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Insights Into Isolation and Molecular Identification of Newcastle Disease Virus Circulating in Chicken Flocks in Egypt from 2023 To 2024

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Abstract

NEWCASTLE disease (ND) outbreaks associated with significant economic losses in chicken populations, continued in Egypt despite the introduction of comprehensive immunization programs. Enhancing ND control methods requires knowledge of the genetic mapping of Newcastle disease virus (NDV) strains isolated from chicken populations immunized against ND. Thus, this study aimed to molecular detection of the circulated NDV in Egypt. As a result, during 2023–2024, outbreaks in broilers that had received the NDV vaccine under various regimes in seven Egyptian governorates including Beni Suef, Fayoum, Dakahlia, Giza, Sharqia, Menoufia, and Qalubia were investigated led to the emergence of 19 NDV strains. The investigated broiler chickens had mortality rates varied from 8% to 20% at the age of 25-40 days. Collected samples were subjected to extraction of nucleic acid (RNA), quantitative reverse transcriptase-PCR (qRT-PCR), isolation of viruses as well as phylogenetic inquiry and the entire sequencing of fusion (F) genes. Results of qRT-PCR displayed that all 19 samples positive for velogenic NDV (vNDV) with cycle threshold between 15 and 33, none of the farms investigated showed avian influenza virus infection utilizing primer probe set for matrix gene. The analysis of the entirely coding (F) gene executing phylogeny for three isolates that were sequenced and then submitted in the GenBank with accession numbers PV334946, PV334947 and PV334948 for the isolates vNDV_02_VSVRI_2023, vNDV_06_VSVRI_2023 vNDV_19_VSVRI_2023, respectively. Being evolutionary dissimilar from vaccine viruses especially Clone 30 and LaSota (genotype II), which have been routinely used to protect chicken farms, these isolates grouped into VII.1.1 sub-genotype. Three strains of the current investigation had the vNDV hallmark of cleavage site motif 112 RRQKRF117. Numerous domains of the F gene were thoroughly examined, and the results showed that most of the gene's domains were conserved in genotype VII 1.1. Continuous monitoring of the circulating virulent NDV is recommended beside evaluation of vaccine and the vaccination programs.

Keywords: Chickens, Egypt, Phylogeny, qRT-PCR, Virus isolation, vNDV.

serious endemic diseases is Newcastle Disease (ND) that carries on to detract from poultry performance by causing severe outbreaks in poultry flocks, even in vaccinated populations [1, 2, 3].

Introduction

In Egypt, poultry industry has contributed as a significant source of animal protein. Many challenges are exhibited by devastating pathogens that trust such an enormous industry. Among the

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diseased pigeons in Egypt. [12, 13]. However, sub-genotype VII1.1 of class II genotype VII is a predominant genotype associated with several NDV outbreaks in poultry farms despite stringent vaccination schedules [14]. Since, genotype VII isolated from different outbreaks in different localities in Egypt ([15, 16, 17, 18, 19].

In this study, the prevalence of NDV was examined in six Egyptian governorates using molecular tools (qRT-PCR) together with virus isolation. Moreover, some viruses were selected for their molecular identification and sequencing analysis to track the circulating viruses' genomic mutations.

Material and Methods

Ethical approval

The Institutional Animal Care and Used Committee The Institutional Animal Care and Used Committee (CU-IACUC) at Cairo University has granted ethical approval for this experiment, which has code CU II F 17 25.

Field samples

Between November 2023 and March 2024, trachea, lung, and spleen tissues were aseptically collected from 19 broiler chicken flocks (10 samples each) exhibiting respiratory problems. Samples were collected in both the fall and winter seasons, with the most samples being taken in the latter (16 flocks) from seven Egyptian governorates including (Beni Suef, Fayoum, Dakahlia, Giza, Sharqia, Menoufia and Qalubia) (Table 1). Tissue samples were ground in a pH 7.0 phosphate buffer saline with 1,000 units/mL of mycostatin and 50 µg/mL of gentamycin diluted 1:5 (w/v). The tissue supernatant was gathered and preserved at -20°C until isolation [20].

Nucleic acid (RNA) Extraction and qRT-PCR

The ViroGeneR Viral RNA/DNA Extraction Kit (ViroGene, Egypt) was used to extract the viral RNA utilizing oligonucleotide primers and probe for the target virus as suggested by the manufacturer. single step rRT-PCR experiments were carried out using TransScriptR Probe One-Step qRT-PCR SuperMix (TRANSGEN; China). Add 4.4 µL of RNase-free water, 0.4 µL of TransScriptR Probe One-Step RT/RI Enzyme Mix, 10 µL2x PerfectStartR Probe One-Step qPCR SuperMix, 0.4 µL for each forward and reverse primer of vNDV [21] and common AIV (Matrix gene) [22] and 4 µL RNA template were all included in the final reaction volume of 20 µL. Thermo-cycling qRT-PCR profile was 45◦C for 5 min, 94◦C for 30 sec, followed by 40 cycles at 94◦C for 5 sec and 30 sec at 60◦C (for common AIV) or 30

The enveloped negative-sense, single-stranded RNA Newcastle Disease Virus (NDV), additionally recognized as Avian Orthoavulavirus 1 (AOaV-1), relates to the order Mononegavirales and family Paramyxoviridae [4]. Haemagglutinin–neuraminidase (HN), matrix protein (M), large RNA polymerase (L), fusion protein (F), phosphoprotein (P), and nucleocapsid protein (NP) are the six distinct proteins that are encoded by the viral genome, which is approximately 15,200 base pairs (bp) long. P protein mRNA editing may additionally encode two additional proteins, V and W [4, 5].

Molecular study of fusion (F) gene sequences resulted in two major groups of NDV strains with different genotypes [6], even though a single serotype is shared by all NDV strains [5], this is because substantial variation in genes has been identified across various NDV isolates [5]. Only genotype I viruses, which are primarily avirulent and isolated from wild birds, make up Class I. Class II comprises both. avirulent and virulent isolation from wild birds and domestic poultry [4, 5]. There are 21 genotypes (I–XXI) of viruses in Class II, which have more genetic and pathogenic variations, genotype VII is one of the genotypes that are in circulation globally. According to the most recent nomenclature and categorization of NDV, the previously identified NDV VII genotypes were divided into sub-genotypes VII.1.1 and VII.1.2 [4, 5]. Sub-genotype VII.1.1 combines the previously identified sub-genotypes VIIb, VIId, VIIe, VIIj, and VIIl. The previous sub-genotype VIIf was reclassified as sub-genotype VII.1.2. VII.2, which is now a single sub-genotype, was created by combining the sub-genotypes VIIa, VIIh, VIIi, and VIIk [4].

The three pathotypes of NDVs lentogenic, mesogenic, and velogenic are categorized according to their pathogenicity in ascending order of virulence. Most velogenic strains result in high rates of chicken mortality (up to 100%). exhibiting noticeable neurological and/or Gastrointestinal tract signs [7]. Based on molecular pathogenicity, the amino acid (aa) sequences of the F protein cleavage site were used to distinguish between virulent and avirulent NDV strains. This method states that pathogenic viruses have the aa sequence motif 112 R/G/K-R-Q/K-K/R-R-F 117 close to the F cleavage site. Nonetheless, the sequence 112G–R/K–Q–G–R–L117 is present in avirulent NDV strains [8, 9].

In 1948, NDV had been reported for the first time in Egypt [10] and by the early 1960s, the disease had been verified as endemic [11]. Since then, several destructive NDV outbreaks are reported in Egypt. In early 1981, genotype VI has been identified in

specified samples isolated in SPF ECE based on rRT-PCR result, season, and geographic variation in compliance with the manufacturer's instructions. The entire fusion (F) gene for vNDV is amplified using oligonucleotide primers, with sequencing primers aiming for 1600-bp, in RT-PCR, which is utilized for molecular characterizations of individual viruses [25]. The following set of components use a twenty μL reaction volume: 5 μL RNA template, 0.4 μL of each forward and reverse primer, 10 μL 2× ES One-Step Reaction Mix, 0.4 μL EasyScriptR One Step Enzyme Mix, and 3.8 μL RNase-free water. The EasyScriptR One Step RT-PCR SuperMix (TRANSGEN, China) was utilized. Thermocycling RT-PCR procedures included 45◦C for 30 min, 94◦C for 5 min, 35 cycles of 30 sec at 94◦C and 30 sec at 55◦C annealing temperature, and a final extension at 72◦C for 10 minutes.

Thermo Scientific's PCR purification Kit was used to purify the amplified RT-PCR products follow the manufacturer's directions. The ABI Prism 3100 automated sequencer system (Applied Biosystems, Foster City, CA) was then used to directly sequence the products. Every sequence was searched using BLAST (http://www.ncbi.nlm.nih.gov/BLAST). Following guidelines provided by the Gene Bank's BankIt tool (http://www.ncbi.nlm.nih.gov/ WebSub/tool=genbank), the collected sequences were edited and aligned using Bio Edit Version 7 and MegAlign (DNASTAR) before being submitted to the Gene Bank, using the Clustal W alignment tool , MEGA Version 6 software and a bootstrap of 1,000 trials was performed to generate a maximum likelihood phylogenetic tree of matched sequences [26].

Biological Pathogenicity Testing

The three sequenced NDV strains were evaluated for pathogenicity using mean death time (MDT) and intracerebral pathogenicity index (ICPI). MDT was estimated as follows: sterile phosphate-buffered saline was used to prepare new infectious allantoic fluid in 10-fold successive dilutions (10-1 to 10-9). At 10–11 days of age, five SPF ECEs were infected with 0.1 mL of each dilution via the allantoic sac method. For 5–7 days, the infected eggs were monitored every day at 37°C to determine the mortality rate. According to Alexander and Senne's description, the embryonic MDT was established [7]. To determine the ICPI, one-day-old chicks (10 chicks/sequenced NDV strain) were intracerebrally inoculated with 50 uL of a 10-fold dilution prepared from the freshly infective allantoic fluid of each strain. The experimentally infected chicks were daily observed for eight days following injection. OIE sec at 54◦C (for vNDV), with fluorescence reading in this stage.

Virus Isolation

Nineteen specimens were isolated in specific pathogen free embryonated chicken eggs (SPF ECE) after rRT-PCR identified a single vNDV infection. Tissue suspensions (spleen, lung, and trachea) were extracted from freshly dead suspected chickens and prepared for inoculation using 10-day-old SPF-ECE via allantoic route then incubated for 96 hours at 37°C, and their embryo viability was checked every day in accordance with OIE 2021 [23]. Hemagglutination was assessed using washed chicken red blood cells (RBCs) with concentration10% and the rapid slide hemagglutination (HA) test utilizing allantoic fluids extracted from both dead and living embryos.

Serological identification of the isolated virus

The hemagglutination inhibition test (HI) was then applied on all positive hemagglutinating allantoic fluids using specific antisera against NDV, highly pathogenic avian influenza (HPAI H5N1) and low pathogenic avian influenza (LPAI H9N2) to exclude the contamination of the isolated virus with any other HA extraneous virus [23].

Molecular identification of the isolated virus

The ViroGeneR Viral RNA/DNA extraction Kit (ViroGene, Egypt) was employed for RNA extraction from infected allantoic fluid of 19 samples that were isolated in SPF ECE in compliance with the manufacturer's recommendations. RT-PCR targets a 921-bp segment of the fusion gene (F gene) and uses certain oligonucleotide primers to partially amplify the vNDV F gene for molecular identification of isolated viruses [24]. The following ingredients were employed in a total reaction volume 20 μL: add 5 μL RNA template, 10 μL 2× ES One-Step Reaction Mix, 0.4 μL of each forward and reverse primer, 0.4 μL EasyScriptR One Step Enzyme Mix, and 3.8 μL RNase-free water. The EasyScriptR One Step RT-PCR SuperMix (TRANSGEN, China) was utilized. The thermocycling RT-PCR settings were 45◦C for 30 min, 94◦C for 5 min, followed by 35 cycles at 94◦C for 30 sec and 30 sec at 57◦C annealing temperature with final extension was performed at 72◦C for 10 min.

Fusion gene sequencing from three isolated virulent NDVs

Using ViroGeneR Viral RNA/DNA extraction Kit (ViroGene, Egypt), nucleic acid RNA was extracted from infected allantoic fluids of three

specific NDV antisera, and the positive one had an HI titer ranged between of 6:7 log2, while when tested with other HA viruses' antisera, it was negative.

Molecular characterization of detected viruses by RT-PCR

Isolated viruses were positive PCR amplification of 921 bp amplicon targeting the specific size of the NDV primers. While the PCR test was negative with other HA tested viruses.

Full fusion (F) gene sequence analysis for specific NDV isolates

Full-length F was successfully amplified in 3 isolates and the consequent gene sequences have been deposited to GenBank and assigned accession numbers. PV334946, PV334947 and PV334948 for vNDV_02_VSVRI_2023, vNDV_06_VSVRI_2023 and vNDV_19_VSVRI_2023 isolates respectively (Table 2).

Analysis of sequences encoding F gene, including the cleavage site motif of F0 protein, the three NDV strains were identified as genotype VII1.1. (Figure D). Deduced aa alignment and nucleotide alignment revealed that the three NDV strains showed 100% identity to each other. In comparison to other NDV strains isolated in Egypt from 2011-2022 retrieved from GenBank database, the identity ranged between 98 and 100% with these strains (Figure C).

The aa sequences of the F gene's proteolytic cleavage site motifs (residues 112 - 117) in the F0 protein correspond to the NDV strain. The three strains shared the vNDV signature, which is the cleavage site motif 112 RRQKRF 117. (Figure E). Numerous domains of the F gene were thoroughly examined, and the results showed that most of the gene's domains were conserved in genotype VII 1.1.

Discussion

According to epidemiological research and the global distribution of NDV, genotype VII is the most prevalent one and accountable for the fourth serious NDV pandemic in history. It is associated with catastrophic outcomes after infecting susceptible birds [27]. Egyptian poultry enterprise is facing enormous economic losses due to NDV GVII outbreaks, even though all commercial flocks are subject to mass vaccination programs. By examining genetic variety of NDV field strains in diverse Egyptian geographic sites, the current research is designed to map out the epidemiology pattern and the spread of NDV in chicken populations in some Egyptian governorates in the period from 2023-2024.

analysed chicks' ICPI value and observation welfare score [23].

Results

Clinical findings, Postmortem examination and Mortality rates of the investigated chicken Flocks

The main clinical signs recorded in investigated flocks were respiratory signs including gasping, rales, cough, sneezing, nasal and ocular discharges, along with some flocks exhibited greenish diarrhea and nervous signs including circling, twisted necks (torticollis), tremors, paralysis of legs and wings, and general paralysis of the entire body. The major pathogenic features revealed by post-mortem examination of the investigated flocks were cyanotic comb and wattles, generalized septicemia and haemorrhages in lymphoid aggregations in the gastrointestinal tract, with differing degrees of severity including hemorrhages on proventriculus, gizzard (Figure 1A), cecal tonsils (Figure 2A), rectum (Figure 3A) and spleen (Figure 4A), as well as tracheitis with severe congestion (Figure 5A) and pneumonia. The investigated chicken flock’s size in the present study, ranged between 10000 and 50000 bird//flock and the investigated chickens were vaccinated against AIV-H9N2, AIV-H5N1, NDV and IBV with different vaccination schedule. Mortality rates in the examined flocks (n=19) varied from 8% to 20% at the age of 25-40 days old with high mortality rate (20%) recorded in a flock at the age of 28 days old though previously vaccinated against NDV (at 1 and 10 days- old) with live and inactivated vaccines.

Newcastle Disease Virus pathogen detected by PCR

As indicated in (Table 1), all collected samples (19) tested positive for vNDV, with a threshold cycle ranging from 15 to 33. RT-PCR amplification using specially designed primers for F genes revealed specific bands at 921-bp (partial amplification). None of the farms investigated showed AIV infection using specific primers and probe for AIV for matrix gene amplification by rRT-PCR.

Virus isolation

Isolation of vNDV on SPF ECE via allantoic sac inoculation showed that the embryo mortality occurred between 44:46 hrs.MDTafter inoculation (Table 2). The embryo was congested and had hemorrhagic spots on the body surface (Figure B).

Virus identification by HI test

The collected allantoic fluid showed positive slide HA activity with rapid elution. The collected allantoic fluid showed positive HI activity with

studies [30, 31]. Alteration in signal peptide and F peptide motifs may also restrict F proteins from linking the envelope of the virus to the cell membrane in accordance with another investigation [32, 33]. Also, entire F protein phylogeny of vNDV isolates confirmed that all strains were velogenic and assigned to genotype VII as well as genetically distant from vaccinal strains but closely related to other Egyptian isolates. The vNDV isolates used in the current study exhibit 100% identity with one another, according to the results of the deduced aa sequences, which showed no substitution events or even any mutation., while the identity of other strains isolated between 2011 and 2022 ranges between 98 and 100%, indicating minor genetic variation.

The genetic distance between Genotype II vaccines and Genotype VII field viruses reflects amino acid changes in F and HN epitopes, which cause partial antigenic mismatch. This mismatch does not eliminate protection against mortality but compromises the vaccine's ability to prevent infection, shedding, and transmission explaining "vaccine failure" in the field.

Conclusion

The current study revealed that the vNDV genotype VII strains are circulating in Egypt and still can break the vaccine efficacy and there are no genetic changes observed among the three isolated virulent strains. Continuous monitoring of the circulating virulent NDV is recommended beside evaluation of vaccine and the vaccination programs. If local production of genotype-matching inactivated vaccines is possible parallel with application of strict biosecurity measures, it is often the fastest scalable solution to overcome such a problem.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

The Institutional Animal Care and Used Committee (CU-IACUC) at Cairo University has granted ethical approval for this experiment, which has code CU II F 17 25.

Clinical signs revealed that the vNDV-infected chicken flocks under investigation were respiratory signs including gasping, rales, cough, sneezing, nasal and ocular discharges, along with some flocks exhibited greenish diarrhoea and nervous signs including circling, twisted necks (torticollis), tremors, paralysis of legs and wings, and general paralysis of the entire body. The major pathogenic features revealed by post-mortem examination of the investigated flocks were cyanotic comb and wattles, generalized septicaemia and haemorrhages in lymphoid aggregations in the gastrointestinal tract, with differing degrees of severity including haemorrhages on proventriculus, gizzard, cecal tonsils, rectum and spleen, as well as tracheitis with severe congestion and pneumonia. These clinical signs and postmortem lesions are consistent with vNDV-infected birds of the earlier investigations [12, 28]. Mortality rates in the examined flocks (n=19) varied from 8% to 20% and these findings correlate with another study [17] which showed mortality rate ranges from 15%–20% in vaccinated- vNDV infected broiler chickens in the period from 2014–2016. In several Egyptian governorates, broiler chicken flocks were shown to have an ongoing circulation associated with NDV sub-genotype VII.1.1 throughout 2023–2024. It is noteworthy that despite getting vaccinations using Hitchner B1, La Sota, or Clone 30 strains, all chicken flocks' experienced NDV outbreaks with losses ranging from 8% to 20%. The genetic identification of strains recovered from NDV immunized flocks may provide with information on how to strengthen NDV control efforts as comprehensive vaccination schedules have not been available.

For molecular pathotyping, the aa sequences of the NDV strains' F0 protein cleavage site motifs (residues 112-117) were adopted as a faster and and accurate in comparison with biological assessments for pathogenicity index. [29]. In the present investigation, all of the isolated NDV strains exhibited the cleavage site motif 112 RRQKRF117, which is considered distinctive to velogenic strains.

Deduced aa sequence of the F protein was examined, especially the functional domains, to comprehend the genetic characteristics of NDV that impact chicken flocks despite vaccination. The functional domains of F proteins were substituted in three strains evaluated here as opposed to lentogenic viruses. The fusion activity of NDV may be impacted by aa changes in the heptad repeat (HR) regions of the F protein and F peptide, as suggested by previous

TABLE 1. Field data & results of real time RT-PCR of the investigated flocks.

Species & aim of rearing	Flock no.	Government	Date of sample collection	Age (day)	Mortality %	Real time RT-PCR		Accession numbers
						vNDV	AIV	
CHICKEN (BROILER)	1	Giza	November 2023	35	15	Positive CT: 15	Negative	PV334947
	2	Menoufia	November 2023	34	8	Positive CT: 24	Negative	-
	3	Dakahlia	December 2023	28	20	Positive CT: 15	Negative	PV334946
	4	Beni suef	December 2023	25	9	Positive CT: 26	Negative	-
	5	Sharkia	December 2023	28	18	Positive CT: 18	Negative	PV334948
	6	Giza	January 2024	30	12	Positive CT: 28	Negative	-
	7	Giza	February 2024	33	10	Positive CT: 21	Negative	-
	8	Giza	February 2024	29	10	Positive CT: 31	Negative	-
	9	Fayum	February 2024	25	18	Positive CT: 23	Negative	-
	10	Beni suef	February 2024	28	12	Positive CT: 28	Negative	-
	11	Menoufia	February 2024	30	10	Positive CT: 18	Negative	-
	12	Giza	February 2024	35	15	Positive CT: 33	Negative	-
	13	Qalubia	February 2024	27	12	Positive CT: 30	Negative	-
	14	Menoufia	March 2024	30	12	Positive CT: 16	Negative	-
	15	Qalubia	March 2024	28	8	Positive CT: 22	Negative	-
	16	Giza	March 2024	40	10	Positive CT: 17	Negative	-
	17	Dakahlia	March 2024	29	10	Positive CT: 24	Negative	-
	18	Sharkia	March 2024	30	8	Positive CT: 23	Negative	-
	19	Menoufia	March 2024	33	15	Positive CT: 25	Negative	-

TABLE 2. Pathogenicity of isolated Egyptian NDV-2023.

Strain name	Pathotype	MDT (hours)	ICPI	Cleavage motif	Accession Numbers
VNDV_02_VSVRI_2023	VELOGENIC	44 HR.	1.7	112RRQKR*F117	PV334946
VNDV_06_VSVRI_2023	VELOGENIC	46 HR.	1.7	112RRQKR*F117	PV334947
VNDV_019_VSVRI_2023	VELOGENIC	48 HR.	1.7	112RRQKR*F117	PV334948

ICPI=INTRACEREBRAL PATHOGENICITY INDEX, MDT= MEAN DEATH TIME

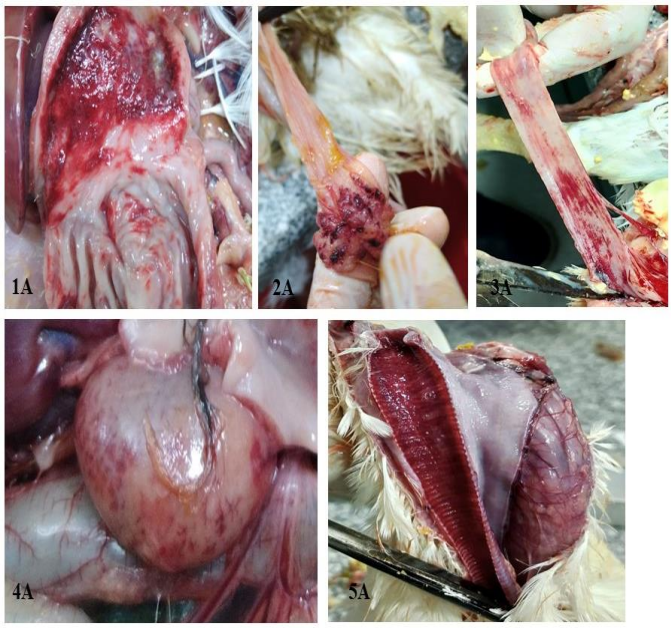

Fig. A. Pathological lesions observed in the chicken flocks investigated; Fig.1A: severe haemorrhages on proventriculus, gizzard, Fig.2A: haemorrhage in caecal tonsils, Fig.3A: haemorrhage in rectum, Fig.4A: haemorrhage in spleen, & Fig.5A: tracheitis with severe congestion.

Fig. B. Haemorrhagic and congested embryos containing haemorrhagic spots on the body surface.

Distance — Metric: Uncorrected Pairwise Distance — Sequences: 23 — Global gap removal — Residues considered: 550

		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
A	CHINA 2011 AEW24467.fasta		0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.11	0.11	0.12
B	CHINA 2014 AIM47238.fasta	0.00		0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.11	0.11	0.12
C	CHINA2011 AEU17194.fasta	0.00	0.00		0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.11	0.11	0.12
D	CHINA2011 AIM47281.fasta	0.00	0.00	0.00		0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.11	0.11	0.12
E	CHINA2012 AIM47276.fasta	0.00	0.00	0.00	0.00		0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.11	0.11	0.12
F	EDYPT DU2023 XU01353.fasta	0.01	0.01	0.01	0.01	0.01		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.12	0.12	0.13
G	egypt 2016 APT68954.fasta	0.00	0.00	0.00	0.00	0.00	0.01		0.00	0.01	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.11	0.11	0.12
H	EGYPT 2016 QCF42117.fasta	0.00	0.00	0.00	0.00	0.00	0.01	0.00		0.01	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.11	0.11	0.12
I	EGYPT 2016 QCF42125.fasta	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01		0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.11	0.12	0.13
J	Egypt 2019 WMQ57589.fasta	0.00	0.00	0.01	0.00	0.00	0.01	0.01	0.01	0.01		0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.11	0.11	0.12
K	egypt 2022 XCZ58535.fasta	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.01		0.01	0.01	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.11	0.11	0.12
L	EGYPT2016 AYN07307.fasta	0.00	0.00	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01		0.01	0.00	0.01	0.01	0.01	0.02	0.02	0.02	0.11	0.11	0.12
M	EGYPT2020 UYK55179.fasta	0.00	0.00	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01		0.00	0.01	0.01	0.01	0.02	0.02	0.02	0.11	0.11	0.12
N	EGYPT2021 QZI95197.fasta	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.01	0.01	0.01	0.11	0.11	0.12
O	ISRAEL 2013 AXE73948.fasta	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.01	0.00	0.01	0.01	0.00		0.00	0.01	0.01	0.01	0.01	0.11	0.11	0.12
P	ISRAEL2012 AXE73912.fasta	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.01	0.00	0.01	0.01	0.00	0.00		0.01	0.01	0.01	0.01	0.11	0.11	0.12
Q	ISRAEL2012 AXE73924.fasta	0.00	0.00	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01		0.02	0.02	0.02	0.11	0.11	0.12
R	vNDV-02 VSVRI -2023.sbd	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.02		0.00	0.00	0.11	0.12	0.13
S	vNDV-06-VSVRI -2023.sbd	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.02	0.00		0.00	0.11	0.12	0.13
T	vNDV-019-VSVRI-2023.sbd	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.02	0.00	0.00		0.11	0.12	0.13
U	CLONE 30 Y18898.fasta	0.11	0.11	0.11	0.11	0.11	0.12	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11		0.00	0.01
V	LASOTA AF077761.fasta	0.11	0.11	0.11	0.11	0.11	0.12	0.11	0.11	0.12	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.12	0.12	0.12	0.00		0.01
W	turkeyUSAVGGA89.fasta	0.12	0.12	0.12	0.12	0.12	0.13	0.12	0.12	0.13	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.13	0.13	0.13	0.01	0.01	

Fig. C. Nucleotide divergence of the three isolated vNDV in comparison to Egyptian isolates and vaccine strains.

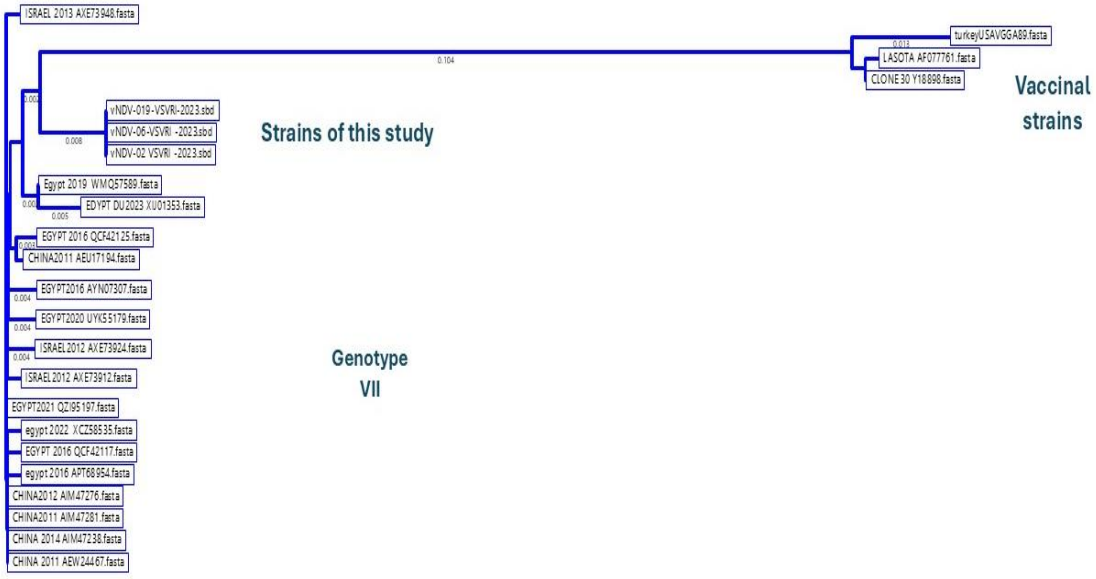

Fig. D. Phylogenetic analysis of the full-length F-gene sequence of the three isolated vNDV strains with representative GVII strains and GII (vaccine strains)

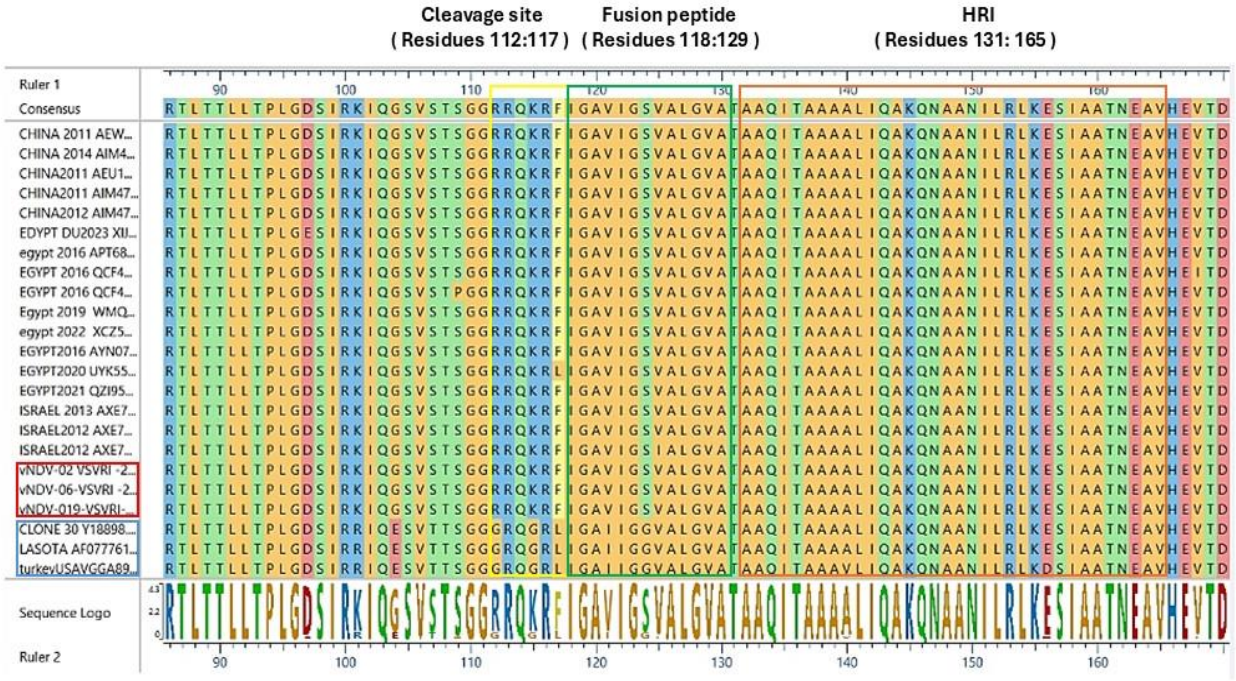

Fig. E. Deduced amino acid sequences alignment of F gene of the three isolated vNDV strains (red box) in comparison to selected Egyptian strains and vaccine strains (blue box); Cleavage site (Residues 112:117) illustrated in (yellow box), Fusion peptide (Residues 118:129) illustrated in (green box) and HRI (Residues 131: 165) illustrated in (orange box).

References

7. Alexander, D.J. and Senne, D.A. Newcastle disease. *Diseases of Poultry*, **12**, 75-100 (2008).
8. Collins, M.S., Bashiruddin, J.B. and Alexander, D.J. Deduced amino acid sequences at the fusion protein cleavage site of Newcastle disease viruses showing variation in antigenicity and pathogenicity. *Archives of Virology*, **128** (3-4), 363-370 (1993).
9. Wang, Y., Yu, W., Huo, N., Wang, W., Guo, Y., Wei, Q., Wang, X., Zhang, S., Yang, Z. and Xiao, S. Comprehensive analysis of amino acid sequence diversity at the F protein cleavage site of Newcastle disease virus in fusogenic activity. *PloS one*, **12**(9), p.e0183923 (2017).
10. Daubney, R. and Mansy, W. The occurrence of Newcastle disease in Egypt. *J. Comp. Pathol. Ther.*, **1** (58), 189-200 (1948).
11. El-Nassary, B.B. and Eskarous, J.K. The distribution of fowl plague and Newcastle disease in upper Egypt. *Archiv. Mikrobiol.*, **36**(2), 147-150 (1960).
12. Megahed, M.M., Eid, A.A., Mohamed, W. and Hassanin, O. Genetic characterization of Egyptian Newcastle disease virus strains isolated from flocks vaccinated against Newcastle disease virus. *Slov. Vet. Res.*, **55**(20), 17–29 (2018).
13. Sabra, M., Dimitrov, K.M., Goraichuk, I.V., Wajid, A., Sharma, P., Williams-Coplin, D., Basharat, A., Rehmani, S.F., Muzyka, D.V., Miller, P.J. and Afonso,
1. Alexander, D.J. Newcastle disease and other avian paramyxoviruses. *Revue Scientifique et Technique-Office International des Epizooties*, **19** (2), 443-455 (2000).
2. Miller, P.J., Decanini, E.L. and Afonso, C.L. Newcastle disease: evolution of genotypes and the related diagnostic challenges. *Infection, Genetic and Evolution*, **10** (1), 26-35 (2010).
3. Dimitrov, K.M., Ramey, A.M., Qiu, X., Bahl, J. and Afonso, C.L. Temporal, geographic, and host distribution of avian paramyxovirus 1 (Newcastle disease virus). *Infection, genetic and evolution*, **1** (39), 22-34 (2016).
4. 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. and Diel, D.G. Updated unified phylogenetic classification system and revised nomenclature for Newcastle disease virus. *Infection, Genetic and Evolution*, **1** *(*74), 103917 (2019).
5. Diel, D.G., da Silva, L.H., Liu, H., Wang, Z., Miller, P.J. and Afonso, C.L. Genetic diversity of avian paramyxovirus type 1: proposal for a unified nomenclature and classification system of Newcastle disease virus genotypes. *Infection, Genetic and Evolution*, **12** (8), 1770-1779 (2012).
6. Sinkovics, J.G. and Horvath, J.C. Newcastle disease virus (NDV): brief history of its oncolytic strains. *J. Clin. Virol.*, **16** (1), 1-15 (2000).

24. Mase, M., Imai, K., Sanada, Y., Sanada, N., Yuasa, N., Imada, T., Tsukamoto, K. and Yamaguchi, S. Phylogenetic analysis of Newcastle disease virus genotypes isolated in Japan. *J. Clin. Microbiol.*, **40**(10), 3826-3830 (2002).

25. Nagy, A., Ali, A., Zain El-Abideen, M.A., Kilany, W. and Elsayed, M. Characterization and genetic analysis of recent and emergent virulent newcastle disease viruses in Egypt. *Transbound. Emerg. Dis.*, **67**(5), 2000-2012 (2020).

26. Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.*, **30**(12), 2725-2729 (2013).

27. Miller, P.J., Haddas, R., Simanov, L., Lublin, A., Rehmani, S.F., Wajid, A., Bibi, T., Khan, T.A., Yaqub, T., Setiyaningsih, S. and Afonso, C.L. Identification of new sub-genotypes of virulent Newcastle disease virus with potential panzootic features. *Infect. Genet. Evol.*, **1** (29), 216-229 (2015).

28. 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*, **11**(12), 3567 (2021).

29. Ganar, K., Das, M., Sinha, S. and Kumar, S. Newcastle disease virus: current status and our understanding. *Virus Res.*, **12** (184), 71-81 (2014).

30. McGinnes, L.W., Sergel, T., Chen, H., Hamo, L., Schwertz, S., Li, D. and Morrison, T.G. Mutational analysis of the membrane proximal heptad repeat of the Newcastle disease virus fusion protein. *Virology*, **289**(2), 343-352 (2001).

31. Sergel-Germano, T., McQUAIN, C.A.T.H.E.R.I.N.E. and Morrison, T. Mutations in the fusion peptide and heptad repeat regions of the Newcastle disease virus fusion protein block fusion. *J. Virol.*, **68**(11), 7654-7658 (1994).

32. White, J.M., Delos, S.E., Brecher, M. and Schornberg, K. Structures and mechanisms of viral membrane fusion proteins: multiple variations on a common theme. *Crit. Rev. Biochem. Mol. Biol*, **43** (3),189-219 (2008).

33. Guseva, N. A., Kolosov, S. N., Zinyakov, N. G., Kozlov, A. A., Shcherbakova, L. O., Chvala, I. A. and Chvala, I. A. Subgenotype VII. 1.1 Newcastle Disease Virus Evolution and Spread in the Russian Federation in 2019–2023. *Viruses*, **17**(10), 1319 (2025). C.L. Phylogenetic assessment reveals continuous evolution and circulation of pigeon-derived virulent avian avulaviruses 1 in Eastern Europe, Asia, and Africa. *BMC Veterinary Research*, **13**(1), 291 (2017).

14. 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**(2), 311-316 (2013).

15. Hussein, H.A., Emara, M.M. and Rohaim, M.A. Molecular characterization of Newcastle disease virus genotype VIID in avian influenza H5N1 infected broiler flock in Egypt. *Int. J. Virol.*, **10**(1), 46-54 (2014).

16. Abd El Aziz, M., Abd El-Hamid, H., Ellkany, H., Nasef, S., Nasr, S. and El Bestawy, A. Biological and molecular characterization of Newcastle disease virus circulating in chicken flocks, Egypt, during 2014-2015. *Zag. Vet. J.*, **44**(1), 9-20 (2016).

17. Ewies, S.S., Ali, A., Tamam, S.M. and Madbouly, H.M. Molecular characterization of Newcastle disease virus (genotype VII) from broiler chickens in Egypt. *Beni-Suef University Journal of Basic And Applied Sciences*, **6**(3), 232-237 (2017).

18. Selim, K.M., Selim, A., Arafa, A., Hussein, H.A. and Elsanousi, A.A. Molecular characterization of full fusion protein (F) of Newcastle disease virus genotype VIId isolated from Egypt during 2012-2016. *Veterinary World*, **11**(7), 930 (2018).

19. Abdel-Latif, S., Atef, A., MA Abdel-Aleem, A., M Dahshan, A.H. and Ali, A. Characterization of avian influenza H9N2 and Newcastle disease virus isolated from vaccinated chickens in Upper Egypt. *J. Vet. Med. Res*, **27**(1), 90-108 (2020).

20. OIE, A.H.S. *Manual of diagnostic tests and vaccines for terrestrial animals*. Office international des epizooties, Paris, France, 2002, pp.1092-1106 (2008).

21. 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. *J. Clin. Microbiol.*, **42**(1), 329-338 (2004).

22. Spackman, E., Senne, D.A., Bulaga, L.L., Myers, T.J., Perdue, M.L., Garber, L.P., Lohman, K., Daum, L.T. and Suarez, D.L. Development of real-time RT-PCR for the detection of avian influenza virus. *Avian Diseases*, **47**(s3), 1079-1082 (2003).

23. Manual, O.T. Newcastle disease (infection with Newcastle disease virus). *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals;* OIE: Paris, France, 14 (2021).

رؤى حول عزل وتحديد فيروس مرض نيوكاسل المنتشر في قطعان الدجاج في مصر من عام 2023 إلى عام 2024

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الملخص

يستمر مرض النيوكاسل في التسبب بخسائر اقتصادية كبيرة في قطعان الدجاج في مصر على الرغم من إدخال برامج تحصين شاملة. ويتطلب تعزيز طرق مكافحة المرض فهماً للخريطة الجينية لعترات فيروس النيوكاسل (NDV) المعزولة من الدجاج المحصن ضد المرض. وبناءً عليه، تم في هذه الدراسة عزل 19 عترة من الفيروس نتجت عن تفشيات في دجاج لاحم كان قد تلقى لقاح النيوكاسل وفق أنظمة تحصين مختلفة في سبع محافظات مصرية شملت (بني سويف، الفيوم، الدقهلية، الجيزة، الشرقية، المنوفية والقليوبية) خلال عامي 2023–2024. تراوحت معدلات النفوق في الدجاج المصاب بين 8% و20% في أعمار تتراوح من 25 إلى 40 يوماً. خضعت العينات المجمعة لاستخلاص الحمض النووي الريبي(RNA) ، وفحص تفاعل البلمرة المتسلسل الكمي بالزمن الحقيقي(qRT-PCR) ، وعزل الفيروس، بالإضافة إلى التحليل الفيلوجيني والتسلسل الكامل لجين الاندماج .(F) أظهرت نتائج فحص qRT-PCR أن جميع العينات التسعة عشر كانت إيجابية لعترات نيوكاسل شديدة الضراوة (velogenic NDV) بقيم Ct تراوحت بين 15 و33، في حين لم تُظهر أي من المزارع وجود إصابة بفيروس إنفلونزا الطيور عند استخدام بادئات لجين.matrix و أُجري التحليل الفيلوجيني للجين المشفر بالكامل (F) لثلاث عزلات تم تسلسلها) أرقام الإيداع في بنك الجينات : PV334946, PV334947, PV334948 للعزلات vNDV_02_VSVRI_2023 ، vNDV_06_VSVRI_2023، vNDV_19_VSVRI_2023 على التوالي .(وقد أظهرت النتائج أن هذه العزلات تختلف تطورياً عن سلالات اللقاحات المستخدمة بشكل روتيني مثل Clone 30 و) Lasotaالنمط الجيني(II ، حيث تم تجميعها ضمن تحت النمط الجيني .VII.1.1 كما تميزت السلالات الثلاثة بعلامة مميزة لعترات النيوكاسل شديدة الضراوة في موقع الانقسام (cleavage site motif) وهي RRQKRF117.112 .تم فحص العديد من مناطق جين الاندماج (F) بشكل تفصيلي، وأظهرت النتائج أن معظم مناطق الجين كانت محفوظة في النمط الجيني VII.1.1. وبناءً على ذلك، توصي الدراسة بالاستمرار في المراقبة المستمرة للعترات المنتشرة من فيروس النيوكاسل شديد الضراوة، إلى جانب تقييم فعالية اللقاحات وبرامج التحصين المستخدمة حالياً.

الكلمات الداله: الدجاج، مصر، علم النشوء والتطور qRT-PCR، عزل الفيروس، vNDV.