

## PHYLOGENETIC AND HISTOPATHOLOGICAL CHARACTERIZATION OF NEWCASTLE DISEASE VIRUSES REVEALS HIGH PREVALENCE OF VERY VIRULENT STRAINS OF GENOTYPE VIIID IN EGYPT

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### ABSTRACT

Newcastle disease virus (NDV) is a highly contagious disease in poultry, also considered as a major challenge for the commercial and traditional poultry industry in Egypt. In this study clinicopathological and immunohistochemistry examination of fourteen flocks from different provinces in Egypt during 2013 and 2014 revealed velogenic ND features and positive staining for ND virus (NDV) antigen in pantropic organs of all strains which also revealed positive NDV by RRT-PCR, as it is already convinced that the fusion protein cleavage site has been deemed as responsible for the pathogenicity of NDV and selected as a target to identify and type lentogenic and velogenic strains, viral Genomic RNAs were extracted from representing swabs and organs of examined flocks, and partially amplified for fusion gene of NDV using RT-PCR, then forwarded to gene sequencing. Four chosen samples amplified for full fusion gene sequence using specific sets of newly designed primers from the available sequence data of NDV strains in Gen Bank, The resulted sequence showed motif <sup>112</sup>RRQKRF<sup>117</sup> which is indicative of velogenic character NDV strains, and indicate presence of 2 mutations E74D and D170N in two strains. Phylogenetic analysis revealed that Egyptian strains are closely related to NDV genotype VIIId strains of south East Asian countries based on the high nucleotide and amino acid similarity.

**Key words:** NDV, RRT-PCR, RT-PCR, Full Fusion gene (F gene), Immunohistopathology, and Molecular evolution.

### INTRODUCTION

Newcastle disease (ND) is one of the major concerns of poultry industry in the world. It is caused by Newcastle disease virus (NDV), a member of avian paramyxovirus (APMV), which belongs to the genus Avulavirus, sub-family Paramyxovirinae, family Paramyxoviridae, and order Mononegavirales (Mayo, 2002). Its single-stranded RNA genome is composed of 6 genes, -NP-P-M-F-HN-L, encoding 6 major proteins nucleoprotein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase (HN), and large protein (L), respectively. The hemagglutinin-neuraminidase (HN) and the fusion (F) proteins are both glycoproteins expressed at the surface of the enveloped virus. They mediate attachment of the viral particle to

sialic acid-containing cell receptors, its fusion with the plasma cell membrane, and the release of progeny virions from the surface of infected cells (Lamb and Parks, 2007). Both proteins also induce virus-neutralizing antibody responses; F protein is regarded as an important pathogenic marker of NDV (Rott *et al.*, 1988). This important pathogen remains havoc for the poultry industry and causes a severe economic impact in terms of heavy production losses and high mortality. Newcastle disease virus (NDV) has a wide host range, including approximately 241 species of 27 orders, out of known 50 orders of birds (Madadger *et al.*, 2013). The most susceptible avian species to this disease are chickens (Rezaeianzadeh *et al.*, 2011). Depending upon the pathotype and susceptibility the mortality varies from zero to 100% (Nanthakumar *et al.*, 2000).

Several pathotypes (asymptomatic enteric, lentogenic, mesogenic, viscerotropic velogenic, or neurotropic velogenic) of NDV are recognized depending on the clinical symptoms observed in chickens (OIE, 2012).

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A virulent and virulent strain may also be distinguished on the basis of the cleavage site sequence of their F protein. During replication, the fusion gene is translated into a precursor protein, F0, which must be cleaved by host cell proteases into F1 and F2 subunits for viral particles to become infectious (Morrison *et al.*, 1993). Most virulent strains exhibit the consensus sequence <sup>112</sup>(R/K) RQ (R/K) R\*F<sup>117</sup> at the cleavage site of the F0 precursor, in contrast to <sup>112</sup>(G/E) (K/R) Q (G/E) R\*L<sup>117</sup> in a virulent viruses (Collins *et al.*, 1993; OIE, 2012). The genomic diversity of NDV increases the possibility of diagnostic failures, resulting in unidentified infections (Miller *et al.*, 2010).

In Egypt, velogenic strains of NDV were isolated and clustered as class II genotype VII sub-genotype d and closely related to Middle East isolates (Radwan *et al.*, 2013). The predominant NDV isolates circulating among chickens are virulent and associated with outbreaks in commercial poultry farms and backyard reared chickens in Egypt (Osman *et al.*, 2014). Phylogenetic analysis of some strains isolated from Egypt was closely related to the NDV strains isolated previously in China. Determination of complete genome sequences of additional NDV strains from Africa is necessary to understand the epidemiology of currently circulating viruses in Africa (Mohamed *et al.*, 2009).

Herein, detection and characterization of Newcastle disease virus (NDV) from recent outbreaks affecting poultry farms in Egypt between 2011 and 2014 were conducted. Partial amplification and sequence of the F gene covering the cleavage site for 14 isolate were done, also full fusion sequence for 4 selected isolates were also done. In the current work we aimed to (a) Determine the circulating NDV genotype and causing the severe outbreaks in poultry farms. (b) Phylogeny of Egyptian strains and determine the potential source of these strains. (c) Determine the genetic relation between different NDV vaccinal strains and the current circulating strains.

## MATERIALS AND METHODS

### Sample collection:

The samples; organs (Brain, trachea, lungs, proventriculus, small intestine and pancreas) and swabs (cloacal and tracheal) from fourteen chicken broiler farms including different Egyptian governorates showed respiratory, nervous signs and diarrhea, sudden onset high mortality. These samples were labeled and transported immediately on ice to the reference laboratory for quality control on poultry

production (RLQP) to be processed immediately for RRT-PCR.

### Detection of NDV by RRT-PCR

The viral genomic RNAs of 14 sample were extracted from the infective cloacal and tracheal swabs and also from organs using QiAmp Viral RNA Mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions, RRT-PCR was carried out using a commercial kit Quantitect probe RT-PCR kit (Qiagen, Inc. Valencia CA). Primers used NDV-F (F+4829) 5'-GGT GAG TCT ATC CGG ARG ATA CAA G-3', NDV-R (F-4939) 5'-TCA TTG GTT GCR GCA ATG CTC T-3' and probe- (F+4894) 5'-FAM-AAG CGT TTC TGT CTC CTT CCT CCA-BHQ-3' (Creelan *et al.*, 2002 and Ausvetplan, 2006). RRT-PCR was conducted in the Stratagene 3005P MXpro Real-Time PCR System (Stratagene, USA) according to manufacture instructions.

### Histopathology and immunohistochemistry (IHC)

Brain, trachea, lungs, proventriculus, small intestine and pancreas were collected tissues were fixed by submersion in 10% neutral buffered formalin and embedded in paraffin. Sections were made at 5 µm and were stained with hematoxylin and eosin (HE). For immunohistochemistry a 4µm section was antigen retrieved by first microwaving the sections in Antigen Retrieval Citra Solution (Biogenex) for antigen exposure. Nonspecific protein and endogenous peroxidase were blocked by incubation for 5 minutes with blocking reagents from the polymer kit. A 1:500 dilution of a mouse-derived monoclonal antibody (Novus Bio) specific for NDV nucleoprotein was applied and allowed to incubate for 30min. at 37°C. The primary antibody was then detected by the application of peroxidase rabbit anti-mouse IgG secondary antibody using Mouse and Rabbit Specific HRP/3, 3' Diaminobenzidine DAB Detection IHC (Abcam) and hematoxylin was used as a counterstain then mounted by depex (Susta *et al.*, 2011).

### Primer design for RT-PCR and sequence of fusion gene

PCR amplification was performed by using Qiagen One Step Enzyme Mix according to the manufacturer's instructions, using primer sets designed from the available sequences of NDV strains from Gen Bank (Table 1). Gel containing DNA band of the expected size (766 bp for partially sequenced samples and 1700bp for full fusion sequence) was excised and purified with the QIAquick Gel Extraction Kit (Qiagen) according to the manufacturer instruction.

**Table 1:** Primers used for sequence of partial and complete fusion gene

Primer name	Direction	Primers	Location	Reference
NDV-F330	<b>Forward</b>	AGG AAG GAG ACA AAA ACG TTT TAT AGG	<b>330-357</b>	<b>This study</b>
NDV-R700	<b>Reverse</b>	TCA GCT GAG TTA ATG CAG GGG AGG	<b>700-676</b>	<b>This study</b>
NDV-F640	<b>Forward</b>	CTA ACT GAA TTA ACT ACA GTA TTC GGG	<b>640-667</b>	<b>This study</b>
NDV-R1290	<b>Reverse</b>	GTC TAA TGA TAA GAC ATT GCA CGA ATG	<b>1279-1290</b>	<b>This study</b>
NDV-F1200	<b>Forward</b>	ATG TAC AGA CCC TCC TGG TAT CAT ATC	<b>1200-1227</b>	<b>This study</b>
NDV-R1740	<b>Reverse</b>	CTT AAG TCT TAT ACT TGA CAG GTT ATC	<b>1713-1740</b>	<b>This study</b>
NDV-M2	<b>Forward</b>	TGG AGC CAA ACC CGC ACC TGCGG		<b>Mase et al., 2002.</b>
NDV-F2	<b>Reverse</b>	GGA GGA TGT TGG CAG CATT		<b>Mase et al., 2002.</b>

### Sequence and phylogenetic analysis

Purified RT-PCR products were sequenced using Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer, Foster city, CA) and Applied Biosystems 3130 genetic analyzer (ABI, USA). Sequences similarities and relationships of the partial (14 samples) and full fusion gene (4 samples) obtained in this study were compared with previously published NDV vaccine and references strains available in the public database (BLAST, NCBI, USA). Amino acid phylogenetic relationship was constructed for the sequenced samples and other

vaccine, reference and Egyptian strains previously published and available on the Gen Bank database using MEGA version 6 (Tamura *et al.*, 2013). A comparative analysis of deduced amino acids and nucleotides sequences of the sequenced fusion gene were created using the CLUSTAL W Multiple Sequence Alignment Program, version 1.83 of Meg Align module of Lasergene DNA Star software. Sequences generated in the frame of this study were submitted to the Gen Bank database with accession numbers as showed in table (2).

**Table 2:** Epidemiological and genetic data of the sequenced strains in the current study

	Sample code	Governorates	production	Collection date	Accession number
1	NDV-FU4-EGYPT-NLQP-2013	Al-Gharbia	Backyard	4-2013	KU365650
2	NDV-FU5-EGYPT-NLQP-2014	Behera	Backyard	8-2014	KU365653
3	NDV-FU8-EGYPT-NLQP-2014	Monofia	Farm	9-2014	KU365651
4	NDV-FU13-EGYPT-NLQP-	Al-Gharbia	Farm	4-2014	KU365652
5	NDV-1-EGYPT-NLQP-2014	Al-Gharbia	Farm	6-2014	KU365654
6	NDV-2-EGYPT-NLQP-2014	Monofia	Farm	3-2014	KU365655
7	NDV-3-EGYPT-NLQP-2013	Behera	Backyard	8-2013	KU365656
8	NDV-4-EGYPT-NLQP-2013	Sharkia	Backyard	6-2013	KU365657
9	NDV-5-EGYPT-NLQP-2013	Cairo	Farm	8-2013	KU365658
10	NDV-6-EGYPT-NLQP-2014	Al-Gharbia	Backyard	9-2014	KU365659
11	NDV-7-EGYPT-NLQP-2013	Behera	Farm	5-2013	KU365660
12	NDV-8-EGYPT-NLQP-2014	Giza	Backyard	10-2014	KU365661
13	NDV-9-EGYPT-NLQP-2014	Bani-suaif	Farm	11-2014	KU365662
14	NDV-31-EGYPT-NLQP-2014	Sharkia	Backyard	8-2014	KU365663

## RESULTS

### Clinical and necropsy findings

Clinically, the most common clinical signs were greenish diarrhea surrounding the cloaca, respiratory (sneezing, gasping for air, nasal discharge and coughing), nervous signs (incoordination, tremors, torticollis, opisthotonusparalysis of wings and/or legs, twisting of head and neck or complete paralysis) and death. Most commonly observed PM lesions were varying degrees of congestion and swelling of respiratory and digestive tract, trachea and lungs

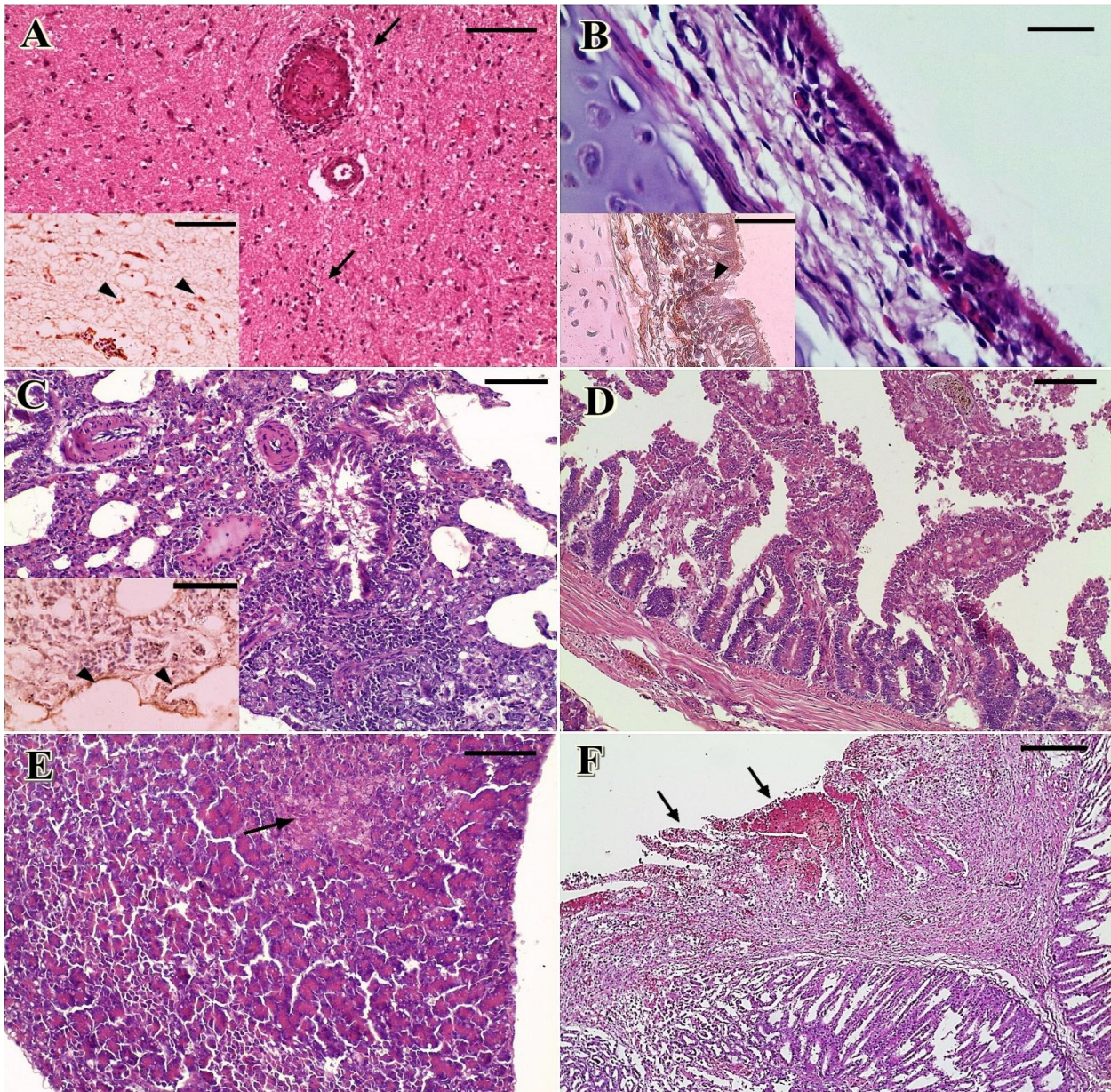
showed congestion and hemorrhages in the mucosa. Marked hemorrhages on the tips of the proventriculus, button-like ulcers in the wall of intestine, cecal tonsils were enlarged, thickened, necrotic and hemorrhagic.

### Histopathology and immunohistochemistry

The histopathological examination of brain, trachea, lungs, proventriculus, pancreas and small intestine of naturally infected birds in the current study in which brain showed congested blood vessels with edematous vacuoles, lymphocytic perivascular

cuffing and diffuse gliosis in cerebral tissue was shown (Fig. 1A). Trachea, showed mild congested blood capillaries with mild edema in the mucosa with desquamation of epithelium and congested blood capillaries. The invading inflammatory cells were mononuclear (Fig.1B). Lungs, bronchial mucosa showed severe epithelial ulceration and necrosis. The invading inflammatory cells were rounding mononuclear in high populations which filled the parabronchial lumen. Blood vessels and alveolar

blood capillaries were severely congested (Fig.1 C). Small intestine especially the duodenum showed severe epithelial ulcerations and sloughing of intestinal villi (Fig.1D). Pancreas showed multifocal necrosis of pancreatic acini (Fig.1E). Proventriculus, showed erosion and ulceration severe congestion and haemorrhage in mucosal epithelium. Diffuse infiltrations of invading round inflammatory cells made thickening of mucosal and submucosal layers (Fig. 1 F).



**Fig.1:** Histopathology and immunohistochemical staining for Newcastle virus antigen in tissues of chickens.

(A) Brain showing perivascular cuffing and gliosis (arrow) scale bar 100 $\mu$ m, (insert) viral antigen (head arrow) is seen in neuron and glial cells in cerebrum scale bar 50 $\mu$ m. (B) Trachea showing mild catarrhal tracheitis scale bar 200 $\mu$ m, (insert) viral antigen in brown (head arrow) in epithelium scale 50 $\mu$ m. (C) Lung showing severe interstitial pneumonia 100 $\mu$ m,

(insert) viral antigen in brown (head arrow) seen in alveolar (air) capillaries. (D) Small intestine showing severe necrosis of intestinal villi scale 100 $\mu$ m. (E) Pancreas showing necrosis of pancreatic acinar epithelium (arrow) scale 100 $\mu$ m. (F) Proventriculus showing mucosal haemorrhages (arrows) scale 40 $\mu$ m.

### Detection and amplification of NDV by PCR

All collected samples (14) are positive by RRT-PCR using specific primers and probe for NDV with threshold cycle between 16.4 and 24.38, By using specific designed primers for fusion gene RT-PCR amplification was done showing specific bands at 766 bp (Partial amplification) and 1700bp for full fusion on agarose gel.

### Sequence and phylogenetic analysis

Sequence analysis of the PCR products revealed that all examined samples are closely related to the very virulent strains of NDV (Genotype VIIId) and are compared with other strains published on Gene Bank on NCBI website indicate that all of them were closely related to Chicken/China/SDWF07/2011 strain with identity (98%-99%) (Table3). Analyses of these samples indicate presence of motif <sup>112</sup>RRQKR<sup>F117</sup> in hypervariable region which is indicative of velogenic character of NDV strains.

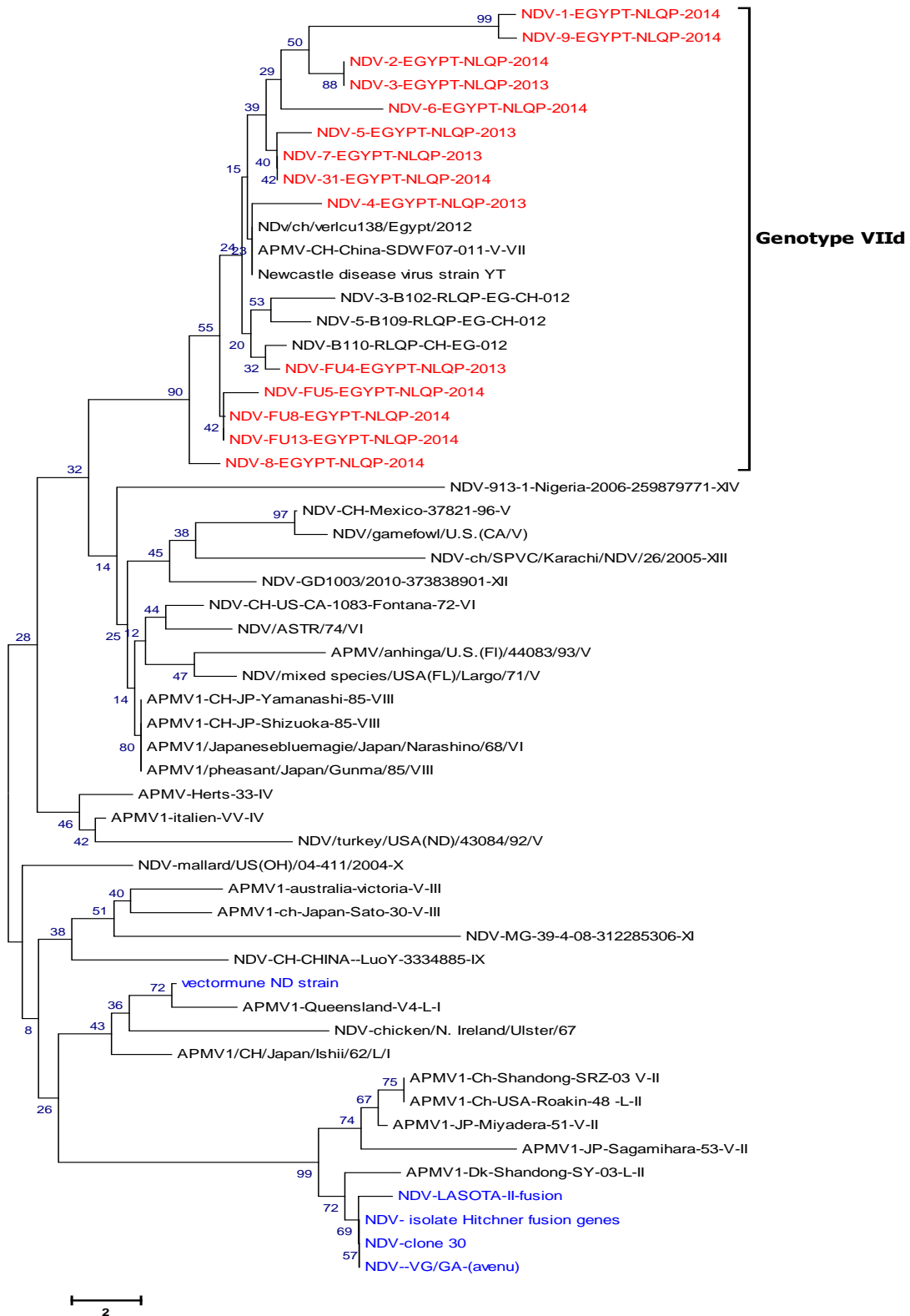
Analysis of four samples of a total 1662 nucleotides encoding for 553 amino acid residues were identified in the complete coding region of the F-gene. Six potential N-glycosylation sites (Asn-X-Ser/Thr where

X is any amino acid except proline or aspartic acid) located at positions 85 to 87, 191 to 193, 366 to 368, 447 to 449, 471 to 473 and 541 to 543 were recognized. Twelve cysteine residues located at positions 25, 76, 199, 338, 347, 362, 370, 394, 399, 401, 424 and 523 were identified. Comparison of glycosylation sites and cysteine residues showed no changes in the amino acid sequence in all field strains, which may indicate that these sites were highly conserved. Analysis of seven neutralizing epitopes located at positions 72, 74, 75, 78, 79, 157 to 171 and 343 of the F-protein showed presence of these substitution mutations in the examined strains L13P, E74D and D170N (Table 5).

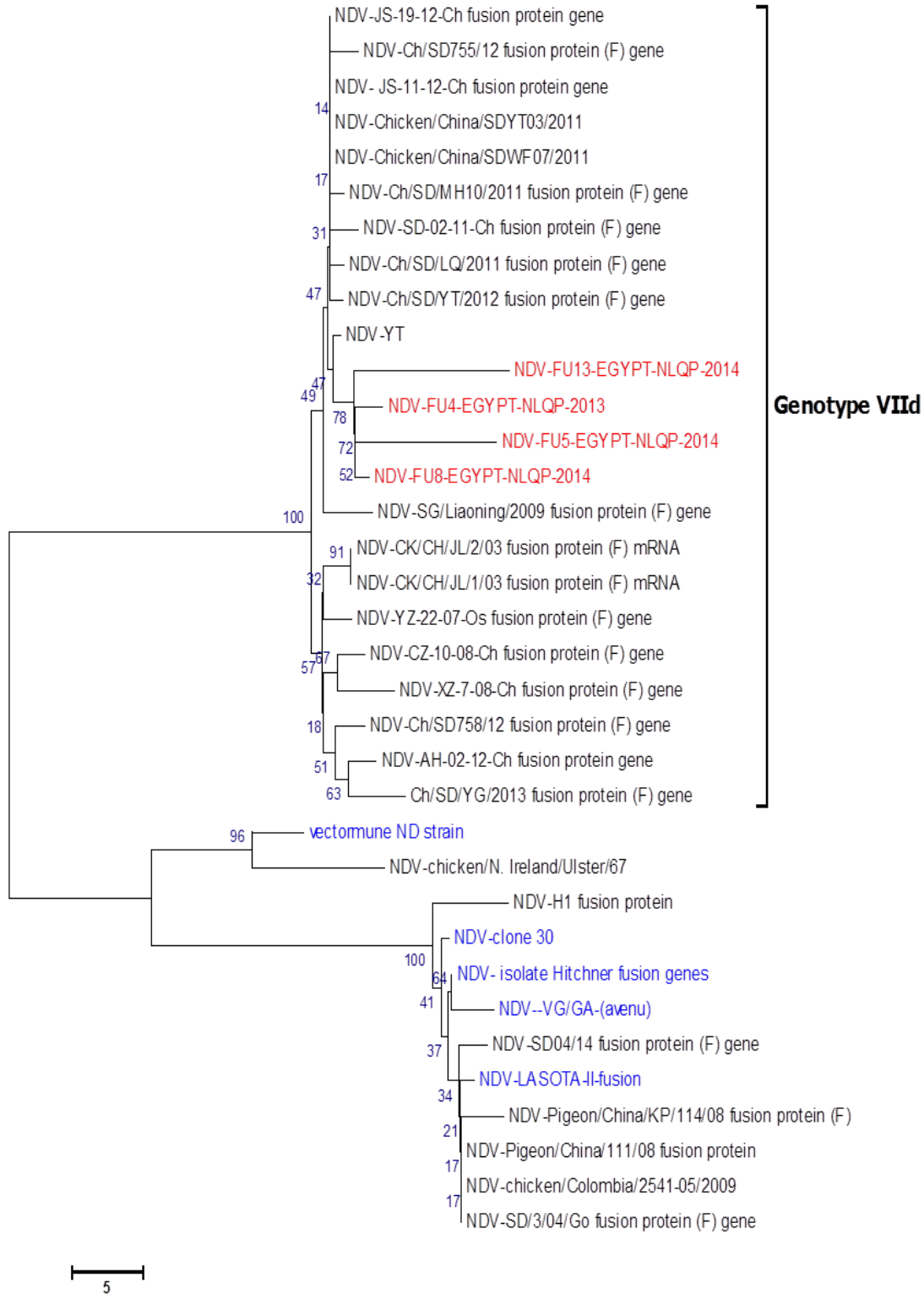
In relation to vaccinal NDV strains (Lasota, Hitchner, clone 30 and Avenu) used in Egypt which genetically genotyped as lentogenic strains of NDVs (class II genotype II), the strains obtained in this study (class II Genotype VIIId) show distant degree of amino acids homology ranged from 82% to 83%. While for the Vectormune ND vaccine (class II genotype I) the amino acids identity ranged from 85%-86%, according to the full fusion amino acid sequences (Table 3).

**Table 3:** Identity % of strains under study with other vaccinal and Egyptian strains

Strain name (Amino acids identity are marked with bold numbers)	Chicken/China/SDWF07/2011	NDV-LASOTA-II-fusion	NDV- isolate Hitchner fusion	NDV-clone 30	NDV--VG/GA-(avenu)	vectormune ND strain	NDV-chicken/N. Ireland/Ulster/67	NDV-FU4-EGYPT-NLQP-2013	NDV-FU8-EGYPT-NLQP-2014	NDV-FU13-EGYPT-NLQP-2014	NDV-FU5-EGYPT-NLQP-2013
Chicken/China/SDWF07/2011		88%	89%	88%	88%	91%	90%	99%	99%	96%	97%
NDV-LASOTA-II-fusion	<b>84%</b>		100%	98%	99%	93%	93%	87%	88%	86%	86%
NDV- isolate Hitchner fusion	<b>84%</b>	<b>99%</b>		98%	99%	93%	93%	88%	88%	86%	86%
NDV-clone 30	<b>83%</b>	<b>98%</b>	<b>98%</b>		98%	92%	92%	87%	87%	85%	85%
NDV--VG/GA-(avenu)	<b>84%</b>	<b>99%</b>	<b>100%</b>	<b>97%</b>		93%	92%	87%	88%	85%	86%
Vectormune ND strain	<b>86%</b>	<b>89%</b>	<b>90%</b>	<b>88%</b>	<b>90%</b>		97%	90%	90%	88%	88%
NDV-ch/N. Ireland/Ulster/67	<b>86%</b>	<b>90%</b>	<b>91%</b>	<b>89%</b>	<b>90%</b>	<b>95%</b>		88%	89%	87%	87%
NDV-FU4-EGYPT-NLQP-2013	<b>99%</b>	<b>83%</b>	<b>83%</b>	<b>82%</b>	<b>83%</b>	<b>86%</b>	<b>85%</b>		99%	97%	97%
NDV-FU8-EGYPT-NLQP-2014	<b>99%</b>	<b>83%</b>	<b>84%</b>	<b>83%</b>	<b>83%</b>	<b>86%</b>	<b>85%</b>	<b>99%</b>		97%	97%
NDV-FU13-EGYPT-NLQP-2014	<b>98%</b>	<b>83%</b>	<b>83%</b>	<b>82%</b>	<b>83%</b>	<b>85%</b>	<b>84%</b>	<b>98%</b>	<b>98%</b>		95%
NDV-FU5-EGYPT-NLQP-2013	<b>98%</b>	<b>83%</b>	<b>83%</b>	<b>82%</b>	<b>83%</b>	<b>85%</b>	<b>84%</b>	<b>98%</b>	<b>98%</b>	<b>98%</b>	



**Fig. 2:** Phylogenetic tree of partially sequenced NDV strains with other Egyptian and vaccinal strains and other strains from Middle East.



**Fig. 3:** Phylogenetic tree of fully sequenced NDV fusion gene strains with other vaccinal and reference strains.

**Table 4:** Amino acid substitution in the variable region and neutralizing epitopes of the F-gene sequences of Egyptian strains.

Virus	Hyper-variable region													Neutralizing epitopes						
	4	10	11	13	20	21	27	52	63	78	93	101	121	72	74	75	78	79	157-171	343
Consensus	K	P	A	L	M	L	R	V	V	R	T	K	V	D	E	A	R	A	SIAATNEAVHEVTD	L
NDV-FU4-EGYPT-NLQP-2013	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-----	-
NDV-FU8-EGYPT-NLQP-2014	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	SIAATNEAVHEVTN	-
NDV-FU13-EGYPT-NLQP-2014	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-----	-
NDV-FU5-EGYPT-NLQP-2013	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D	-	-	-	-----	-

## DISCUSSION

Newcastle disease (ND) is the most devastating disease affecting poultry especially in large scale production (Alexander *et al.*, 2003). Egypt is endemic for Newcastle disease virus (NDV) with continuous long-lasting outbreaks causing significant economic losses in the poultry industry due to high mortality which may reach 100% in very virulent strains of NDV, despite the intensive vaccination programs (Mohamed *et al.*, 2011; Radwan *et al.*, 2013; Nabila *et al.*, 2014 and El Bagoury *et al.*, 2015). The use of rRT-PCR and other similar techniques for the determination of the virulence of ND viruses and for Phylogenetic studies has been reported (Farkas *et al.*, 2009), there is increasing use of such molecular techniques to detect NDV in clinical specimens with its advantage being the extremely rapid demonstration of the presence of virus (OIE, 2009).

Based on clinic-pathological findings indicate that examined NDV strains of considerable virulence and is comparable to many other highly velogenic viscerotropic NDV strains. Principal gross lesions consisted of severe nervous manifestation, visceral hemorrhages, respiratory symptoms and splenic necrosis all of which are consistent with other velogenic viscerotropic NDV strains (Cattoli *et al.*, 2011). Histologically, NDV strains behaved as a typical highly virulent strain, with extensive interstitial pneumonia, severe enteritis, gliosis with neuronal degeneration and necrosis of lymphoid tissues, especially those of the spleen. By immunohistochemistry, showed positive tissues indicating a marked systemic pantropism, which is also typical of highly virulent NDV strains (Susta *et al.*, 2011 and cattoli *et al.*, 2011).

Evolution of NDV may be related to the accumulation of point mutations that induce amino

acid substitution in the neutralizing epitopes as the N-linked glycosylation sites of the F protein (Chambers *et al.*, 1986). F-Protein of virulent strains differ from those of a virulent virus by virtue of possessing a pair of dibasic amino acids at the carboxyl (C) terminus of F2 and phenylalanine at residue 117 which is the N-terminus of the F1 protein. The term 'multiple basic amino acids' refers to at least three arginine (R) or lysine (K) residues between residues 113 and 116 while a virulent viruses have Leu (leucine) at amino acid no.117 cleavage site in their fusion protein (Collins *et al.*, 1993 and Zhu *et al.*, 2010).

Genotype VII (class II genotype VII) was firstly categorized into two subgenotype: VIIa, which represents viruses that emerged in the 1990s in the Far East and spread to Europe and Asia; and VIIb, which represents viruses that emerged in the Far East and spread to South Africa. Later, the two subgenotypes of VII are classified into VIIc, d, and e, which represents isolates from China, Kazakhstan and South Africa; and VIIf, g, h, and i, which represent African isolates (De Almeida *et al.*, 2009 and Miller *et al.*, 2010).

Analysis of the deduced amino acid sequence and phylogenetic analysis of the NDV F-gene of the strains isolated from Egypt in 2013 and 2014 suggests that all examined samples close to genotype VIId circulating in the Middle Eastern countries and China, which is responsible for the continuing outbreaks of ND in Egypt (Nabila *et al.*, 2014 and Hussien *et al.*, 2014). This is confirmed by presence of Serin at site 176 which is recorded as specific signature to cluster VII. Exclusive Amino acid and nucleotide signatures of genotype VIId are V52, I255, Y314, T1044, G1608 (De Almeida *et al.*, 2013) that are recorded in strains under study. Seven major epitopes have been identified involving the fusion inhibition and neutralization of F-protein (Neyt *et al.*, 1989 and



Yusof *et al.*, 1989). Individual amino acids at 72, 74, 75, 78, 79 and 343 and a stretch of amino acids from residues 157–171 were identified to be critical for both structures and functions of the F-gene (Yusoff K *et al.*, 1989). In this study, nucleotide substitution in one of the fusion inhibition and neutralizing epitope (K78R) was identified in all VIIId strains and present in 4 strains under study. Comparison with sequence data from reference strains showed that among these mutations, K4I were conserved only in NDV strains originating from Japan while L21P, I52V, K78R and R101K were conserved in strains originating from the Far East Asia (Japan, China and Taiwan). These substitutions maybe used as crude molecular markers of geographic origins of NDVs (Dennis *et al.*, 2013).

When compared the F gene of strains under study (genotype VII) with vaccine strain like La Sota, two of these epitopes were changed as follows: E74D in NDV-FU5-EGYPT-NLQP-2014 and D170N in NDV-FU8-EGYPT-NLQP-2014, which displayed the antigenic difference and considered as escape mutants that may affect antigenicity of these viruses (Renata *et al.*, 2013).

A point mutation in the F-gene may have resulted to neutralizing epitope variants. However, it is unclear if these mutations were a part of adaptive mechanism of NDVs to evade the immune response to be able to infect vaccinated chickens, or whether this mutation was actually the effect of selective immune pressure exerted on ND viral particles as a consequence of vaccination. To understand how wild NDVs infect vaccinated chickens, this identified mutation may be useful for future site-directed mutagenesis studies (Dennis *et al.*, 2013).

The NDV can occur in vaccinated birds because some of the birds will have had a poor vaccine response and will be susceptible to infection. This attributed to ND vaccines do not protect vaccinates from infection and viral shedding. Also, parental immunity contributed to vaccination inefficiency in young chicks (Kapczynski and King, 2005). Vaccination failure due to inefficient vaccination or virus genetic drift should be considered to draft the efficiency of the commercial available vaccines against these isolates or to prepare a new one.

In conclusion, data obtained here confirm high circulation of genotype VII which became the predominant strain and causing the severe outbreaks in poultry farms, which may be originated from south East Asian countries. Vaccines and vaccination programs evaluation should be repeated. Continuous monitoring of the flocks' immunological status should be carried out to evaluate the antibody response to currently used vaccines.

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## التوصيف الجيني والباثولوجي لفيروس النيوكاسل يكشف انتشار العترة شديدة الضراوة من النوع ٧

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فيروس النيوكاسل يسبب مرض شديد العدوى وذو اهمية اقتصادية كبيرة في الدواجن. و أيضا يمثل تحديا كبيرا لصناعة الدواجن في مصر ، ويعتبر جين fusion هو المسئول عن احداث الصفة المرضية للفيروس في الدجاج. في هذه الدراسة، تم استخراج الحمض الجينوم الفيروسي RNA من مسحات و اعضاء ، وتم عملية اكنثار F جين باستخدام مجموعات من ال primers تم تصميمها من العترة المتاحة من سلالات فيروس نيوكاسل من بنك الجينات ، تم عمل تحليل جيني جزئي لعدد ١٤ عينة من محافظات مختلفة في عامي ٢٠١٣ و ٢٠١٤. تم اختيار عدد ٤ عينات لعمل تحليل جيني لجين F كاملا. وقد وجد ان جميع الحالات تتماثل مع العترة شديدة الضراوة تنتمي للعترة الصينية، والاسيوية والتي تتبع GENOTYPE 7D ووجد ان هذه العينات شديدة الضراوة يعيده عن العترة المستخدمة في التحصين ولذلك فانه يجب علينا مراجعه التحصينات الموجودة في مصر.