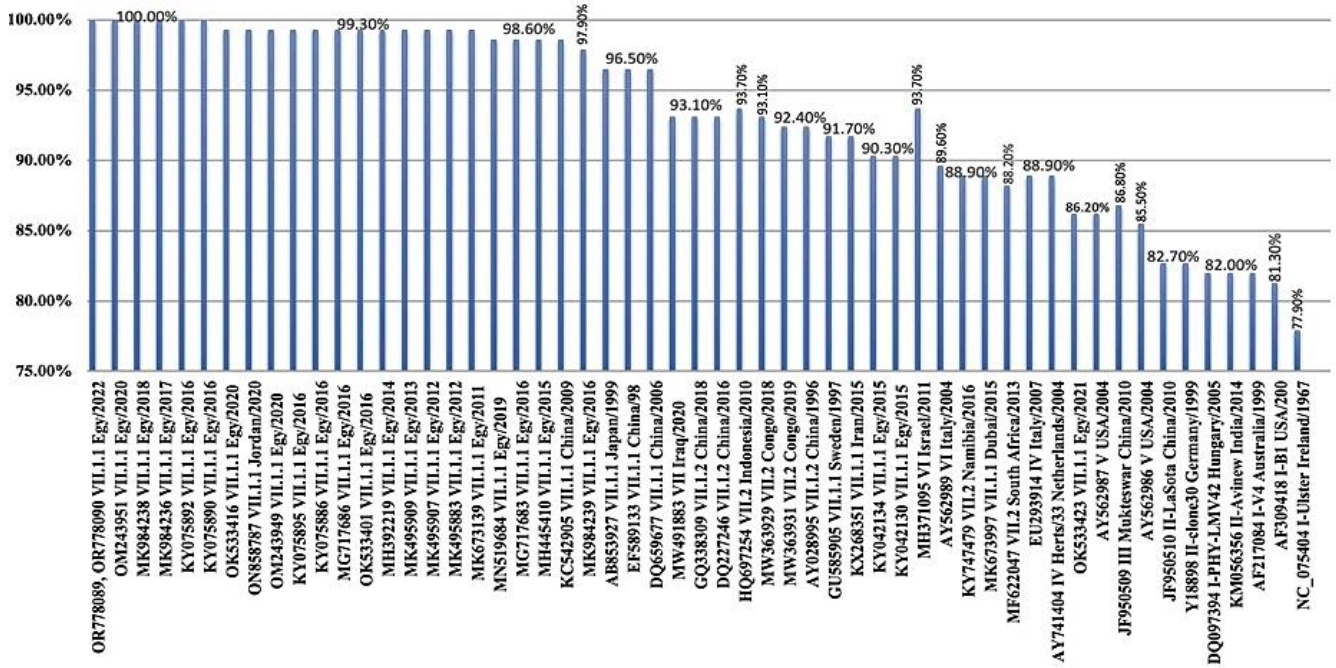

Figure 3: Comparative percent similarity between the 2 obtained vNDV-VII.1.1 field isolates (20: OR778089 and 44: OR778090) and other NDV isolates resembling genotypes I-VII.

Comparative alignment of the deduced amino acid of the 2 vNDV-VII.1.1 isolates (OR778089 and OR778090) at positions 112 to 117 (the cleavage site motif) clarified the presence of multiple basic amino acids (RRQKRF) along with conserved amino acids of K101R and V121I, both of which are features of more virulent AOAV-1 viruses, molecularly confirming the velogenic pathotyping of both isolates. Some deduced amino acid substitutions were also identified in the 2 vNDV-VII.1.1 isolates, such as L13P, I19V, T22I, R27C, N30S, V52I, R71K, R78K, K101R and K145N. The fusion peptides (aa 117-136) showed similar aa sequence to all sequences of vNDV-VII.1.1 except V121I and A132S compared to VII.1.1 of Egy/BSU-24/2021 (OK533423), Egy/El-Fayom2015 (KY042134), Egy/Helwan/2015 (KY042130) and Camel/Dubai/2015 (MK673997). Both isolates sequenced in this study (OR778089 and OR778090) contained 5 conserved aa-neutralizing epitopes important for the function of the fusion protein at positions D72, E74, A75, R78, A79. The detailed aa alignments are shown in **Figures 4a and b**

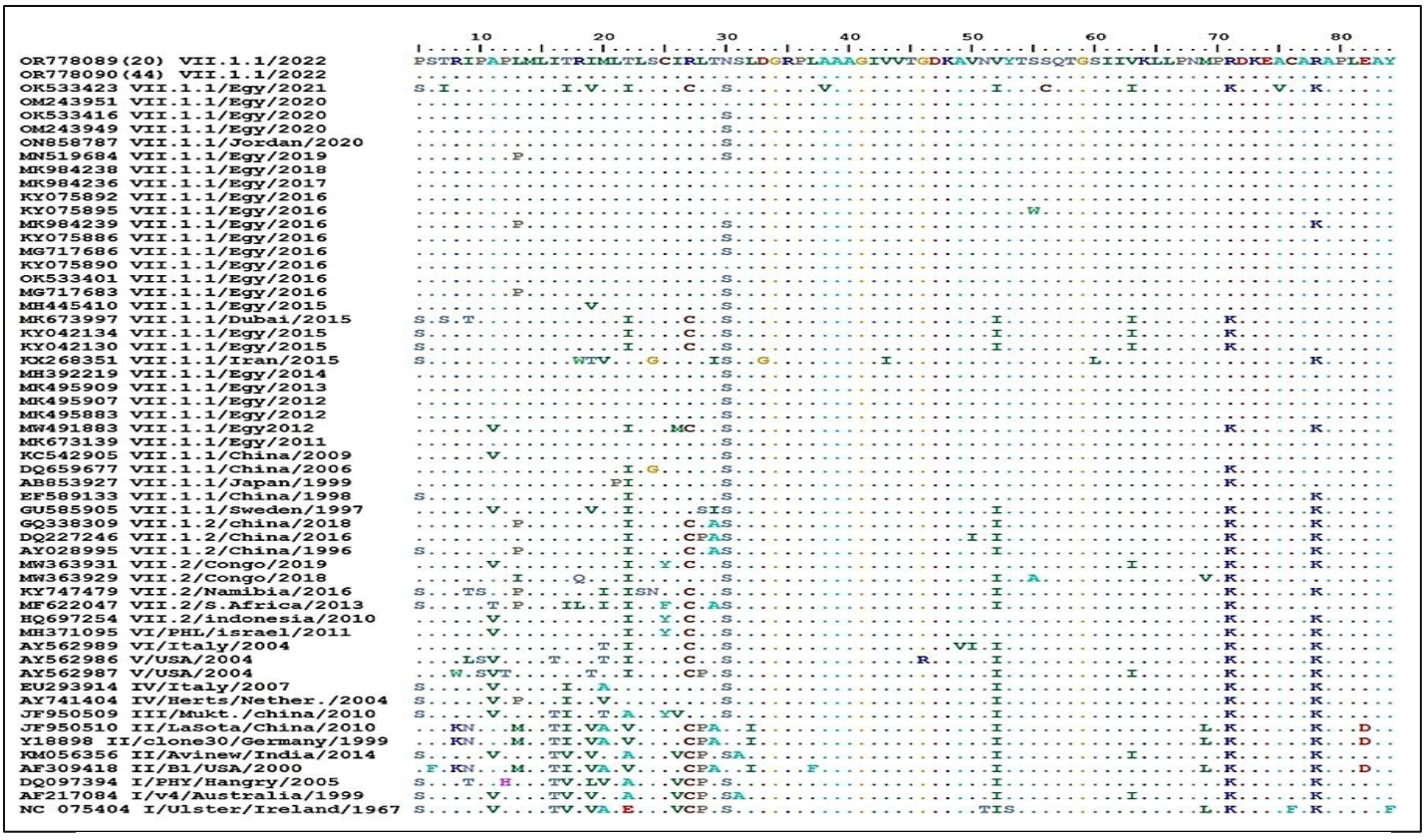

Figure 4a: Amino acid **(**aa 5-84) alignments between the 2 vNDV-VII.1.1 isolates obtained (20: OR778089 and 44: OR778090) and other NDV isolates resembling genotypes I-VII.

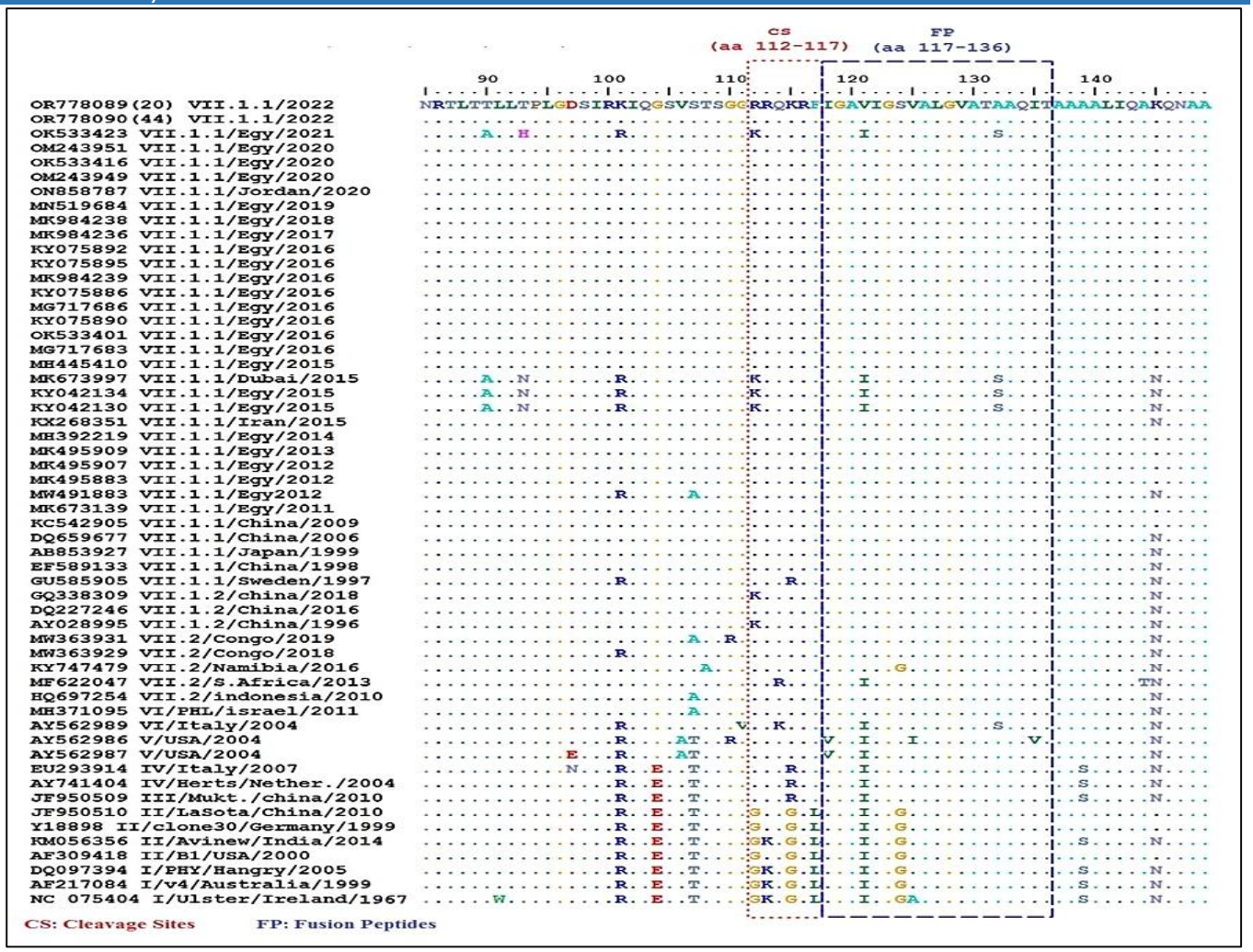

Figure 4b: Amino acid (aa 85-149) alignments between the 2 vNDV-VII.1.1 isolates obtained (20: OR778089 and 44: OR778090) and other NDV isolates resembling genotypes I-VII.

Intracerebral pathogenicity index (ICPI) of vNDV-VII.1.1 isolate (Accession No OR778090):

The ICPI score of vNDV-VII.1.1 wild duck isolate no. 44 (OR778090) was 1.82.

DISCUSSION

Newcastle is an enzootic disease in many countries and shows variable outcomes and consequently variable economic losses according to several factors, particularly vaccination and the host's immunity ***(Miller et al., 2015)***. To date, 20 different genotypes of AOAV-1 class II have been identified ***(Dimitrove et al., 2019)***, including both virulent and non-virulent viruses. Velogenic NDV genotype VII has been associated with recent outbreaks in Asia, Europe, Africa, the Middle East, and South America ***(Lomniczi et al., 1998; Miller et al., 2009; Khan et al., 2010; Zhang et al., 2011 & Perozo et al., 2012)***. Genotype VII can be further subdivided into 3 major subgenotypes according to amino acid substitutions (VII.1.1, 1.2 and 2) ***(Dimitrove et al., 2019)***. In Egypt, a highly pathogenic AOAV-1 (genotype-VII) is still causing several outbreaks in poultry since 2011 (Radwan et al., 2013). All genotype VII of vNDV that so far detected in Egypt have been clustered into one sub-genotype (VII.1.1) ***(Radwan et al., 2013; Abdel-Glil et al., 2014; Awad et al., 2015; Orabi et al., 2017; Saad et al., 2017; Amer et al., 2018; El Naggar et al., 2018; Moharam et al., 2019; Abd El-Hamid et al., 2020; Mansour et al., 2021; Abozaid and Abdel-Moneim, 2022 & Eid et al., 2022)***.

A total of 7/51 (13.7%) of vNDV-VII.1.1 positive samples were confirmed by rRT-PCR. Five of them were isolated from commercial broiler chickens that were vaccinated against NDV with genotype II-based vaccines and 2 of them were isolated from wild marbled ducks (*Marmaronetta angustirostris*) for the first time in Matrouh governorate. This may indicate the potential role of these wild birds in the transmission of vNDV to commercial chicken farms, especially under poor biosecurity measures. The wild marbled duck (*Marmaronetta angustirostris*) is known to be farmed in southern Spain, southern Italy, north-west Africa, and the wider Levant. Further east it survives in southern Iraq, Iran, Armenia, Azerbaijan, southern European Russia, western India, and western China ***(Bird Life International, 2017)***, so we can say that vNDV-VII.1.1 could be transmitted from and to all of these countries.

Previously, ***El Naggar et al. (2018)*** genetically identified a total of 3.6% (4 out of 112) positive samples collected from different families of wild birds arranged as follows: 2/19 from Anseriformes (*A. crecca* or Eurasian teal),

1/24 from Galliformes (coturnix quails), and 1/40 from Pelecaniformes (*Bubulcus ibis* or cattle egret), while all the 29 samples from Passeriformes (*Passer domesticus* or house sparrow) revealed negative results. Furthermore, ***Mohammed et al. (2020)*** investigated the prevalence of NDV circulating in wild birds within live bird markets in 3 different cities in Egypt. They reported that 6/159 (3.77%) samples (5 from Damietta and 1 from Matrouh) were collected from eight different wild bird species and tested positive for NDV genotype I (non-virulent strains). Interestingly, wild Anseriformes accounted for the largest percentage of detections (5/6 samples, 2 from Pintail and 3 from Northern Shoveler) and one sample was isolated from wild Columbiformes (Laughing Dove). In addition, the experimental study by ***Elbestawy et al. (2019)*** investigated the role of domestic ducks as efficient vectors for vNDV-VII.1.1 and demonstrated that Muscovy ducks that were infected with velogenic NDV (AOAV-1) genotype VIId (vNDV-VII.1.1) via the intranasal route, could transmit the virus to their in-contact chickens, resulting in clear characteristic signs of ND and 20% mortality. In addition, these in-contact chickens continued to shed the virus in their oropharyngeal and cloacal excreta for up to 11 days after exposure to infected ducks.

The results obtained also clearly indicate the continuous vNDV-VII.1.1 challenge to commercial broiler chickens despite the vaccinations used, especially genotype II-based vaccines. In Egypt, ***Shakal et al. (2022)*** molecularly identified a v NDV strain (with polybasic amino acid sequences in the F gene cleavage sites) that was isolated from a vaccinated commercial broiler flock with a mortality rate of 35.8%. Interestingly, this flock had received 2 live vaccinations with LaSota. In a previous study of ***Abd El-Hamid et al. (2020)***, the authors reported a high prevalence of AOAV-1 genotype VII.1.1 in Egyptian chicken flocks despite intensive vaccination with live and killed NDV vaccines based on genotype II. Moreover, the authors recommended that the use of genetically related vaccines to genotype VII.1.1 will reduce its multiplication and environmental shedding and minimie the spread of this devastating viral infection among poultry flocks.

The partial F gene sequences (1-149) of 2 obtained vNDV (OR778089 and OR778090) showed 100% identity with the previous isolates of vNDV-VII.1.1 in Egypt during 2016-2020, sharing the same cleavage site motif of RRQKRF at positions (112-117 aa), and had 77.9-82.7% similarity with the genotypes I and II that are used as commercial vaccines in Egypt. Recently, an attractive research work that was done by ***Elbestawy et al. (2023)*** showed superior efficacy of apathogenic genotype I (V4) over lentogenic genotype II (LaSota) live vaccination against vNDV-VII.1.1 in broiler chickens that were vaccinated with a pathogen-associated molecular pattern H9N2 inactivated vaccine. They confirmed the theory explained by ***Tabatabaeizadeh (2021),*** who mentioned that the higher the match of linear and conformational neutralizing epitopes of F and HN proteins (n = 15) of the vaccine strains and vNDV-VII.1.1 (V4 = 14 > LaSota = 7), the higher obtained protection in the vaccinated birds.

In addition, the comparative alignments of the cleavage site motif of the F1-2 gene in the vNDV-VII.1.1 isolate (OR778090) isolate revealed the conserved amino acids at positions K101R and V121I of the F protein as a unique feature of genotype VII, in agreement with ***Lien et al. (2007)***. The presence of Q in the cleavage site motif of RRQKRF of vNDV-VII.1.1 here enhances and increases the virulence ***(Wang et al., 2017)***. All signal peptides in both vNDV-VII.1.1 sequences (OR778089 and OR778090) contained the same amino acid features for vNDV-VII.1.1 sub-genotypes representing viruses of the 4th panzootic at positions I9, A11, R18, C25, whereas the 5th panzootic of AOAV-1 infections belonged to viruses of sub-genotype VII.1.2 ***(Miller et al., 2015 & Liu et al., 2019)***. Again, a signal peptide region N30 present in both isolates is the same as in genotype II of vaccinal strains (LaSota, clone-30, and B1), which is different from the previously studied Egyptian strain vNDV-VII.1.1 that recorded a substitution S30N ***(Orabi et al., 2017)***. During NDV replication, F0 is synthesized as an inactive precursor and subsequently cleaved by cellular proteases into a disulfide-linked F1-F2 complex ***(Sergel-Germano et al., 1994)***. The F1 subunit containing the fusion peptide FP from position 117-136 aa had a similar aa sequence between both isolates in this study and all vNDV-VII.1.1 except aa V121I and A132S in comparison to VII.1.1 of Egy/BSU-24/2021 (OK533423), Egy/El-Fayom2015 (KY042134), Egy/Helwan/2015 (KY042130) and UAE/Dubai/2015 (MK673997).

According to the ***WOAH (2021)***, the virulence of NDV strains is mainly based on 2 points: 1- The presence of the 112R/K-R-Q-R/K-R-F117 motif with two pairs of basic amino acids and a phenylalanine at the N-terminus of F1 (residue 117). 2- The ICPI of AOAV-1 in one-day-old chicks, if the score is equal or greater than 0.7, it is classified as a virulent pathotype. In the current study, the velogenic motif of multiple basic amino acids (RRQKRF) was detected by partial F gene sequence analysis. The ICPI value for the confirmed vNDV-VII.1.1 No. 44 that isolated from wild marbled ducks (GenBank accession No. OR778090) was 1.82, indicating its virulent pathotype nature. Previously, the ICPI scores of most of the Egyptian AOAV-1 genotype VII viruses were reported to be between 1.66 and 1.98 ***(Saad et al., 2017; El Naggar et al., 2018; Selim et al.,2018; Moharam et al., 2019; Abd El-Hamid et al., 2020)***.

CONCLUSION

This study clarifies the potential role of wild marbled ducks in the transmission of vNDV-VII.1.1 to commercial poultry flocks. Also, it highlights the continuity of isolation of vNDV-VII.1 .1 from commercial broiler flocks despite extensive vaccination programs (genotype II based live and inactivated vaccines) in Matrouh Governorate. In addition, there is a need to change the vaccines used into more genetically and antigenically matched vaccines to reduce virus replication and spread of this devastating viral infection between poultry

flocks. Finally, the full sequences of F and HN genes will be the following study in order to identify any other AA mutations.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Abd El-Hamid, H.S., Shafi, M.E., Albaqami, N.M., Ellakany, H.F., Abdelaziz, N.M., Abdelaziz, M.N., Abd El-Hack, M.E., Taha, A.E., Alanazi, K.M. & Elbestawy, A.R. (2020). Sequence analysis and pathogenicity of Avian Orthoavulavirus 1 strains isolated from poultry flocks during 2015–2019. BMC Veterinary Research, 16, 253. https://doi.org/10.1186/s12917-020-02470-9

Abd Elfatah, K.S., Elabasy, M.A., El-khyate, F., Elmahallawy, E.K., Mosad, S.M., El-Gohary, F.A., Abdo, W., Al-Brakati, A., Seadawy, M.G., Tahoon, A.E. & El-Gohary, A. (2021). Molecular Characterization of Velogenic Newcastle Disease Virus (Sub-Genotype VII.1.1) from Wild Birds, with Assessment of Its Pathogenicity in Susceptible Chickens. Animals, 11, 505. Doi: 10.3390/ani11020505

Abdel-Glil, M.Y., Mor, S.K., Sharafeldin, T.A., Porter, R.E. & Goyal, S.M. (2014). Detection and Characterization of Newcastle Disease Virus in Formalin-Fixed, Paraffin-Embedded Tissues from Commercial Broilers in Egypt. Avian Diseases, 58 (1):118-123. doi: 10.1637/10616-071813-Reg.1

Abozaid, K.G.A. & Abdel-Moneim A. (2022). Epidemiological surveillance of Newcastle disease virus in Egypt — a 6-year cohort study. Tropical Animal Health and Production 54(4):243. doi: 10.1007/s11250-022-03234-3

Acevedo, A.M., Perera, C.L., Vega, A., Ríos, L., Coronado, L., Relova, D., Frías, M.T., Ganges, L., Núñez, J.I. & Pérez, L.J. (2013). A duplex SYBR Green I-based real-time RT-PCR assay for the simultaneous detection and differentiation of Massachusetts and non-Massachusetts serotypes of infectious bronchitis virus. Molecular Cell Probes 27, 5–6, 184-192. doi: 10.1016/j.mcp.2013.06.001

Amer, M.M., Maatouq, A.M., Abdel-Alim, G.A., Awaad, M.H.H. & Kutkat, M.A. (2018). Isolation and Identification of H9N2 Avian Influenza and Newcastle Disease Viruses Co-Infections in Chicken. Egyptian Journal of Veterinary Science, 49 (2): 135-146. doi: 10.21608/ejvs.2018.4963.1043

Awad, A.M., Sedeik, M.E. & Abdelkariem, A.A. (2015). Isolation, molecular characterization and pathotyping of Newcastle disease viruses from field outbreaks among broiler flocks in Egypt from 2014–2015. International Journal of Current Research 7 (02): 12925-129347. http://www.journalcra.com

Bird Life International (2017). "Marmaronetta angustirostris". IUCN Red List of Threatened Species. e.T22680339A110054350. doi: 10.2305/IUCN.UK.20171.RLTS.T22680339A110054350.en.

Eid, A.A.M., Hussein, A., Hassanin, O., Elbakrey, R.M., Daines, R., Sadeyen, J.-R., Abdien, H.M.F., Chrzastek, K. & Iqbal, M. (2022). Newcastle Disease Genotype VII Prevalence in Poultry and Wild Birds in Egypt. Viruses, 14, 2244. doi.org/10.3390/v14102244

Dimitrov , K.M., Abolnikb, C., Afonsoa, C.L., Albinac, E., Bahle, J., Bergf, M., Briandg, F., Brownh, I.H., Choii, K., Chvalaj, I., Dielk, Durrl, P.A., Ferreiraa, H.L., Fusaron, A., Gild, P., Goujgoulovap, G.V., Grundq, C., Hickse, J.T., Joannisr, T.M., Torchettis, M.K., Kolosovj, S., Lambrechtt, B., Lewish, N.S., Liuv, H., Liuw, H., McCulloughl, S., Millerx, P.J., Monnen, I., Mullery, C.P., Munirz, M., Reischakaa, D., Sabraab, M., Samalac, S.K., de Almeidad, R.S., Shittur, I., Snoecky, C.J., Suareza, D.L., Bormt, S.V., Wangw, Z. & Wong, F.Y.K. (2019). Updated unified phylogenetic classification system and revised nomenclature for Newcastle disease virus. Infection, Genetics and Evolution, 74: 103917-103932. doi: 10.1016/j.meegid.2019.103917.

Elbahnasawy, R., Abouelazm, K.I., Fahmy, H.A., Awad, S.S. (2023). Prevalence of circulating pigeon paramyxoviruses (velogenic PPMV-1). Mansoura Veterinary Medical Journal, Article in Press, ISSN: 1110-7219, doi: 10.21608/MVMJ.2023.97195.1082

Elbestawy, A., Ellakany, H., Sedeik, M., Gado, A., Abdel-Latif, M., Noreldin, A., Orabi, A., Radwan, I. & El-Ghany, W.A. (2023). Superior Efficacy of Apathogenic Genotype I (V4) over Lentogenic Genotype II (LaSota) Live Vaccines against Newcastle Disease Virus Genotype VII.1.1 in Pathogen-Associated Molecular Pattern-H9N2 Vaccinated Broiler Chickens. Vaccines, 11, 1638. doi: 10.3390/vaccines11111638

El Naggar, R.F., Rohaim, M.A., Bazid, A.H., Ahmed, K.A., Hussein, H.A. & Munir, M. (2018). Biological characterization of wild-bird-origin avian avulavirus 1 and efficacy of currently applied vaccines against potential infection in commercial poultry. Archives of Virology, 163: 2743-2755. doi: 10.1007/s00705018-3916-5

Günes, A., Marek, A., Grafl, B., Berger, E. & Hess, M. (2012). Real-time PCR assay for universal detection and quantitation of all five species of fowl adenoviruses (FAdV-A to FAdV-E). Journal of Virological Methods, 183, 2, 147-153. doi: 10.1016/j.jviromet.2012.04.005.

Hoffmann, B., Hoffmann, D., Henritzi, D., Beer, M. & Harder, T. C. (2016). Riem's influenza a typing array (RITA). An RT-431 qPCR-based low-density array for subtyping avian and mammalian influenza a virus. Scientific Report, 6, 27211. doi: 10.1038/srep27211

Khan, T.A., Rue, C.A., Rehmani, S.F., Ahmed, A., Wasilenko, J.L., Miller, P.J. & Afonso, C.L. (2010). Phylogenetic and biological characterization of Newcastle disease virus isolates from Pakistan. Journal of Clinical Microbiology, 48 (5): 1892-1894. doi: 10.1128/JCM.00148-10

Kumar S., Stecher, G., Li, M., Knyaz, C. & Tamura K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution, 35: 1547-1549. doi:10.1093/molbev/msy096

Lien, Y.Y., Lee, J.W., Su, H.Y., Tsai, H.J., Tsai, M.C. & Hsieh, C.Y. (2007). Phylogenetic characterization of Newcastle disease viruses isolated in Taiwan during 2003–2006. Veterinary Microbiology, 123: 194-202. doi: 10.1016/j.vetmic.2007.03.006

Liu, H., Wang, J., Ge, S., Lv, Y., Li, Y., Zheng, D., Zhao, Y., Castellan, D. & Wang, Z. (2019). Molecular characterization of new emerging sub-genotype VIIh Newcastle disease viruses in China. Virus Genes, 55: 314–321. doi: 10.1007/s11262-019-01651-5

Lomniczi, B., Wehmann, E., Herczeg, J., Ballagi-Pordny, A., Kaleta, E.F., Werner, O., Meulemans, G., Jorgensen, P.H., Manté, A.P., Gielkens, A.L., Capua, I. & Damoser, J. (1998). Newcastle disease outbreaks in recent years in Western Europe were caused by an old (VI) and a novel genotype (VII). Archives of Virology, 143: 49-64. doi.org/10.1007/s007050050267

Mansour, S.M.G., ElBakrey, R.M., Mohamed, F.F., Hamouda, E.E., Abdallah, M.S., Elbestawy, A.R., Ismail, M.M., Abdien, H.M.F & Eid, A.A.M (2021). Avian Paramyxovirus Type 1 in Egypt: Epidemiology, Evolutionary Perspective, and Vaccine Approach. Front. Vet. Sci. 8:647462. doi: 10.3389/fvets.2021.647462

Miller P.J. & Koch, G. (2020). Newcastle disease. Diseases of Poultry. 14thed. editor-in-chief, David E. Swayne; associate editors, Martine Boulianne [and 12 others], Hoboken, NJ: Wiley-Blackwell, p: 112-129. doi:10.1002/9781119371199

Miller, P.J., Kim, L.M., Ip, H.S. & Afonso, C.L. (2009). Evolutionary dynamics of Newcastle disease virus. Virology, 391 (1): 64-72. doi: 10.1016/j.virol.2009.05.033.

Miller, P.J., R. Haddas, L., Simanov, A., Lublin, S.F., Rehmani, A., Wajid, T., Bibi, T.A., Khan, T., Yaqub, S., Setiyaningsih & Afonso, C.L. (2015). Identification of new sub-genotypes of virulent Newcastle disease virus with potential panzootic features. Infection, Genetics and Evolution, 29: 216-229. doi: 10.1016/j.meegid.2014.10.032

Mohammed, M.H., Kandeil, A., Alkhazindar, M., AbdElSalam, E.T. & Ali, M.A. (2020). Isolation of Newcastle Disease Virus from Wild Migratory Birds in Egypt. J. World Poult. Res., 10 (3): 520-526. doi: 10.36380/jwpr.2020.60

Moharam, I., Abd el Razik, A., Sultan, H., Ghezlan, M., Meseko, C., Franzke, K., Harder, T., Beer, M. & Grund, C. (2019). Investigation of suspected Newcastle disease (ND) outbreaks in Egypt uncovers a high virus velogenic ND virus burden in small-scale holdings and the presence of multiple pathogens. Avian Pathology, 48 (5): 406-415. doi: 10.1080/03079457.1612852.

Naguib, M.M., Höper, D., Elkady, M.F., Afifi, M.A., Erfan, A., Abozeid, H.H., Hasan, W.M., Arafa, A.S., Shahein, M., Beer, M., Harder, T.C., Grund, C. (2022). Comparison of genomic and antigenic properties of Newcastle Disease virus genotypes II, XXI and VII from Egypt do not point to antigenic drift as selection marker. Transboundary and Emerging Diseases, 69(2):849-863. doi: 10.1111/tbed.14121.

World Organization of Animal Health (WOAH) Terrestrial Manual (2021). Newcastle Disease (Infection with Newcastle Disease Virus). Chapter 2.3.14. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. www.woah.org. https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.03.14_NEWCASTLE_DIS.pdf

Orabi, A., Hussein, A., Saleh, A.A., Abu El-Magd, M. & Munir, M. (2017). Evolutionary insights into the fusion protein of Newcastle disease virus isolated from vaccinated chickens in 2016 in Egypt. Archives of Virology, 162: 3069-3079. doi:10.1007/s00705-017-3483-1.

Perozo, F., Marcano, R. & Afonso, C.L. (2012). Biological and phylogenetic characterization of a genotype VII Newcastle disease virus from Venezuela: Efficacy of field vaccination. Journal of Clinical Microbiology, 50 (4): 1204-1208. doi: 10.1128/JCM.06506-11

Radwan, M.M., Darwish, S.F., El-Sabagh, I.M., El-Sanousi, A.A. & Shalaby, M.A. (2013). Isolation and molecular characterization of Newcastle disease virus genotypes II and VIId in Egypt between 2011 and 2012. Virus Genes, 47 (2): 311-316. doi: 10.1007/s11262-013-0950-y

Rohaim, M.A., El-Naggar, R.F., Helal, A.M., Hussein H.A. & Le-Blanc, N. (2016). Genetic characterization of pigeon paramyxovirus type 1 in Egypt. British Journal of Virology, 3(2): 27-32.

Saad, A.M., Samy, A., Soliman. M.A., Arafa, A., Zanaty, A., Hassan, M.K., Sultan, A.H., Bazid, A.I. & Hussein, H. (2017). Genotypic and pathogenic characterization of genotype VII Newcastle disease viruses isolated from commercial farms in Egypt and evaluation of heterologous antibody responses. Archives of Virology, 162 (1). doi:10.1007/s00705-017-3336-y.

Samal, S.K. (2008). Paramyxoviruses of Animals. Encyclopedia of Virology. Elsevier Ltd, P: 40–7. doi: 10.1016/B978-012374410-4.00460-X.

Selim, K.M., Selim, A., Arafa, A., Hussein, H.A. & Elsanousi, A.A. (2018). Molecular characterization of full fusion protein (F) of Newcastle disease virus genotype VIId isolated from Egypt during 2012-2016. Veterinary World, 11(7): 930-938. doi: 10.14202/vetworld.2018.930-938.

Sergel-Germano, T., McQuain, C. & Morrison, T. (1994). Mutations in the fusion peptide and heptad repeat regions of the Newcastle disease virus fusion protein block fusion. Journal of Virology, 68 (11): 7654-7658. doi: 10.1128/JVI.68.11.7654-7658.1994

Shakal, M., Maher, M., Metwally, A.S., AbdelSabour, M.A., Madbbouly, Y.M., Safwat, G. (2020). Molecular Identification of a velogenic Newcastle Disease Virus Strain Isolated from Egypt. Journal of World Poultry Research, 10 (2S): 195-202. doi/10.36380/jwpr.2020.25

Shehata, A., Seddik, M., Elbestawy, A., Zain El-Abideen, M., Hammam, H., Kilany, W., Ali, A. (2019). Co-infections, genetic and antigenic relatedness of avian influenza H5N8 and H5N1 viruses in domestic and wild birds in Egypt. Poultry Science, 98 (6): 2371–2379 doi: 10.3382/ps/pey559

Tabatabaeizadeh, S.E. (2021). Immunoinformatic analysis and antibody epitope comparison of Newcastle disease virus classical vaccines with a virus involved in the fifth NDV panzootic. Mapp. Intimacies, 1, 1–16. doi: 10.21203/rs.3.rs-456948/v1

Tamura, K., Stecher G., Peterson D., Filipski A., & Kumar S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution, 30: 2725-2729. doi: 10.1093/molbev/mst197

Terregino, C. & Capua, I. (2009). Conventional diagnosis of Newcastle disease virus infection. Pages 123–125 in Avian Influenza and Newcastle Disease. A Field and Laboratory Manual. I. Capua, and A.J. Alexander, eds. Springer, Italy. https://www.springer.com/gp/book/9788847008250

Tomás, G., Hernández, M., Marandino, A., Panzera, Y., Maya, L., Hernández, D., Pereda, A., Banda, A., Villegas, P., Aguirre, S. & Pérez, R. (2012). Development and validation of a TaqMan-MGB real-time RT-PCR assay for simultaneous detection and characterization of infectious bursal disease virus. Journal of Virological Methods, 185, 101-107. doi: 10.1016/j.jviromet.2012.06.012.

Wang, Y., Yu, W., Huo, N., Wang, W., Guo, Y. & Wei, Q. (2017). Comprehensive analysis of amino acid sequence diversity at the F protein cleavage site of Newcastle disease virus in fusogenic activity. PLoS ONE, 12 (9): 1-17. e0183923. doi.org/10.1371/journal.pone.0183923.

Yehia, N., Salem, H.M., Mahmmod, Y., Said, D., Samir, M., Mawgod, S.A., Sorour, H.K., AbdelRahman, M.A.A., Selim, S., Saad, A.M., El-Saadony, M.T., El-Meihy, R.M., Abd El-Hack, M.E., El-Tarabily, K.A. & Zanaty, A.M. (2023). Common viral and bacterial avian respiratory infections: an updated review. Poult Science, 102(5):102553. doi: 10.1016/j.psj.2023.102553

Zhang, S., Wang, X., Zhao, C., Liu, D., Hu, Y., Zhao, J. & Zhang, G. (2011). Phylogenetic and pathotypical analysis of two virulent Newcastle disease viruses isolated from domestic ducks in China. PLoS One, 6 (9). 1-9. e25. doi: 10.1371/journal.pone.0025000

Zhao, H. & Peeters, B.P.H. (2003). Recombinant Newcastle disease virus as a viral vector: effect of genomic location of foreign gene on gene expression and virus replication. Journal of General Virology, 84 (4): 781–788.

Cite this Paper

Ahmed R. Elbestawy, Ahmed R. Elnagar, Adel H. Saad, and Eman Khalifa (2024). Isolation and molecular characterization of velogenic Newcastle disease virus genotype VII.1.1 from commercial broilers and wild birds in Matrouh governorate, Egypt. MJVM., Vol. (4), Issue (1), pages (16-25).
DOI: *10.21608/MJVM.2023.248574.1021*

About the Journal

Matrouh Journal of Veterinary Medicine (MJVM)
The official journal of the faculty of veterinary medicine, Matrouh University, Egypt.
Publisher: *Matrouh University, Egypt.*
ISSN (Online):2735-458X
ISSN (Print): 2735-4903
Indexed in EKB Database