VacciMonitor 2025;34:e093425
www.vaccimonitor.finlay.edu.cu
Artículo Original

Isolation and characterization of a recent Newcastle disease virus from infected backyard chickens in Egypt

Samir A. Nassif ORCID: https://orcid.org/0000-0002-7907-0102
Reem A. Soliman ORCID: https://orcid.org/0000-0003-4074-4584
Marwa Fathy ORCID: https://orcid.org/0000-0002-3794-9071
Ahlam Mourad ORCID: https://orcid.org/0000-0002-4322-6865
Esraa Fouad ORCID: https://orcid.org/0000-0003-3236-512X
Hala Ahmad ORCID: https://orcid.org/0009-0007-2594-6125
Mahmoud M. Abotaleb* ORCID: https://orcid.org/0000-0001-9303-539X

* Central Laboratory for Evaluation of Veterinary Biologics. Agricultural Research Center (ARC), Cairo, Egypt.

Corresponding author: M.abotaleb84@yahoo.com

Genomic surveillance of Newcastle disease virus is critical for determining the genetic diversity of circulating strains and improving preparedness and response to potential outbreaks. Backyard poultry continue to present significant challenges to Newcastle disease virus control due to inadequate biosecurity, poor vaccination practices, and close interactions with migratory birds. This study was designed to isolate and characterize Newcastle disease virus from infected backyard chickens in Qalubia governorate, Egypt. The hemagglutination test and reverse transcription-polymerase chain reaction, targeting a partial segment of the F-gene, confirmed an isolate as Newcastle disease virus. Positive Newcastle disease virus isolate was sequenced and phylogenetically analyzed, revealing that it belongs to Newcastle disease virus genotype VII.1.1. It was deposited in the NCBI GenBank (CLEVB1/2024) under Accession Number PP130129. The data further confirmed that the CLEVB1 isolate had the cleavage site motif ^{112}RRQKRF^{117}, characteristic of velogenic Newcastle disease virus strains. Based on amino acid sequence comparison, the CLEVB1 isolate shared 99.2 % homology with strains teal/Egypt/SDU-3/2016 and quail/Egypt/SDU-2/2016, which were both isolated from migratory birds in 2016. Therefore, the CLEVB1 isolate is thought that it was originated from two previously identified Newcastle disease virus strains, highlighting the results of ongoing interactions between migratory birds and backyard poultry. It is recommended that continuous Newcastle disease virus surveillance and routine vaccination in backyard poultry sectors are mandatory to reduce the risk of future outbreaks.

Keywords: Newcastle disease virus; viral fusion protein; PCR; Egypt.

Introduction

The poultry industry faces numerous challenges, including viral and bacterial diseases, as well as management problems.[1] Among these, Newcastle Disease (ND) are particularly problematic due to their high mortality and morbidity rates;[2] it is caused by Newcastle Disease virus (NDV), a highly contagious virus that is responsible for severe economic losses for poultry production.[2] It belongs to the genus *Avulavirus* in the subfamily *Paramyxovirinae* within the family *Paramyxoviridae*.[3] Based on the severity of the disease, NDV can be categorized into five pathotypes, designated a) velogenic neurotropic b) velogenic viscerotropic c) mesogenic d) lentogenic and d) asymptomatic.[3] The NDV genome consists of negative -sense, non-segmented, single-stranded RNA encoding eight essential genes: nucleocapsid (N), matrix protein (M), phosphoprotein (P), fusion protein (F), haemagglutinin-neuraminidase (HN), large polymerase (L), and the V and W proteins.[3] The F and HN

* Associate Professor, Central Laboratory for Evaluation of Veterinary Biologics (CLEVB), Agricultural Research Center (ARC), Cairo, Egypt .

glycoproteins are responsible for virus attachment, fusion, and release from the host cell membrane, making the F-protein an important target for NDV diagnosis and serotyping.[4] The F-protein is initially synthesized as an inactive precursor (F0) and is proteolytically cleaved by host cell proteases into F1 and F2 polypeptides, which are necessary for the virus to become infectious.[5] Cleavage of the F-protein in various tissues allows systemic dissemination of NDV, contributing to its virulence.[2] The World Organization for Animal Health (WOAH) defines virulent strains of NDV by an intracerebral pathogenicity index (ICPI) of 0.7 or above, or by specific amino acid sequences at the fusion cleavage site, such as ^{113}RQK/RRF^{117}.[2] In comparison, lentogenic strains have the sequence ^{113}K/RQG/ERL^{117}, which restricts their ability to replicate to tissues with trypsin-like enzymes, like those found in the respiratory and intestinal systems.[2] Virulent strains can infect a broader range of tissues when compared to other strains, leading to severe systemic infections.[3] Despite extensive vaccination programs, NDV remains prevalent, with continuous outbreaks worldwide, especially in parts of Asia, the Middle East, and Africa, where it is endemic.[6] In Africa, local poultry production largely consists of free-range indigenous chickens**,** many of which are multi-age flocks that have not been vaccinated against NDV.[7] These chickens may also originate from different geographic locations and some are sold in live bird markets, where the birds are housed in close proximity and with other avian species and migratory birds. Especially, Egypt constitutes part of a land bridge between Africa and Eurasia,[8] therefore a huge number of migratory birds pass from Asia and Europe to Africa and vice versa during the annual migration, where birds stop at the northern coast of Egypt and may be critical for new entry and epidemiology of the virus.[9] Given the high mutation rate of NDV, there is a global initiative aimed at controlling or eradicating the virus through continuous virological surveillance and phylogenetic analysis of the viral F-protein. This study seeks to isolate and characterize NDV from a suspected case in a backyard environment, and to examine its genetic relationship with previously identified strains in GenBank. Such efforts are vital for developing a comprehensive understanding of the current epidemiological situation. Therefore, knowledge of the NDV strains circulating in an area is essential for the development of effective control strategies, including the selection of appropriate vaccines to be used.

Materials and Methods

Specific pathogen free embryonated chicken eggs (SPF-ECE)

One hundred (one day old) SPF-ECE were obtained from the national project for production of specific pathogen free (SPF) eggs, Koum Oshim, El-Fayom, Egypt. They were kept in the egg incubator at 37 ºC with humidity 40-60 % until the age of 9-11 day old and used for isolation and propagation of the virus.

Specific pathogen free chickens

Thirty SPF-chicks, 3 weeks old purchased from Khom-Oshem farm, El Fayoum, were housed in positive pressure stainless steel isolation cabinets at the Central Laboratory for Evaluation of Veterinary Biologics (CLEVB) with continues light exposure. They were used for blood collection to prepare chicken red blood cell suspension.

Chicken red blood cells suspension (10%)

It was prepared according to WOAH[3] to be used in Hemagglutination (HA) test.[3]

Sample collection and preparation

Oropharyngeal and cloacal swabs were collected from diseased and freshly dead backyard poultry raised in Qalubia governorate during August 2023. Diseased birds suffered from respiratory distress including rales, nasal discharge, watery eyes and nervous impairment as head deviation. Dead bird showed air-saculitis, visceral intestinal lesions and pin point hemorrhages at the tip of proventriculus gland. Collected swabs were gathered in 2 mL cryovials containing sterile saline. Then the samples were labeled and sent to the laboratory in icebox. The collected samples were kept at -80 ºC for further analysis and isolation.

Virus isolation

The procedure was conducted following the protocol established by WOAH.[3] A volume of 0.2 mL from the pooled swab suspension was inoculated into the allantoic cavity of 9-day-old SPF-ECE. The eggs were candled twice daily to check embryo mortality. Deaths that occurred within the first 24 hours post-inoculation (PI) were deemed nonspecific, while those occurring between the 2nd and 4th days PI were considered specific results. The allantoic fluid collected was then tested for hemagglutinating activity using a slide HA test.[3]

Virus identification and characterization

Molecular characterization of the isolated virus by reverse transcription-polymerase chain reaction (RT-PCR)

RNA extraction

Viral RNA was extracted from infected allantoic fluids using the QIAamp viral RNA Mini kit (Qiagen, Germany, GmbH) following the manufacturer's instructions. Briefly, 140 µL of the sample suspension was incubated with 560 µL of AVL lysis buffer and 5.6 µL of carrier RNA at room temperature for 10 min. After incubation, 560 µL of 100 % ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 60 µL of elution buffer provided in the kit.

RT-PCR

RT-PCR was used for the detection of partial F-gene of NDV using the following primers (Table 1).

Primers were utilized in a 25 µL reaction containing 12.5 µL of Quantitect probe rt-PCR buffer (QIAgen, Gmbh), 1 µL of each primer of 20 pmol concentration, 0.25 µL of rt-enzyme 4.25 L of water, and 6 µL of template. The reaction was performed in a Biometra thermal cycler. Reverse transcription was applied at 50 °C for 30 min, a primary denaturation step was done at 95 °C for 5 min, followed by 35 cycles of 94 °C for 30 sec, 50 °C for 40 sec and 72 °C for 45 sec. A final extension step was done at 72 °C for 10 min. All RT-PCR products were subjected to electrophoresis in a 1 % agarose gel (0.5 X TBE) and the molecular marker was added; it was then visualized by ultraviolet (UV) transillumination.

Gene sequencing and phylogenetic analysis

The appropriately sized DNA band was excised from the gel and purified using QIAquick PCR Product extraction kit (Qiagen, Valencia). Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer) was used for the sequence reaction and then it was purified using Centrisep spin column. DNA sequences were obtained by Applied Biosystems3130 genetic analyzer (HITACHI, Japan); a BLAST® analysis (Basic Local Alignment Search Tool)[11] was initially performed to the phylogenetic tree created by the MegAlign module of LasergeneDNAStar version 12.1[12] and the phylogenetic analyses was done using maximum likelihood, neighbor joining and maximum parsimony in MEGA6.[13]

Results

Virus isolation

As shown in Figure 1 (A, B, C), post-mortem findings and clinical signs of the diseased and freshly dead chickens pointed to NDV infection. Swab samples from these chickens inoculated on SPF-ECE and allantoic fluids extracted from eggs demonstrated positive HA results.

Table 1. Oligonucleotide primers used in RT-PCR for detection of the NDV F-protein gene.

Target gene	Primer sequence (5'-3')	Length of amplified product (bp)	Reference
M and F	M2: TGG-AGC-CAA-ACC-CGC-ACC-TGC-GG F2: GGA-GGA-TGT-TGG-CAG-CAT-T	766 bp	(10)

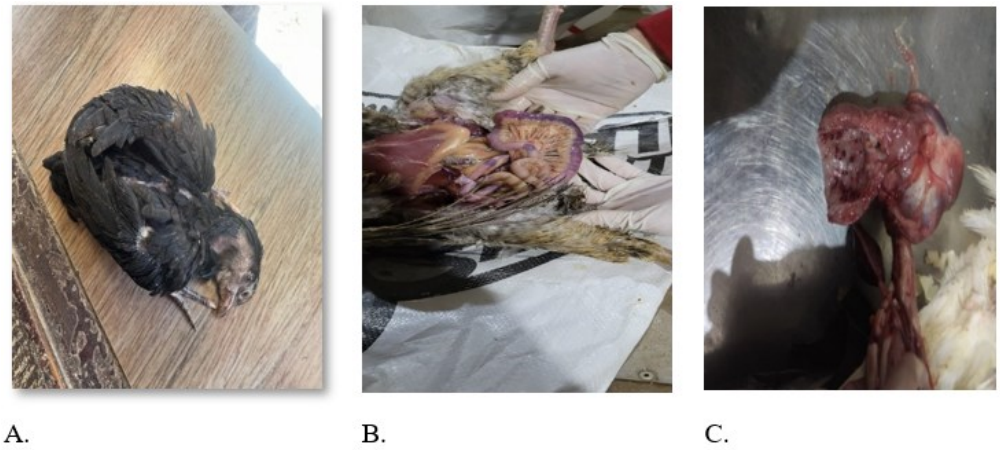

Fig. 1. Post-mortem findings and clinical signs of sick and freshly dead chickens. A. The clinically diseased bird suffered from depression and twisting of the head and neck. B: visceral intestinal lesions showing multiple hemorrhagic foci. C. gross pathological lesion with pinpoint hemorrhages at the tip of the proventricular gland.

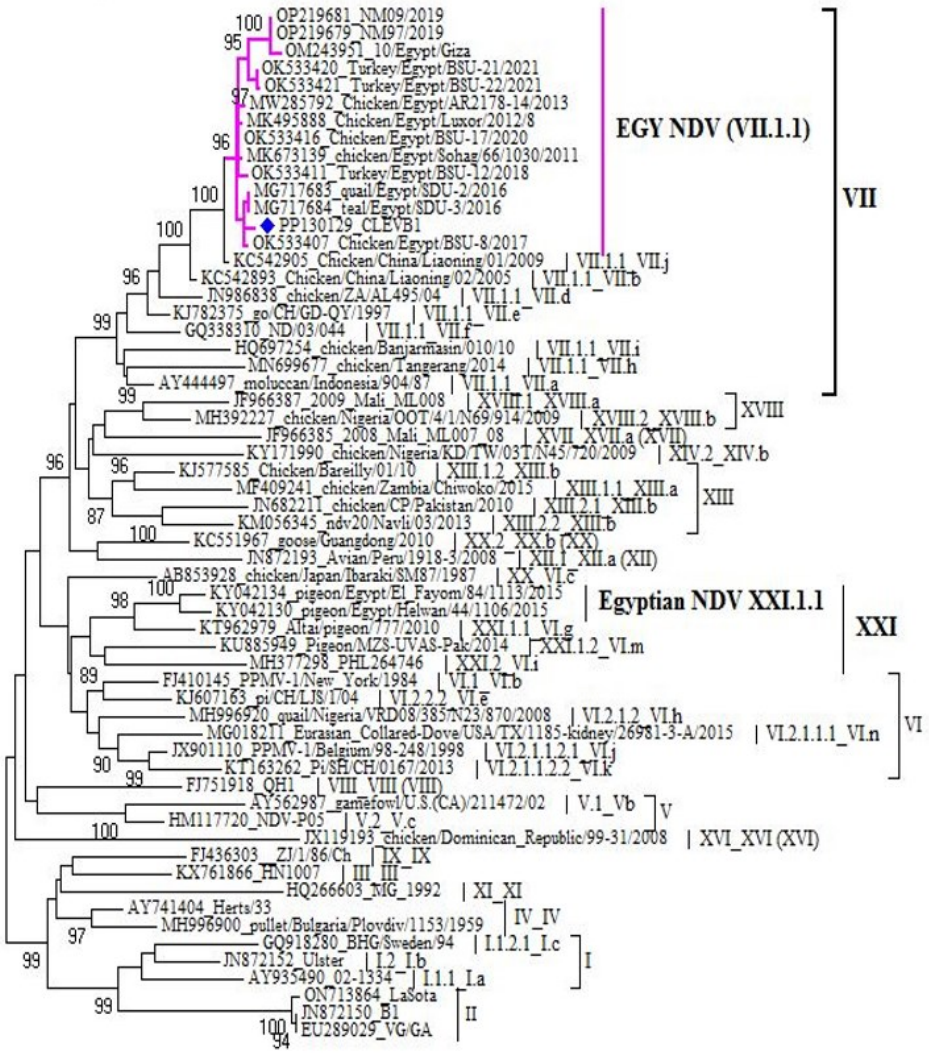

Fig. 2. Phylogenetic tree of NDV based on partial nucleotide sequences of the F gene. The isolates from this study are indicated by a circle.

Virus identification and characterization

Molecular characterization of the isolated virus by RT-PCR

The positive results from the HA test were corroborated by RT-PCR, which successfully detected the F gene of NDV, resulting in an amplified product measuring 766 bp.

Sequence and phylogenetic analysis of isolated NDV

A positive PCR viral isolate was subjected for sequencing and phylogenetic analysis. The sequence of this strain was submitted to NCBI GenBank and designated as CLEVB1/2024 under Accession Number PP130129. The phylogenetic tree (Fig. 2) indicates that this NDV isolate is classified under genotype VII.1.1.

In comparison to reference strains in the GenBank database, the CLEVB1 isolate was closely related to NDV genotype VII.1.1 strains, including chicken/Egypt/BSU-8/2017, teal/Egypt/SDU-3/2016, and quail/Egypt/SDU2/2016, with homology percentages of 99.3 %, 99.2 %, and 99.2 %, respectively. Conversely, there was a significant nucleotide divergence of 0.7 % to 29.4 % between the CLEVB1 isolate and other reference strains. The amino acid sequence at the fusion protein cleavage site of the isolate features the motif ^{112}RRQKRF^{117}, which is characteristic of velogenic strains. Additionally, an amino acid substitution from threonine (T) to cysteine (C) was detected at position 15 (T15C), as illustrated in Figure 3, Figure 4 and Figure 5.

Discussion

NDV is still considered a major threat to poultry industry, affecting both backyard and commercial sectors.[2] In Egypt, the high prevalence of NDV in

Percent Identity

Divergence

	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	
1		99.4	99.4	97.3	97.9	97.2	97.3	97.8	97.5	97.3	97.8	97.6	96.8	91.7	87.1	92.6	90.2	86.6	90.2	95.1	79.6	77.0	77.0	76.9	83.2	96.8	1
2	0.6		100.0	97.6	98.5	97.8	97.6	98.3	98.0	97.9	98.3	98.2	97.3	92.0	87.3	93.0	90.5	86.8	90.5	95.4	79.6	77.3	77.3	76.9	83.5	97.3	2
3	0.6	0.0		97.6	98.5	97.8	97.6	98.3	98.0	97.9	98.3	98.2	97.3	92.0	87.3	93.0	90.5	86.8	90.5	95.4	79.6	77.3	77.3	76.9	83.5	97.3	3
4	2.7	2.4	2.4		98.3	99.6	100.0	99.3	99.0	98.9	98.2	99.2	98.3	93.8	88.5	93.7	91.9	88.5	92.0	96.9	80.0	77.5	77.5	77.3	83.3	99.2	4
5	2.1	1.6	1.6	1.7		98.5	98.3	99.0	98.7	98.6	99.9	98.9	98.0	93.0	87.7	93.4	91.0	87.5	91.2	96.4	79.7	77.5	77.5	77.0	83.1	98.0	5
6	2.9	2.3	2.3	0.4	1.6		99.6	99.4	99.2	99.0	98.3	99.3	98.5	93.4	88.1	93.6	91.5	88.1	91.6	96.5	79.8	77.6	77.6	77.2	83.2	99.3	6
7	2.7	2.4	2.4	0.0	1.7	0.4		99.3	99.0	98.9	98.2	99.2	98.3	93.8	88.5	93.7	91.9	88.5	92.0	96.9	80.0	77.5	77.5	77.3	83.3	99.2	7
8	2.3	1.7	1.7	0.7	1.0	0.6	0.7		99.7	99.6	98.9	99.9	99.0	93.7	88.4	94.1	91.7	88.1	91.9	97.1	80.1	77.9	77.9	77.5	83.8	99.0	8
9	2.6	2.0	2.0	1.0	1.3	0.8	1.0	0.3		99.3	98.6	99.6	98.7	93.4	88.1	93.8	91.5	88.1	91.6	96.8	79.8	77.6	77.6	77.2	83.5	99.0	9
10	2.7	2.1	2.1	1.1	1.4	1.0	1.1	0.4	0.7		98.5	99.4	98.6	93.3	88.0	93.7	91.3	87.7	91.5	96.6	79.7	77.7	77.7	77.3	83.3	98.6	10
11	2.3	1.7	1.7	1.9	0.1	1.7	1.9	1.1	1.4	1.6		98.7	97.9	93.1	87.5	93.3	90.9	87.4	91.0	96.2	79.6	77.3	77.3	76.9	82.9	97.9	11
12	2.4	1.8	1.8	0.8	1.1	0.7	0.8	0.1	0.4	0.6	1.3		98.9	93.7	88.4	94.1	91.7	88.1	91.9	96.9	80.1	77.7	77.7	77.5	83.8	98.9	12
13	3.3	2.7	2.7	1.7	2.0	1.6	1.7	1.0	1.3	1.4	2.1	1.1		94.3	89.2	94.7	92.0	88.1	92.4	97.6	80.7	78.2	78.2	77.7	84.3	98.0	13
14	8.9	8.6	8.6	6.5	7.5	7.0	6.5	6.7	7.0	7.2	7.3	6.7	6.1		90.8	95.8	93.7	89.1	94.3	95.8	81.8	79.6	79.6	79.7	84.7	93.0	14
15	14.7	14.5	14.5	12.9	14.0	13.5	12.9	13.1	13.5	13.7	14.2	13.1	12.1	10.1		92.0	91.5	88.9	93.4	90.8	81.2	77.6	77.6	77.5	84.5	88.0	15
16	8.0	7.5	7.5	6.7	7.0	6.9	6.7	6.2	6.6	6.7	7.2	6.2	5.6	4.4	8.6		95.4	90.1	95.8	96.5	82.1	80.1	80.1	80.0	85.6	93.1	16
17	10.7	10.4	10.4	8.8	9.8	9.3	8.8	8.9	9.3	9.4	9.9	8.9	8.6	6.7	9.2	4.8		90.6	95.4	93.8	81.4	79.1	79.1	79.4	86.3	91.0	17
18	15.4	15.0	15.0	12.9	14.1	13.4	12.9	13.4	13.4	14.0	14.3	13.4	13.4	12.2	12.3	10.9	10.2		92.9	89.8	80.5	77.9	77.9	77.7	82.8	87.7	18
19	10.8	10.5	10.5	8.7	9.7	9.2	8.7	8.8	9.2	9.3	9.8	8.8	8.2	6.1	7.0	4.4	4.8	7.6		94.8	81.8	79.6	79.6	79.7	86.4	91.2	19
20	5.1	4.8	4.8	3.2	3.8	3.6	3.2	3.0	3.3	3.5	3.9	3.2	2.4	4.4	10.1	3.6	6.5	11.3	5.5		81.1	79.1	79.1	79.0	84.7	96.1	20
21	25.5	25.5	25.5	24.9	25.4	25.2	24.9	24.7	25.2	25.4	25.6	24.7	23.9	22.1	22.9	21.7	22.6	23.9	22.1	23.2		88.8	88.8	88.7	81.0	79.4	21
22	29.1	28.7	28.7	28.6	28.5	28.3	28.6	27.9	28.3	28.1	28.8	28.1	27.4	25.1	28.3	24.2	25.7	27.7	25.2	25.8	12.4		100.0	99.3	78.4	77.3	22
23	29.1	28.7	28.7	28.6	28.5	28.3	28.6	27.9	28.3	28.1	28.8	28.1	27.4	25.1	28.3	24.2	25.7	27.7	25.2	25.8	12.4	0.0		99.3	78.4	77.3	23
24	29.3	29.4	29.4	28.8	29.2	29.0	28.8	28.5	29.0	28.8	29.4	28.5	28.1	24.8	28.5	24.4	25.2	27.9	24.9	26.0	12.6	0.7	0.7		78.4	76.9	24
25	20.0	19.6	19.6	19.9	20.3	20.1	19.9	19.3	19.7	19.9	20.5	19.3	18.5	17.9	18.2	16.7	15.7	20.6	15.6	17.9	23.3	26.8	26.8	26.8		82.8	25
26	3.3	2.7	2.7	0.8	2.0	0.7	0.8	1.0	1.0	1.4	2.1	1.1	2.0	7.5	13.6	7.3	9.8	13.9	9.7	4.1	25.8	28.8	28.8	29.4	20.7		26
	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	

1 OM243951 10/Egypt/Giza
2 OP219681 NM09/2019
3 OP219679 NM97/2019
4 MG717683 quail/Egypt/SDU-2/2016
5 OK533420 Turkey/Egypt/BSU-21/2021
6 OK533407 Chicken/Egypt/BSU-8/2017
7 MG717684 teal/Egypt/SDU-3/2016
8 OK533416 Chicken/Egypt/BSU-17/2020
9 MW285792 Chicken/Egypt/AR2178-14/2013
10 OK533411 Turkey/Egypt/BSU-12/2018
11 OK533421 Turkey/Egypt/BSU-22/2021
12 MK495888 Chicken/Egypt/Luxor/2012/8
13 KC542905 Chicken/China/Liaoning/01/2009
14 JN986838 Chicken/ZA/AL495/04
15 HQ697254 Chicken/Banjarmasin/010/10
16 KJ782375 go/CH/GD-QY/1997
17 GQ338310 ND/03/044
18 MN699677 Chicken/Tangerang/2014
19 AY444497 moluccan/Indonesia/904/87
20 KC542893 Chicken/China/Liaoning/02/2005
21 JN872152 Ulster
22 JN872150 B1
23 EU289029 VG/GA
24 ON713864 LaSota
25 KY042130 pigeon/Egy/Helwan/44/1106/2015
26 PP130129 CLEVB1

Fig. 3. Results of sequence identity Matrix of the present NDV isolate and other published available sequence on Genbank.

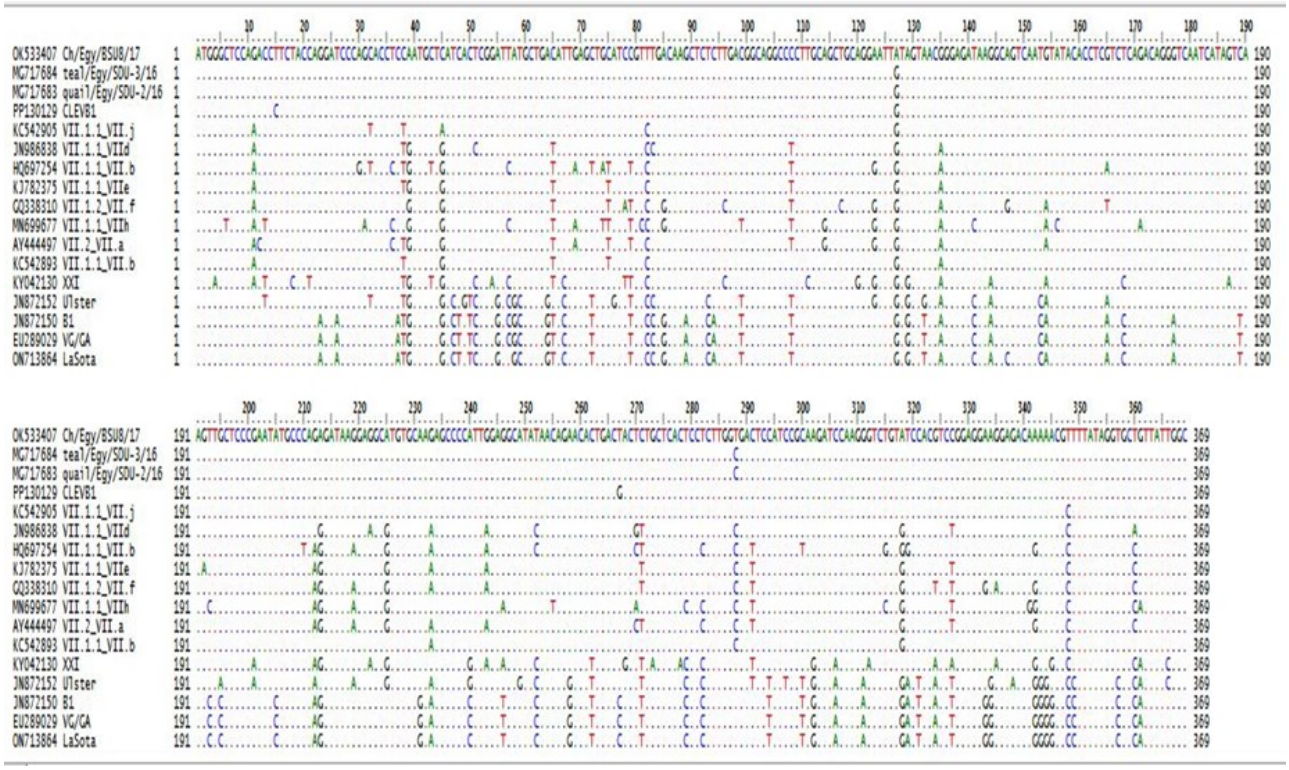

Fig. 4. Amino acid sequences of NDV isolates, reference strains and vaccine strain (on the gene bank). Dots denote identical amino acid sequence.

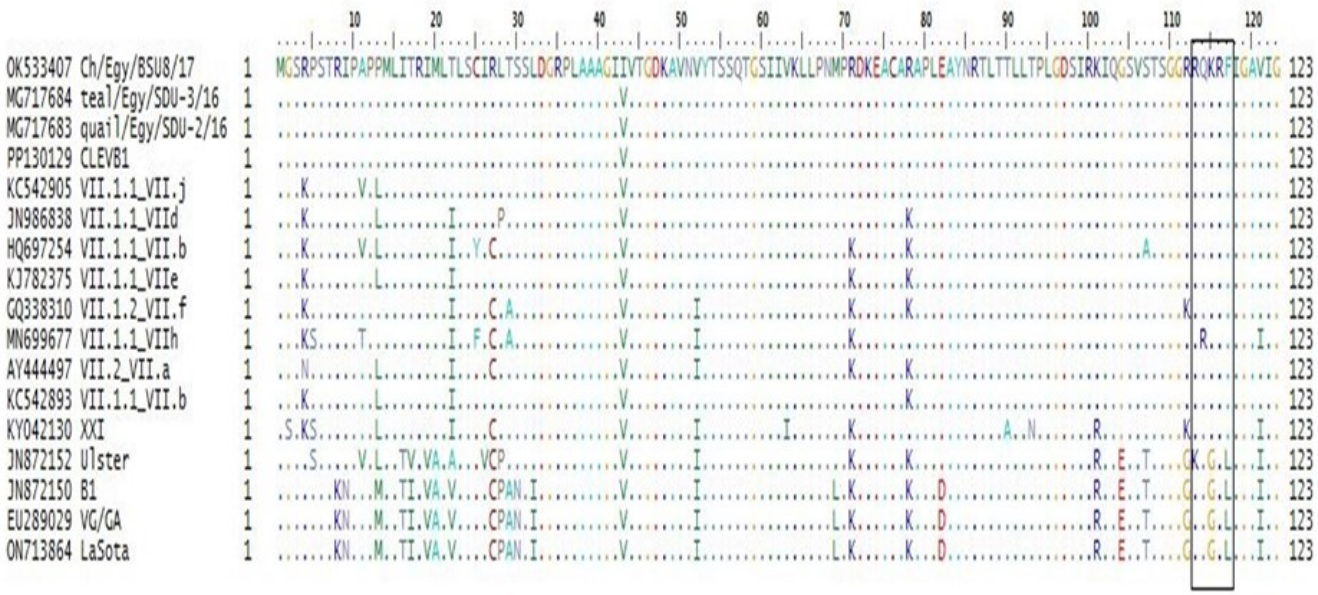

Fig. 5. Nucleotide sequences of the F gene of NDV isolates. Dots indicate position where the sequence is identical to the consensus. Proteolytic cleavage site motifs (residues 112 to 117) of the NDV strains F-gene were compared.

backyard poultry has resulted in significant genetic diversity and emerging of new genotypes, complicating control measures. Continuous virological surveillance and phylogenetic analysis of the viral F-protein is very important to understand the virus's geographical spread, predicting outbreaks, and informing biosecurity and vaccination strategies. In the present study, oropharyngeal and cloacal swabs were collected from diseased and freshly dead backyard chickens in Qalubia governorate. Clinical signs that appeared on these chickens, including depression, twisting of head and neck, ruffled feathers, and gasping with high mortality, were consistent with a previous report[14] indicating the appearance of signs related to NDV infection. Postmortem examinations of the same infected cases revealed pinpoint hemorrhages in the tip of the proventricular gland, hemorrhagic intestinal lesions, and splenomegaly.[14] HA testing revealed that allantoic fluid collected from the suspected samples tested positive, as NDV isolates are known to agglutinate red blood cells.[3] RT-PCR was used due to more sensitive, high specific, rapid and low cost tool for diagnosis and subtyping for NDV. Because of the highly mutable nature of the NDV RNA genome,[15] strain characterization involved sequencing and phylogenetic analysis of the F-protein cleavage sites.[16] Selected positive PCR sample was sequenced and deposited in the NCBI GenBank with the accession number PP130129 (CLEVB1/2024). Phylogenetic analysis revealed that the recent NDV isolate belongs to class II genotype VII.1.1, according to a study[17] that stated that NDV was divided into two main groups: class I and II. Viruses from class I belong to a single genotype which are virulent strains, whereas class II viruses are mostly virulent strains and classified into 21 genotypes (I-XXI) and many sub-genotypes. In addition,[17] genotype VII was clustered into three subgenotypes (VII.1.1, VII.1.2, and VII.2) based on nucleotide distances, branch support and number of independent isolates. Diversity of genotype VII strains among class II genotypes was reported resulting in outbreaks in commercial flocks even though they were vaccinated.[6] Genotype VII.1.1 emerged around 1985 in the Far East and rapidly spread to Asia, the Middle East, Europe, and Africa. Pathotyping of the CLEVB1 isolate using the amino acid sequence of the F0 protein cleavage site revealed the presence of the ^{112}RRQKRF^{117} motif, which is characteristic of velogenic NDV strains.[2] The phenylalanine residue at position 117 is linked to neurological symptoms in infected birds.[18] Sequence analysis also revealed a high degree of similarity (99.2 %) between the CLEVB1 isolate and two other NDV strains (teal/Egypt/SDU-3/2016 and quail/Egypt/SDU-2/2016) isolated from migratory birds in El-Fayoum, in 2016. These findings support previous research indicating that migratory birds, such as the green-winged teal, play an important role in the introduction of new viruses into Egypt.[19] The risk of NDV introduction from migratory birds is particularly high in backyard poultry systems, where birds have frequent contact with wild birds due to lack of biosecurity measures and poor vaccination practices.[20] In 2017, chicken/Egypt/BSU-8/2017 isolated from Giza governorate had the greatest identity percentage (99.3 and 99.6 %) with the recent new isolate which submitted in 2024 and 2016, respectively, which agreed other authors[21] who said that once introduced and adapted to poultry, viruses are able to keep circulating among domestic bird populations. The findings of the present study confirm the persistent risk of NDV introduction via wild birds, as demonstrated by previous epidemiological studies linking avian paramyxoviruses in Europe and Africa. Therefore, continuous surveillance and genetic monitoring are required for the effective control of NDV in Egypt and elsewhere. The obtained results confirm the ongoing risk of virus transmission from wild birds, as indicated by previous studies on the epidemiology of avian paramyxoviruses in Europe and Africa, in which similar results were found.[22]

Conclusions

A recent NDV strain was successfully isolated and identified from infected backyard chickens in Qalubia governorate, Egypt. The strain, CLEVB1/2024, was determined to be a velogenic strain belonging to class II genotype VII.1.1. The genetic analysis revealed a high identity between the recent isolated NDV and the previously isolated from migratory birds, highlighting the possibility of continued virus transmission through interactions between backyard and wild bird populations. Moreover, the results clarify the

significance role of backyard poultry vaccination to prevent and control the spread of NDV in Egypt.

Conflict of interest

The authors declare that there is no conflict of interest.

Author's contributions

Samir A. Nassif: conducted the experiment, drafted the manuscript. Designed the study and followed up the experiment and critically reviewed the manuscript. Participated in study design and followed up on the practical work.

Reem A. Soliman: designed the study and followed up the experiment and critically reviewed the manuscript. Participated in study design and followed up on the practical work.

Marwa Fathy: designed the study and followed up the experiment and critically reviewed the manuscript. Participated in study design and followed up on the practical work.

Ahlam Mourad: designed the study and followed up the experiment and critically reviewed the manuscript. Participated in study design and followed up on the practical work.

Esraa Fouad: designed the study and followed up the experiment and critically reviewed the manuscript. Participated in study design and followed up on the practical work.

Hala Ahmad: designed the study and followed up the experiment and critically reviewed the manuscript. Participated in study design and followed up on the practical work.

Mahmoud M. Abotaleb: conducted the experiment, drafted the manuscript. Designed the study and followed up the experiment and critically reviewed the manuscript. Participated in study design and followed up on the practical work.

All authors have read and agreed to the published version of the manuscript.

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Aislamiento y caracterización de un virus reciente de la enfermedad de Newcastle, proveniente de pollos de traspatio, en Egipto

Resumen

La vigilancia genómica del virus de la enfermedad de Newcastle es fundamental para determinar la diversidad genética de las cepas circulantes y mejorar la preparación y respuesta ante posibles brotes. Las aves de traspatio siguen planteando importantes problemas para el control del virus de la enfermedad de Newcastle debido a la bioseguridad inadecuada, las prácticas de vacunación deficientes y la estrecha interacción con aves migratorias. El objetivo de este estudio fue aislar y caracterizar el virus de la enfermedad de Newcastle, a partir de pollos de traspatio infectados en la gobernación egipcia de Qalubia. La prueba de hemaglutinación y la reacción en cadena de la polimerasa con transcripción inversa, dirigida a un segmento parcial del gen F, confirmaron que se aisló el virus de la enfermedad de Newcastle. Un aislamiento positivo del virus de la enfermedad de Newcastle fue secuenciado y analizado filogenéticamente, revelando que pertenece al genotipo VII.1.1 del virus de la enfermedad de Newcastle. Se depositó en el NCBI GenBank (CLEVB1/2024) con el número de acceso PP130129. Los datos confirmaron, además, que el aislamiento CLEVB1 tenía el motivo ^{112}RRQKRF^{117} del sitio de clivaje, característico de las cepas velogénicas del virus de la enfermedad de Newcastle. Basándose en la comparación de la secuencia de aminoácidos, el aislado CLEVB1 compartió un 99,2 % de homología con las cepas teal/Egypt/SDU-3/2016 y quail/Egypt/SDU-2/2016, ambas aisladas de aves migratorias en 2016; de ahí que se cree que el aislado CLEVB1 se originó a partir de dos cepas del virus de la enfermedad de Newcastle previamente identificadas, lo que pone de relieve los resultados de las interacciones en curso entre las aves migratorias y las aves de traspatio. Se recomienda que la vigilancia continua del virus de la enfermedad de Newcastle y la vacunación sistemática en los sectores con aves de traspatio sean obligatorias para reducir el riesgo de futuros brotes.

Palabras clave: virus de la enfermedad de Newcastle; proteínas virales de fusión; reacción en cadena de la polimerasa; Egipto.

Received: March 13, 2025 *Accepted: April 28, 2025*