Journal of Current Veterinary Research

ISSN: 2636-4026

Journal homepage: http://www.jcvr.journals.ekb.eg

Virology

Characterization of Newcastle Disease Virus Circulating in Vaccinated Poultry Farms in Egypt During 2020

Asmaa Ibrahim Abdelaziz Zain Eldeen[1*], Abdelgayed M. Younes[2], Khalid Mohammad Elbayoumi[3], Abdel Hamid Ismael Mussa[4], Mohammad Mahmoud Effat[1] and Mohammad Abdel Hamid AboElkhair[4].

(1)Department of Microbiology and Immunology, National Research Centre, Dokki, Giza, Egypt.
(2)Hydrobiology Department, National Research Centre, Dokki, Giza, Egypt.
(3)Department of Poultry Diseases, Veterinary Research Division, National Research Centre, Dokki, Giza, Egypt.
(4)Department of Virology, Faculty of Veterinary Medicine, University of Sadat City, Egypt.

**Corresponding author:* asmaa_ay@yahoo.com *Received: 25/2/2022* *Accepted: 28/4/2023*

ABSTRACT

Newcastle disease virus (NDV) is responsible for huge economic losses in birds worldwide. This study aims to isolate, identify, pathotype, and genetically characterize field isolates of NDV from various governorates in Egypt in 2020 (Giza, Gharbya, Kalyobiya, Cairo, and Menofiya). After the third passage, 23 of 50 infected samples (46%) showed positive hemagglutination activity in the collected allantoic fluid. The results revealed that all isolates from the governorates of Menoufia, Cairo, and Giza were velogenic, with a Mean death time (MDT) of 48 hours and an Intracerebral pathogenicity index (ICPI) ranging from 1.55-1.65. Sequencing the 766-bp segment, including the 3' end of the matrix gene and the 5' end of the fusion gene from three different isolates, indicated that they all possessed the 112RRQKRF117 motif typical of the virulent isolates. The sequenced isolates were grouped with class II genotype VIIb and had a high genetic distance to the LaSota reference strain. It is concluded that NDV strains circulating among vaccinated poultry farms in Egypt are virulent genotype VII and had a high genetic distance to the LaSota reference strain, resulting in significant economic losses.

Keywords: Characterization, Egypt, Genotype VII and Newcastle disease virus.

INTRODUCTION

A highly contagious viral disease of birds called Newcastle disease (ND) significantly harms the global economy. Avian Paramyxovirus Type 1, often known as the Newcastle disease virus (NDV), is the disease's origin. NDV was recently reclassified as avian orthoavulavirus-1, a member of the *Paramyxoviridae* family, based on a phylogenetic examination of the large RNA-dependent polymerase protein (L-protein) (Rima et al., 2019).

NDV contains a 15 kb negative polarity single-stranded RNA genome. It encodes six

structural proteins. They include the genes for hemagglutinin-neuraminidase, matrix protein, phosphoprotein, nucleocapsid protein, and RNA-dependent polymerase from the 5' to the 3' ends (Alexander, 2003).Several genes lead to NDV virulence, although the crucial region for significant virulence changes is the amino acid sequence motif at the precursor F-cleavage glycoprotein's site (Romer-Oberdorfer et al., 2003). NDV's pathogenicity is connected to the amino acid sequence motif of the fusion protein's protease cleavage site, as well as the capacity of certain cellular proteases to break this protein. Furthermore, NDV can be classified into 21 genotypes (2.I-2.XXI) (Dimitrov et al., 2019). A broad variety of bird hosts (27 of the 50 orders) are prone to infection, with chickens being the most vulnerable (Aldous et al., 2007).

Egypt has been designated as an NDV endemic nation. Despite continued vaccination campaigns, outbreaks continue to occur in vaccinated chicken flocks, posing a danger to the national poultry sector (Nabila et al., 2014). Many NDV outbreaks may be caused by the haphazard use of intense vaccinations, frequent mutations, and the emergence of novel pathotypes of NDV.NDV genotypes II and VII has been reported in Egypt (Naguib et al., 2021). ND is a severe threat to the growth of the poultry industry since it affects both domestic and wild birds. Doyle (1927) recognized ND for the first time in England, while Doubney and Mansi (1947) reported an enzootic of the illness in Egypt. The severity of the disease produced by a virulent strain of NDV has been found to be greatly influenced by the immune status of the host. It affects a number of bird species and can result in significant mortality rates, respiratory distress, diarrhoea, loss of egg production, and neurological symptoms (Alexander, 1991).Respiratory discomfort and neurological problems were the most common clinical findings in the impacted farms. Respiratory symptoms included wet eyes, sneezing, rales, and dyspnea. Neurological signs included tilting of the head and torticollis. Mortality varied, ranging from 15–20 percent in most cases to 80 percent or more in some reports (Zanaty et al., 2019).

Based on the data presented above, the primary purpose of this research is to identify the NDV strains responsible for repeated outbreaks on vaccinated Egyptian farms. The isolates are then identified using haemagglutination (HA), pathotyped using mean death times (MDT) in chicken embryonated eggs, and intracerebral pathogenicity index (ICPI) in one-day old chicks. The secondary goal is to genetically characterize field isolates of NDV acquired from different governorates in Egypt in 2020 and examine their genetic distance from related strains.

MATERIAL AND METHODS

Samples collection:

From five Egyptian governorates (Menoufia, Cairo, El-Gharbia, El-Kalyobiya, and Giza), fifty tissue sample pools (trachea, proventriculus, lung, gut, cecal tonsils, spleen, and liver) were collected from ND suspected chicken farms under strictly aseptic conditions(Table 1).The all flocks had been immunized against NDV and other viral agents. Clinical symptoms included difficulty breathing, green diarrhea, ruffled feathers, anxious symptoms, a decline in productivity, and a high death rate. These samples were tagged and brought to the virology lab at National Research Centre as soon as possible on ice. Finally, they were kept at -80°C until processing and isolation.

Table (1): Data of collected samples from Egyptian governorates chicken flocks for NDV detection:

location	Samples ID	Type of bird	Flocks no.	Age	Clinical signs and lesions	vaccination	Mortality	Date of collection
Giza	1-7	Broiler	1000	40 days	greenish diarrhea, drop of production	**vaccinated**	300	7/2019
	8-11	Layers	900	1 year	proventricular hemorrhage, congested spleen		250	9/2020
Cairo	12-17	Broiler	1500	36 days	greenish diarrhea, sneezing		350	12/2020
	18-20	Layers	1200	230days	paralysis, Enlarged hemorrhagic cecal tonsils, hemorrhage on the tips of the proventriculus		200/3day	4/2019
Kalyobiya	21-31 32-37	Breeders Broiler	800 2000	360 days 32days	respiratory difficulties, greenish diarrhea, Tracheitis and congestion of the liver, and cecal tonsils		50 100	11/2019 9/2019
Gharbiya	38-42	Layers	2500	300 days	Nasal discharge, greenish diarrhea, high mortality		90	10/2020

Menoufia	43-50	Broiler	3000	28 days	Mottled enlarged spleen, proventricular hemorrhage		100/day	11/2020

Isolation of NDV on SPF-ECE:

Embryonic chicken eggs (ECE) from a specific pathogen-free (SPF) production farm were obtained (Koum Oshiem, Fayoum, Egypt). By calculating the mean death time (MDT) of egg embryos, it was utilized for isolation, infectivity titration, and pathotyping of NDV until it was 10 days old. Fertile eggs were set in a humid incubator at 37–38°C and turned twice daily**.**

Virus isolation was carried out by the procedure established by (OIE, 2012). Briefly, organ suspensions were first centrifuged for 5 minutes at 4000 rpm in a bench-top centrifuge. The supernatants were given antibiotics (penicillin 2000 units/ml, streptomycin 0.01 l/ml, gentamycin 50 g/ml, and mycostatin 1000 units/ml) and incubated for an hour at 4 ℃. Following that, 0.2 ml of the supernatant was injected into the allantoic cavity of five 10-day-old SPF-ECE. Deaths that occurred after that (from the second to fourth day after inoculation) were regarded as specific. Deaths that occurred during the first 24 hours of inoculation (PI) were referred to as nonspecific. Allantoic fluid (AF) was collected, centrifuged at 3000 rpm for five minutes, divided into aliquots, and kept in sterile screw-capped vials at -80°C until needed. In SPF-ECE, four basic viral passages were executed. The HA test given evidence that the virus was present.

Pathogenicity tests:

Thirty one-day-old SPF chicks kept in a strictly isolated, mosquito-proof environment performed pathotyping of the NDV isolates using Mean Death Time (MDT) and Intracerebral Pathogenicity Index (ICPI) calculations in accordance with (OIE, 2012).

Serological identification of NDV:

For the creation of hyperimmune serum, 20 unvaccinated SPF chickens were given subcutaneous injections of killed-oil NDV vaccine (VET.SER and VACC.RES. Inst., Egypt) on the first day of age, followed by injections of Lasota NDV vaccine on the 7^{th}, 14^{th}, 21^{st}, 28^{th}, and 42^{nd}days of age. Chickens that had received vaccinations had their blood isolated into hyper-immune serum, which was then stored at -20°C for later use (Iqbal et. al., 2003).

Molecular identification:

Using QIAamp viral RNA extraction Kits in accordance with the manufacturer's instructions, the genomic viral RNA was isolated from collected HA positive allantoic fluid. The QIAGEN® OneStep RT-PCR kit was used to perform one-step RT-PCR (QIAGEN, USA).

The Oligonucleotide primersM2: TGGAGCCAAACCCGCACCTGCGG, and F2: GGAGGATGTTGGCAGCATT (Metabion, Germany) were used to amplify 766 bp of NDV (Mase et al., 2002). The 1.5% agarose gel in TAE buffer was used for gel electrophoresis to separate the PCR products. Ethidium bromide (Merck, Germany) was used for staining, and the results was shown under UV trans-illumination. The QIAquick gel extraction kit (Qiagen, USA) was used to purify the PCR products before they were sequenced in both directions on an automated ABI 3730 DNA sequencer (Applied Biosystems, USA).

Phyllogenetic tree:

MEGAX software (Tamura et al., 2013) was used to align the sequences using the ClustalW technique. The nucleotide sequences and the NDV sequences available in GenBank were compared. Sequences which were aligned were used to create a phylogenetic tree. The pathotypes of isolated NDV were identified using the deduced amino acid sequences.

RESULTS

Clinical findings:

Clinical signs included depression, ruffled feathers, decreased food consumption, respiratory difficulties, greenish diarrhea, nervous symptoms, a drop of production, and high mortality. Necropsies found septicemia which indicated tracheitis and air-sacculitis, congestion of spleen, lungs, liver, subcutaneous blood vessels, and gallbladder enlargement. There was also an increase in hemorrhage at the tips of the proventriculus glands (Fig. 1).

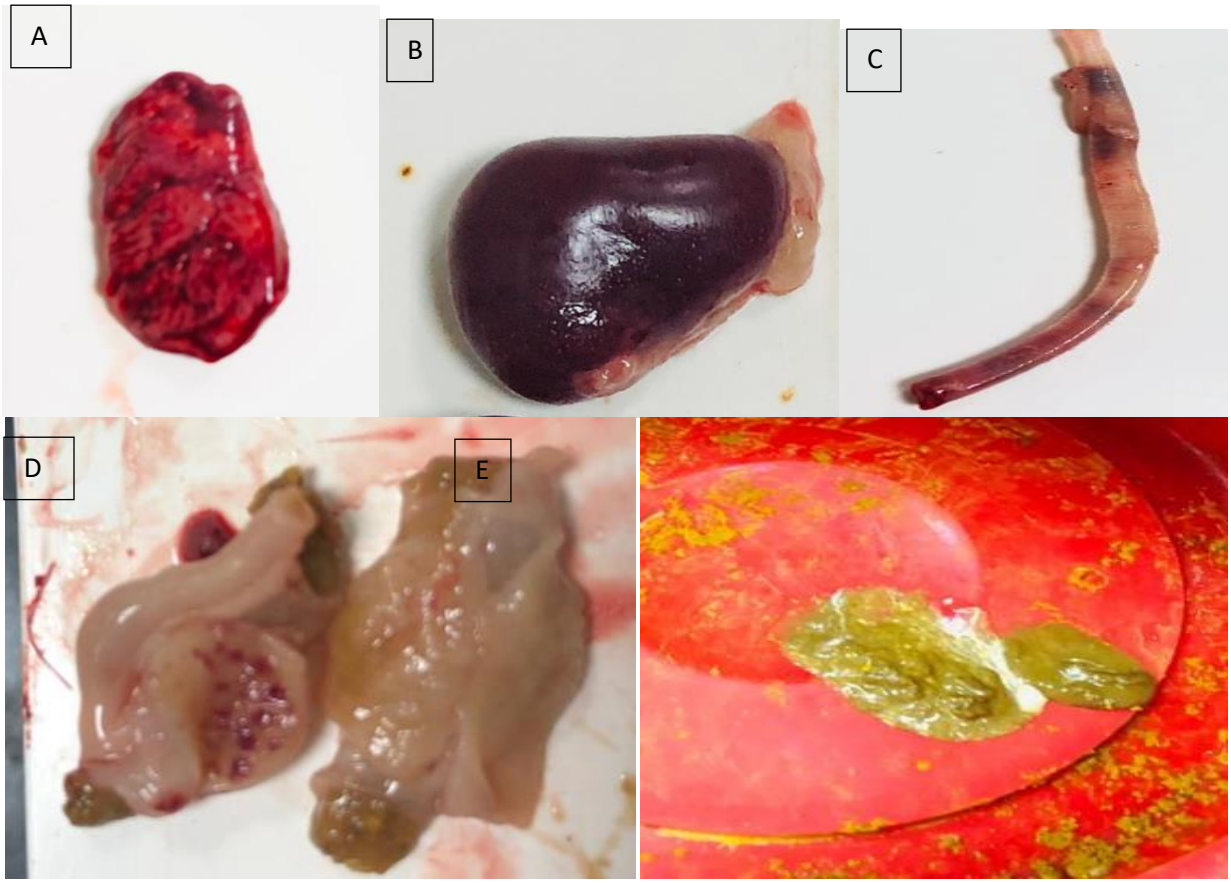

Fig. (1): A) Congested lungs, B) Congested spleen, C) Tracheitis, D) Hemorrhagic cecal tonsils, E) Greenish diarrhea.

Isolation of virus on SPF-ECE from suspected samples:

Table (2) shows that after the third passage, 23 of the 50 infected samples (or 46% of them) showed positive hemagglutination activity in the collected allantoic fluid. Positive isolates were found in three out of five samples from Gharbia, three out of seventeen samples from Kalyobiya, eight out of nine samples from Cairo, five out of eleven samples from Giza, and four out of eight samples from Menofiya. These findings were supported by the use of the HA test on the collected allantoic fluid of

infected ECE, which yielded HA titers of 6 log2, 9 log2, 5 log2, 8 log2, 8 log2, and 7 log2 HA units/ml for Giza, Cairo, Kalyobiya, Gharbiya, Fayoum and Menofiya isolates respectively.

Table (2): Results of HA, HI of infected allantoic fluids for detection of NDV Samples collected from Egyptian governorates chicken's flocks:

Governorate	**No. of samples**	**No. of positive samples**	**% of positive samples**	**HA titer**	**HI results**			**No. of RT-PCR positive Samples for ND**
					H5N1	**H9N2**	**ND**	
Giza	11	5	45.45	6	0	0	5	1
Cairo	9	8	88.88	9	0	0	8	1
Kalyobiya	17	3	17.64	5	0	0	3	0
Gharbiya	5	3	60	8	0	0	3	0
Menoufia	8	4	50	7	0	0	4	1
Total	**50**	**23**	**46**		0	0	**23**	**3**

Pathotyping of NDV isolates:

Pathotyping was performed on isolates from Giza, Cairo, and Menofiya to determine if they were virulent or avirulent (vaccine) strains using the MDT and ICPI tests. Table (3) indicated that all isolates were velogenic with an MDT of 48 hours and an ICPI ranging from 1.55-1.65. Within 48–96 hours of the initial passage, the injected embryos displayed severe bleeding, congestion, and death (Fig.2).

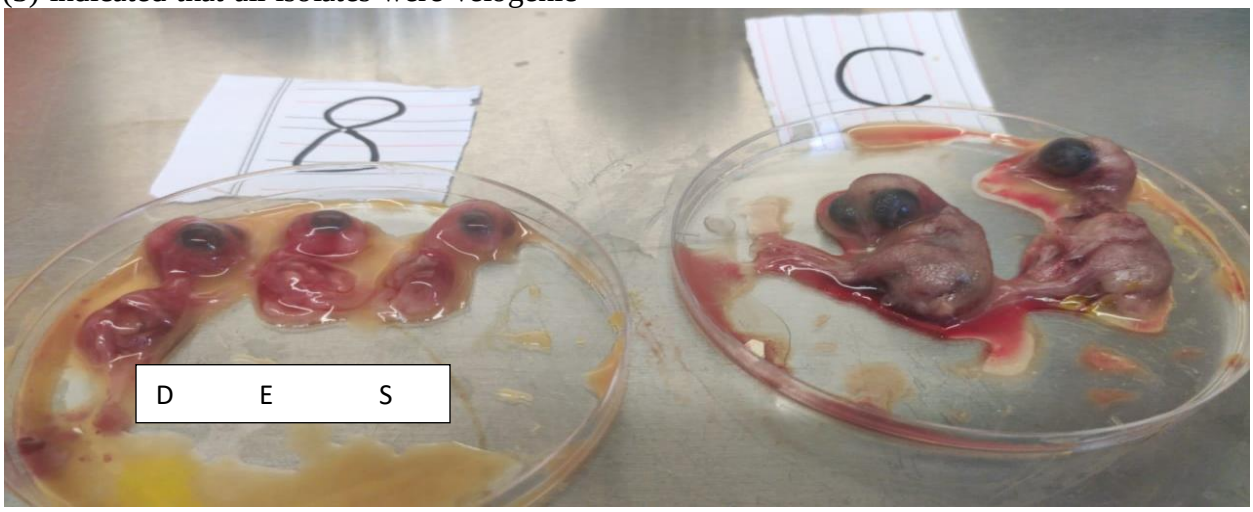

Fig. (2): Inoculated ECE showed the effect of NDV. (E and S): Chicken embryo (59-60 hours Post inoculation) showing diffuse redness and the subcutaneous tissue of the head is filled with blood and the blood vessels over the body were prominent. (D): Chicken embryo (60-62 hours Post inoculation), showing diffuse congestion and hemorrhage in the whole body and edema in the head region. (C): Control non-inoculated NDV chicks.

Table (3) indicated that all isolates were velogenic with an MDT of 48 hours and an ICPI ranging from 1.55-1.65:

The isolates origin	MDT(hours)	ICPI	Pathotype
Cairo	48	1.652	Velogenic
Giza	49	1.601	Velogenic
Menofiya	48	1.55	Velogenic

MDT values: <60 hours denoted for velogenic strains, 60–90 hrs denoted for mesogenic strains and >90hrs denoted for lentogenic strains, while ICPI values <0.5 denoted for lentogenic strains, 0.5–1.5 denoted for mesogenic strains and >1.5 denoted for velogenic strains, according to OIE (2009).

Molecular identification of the NDV isolates:

The virulent NDV strains' matrix and fusion genes were detected using the RT-PCR technique, which amplified a 766-bp fragment. The 766-bp fragment of the M gene and F gene was sequenced and submitted to GenBank under the accession numbers OM243949, OM243950, and OM243951, respectively. As shown in Fig. (3), the sequence of three strains revealed that they carried the 112RRQKRF117 motif, indicative of virulent strains. When the three sequences were aligned, they revealed differences in nucleotide identity. The changes mentioned above in nucleotide identity resulted in many amino acid substitutions, as seen in Table (4). Isolate 10 (accession NoOM243949) shared three amino acid substitutions at positions L15F, A75V, andG110Rwith isolate 5 (accession NoOM243950), while isolate 11 (accession NoOM243951) showed one amino acid substitution at positions (S30N). When the sequenced strains were blasted with other NDV isolated in Egypt, they were closely related to avian avulavirus 1 isolates collected from Luxor in 2012, ranging from 97.9% to 98.17%.

According to the phylogenetic analysis, the three tested isolates grouped with genotype VII of previously described velogenic NDV isolates worldwide (Fig.4). The studied isolates showed nucleotide difference with the LaSota reference strain and clustered away from it. Furthermore, the three investigated isolates grouped apart from earlier strains recovered from the governorates of Giza and Menofiya (Fig. 3).

Table (4) Indicated amino acid substitutions between three isolates:

Position	15	30	75	110
Isolate 5	L	S	A	G
Isolate 11	L	N	A	G
Isolate 10	F	S	V	R
consensus	L	S	A	G

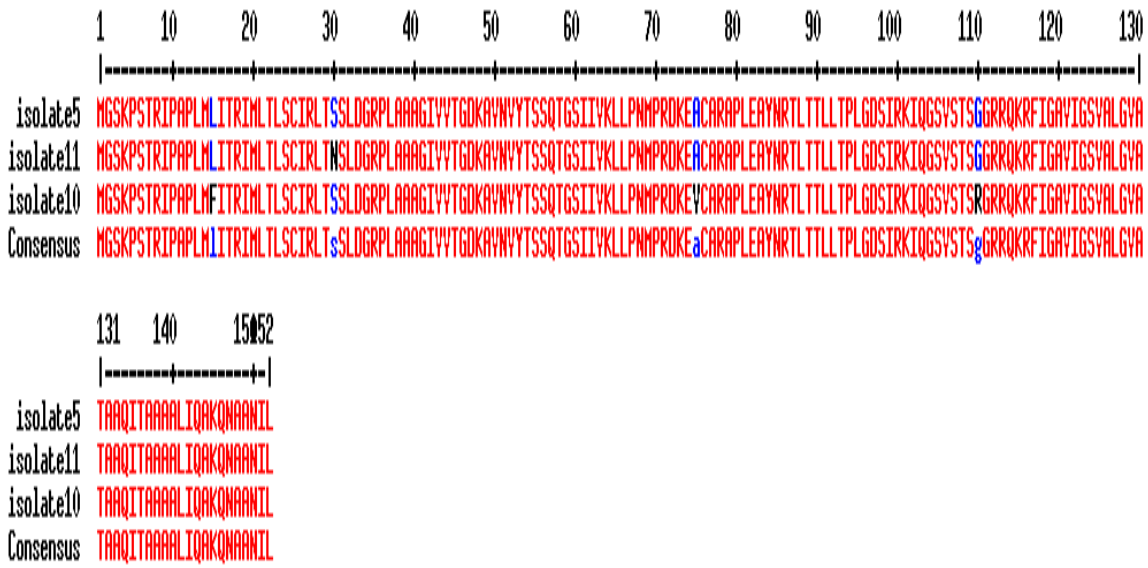

Fig. (3): Amino acid sequence identity showing identity percent based on A. Sequence comparison of F protein. Results confirmed that all strains shared the cleavage site motif 112 RRQKRF117.

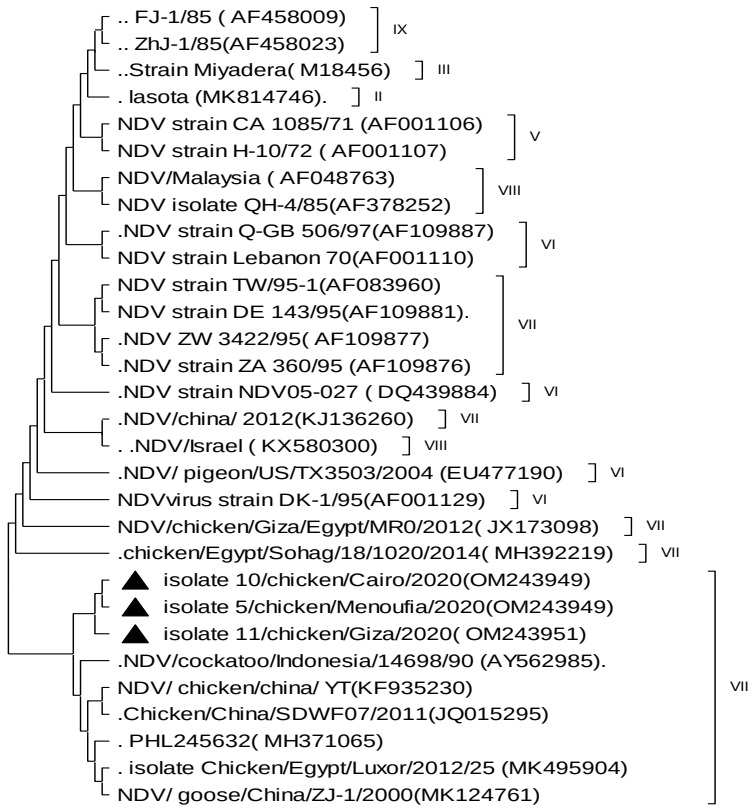

Fig. (4): Phylogenetic tree based on a partial sequence of NDV F gene using the neighbor-joining method for tree construction, showing the relationship between the selected Egyptian NDV isolates in the present study with related NDV strains from a gene bank.

DISCUSSION

In Egypt, outbreaks of NDV are still frequently occurring in vaccinated poultry flocks, despite the intensive vaccination programs (Mohamed et al., 2011 and Nabila et al., 2014). The main objective of the present work was identification of circulating NDV in Egyptian chicken and development of control methods for infection. In the first experiment, the isolation, identification, pathotyping and genotyping of NDV isolates from infected chicken flocks from different governorates Giza, Gharbya, Qulyubia, Cairo, and Menoufiya in Egypt in 2020 was carried out. The study reported virus isolation on SPF-ECE then the detection of HA activity. This was in agreement with that described by OIE (2012) and Abdelrahim and Elhag (2014).

Regarding isolation of ND virus on SPF-ECE egg, isolation of the virus was performed through the inoculation into allantoic cavity of SPF-ECE for three passages. The Samples inoculated in SPF-ECE induced signs in 23 samples out of 50 samples. Similar results were reported by

Hussein et al. (2013); Ahmadi et al. (2014) and Salehinezhad et al. (2014).

Field samples from suspected NDV positive chicken flocks with successful isolation on SPF-ECE were subjected for HA titration after the third passage. HA titers of the isolates ranged from 6 log2 HA units/ml for isolates from Giza, 9 log2 HA units/ml for isolates from Cairo, 5 log2 HA units/ml for isolates from Qulyubia, 8 log2 HA units/ml for isolates from Gharbiya, 7 log2 HA units/ml for isolates from Menoufia. This result agreed with that obtained by Bilal et al. (2014), Mantip et al. (2011) and Pansota et al. (2013). The present study revealed pathotyping of NDV isolates by calculation of the mean death time (MDT) and intracerebral pathogenicity index (ICPI). This was in agreement with that described by OIE (2012) and Munir et al. (2012).

Regarding MDT, the result showed that isolates from Giza and Menoufiya governorates were velogenic with MDT of 48 hours and isolate from Cairo was velogenic with MDT of 49 hours. This result agreed with that obtained by Mantip et al. (2011), Munir et al. (2012), OIE (2012), Abdelrahim and Elhag (2014) and Mehrabanpour et al. (2014). Regarding ICPI, the result showed that isolate from Giza was velogenic with ICPI of 1.652 and isolates from Cairo and Menoufiya governorates were velogenic with ICPI of 1.601 and 1.601 respectively. This result agreed with that obtained by Nabila et al. (2014).

Molecular detection of three isolates NDV using RT-PCR for amplification of the 3' end of the M protein encoding gene and the 5' end of the F protein encoding gene using Taq polymerase enzyme with the upstream and downstream specific primers, revealed the presence of the amplified products of both reference strain and local isolates at the correct expected size (766 bp) on electrophoresis. Results of RT-PCR as a sensitive test for NDV detection confirmed the results of HA assay and agreed with those of Fazel et al. (2012) and Munir et al. (2012). In spite of allantoic fluid with 8 or 5 HA titer and give negative RT-PCR result for NDV, may be *Mycoplasma* infection as cited by Jordan and Amin (1980).

Sequencing was used to characterize isolated NDV field isolates accurately. This procedure is faster and more accurate than the mean death time, intravenous pathogenicity index, or intracerebral pathogenicity index tests for NDV pathotyping (Ganar et al., 2014). The results verified that all strains in this study had the cleavage site motif 112RRQKRF117, which is characteristic of vNDV strains, and that our isolates belonged to class II genotype VII, which is a velogenic genotype producing major outbreaks in China (Eweis et al., 2017).

The NDV isolates in this study (accession numbers OM243949, OM243950, and OM243951, respectively) demonstrated 97.5% -98.2% Egyptian strains and 96% - 99% identity with other world vNDV strains on GenBank. The sequenced strains were grouped with class II genotype VIIb and had a high genetic distance to the LaSota reference strain. It is concluded that NDV isolates circulating among chickens are virulent (Genotype VII).

CONCLUSION

It is established that NDV strains circulating among poultry farms in Egypt are virulent genotype VII, linked to outbreaks, and cause significant losses. Furthermore, it is recommended to use natural products as an immune booster and apply vaccination with similar field strains to limit the spread of these outbreaks.

REFERENCES

Abdelrahim, E.S., Elhag, J. (2014): A Case of Newcastle Disease Virus in Red-Headed Lovebird in Sudan. Case Reports in Veterinary Medicine Volume, Article ID 704239, 2 pages.

AboElkhair M, Bazid AI, Sakr MA, AbdEl-Razak AG, Sultan H (2012) Molecular characterization of fusion protein of Newcastle disease virus in Egypt. Egyptian J Virol 9: 243-254

Ahmadi, E., Seyed, A.P., Malahat, A.,Alireza, T. (2014): Pathotypic characterization of the Newcastle disease virus isolated from commercial poultry in northwest Iran.Turk J Vet Anim Sci 38.10.3906/vet-1311-82.

Aldous, E.W. (2007): Outbreak of Newcastle disease in pheasants (Phasianus colchicus) in south-east England in July 2005, Vet Rec, 160:482-4.

Alexander DJ (1991) Newcastle disease and other paramyxoviridae infections In: Disease of poultry 9th. In: Calnek BW, Barnes HJ, BeardCW, Reid WM, and Yoder HW (eds) Iowa State University press, Ames, Iowa. 506–519

Alexender, D. J. (2003): Newcastle disease. In Diseases of poultry, 11th ed. Y. M. Saif, H. J.Barnes, J. R. Glisson, A. M. Fadly, L. R. McDougal, and D. E. Swayne, eds., IowaState University Press, Ame: pp 64-87.

Bilal, E.S.A., Iman, M. Elnasri, Aymen,M.A., Khalda, A.K., Jedddha, I. Elhag, Selma, O. Ahmed, (2014): Biological Pathotyping of Newcastle Disease Viruses in Sudan 2008–2013.Journal of Veterinary Medicine, Article ID 209357, 4 pages.

Brown, V. R., & Bevins, S. N. (2017). Correction to: A review of virulent Newcastle disease viruses in the United States and the role of wild birds in viral persistence and spread. Veterinary Research, 48(1), 77. https://doi.org/10.1186/s13567-017-0485-7

De Battisti, C., Salomoni, A., Ormelli, S., Monne, I., Capua, I. and Cattoli, G. (2013): Rapid pathotyping of Newcastle Disease Virus by pyrosequencing. *J. Virol. Method*, 188, 13–20.

Doubney R, Mansi W (1947): The occurrence of ND in Egypt. J Comp Path 28:189–200.

Doyle TM (1927) Newcastle disease of fowls. Cited by Doyle TM (1935): J Comp Pathol Therap 48:1–20.

Diel DG, da Silva LH, Liu H, Wang Z, Miller PJ, Afonso CL(2012): Genetic diversity of avian paramyxovirus type 1: proposalfor a unified nomenclature and classification system of Newcastledisease virus genotypes. Infect Genetics Evol 12:1770–1779

Dimitrov, K. M., Lee, D. H., Williams-Coplin, D., Olivier, T. L., Miller, P. J., & Afonso, C. L. (2016). Newcastle disease viruses causing recent outbreaks worldwide show unexpectedly high genetic similarity to historical virulent isolates from the 1940s. Journal of Clinical Microbiology, 54(5), 1228–1235. https://doi.org/10.1128/jcm.0304415

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

Grimes, S.E. (2002): A basic laboratory manual for the small-scale

production and testing of I-2 Newcastle disease vaccine. In FAO Regional Office for Asia and the Pacific publication, Bangkok, Thailand Senior Animal Production and Health Officer and Secretary of APHCA and FAO Regional Office for Asia and the Pacific (RAP), Thailand, pp: 139.

Hussein HA, El-Sanousi AA, Youssif AA, Shalaby MA, Saber MS, Reda IM (2005) Sequence analysis of fusion and matrix protein genes of the velogenic viscerotropic newcastle disease virus egyptian strain sr/76. Int J Virol 1:38

Iqbal, M., K. Mahboob, M. Zulifiqar, A.Haq, G. Nabi and R. Tubssum. (2003): Production of hyperimmune serum against Newcastle disease virus (NDV) in rabbits. Pak.J. Vet. Res. 1:22-25.

Jordan FTW, Amin MM (1980): A survey of Mycoplasma infections in domestic poultry. Research in Veterinary Science 28 96-100.

Liu, X.F., Wan, H.Q., Ni, X.X., Wu, Y.T. and Liu, W.B. (2003): Pathotypical and genotypical characterization of strains of Newcastle disease virus isolated from outbreaks in chicken and goose flocks in some regions of China during 1985-2001. *Archives of Virol.*, 148, 1387–1403.

Mahmoud M. Naguib, M.M.;Höper D.;Elkady M.F.; Afifi M.A.; Erfan A.;Abozeid H.H.; Hasan W.M.;Arafa A.; Shahein M.;Beer M.; Harder T.C.;Grund C. (2021): Comparison of genomic and antigenic properties of Newcastle Disease virus genotypes II, XXI and VII from Egypt do not point to antigenic drift as selection marker. Transbound Emerg Dis. 2021;00:1–15.DOI: 10.1111/tbed.14121.

Mehrabanpour, M.J., Setareh, K., Abdollah,R., Mohammad, B.N., Mohammad,R. K., (2014): Phylogenetic characterization of the fusion genes of the Newcastle disease viruses isolatedin Fars province poultry farms during 2009-2011. Veterinary Research Forum, 5(3):187-191.

Mohamed MH, Kumar S, Paldurai A, Samal SK (2011) Sequence analysis of fusion protein gene of Newcastle disease virus isolated from outbreaks in Egypt during 2006. Virol J 8:237. doi:10.1186/1743-422X-8-237

Nabila, O., Sultan, S., Ahmed, A.I.,Ibrahim, R.S. and Sabra, M., (2014):Isolation and Pathotyping of Newcastle Disease Viruses from Field Outbreaks among Chickens in the Southern Part of Egypt 2011-2012. Global Veterinaria, 12 (2): 237-243.

Nagy, A., Ali, A., Zain El-Abideen, M. A., Kilany, W., & Elsayed, M. (2020). Characterization and genetic analysis of recent and emergent virulent newcastle disease viruses in Egypt. Transbound Emerg Dis, 67, 2000–2012. https://doi.org/10.1111/tbed.13543

OIE (2012): Newcastle disease. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 2.3.14.http://www.oie.int/ internationalstandard-setting/terrestrialmanual/access-online.

Pham H M, Konnai S, Usui T, Chang KS,Murata S, Mase M, Ohashi K, Onuma M (2005): Archive Virol. 150:2429-2438.\

Radwan, M. M., Darwish, S. F., El-Sabagh, I. M., El-Sanousi, A. A., & Shalaby,

M. A. (2013). Isolation and molecular characterization of Newcastle disease virus genotypes II and VIId in Egypt between 2011 and 2012. Virus Genes, 47(2), 311–316. https://doi.org/10.1007/ s11262-013-0950-y

Rima, B.; Balkema-Buschmann, A.; Dundon, W. G.; Duprex, P.; Easton, A.; Fouchier,R.; Kurath, G.; Lamb, R.; Lee, B.; Rota, P.; Wang, L. (2019): Ictv Report, C., ICTV VirusTaxonomy Profile: Paramyxoviridae. *J Gen Virol*, 100, 1593-1594.

Romer-Oberdorfer, A., Werner, O., Veits, J., Mebatsion, T., Mettenleiter, T.C. (2003): Contribution of the length of the HN protein and the sequence of the F protein cleavage site to Newcastle disease virus pathogenicity. J. Gen. Virol. 84: 3121–3129.

Saad, A. M., Samy, A., Soliman, M. A., Arafa, A., Zanaty, A., Hassan, M. K., Sultan, A. H., Bazid, A. I., & Hussein, A. H. (2017). Genotypic and pathogenic characterization of genotype VII Newcastle disease viruses isolated from commercial farms in Egypt and evaluation of heterologous antibody responses. Archives of Virology, 162(7), 1985–1994. https://doi.org/10.1007/s00705-017-3336-y

Saad, A. M., Samy, A., Soliman, M. A., Arafa, A., Zanaty, A., Hassan, M. K., Sultan, A. H., Bazid, A. I., & Hussein, A. H. (2017). Genotypic and pathogenic characterization of genotype VII Newcastle disease viruses isolated from commercial farms in Egypt and evaluation of heterologous antibody responses. Archives of Virology, 162(7), 1985–1994. https://doi.org/10.1007/s00705-017-3336-y

Zanaty, A. M.; Hagag, N. M.; Rabie, N.; M. Saied; Selim, K.; Mousa, S. A.; A. G.Shalaby; Arafa, A.; Hassan, M. K. (2019): Epidemiological, Phylogenetic Analysis andPathogenicity of Newcastle Disease Virus Circulating in Poultry Farms, Egypt during2015-2018. Hosts and Viruses, 6, 50-59.

Zhang, L., Pan, Z., Geng, S., Chen, X., Hu,S., Liu, H., Wu, Y., Jiao, X., Liu, X. (2010): Sensitive, seminested RT-PCR amplification of fusion gene sequences for the rapid detection and differentiation of Newcastle disease virus. Research in Veterinary Science, 89: 282-289.