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Molecular Characterization of Recently Classified Newcastle Disease Virus Genotype VII.1.1 Isolated from Egypt

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ABSTRACT

Newcastle disease virus (NDV) is classified as Avian OrthoAvulavirus-1 (AOAV-1) and is still one of the most important poultry diseases in the world. Collected tissue specimens (trachea, cecal tonsils, and spleen) from 85 chicken flocks (broilers, layers, and native breeds) raised in 5 Egyptian governorates (El-Qalubia, El-Behaira, El-Menofia, El-Gharbia, and Alexandria) in the period between March 2019 to February 2020. The flocks had a history of vaccination against ND and showed clinical signs and lesions of infection. We characterized the isolates molecularly using conventional RT-PCR via amplifying the NDV full Fusion protein gene. The analysis of the full F-gene sequence revealed that our isolates were classified as genotype VII with the characteristic amino acids motif in the fusion protein cleavage site for the velogenic NDV (vNDV) strains. Among the molecularly identified NDV Genotype VII isolates, 5 representative isolates in different 5 Egyptian Governorates were sequenced for full fusion protein gene (sequence). The obtained sequences were submitted on GenBank: MW580389, MW580388, MW580387, MW566177, and MW590306. The five isolates' full-length F protein gene amino acid sequences revealed 99.5% identity with the Reference GenBank NDV/Egypt/ch/MN51/2019 strain isolate. The deduced amino acids sequences' comparative alignment of the five Avian OrthoAvulavirus-1 isolates confirmed the presence of motif of virulent strains (RRQKRF) between 112 and 117 residues of fusion protein cleavage site. All five AOAV-1 isolates from 2019 and 2020 belonged to the NDV genotype classified as genotype VII.1.1 depending on full Fusion gene sequences; those five isolates were grouped with subgenotype VIId. Through significant genetic changes in vNDV observed in our study, continuous monitoring of the multiple vNDV outbreaks in vaccinated chicken and targeted evaluation of the used vaccines.

Key words: NDV, Isolation, RT- PCR, Genotype VII, F gene Sequence; Chick.

INTRODUCTION

Newcastle Disease virus (NDV) is an RNA virus spread worldwide, it is named Avian OrthoAvulaVirus-1 (AOAV-1) which belonged to Avulavirus genus, subfamily Paramyxovirinae, Paramyxoviridae family which affects nearly all species of wild and domestic birds (Susta et al. 2014). The genome length of NDV is a 15 kb consists of six main genes translating six main proteins in the order, 3′-NP-P-M-F- HN-L-5′ (Dimitrov et al. 2016; Gogoi et al. 2017).

NDV is a great threatening pathogen for the poultry production in developing countries and nowadays spread widely in all areas of Egypt; outbreaks in poultry farms increased since NDV genotype VII identified firstly in Egypt at 2012 by Radwan et al. (2013). Isolates of NDV differ in their virulence to chickens: lentogenic, mesogenic and velogenic. There were 2 types of velogenic NDV: neurotropic and viscerotropic (Cattoli et al. 2011; Mousa et al. 2019). Fusion gene is the main determinant of NDV virulence plus, NDV isolates were categorized into genotypes according to this gene sequence; so, the sequencing of full Fusion gene is very important (Aldous et al. 2003; de Leeuw et al. 2003; Panda et al. 2004; Selim et al. 2018; Manar et al. 2020).

Additionally, amino acids sequences of fusion protein cleavage site used to differentiate among a virulent and virulent NDV strains; depending on this base, the sequence motif ^{112}R/G/K–R–Q/K–K/R–R–F^{117} at the F cleavage site characterized virulent strains, while the motif in ^{112}G–R/K–Q–G–R–L^{117} characterized a virulent NDV strains (Collins et al. 1993; Wang et al. 2017).

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Although, NDV of one serotype, it is highly diversified antigenically and genetically; Owing to the economic and clinical importance of NDV effect on poultry production and the wide use of live ND vaccines, the best methods to characterize circulating NDV strains in the field were sequencing and phylogenetic study. To understand NDV genetic diversity and evolution, many categorization systems have been sophisticated depended on the fusion protein gene sequence, based on the partial F gene sequence Czegledi et al. (2006) divided NDV isolates into two distinct classes, each class was furthermore grouped into various genotypes (Kim et al. 2007a; Miller et al. 2010); another classification was carried out by Aldous et al. (2003) based on partial F gene sequences, which divided isolates of NDV into six definite lineages (1 to 6) and 13 sub-lineages. Based on full Fusion protein gene sequence Diel et al. (2012a) developed unified classification system resulting in sorting of NDV isolates into class I with one genotype (genotype 1) and class II with 15 genotypes (I to XV). Shittu et al. (2016) classified isolates of NDV genetically into 2 classes depending on analysis of whole coding region of fusion gene protein of NDV, class I (with one genotype) and class II (include 21 genotypes) (I-XXI), genotype VII which the most predominant virulent NDV genotypes was associated with class II.

According to updated classification of (Dimitrov et al. 2019) genotype VII was divided into sub-genotypes VII.1 and VII.2 depended on full F protein gene sequence analysis. The sub-genotype VII.1 divided into VII.1.1 that includes previous sub-genotypes VIIb, VIId, VIIe, VIIj, and VIIl and sub-genotype VII.1.2 that includes previous sub-genotype VIIf. While, sub-genotype, VII.2 contains previous sub-genotypes VIIa, VIIh VIIi and VIIk (Dimitrov et al. 2019); so, in our study we classified our isolates based on analysis of complete Fusion protein sequence as previously done by Selim et al. (2018).

Genotype VII of NDV was primarily identified in Egypt by Radwan et al. (2013), later on several studies (Hussein et al. 2014; Abd El-Aziz et al. 2016; Ahmed et al. 2017; Ewies et al. 2017; Nagy et al. 2020; Moussa et al. 2021) reported presence of NDV genotype VII isolates associated with concurrent outbreaks. Dimitrov et al. (2019) reported that genomic surveillance of NDV is important to give visions on genetic variability of circulating strains and to improve protection and response to outbreaks. Additionally, updated genotype investigations are necessary for determination of genotypes geographic distributions that develop present molecular classification methods. Therefore, we conducted this study for identification and characterization of NDV strains that responsible for several outbreaks in Egyptian vaccinated farms, in addition to classification of these strains according to recent classification of NDVs.

MATERIALS AND METHODS

Ethical Approval

The study was approved under ethics of Medical Research Ethics Committee (MREC) of the National Research Centre, Egypt with approval number: (7437082021).

Samples Collection

Tissue specimens (trachea, cecal tonsils and spleen) were collected from 85 chicken flock as pool (broiler, layers and native breeds) raised in five Egyptian governorates including El-Qalubia, El-Behaira, El-Menofia, El-Gharbia and Alexandria in the period between March 2019 to February 2020. The flocks had a history of vaccination against ND, however, they exhibited clinical signs including nervous, respiratory signs, diarrhea and increased mortality. Post-mortem lesions including petechial hemorrhage on tips of proventricular glands enlarged mottled spleen, tracheitis, and enteritis with severe ulceration of cecal tonsils; the tissues were aseptically collected from dead and moribund birds, transported on ice and maintained at -80°C till processed.

Virus Isolation and Propagation

Virus isolation was carried out according to OIE (2012). Each tissue pool was homogenized as 1:10 (w/v) suspension in PBS including 10mg/mL streptomycin, 10000IU/mL penicillin, 250µg/mL gentamycin sulphate and 5000IU nystatin. Following centrifugation at 4,000 rpm for 10 min, a volume of 0.2mL from each clear supernatant was injected in 10-day old (ECE) allantoic sacs. The eggs were preserved for 5 days at 37°C with everyday observation to embryo viability; after egg chilling on day 5, harvesting of allantoic fluids and testing for HA activity were carried out.

Hemagglutination (HA) Test

Test procedure was described by OIE (2012); in a V bottomed 96-well micro-titer plate equal volume of a 1% red blood cells were mixed with 2-fold serial dilutions of allantoic fluid in PBS.

Molecular Identification of NDV Isolates

Genomic Viral RNA Extraction

The extraction of genomic viral RNA from harvested HA positive allantoic fluid was carried out according to the manufacture's protocol of QIAamp® viral RNA extraction kits.

Detection of NDV Full Fusion Protein Gene by one step RT-PCR

Detection of full fusion gene of NDV by RT-PCR using the following primers:
F-Primer (28bp) 5'-GTCAGATCTTGATGGGCTCCAA ACCTTC-3' R-Primer (28bp) 5'-ATGAATTCTCACGCT CTTGTGGTGGCTC-3' which flank 1681bp of the full F gene of NDV as previously done by (Wulanjati et al. 2018). One step RT-PCR was done according to protocol of QIAGEN® One Step RT-PCR kit (QIAGEN, Valencia, CA).

Agarose Gel Electrophoresis

The PCR results were screened using 1.5% Agarose gel in TAE buffer with ethidium bromide stain; compared with 1 kp DNA markers and seen by ultraviolet (UV) trans illumination (Wulanjati et al. 2018).

The Sequence of the Full Fusion Gene and Phylogenetic Analysis

The positive bands were excised from the gel and purified according to protocol of QIAquick gel extraction kit (Qiagen, Valencia, CA) then purified DNA was sent to an automated ABI 3730 DNA sequencer (Applied Bio systems, USA), sequences acquired in this study were matched with prior published NDV vaccinal strains and references strains available in Genbank (BLAST, NCBI, USA).

A comparative alignment of nucleotides sequences and deduced amino acids of full F-protein gene sequences was done using the Bioedit Sequence Alignment Program and DNA Star software, then, submission of obtained sequences in this study to the GenBank database with accession numbers.

RESULTS

Virus Isolation and Haemagglutination Activity

Following samples inoculation for one blind passage in 9-11 days-old embryonated chicken eggs (ECE) allantoic sac, embryo mortality was observed in some samples within 24-72hrs post-inoculation. The dead embryos showed congestion and hemorrhage in the whole bodies as shown in Fig.1. The slide HA test was carried out on harvested allantoic fluids for the 85 samples after the passage revealed that: 32 samples were HA positive.

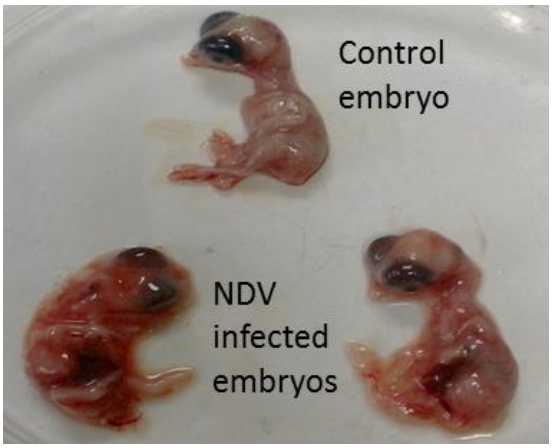

Fig. 1: Embryos of the control non infected embryo and the NDV infected embryos after one blind passage in ECE.

NDV Detection by RT-PCR

All 32 HA +ve allantoic fluids were diagnosed by one step RT-PCR using specific primer to amplify 1681bp of full F gene of NDV (Fig. 2). Twenty-one samples out of 32 samples tested were found positive for NDV.

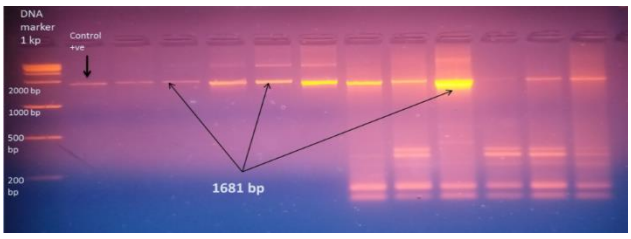

Fig. 2: RT-PCR Products of NDV full Fusion protein gene. Agarose Gel Electrophoresis (AGE) showing 1681 bp fragments of positive samples (Lane 2,3,4,5,6,7,8,9,11,12). Lane 1 DNA marker and lane 2 is a positive control.

Analysis of Fusion Protein Gene Sequences and Phylogenetic Tree of some Positive Isolates

Five isolates (represent five Egyptian provinces) were sequenced for full Fusion protein gene. Their GenBank accession numbers were as follows: MW580389, MW580388, MW580387, MW566177, and MW590306. BLASTN analysis of the five isolates nucleotides sequences revealed presence of 99.5% identity with Reference strain isolate from GenBank NDV/Egypt/ch/ MN51/2019. Amino acids alignment of the five isolates confirmed the presence of the motif of virulent strains RRQKRF at cleavage site (Fig. 3).

The five isolates were grouped within sub genotype VIId and according to the updated classification of Dimitrov et al. (2019) the five isolates from 2019 to 2020 were clustered as genotype VII.1.1. Amino acids sequence's identity and phylogenetic tree revealed the high identity and correlation to the Egyptian isolates and other neighbouring countries from 2011-2019 (Table 1; Fig. 4).

DISCUSSION

Newcastle disease (ND) considered a great serious viral disease affecting poultry industry causing severe economic losses (Orsi et al. 2010). The characteristic clinical signs present in case of NDV infection are marked depression, decrease in feed intake, respiratory and nervous signs, greenish diarrhea as observed by Amer et al. (2018). While the postmortem lesions are petechial hemorrhages on the periventricular gland's tips, congestion of liver, greenish content of intestine, cecal tonsils necrosis and ulceration, and sever tracheitis as observed by Susta et al. (2011), Abd El-Aziz et al. (2016) and Amer et al. (2018).

In Egypt, NDV caused successive outbreaks for commercial poultry farms despite intense vaccination programs with live and inactivated vaccines (Radwan et al. 2013; Hussein et al. 2014; Ahmed et al. 2017; AboElkhair et al. 2019; Mahmood and Sabir 2021), this may be due to vaccination failure (Moharam et al. 2019) and genetic evolution between NDV strains led to concurrent outbreaks and appearance of new strains causing serious infections (Miller et al. 2010; Radwan et al. 2013; Abbas et al. 2021); therefore, the genetic variation of NDV field strains in different locations should be investigated in Egypt for comprehension of the epidemiology of the NDV and determination the genetic relatedness among detected virus strains.

In this study, NDV was investigated in some provinces of Egypt**;** the collected samples were from different chicken flocks (broilers, layers, and breeders) received different vaccination programs against NDV, these samples were collected from farms with high mortality rates in broiler flocks and decrease egg production in breeder and layer flocks; virus isolation and Hemagglutination test were carried out according to OIE (2012). NDV molecularly identified by RT-PCR which considered an accurate method for detection of NDV (Wang et al. 2001; Creelan et al. 2002). The results revealed that 21 samples out of 85 samples tested were positive for NDV by RT-PCR. For determination of NDV pathotypes, analysis of nucleotide sequences of the F-gene cleavage site is carried out as stated by Gould et al. (2001). Virulent NDV characterized by presence of cleavage site

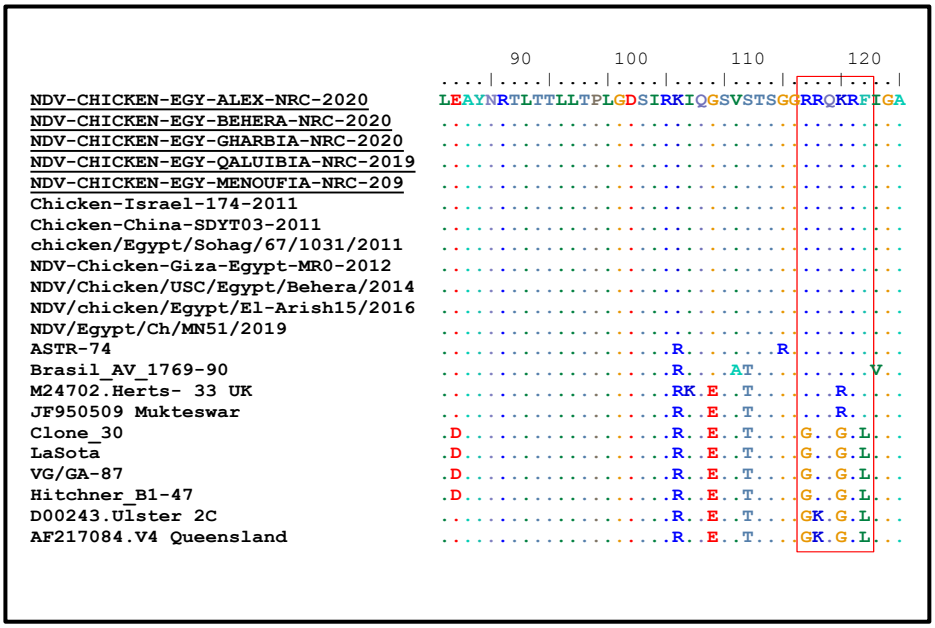

Fig. 3: Amino acids alignment of the amplified F-gene for the identified vvNDV genotype VII.1.1 isolates related to other NDV strains circulating in Egypt and other countries and showing cleavage site identity from AA position 112 to 117 using BioEdit program. Underlined isolates are our study isolate.

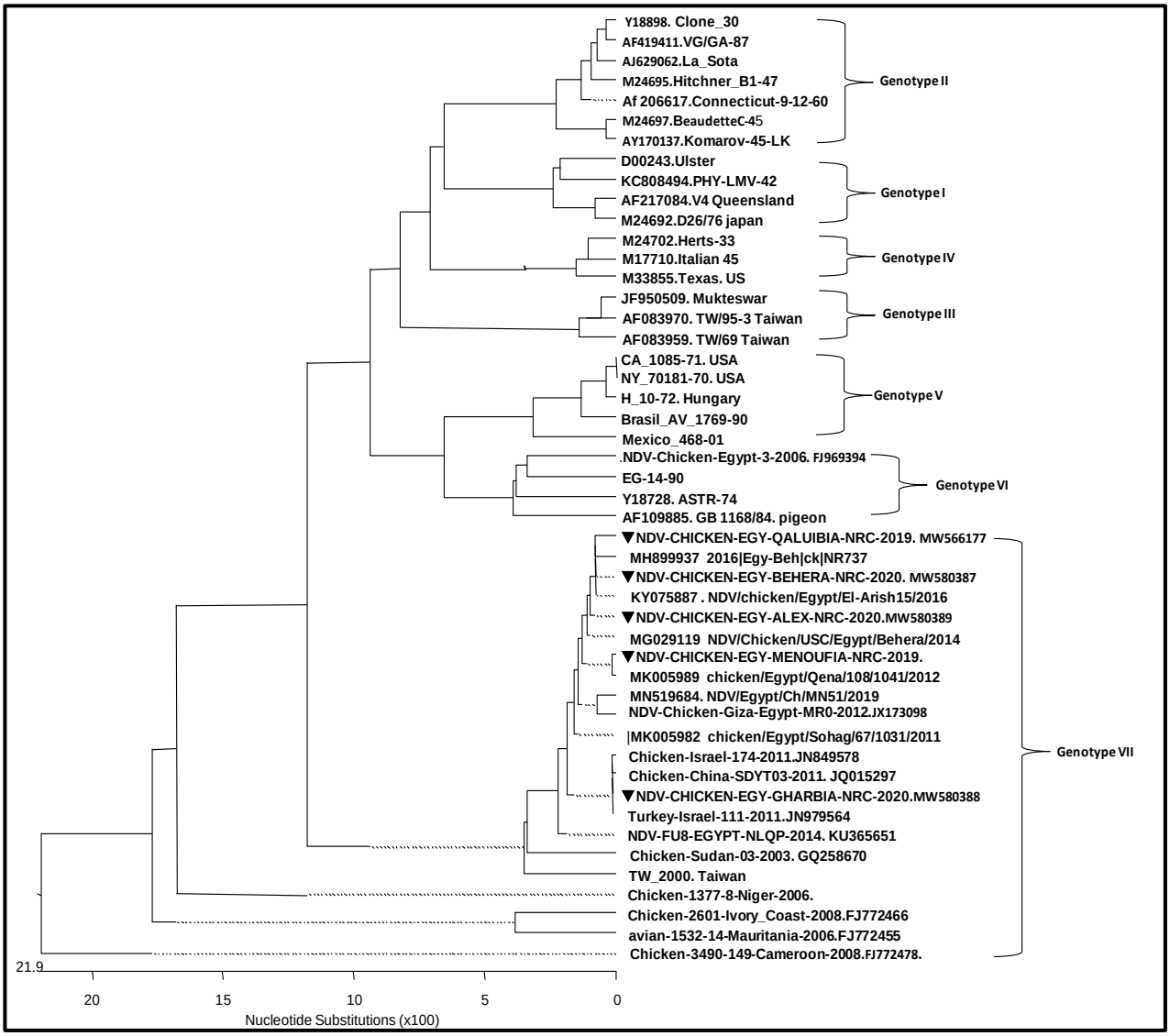

Fig. 4: Phylogenetic tree of the isolates obtained in this study, other NDV isolates in Egypt and other countries and vaccinal, reference strains get back from GenBank using maximum like hood method at 1000 bootstrap replicates. ▼ Isolates are of our study.

Percent Identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
1		99.7	99.2	98.4	99.7	98.9	99.7	99.2	99.2	90.1	86.9	84.8	82.1	84.3	79.2	97.6	98.9	85.9	85.1	83.7	85.1	83.7	64.0	79.2	79.2	79.5	78.9	79.7	79.5	80.8	81.6	1
2	0.3		98.9	98.7	99.5	98.7	100.0	98.9	98.9	89.9	86.7	84.5	81.9	84.0	78.9	97.3	98.7	85.6	84.8	84.0	84.8	83.5	63.7	78.9	78.9	79.2	78.7	79.5	79.2	80.5	81.3	2
3	0.8	1.1		97.6	99.5	98.7	98.9	98.9	100.0	89.9	87.5	85.3	82.4	84.8	79.7	98.4	99.7	86.4	85.6	84.3	85.6	84.3	64.5	79.7	79.7	80.0	79.5	80.3	80.0	81.3	82.1	3
4	1.6	1.4	2.4		98.1	97.3	98.7	97.6	97.6	88.5	85.9	83.2	80.5	82.7	78.4	96.0	97.3	84.3	83.5	83.7	83.5	82.7	63.7	79.2	78.7	78.9	78.9	79.7	79.5	80.3	81.1	4
5	0.3	0.5	0.5	1.9		99.2	99.5	99.5	99.5	90.4	87.2	85.1	82.4	84.5	79.5	97.9	99.2	86.1	85.3	84.0	85.3	84.0	64.3	79.5	79.5	79.7	79.2	80.0	79.7	81.1	81.9	5
6	1.1	1.3	1.3	2.7	0.8		98.7	98.7	98.7	89.6	87.5	85.9	83.2	84.3	79.7	97.1	98.4	86.1	85.6	84.3	85.1	83.7	65.1	80.3	80.3	80.5	80.0	80.8	80.5	81.9	82.1	6
7	0.3	0.0	1.1	1.4	0.5	1.3		98.9	98.9	89.9	86.7	84.5	81.9	84.0	78.9	97.3	98.7	85.6	84.8	84.0	84.8	83.5	63.7	78.9	78.9	79.2	78.7	79.5	79.2	80.5	81.3	7
8	0.8	1.1	1.1	2.5	0.5	1.4	1.1		98.9	90.7	86.7	84.5	81.9	84.0	79.5	97.3	98.7	85.6	84.8	83.5	85.9	84.0	64.3	79.5	79.5	79.7	79.2	80.0	79.7	81.1	81.9	8
9	0.8	1.1	0.0	2.4	0.5	1.3	1.1	1.1		89.9	87.5	85.3	82.4	84.8	79.7	98.4	99.7	86.4	85.6	84.3	85.6	84.3	64.5	79.7	79.7	80.0	79.5	80.3	80.0	81.3	82.1	9
10	2.1	2.4	2.4	3.9	1.8	2.7	2.4	1.5	2.4		78.1	77.3	75.5	76.8	87.5	91.5	89.6	77.6	76.8	75.5	77.3	75.5	72.3	71.7	71.2	71.2	70.9	71.5	71.7	72.5	73.6	10
11	14.9	15.3	14.3	16.4	14.6	14.3	15.3	15.3	14.3	17.6		92.8	88.5	86.9	73.3	85.9	87.2	88.3	85.3	83.7	84.3	83.7	66.7	81.3	81.9	81.9	81.6	81.6	81.1	83.2	83.5	11
12	17.6	18.0	17.0	19.9	17.3	16.2	18.0	18.0	17.0	18.7	7.7		87.2	86.1	73.9	83.7	85.1	88.3	84.8	82.7	84.0	82.7	67.2	81.3	80.8	80.5	81.1	80.8	81.1	81.3	81.1	12
13	21.1	21.5	20.7	23.6	20.7	19.6	21.5	21.6	20.7	21.1	12.3	13.8		85.9	73.3	80.8	82.1	86.1	84.0	81.9	81.9	81.1	65.3	80.5	79.5	79.5	79.2	79.2	80.0	81.1	80.0	13
14	18.1	18.5	17.5	20.4	17.8	18.2	18.5	18.6	17.5	19.1	14.7	15.6	15.4		72.0	83.2	84.5	86.1	83.2	82.1	82.1	81.3	64.8	79.7	78.9	79.2	78.9	79.2	79.5	80.0	79.5	14
15	5.7	6.1	5.0	6.8	5.4	5.0	6.1	5.4	5.0	5.4	14.1	13.2	13.4	15.8		81.3	79.5	74.7	72.8	72.3	73.1	70.9	82.1	69.3	68.5	68.5	68.5	68.5	69.6	69.3	69.3	15
16	0.8	1.1	0.0	2.5	0.5	1.4	1.1	1.1	0.0	2.4	14.6	17.3	21.1	17.8	5.0		98.1	84.8	84.0	82.7	84.0	82.7	66.1	78.1	78.1	78.4	77.9	78.7	78.4	79.7	80.5	16
17	1.1	1.3	0.3	2.7	0.8	1.6	1.3	1.3	0.3	2.7	14.7	17.3	21.1	17.9	5.4	0.3		86.1	85.3	84.0	85.9	84.5	64.8	80.0	80.0	80.3	79.7	80.5	80.3	81.6	82.4	17
18	16.4	16.7	15.7	18.6	16.0	16.0	16.7	16.8	15.7	18.4	13.2	13.1	15.3	15.7	12.1	16.0	16.1		90.4	88.3	89.9	86.4	68.0	84.5	83.5	84.3	83.2	83.5	83.7	86.7	86.9	18
19	17.5	17.8	16.8	19.8	17.1	16.8	17.8	17.9	16.8	19.7	17.1	17.7	18.3	19.7	15.0	17.2	17.2	10.5		93.3	88.5	86.9	66.4	82.1	82.1	82.7	81.9	81.9	81.9	86.4	84.3	19
20	18.9	18.6	18.3	18.9	18.6	18.2	18.6	19.4	18.3	21.3	19.0	20.3	21.0	20.9	16.2	18.6	18.7	12.8	6.8		86.1	84.8	65.6	81.1	80.3	81.1	80.5	80.8	81.1	84.0	83.2	20
21	17.4	17.7	16.7	19.6	17.1	17.4	17.7	16.3	16.7	18.7	18.6	18.7	21.3	21.1	14.5	17.1	16.4	11.1	12.9	15.7		93.9	69.9	85.6	85.1	85.9	84.8	84.5	84.8	90.1	90.1	21
22	19.3	19.7	18.7	20.9	19.0	19.4	19.7	19.0	18.7	21.7	19.4	20.7	22.6	22.4	18.0	19.0	18.3	15.6	15.0	17.5	6.5		69.1	85.9	84.8	85.1	85.1	84.8	85.6	90.1	89.1	22
23	24.6	25.2	23.7	25.2	24.2	22.7	25.2	24.2	23.7	24.2	19.7	18.7	21.2	22.7	20.2	23.7	23.2	17.3	20.2	21.2	14.2	15.6		77.3	79.7	78.9	79.7	78.9	77.1	72.3	71.2	23
24	26.0	26.4	25.2	26.0	25.6	24.3	26.4	25.6	25.2	27.9	22.8	22.5	23.1	24.6	20.5	25.8	24.8	18.1	21.6	22.7	16.5	16.2	3.4		95.7	94.9	96.0	96.3	98.9	88.8	87.2	24
25	25.8	26.2	25.1	26.7	25.4	24.2	26.2	25.4	25.1	28.7	21.8	23.2	24.6	25.8	21.8	25.6	24.7	19.5	21.4	23.8	17.2	17.6	0.3	4.4		98.9	99.2	98.1	95.7	89.6	87.2	25
26	25.4	25.8	24.7	26.2	25.0	23.8	25.8	25.0	24.7	28.6	21.8	23.6	24.6	25.3	21.8	25.2	24.2	18.4	20.7	22.6	16.1	17.2	1.3	5.3	1.1		98.1	98.1	94.9	89.9	87.5	26
27	26.2	26.7	25.5	26.2	25.9	24.6	26.7	25.9	25.5	29.2	22.2	22.9	25.1	25.8	21.8	26.0	25.1	19.9	21.8	23.4	17.5	17.2	0.3	4.1	0.8	1.9		98.4	96.0	89.9	87.5	27
28	25.0	25.4	24.3	25.0	24.7	23.4	25.4	24.7	24.3	28.2	22.3	23.2	25.1	25.4	21.8	24.8	23.9	19.5	21.9	23.1	17.9	17.6	1.3	3.9	1.9	1.9	1.6		96.3	90.1	87.7	28
29	25.2	25.6	24.5	25.2	24.8	23.6	25.6	24.8	24.5	27.5	22.9	22.5	23.6	24.7	20.5	25.0	24.0	18.9	21.6	23.1	17.3	16.2	3.5	0.8	4.2	5.0	3.9	3.6		88.5	86.4	29
30	23.9	24.3	23.2	24.8	23.6	22.3	24.3	23.6	23.2	27.0	20.3	22.8	22.7	24.5	20.9	23.7	22.8	15.4	15.8	18.8	10.9	10.9	10.5	12.4	11.4	11.1	11.1	10.8	12.5		95.2	30
31	22.4	22.9	21.8	23.3	22.1	21.7	22.9	22.1	21.8	25.0	19.7	23.0	24.2	25.2	20.8	22.2	21.4	14.9	18.7	19.8	10.8	12.2	12.1	14.4	14.3	14.0	14.0	13.7	15.1	5.0		31
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	

1 ▼NDV-CHICKEN-EGY-ALEX-NRC-2020
2 ▼NDV-CHICKEN-EGY-BEHERA-NRC-2020
3 ▼NDV-CHICKEN-EGY-GHARBIA-NRC-2020
4 ▼NDV-CHICKEN-EGY-QALUIBIA-NRC-2019
5 ▼NDV-CHICKEN-EGY-MENOUFIA-NRC-2019
6 NDV-FU8-EGYPT-NLQP-2014
7 NDV/chicken/Egypt/El-Arish15/2016
8 NDV/Egypt/Ch/MN51/2019
9 Turkey-Israel-111-2011.
10 NDV-Chicken-Giza-Egypt-MR0-2012
11 Chicken-2601-Ivory_Coast-2008
12 avian-1532-14-Mauritania-2006
13 Chicken-3490-149-Cameroon-2008
14 Chicken-1377-8-Niger-2006
15 Chicken-Sudan-03-2003
16 Chicken-Israel-174-2011
17 Chicken-China-SDYT03-2011
18 ASTR-74
19 Brasil_AV_1769-90
20 Mexico_468-01
21 M24702.Herts-33
22 Mukteshwar EF
23 Connecticut-9-12-60
24 BeaudetteC-45
25 Clone_30
26 La_Sota
27 VGGA-87
28 Hitchner_B1-47
29 Komarov-45-LK
30 Ulster 2C
31 V4 Queensland

Table 1: Amino acids sequence identity of obtained NDV isolates with others circulating in Egypt and other countries and NDV vaccinal strains retrieved from GenBank showing identity and divergence percent based on AA sequence.

motif RRQKRF between 112 and 117 residues (OIE 2012), therefore, in this study F gene cleavage site is detected by RT-PCR and nucleotide sequence analyses, our finding indicated that five NDV isolates were categorized as virulent strains depend on fusion protein cleavage site amino acids sequence motif ^{112}RRQKRF^{117} and this finding is in agreement with (Collins et al. 1993; Wang et al. 2017). Phylogenetic study and sequence analysis of the detected NDV isolates indicated presence of nucleotide similarity between isolated strains reached up to 99-100% and 99.5% identity with NDV/Egypt/ch/MN51/2019 (accession no. MN519684). The amino acids alignment of the 5 isolates confirmed the presence of cleavage site motif RRQKRF between 112 and 117 residues; based on sequencing of complete fusion gene the 5 isolates were classified as genotype VII.1.1 and were clustered with sub genotype VIId. Kim et al. (2007a), Khan et al. (2010) and Liu et al. (2019) reported that genotype VII NDV was responsible for the latest outbreaks in Africa, Asia and the Middle East; in addition, NDV genotype VII.1.1 were detected previously in poultry farms in Egypt (Radwan et al. 2013; Hussein et al. 2014; Ahmed et al. 2017; AboElkhair et al. 2019; Abd El-Hamid et al. 2020; Abd Elfatah et al. 2021).

In our study sequencing of full Fusion protein gene were carried out to classify our isolates according to unified and updated classification of NDV isolates and overcome the challenges that result from classification based on sequencing of partial fusion protein gene; these challenges created in NDV categorization, caused genotypes comparisons difficult, and created confusion in genotypes and sub-genotypes assignment (Dimitrov et al. 2019). According to complete F gene sequence that carried out in this study and based on updated classification of (Dimitrov et al. 2019), the detected NDV isolates were classified as NDV genotype VII.1.1.

Conclusion

From our study, NDV sub-genotype VII.1.1 was found constantly circulated between chicken farms in some governorates of Egypt during 2019-2020. This genotype supposed to cause concurrent outbreaks in chicken flocks and might be responsible for severe losses in poultry industry. Therefore, repeated monitoring for genetic diversity of NDV strains and the characteristics of the virulent NDV as well as assessment of the used vaccination programs are necessary to control NDV infection more efficiently in Egyptian poultry farms.

Authors' Contributions

M.M.A, K.M.A and H.M.A designed and planned this study. H.M.A and S.A A. collect samples and performs all laboratory work. ELbayoumi.Kh.M and K.M.A supplied and supervised laboratory work. All authors shared samples collection, performing the tests, manuscript writing, drafted, revised the manuscript and approved the final manuscript.

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