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Detection of Virulent Genotype VII Newcastle Disease Virus in Lebanese Poultry Using Partial F Gene Sequences Reveals Regional and Global Genetic Relatedness

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Abstract

Newcastle disease virus (NDV) continues to threaten poultry industries worldwide, with genotype VII strains currently dominating outbreaks across the Middle East. In Lebanon, data on circulating NDV lineages remain scarce. This study molecularly characterizes three NDV isolates obtained in Spring 2025 from commercial and backyard poultry farms in Lebanon, where birds exhibited respiratory and/or neurological signs. Virus propagation was conducted in embryonated chicken eggs, and successful replication was confirmed by hemagglutination activity. Reverse transcription PCR targeting a 254 bp fragment of the fusion (F) gene consistently yielded the expected amplicons from all samples. Sequencing revealed the virulent polybasic cleavage site motif ^112RRQKR^117 in all isolates. Phylogenetic analysis placed the Lebanese viruses within genotype VII cluster, showing 100% nucleotide identity with an Indonesian reference strain and close clustering with isolates from neighboring Middle Eastern and North African countries, as well as South and Southeast Asia. Despite different vaccination profiles, all isolates exhibited identical partial F gene sequence. These findings highlight the dominance and persistence of genotype VII NDV in the region, its genetic relatedness to globally circulating strains, and the potential challenges for vaccine efficacy. Continuous molecular surveillance and evaluation of vaccine performance are essential for improving NDV control strategies in Lebanon and the region.

Keywords

Newcastle Disease Virus (NDV), Genotype VII, Fusion Protein Cleavage Site, Phylogenetic Analysis, Lebanon

1. Introduction

Newcastle disease virus (NDV), a member of the genus *Avian orthoavulavirus* (Avian orthoavulavirus 1; AOAV-1), remains one of the most economically devastating diseases in poultry globally. As an enveloped, single-stranded, negative-sense RNA virus within the *Paramyxoviridae* family, NDV causes a broad clinical spectrum ranging from mild respiratory illness to severe systemic disease, depending on viral and host factors. Pathotypic classification of NDV is traditionally based on severity in chickens (lentogenic, mesogenic, velogenic), but current molecular methods have shown that virulence can also be encoded by the fusion (F) protein cleavage site motif [1] [2]. Virulent strains typically possess a polybasic motif at positions 112–116 in F genes, which enables systemic infectivity via ubiquitous host proteases while avirulent (lentogenic) strains exhibit fewer basic residues. These cleavage site motifs are strong predictors of field isolates pathotypes, although additional genes such as the Haemagglutinin-Neuramindase gene (HN) contribute to viral replication, tissue tropism, and overall pathogenicity [1] [3].

Since the late 1980s, genotype VII NDV has emerged as a dominant lineage worldwide, causing repeated outbreaks in poultry populations across multiple continents. This genotype has been particularly associated with velogenic disease, leading to substantial economic losses in the Middle East, North Africa, and parts of Asia. In Egypt, nearly all recent outbreaks are caused by NDV genotype VII, particularly sub-genotype VII.1.1 [4], frequently associated with the RRQKRF cleavage motif of the Fusion protein, paired with high pathogenicity *in vivo* [4] [5].

In the broader Middle Eastern region, genotype VII strains are still predominant. Their circulation has been confirmed in neighboring countries such as Iran (VII.1.1), Iraq, Jordan, and others [6]-[8]. In contrast to the widespread detection of genotype VII, genotype VI has been less frequently reported in Middle Eastern countries like Egypt and Iran [9] [10]. Viruses belonging to both Genotypes (VI and VII), among others, often have high antigenic mismatch with classic vaccine strains, resulting in reduced vaccine efficacy [11]-[13]. Data on NDV in Lebanon remain comparatively limited. However, previous studies indicated that genotype VI velogenic strains have been endemic in broiler farms, and these isolates have served for the development of an autogenous vaccine [14]. Given the dynamic evolution of NDV and the co-circulation of multiple genotypes, understanding the current genotype distribution in Lebanon is critical for designing effective control strategies, optimizing vaccination programs, and evaluating the antigenic match of available vaccines.

In this study, we aimed to characterize NDV isolates from three specific outbreaks in commercial and backyard poultry during the Spring season of 2025, using partial F gene sequencing to assess virulence and facilitate preliminary genotyping. Subsequently, phylogenetic analyses were performed to elucidate the evolutionary relationships of these isolates with global NDV strains and to assess their genetic characteristics in the context of regional and international circulation.

2. Materials and Methods

2.1. History of Field Cases Submitted for Newcastle Disease Investigation

During the Spring season of 2025, the Animal Research and Diagnostics Lab at the Faculty of Agricultural and Food Sciences at the American University of Beirut received samples from poultry flocks with suspected Newcastle disease infections. The submitted cases originated from three different locations (Figure 1):

- A layer farm in Baalbeck region, Bekaa Valley, from which four birds were submitted at the age of 140 days: one dead, one displaying torticollis, and two clinically morbid.
- A commercial broiler farm, also in Baalbeck region, which provided four birds at the age of 28 days: two dead and two morbid.
- A backyard flock of layer chickens from the Baabda region (Chouit) in Mount Lebanon, from which two morbid birds were submitted at the age of around 12 months, including one with torticollis.

Figure 1. Locations of gathered isolates across Lebanon.

The observed mortality rates in these flocks were 7.6% in the commercial layer farm, 8.7% in the broiler farm, and 12.5% in the backyard flock. Both commercial farms reported compliance to routine Newcastle disease vaccination programs, while the backyard chickens were unvaccinated. Upon autopsy, all birds showed tracheitis with mild airsacculitis and lung congestion. It is important to note that these cases represent only the submissions received by the laboratory and do not necessarily reflect the outbreaks that took place nationwide during that period.

2.2. Virus Isolation and Propagation in Embryonated Eggs

Lung tissues from each bird type (commercial layers, commercial broilers, and

backyard layers) were pooled separately and homogenized in viral transport medium [15]. The homogenates were subjected to three freeze-thaw cycles, followed by brief centrifugation at 514 ×g for 10 minutes to remove debris. Supernatants were then inoculated in triplicates into 10-day-old embryonated chicken eggs using 100 μL volumes via the allantoic route. Negative control eggs were inoculated with 100 μL of sterile saline, while positive control eggs were inoculated with 100 μL of a velogenic Newcastle disease virus (NDV) strain previously isolated from Lebanese poultry [14]. All eggs were incubated at 99.5˚F (37.5˚C) for three days. After incubation, the allantoic fluid was collected and tested for hemagglutination (HA) activity using a 1% suspension of chicken red blood cells. A positive HA result in the allantoic fluid indicates the presence of a propagated virus expressing the hemagglutinin protein. Allantoic fluids that exhibited positive HA activity were pooled and stored at −80˚C for further analysis.

2.3. Viral RNA Extraction and RT-PCR Targeting Partial F Gene Sequence

Viral RNA was extracted from the three pooled homogenates that exhibited positive HA response using LabPrep Viral DNA/RNA mini kit (BioVendor LLC, Cat. No. LPVX100). The concentration of the extracted RNA was determined using Implen Nanophotometer N60 (Implen GmbH, Germany). QIAgen one-step RT-PCR kit (QIAgen, Valencia, CA, USA) was used to amplify a 254 bp fragment of the F gene, including the cleavage site. The primers used for the detection of virulent NDV isolates were: F: 5’-TTGATGGCAGGCCTCTTGC-3’ and R: 5’-AGCGT(C/T)TCTGTCTCCT-3’ [16].

The RT-PCR reaction mixture contained 10 μL of 5x reaction buffer, 4 μL of 5 μM concentration of each primer, 40 ng of extracted RNA template, 2 μL of dNTPs and 2 μL of enzyme mix (DNA polymerase and reverse transcriptase). Nuclease free water was added up to 50 μL volume. The thermal cycling program consisted of reverse transcription at 50˚C for 30 min, followed by initial denaturation at 95˚C for 15 min (CX1000 PCR thermal cycler, Bio-Rad) and 35 cycles of denaturation at 94˚C for 1 min, annealing at 53˚C for 1 min, and extension at 72˚C for 1 min followed by a final extension step at 72˚C for 10 min. Amplicons were visualized on a 1% agarose gel using a gel documentation system (Gel-Doc, Bio-Rad).

2.4. RT-PCR Amplicon Sequencing and Phylogenetic Analysis

The obtained 254 bp amplicons were excised from the gel and purified using the QIAquick Gel Extraction Kit (QIAgen, Valencia, CA, USA). This partial F gene fragment, which includes the critical polybasic cleavage site at positions 112–116, is a widely used and reliable target for preliminary virulence prediction and genotyping of NDV. The purified amplicons were subjected to Dideoxy Sanger sequencing using the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems). Upon sequencing, the partial F gene sequences of the three Newcastle Disease Virus (NDV) isolates were submitted to GenBank to obtain accession

numbers. These sequences, derived from backyard chickens (Chicken/Aley_LB/2025/LH1; GenBank accession no. PX215999.1), commercial broilers (Chicken/Baalbeck_LB/2025/LH1; GenBank accession no. PX216000.1), and commercial layer chickens (Chicken/Baalbeck_LB/2025/LH2; GenBank accession no. PX216001.1) (**Table 1**), were subjected to nucleotide similarity analysis using the BLASTn tool available on the NCBI website. Each sequence was compared against the GenBank database to identify closely related international NDV strains. The matching sequences were selected based on high percentage identity and query coverage. The phylogenetic tree was constructed using 26 partial F gene sequences (including the ones identified in this study highlighted in yellow), 10 complete F gene sequences, 6 complete genomes, and 3 partial genome sequences, using version 12 of the Molecular Evolutionary Genetic Analysis (MEGA12) software. The neighbor-joining method was used to visualize the relationships among the isolates and their closest reference sequences.

Table 1. GenBank accession numbers of the partial DNA sequences of the fusion (F) protein gene in Avian paramyxovirus 1 isolates extracted from different strains and their locations in Lebanon.

Isolate Strain	Isolate Location	Accession number
Backyard	Baabda	PX215999.1
Broiler	Baalbeck	PX216000.1
Layers	Baalbeck	PX216001.1

3. Results

3.1. Virus Propagation in Embryonated Eggs

Following inoculation of the triplicate lung homogenate samples from each bird type into 10-day-old embryonated chicken eggs, allantoic fluids were harvested after 72 hours of incubation. Hemagglutination (HA) testing was performed individually on each allantoic fluid sample. For each bird type, HA-positive samples were subsequently pooled and retested to determine the final HA titer. All triplicate samples from each bird type showed positive HA activity, confirming successful virus propagation in embryonated eggs prior to molecular characterization. The pooled allantoic fluid samples had HA titers of 1:32 for backyard and layer chickens and 1:16 for broilers. Negative control eggs, inoculated with 100 µL of sterile saline, showed no HA activity, while the HA titer of the positive control eggs was 1:32.

3.2. Detection of Virulent NDV Isolates By RT-PCR

All three field isolates collected from commercial broilers, commercial layers, and backyard chickens tested positive for Newcastle disease virus using RT-PCR. Amplification was performed using primers previously described [16], which target a region of the fusion (F) gene that includes the cleavage site associated with virulent Newcastle disease virus strains. A single amplicon of 254 bp was observed in

all three samples, consistent with the expected product size for virulent NDV. The amplified products were visualized by agarose gel electrophoresis (**Figure 2**), confirming the presence of virulent NDV RNA in all three isolates. No amplification was observed in the negative control.

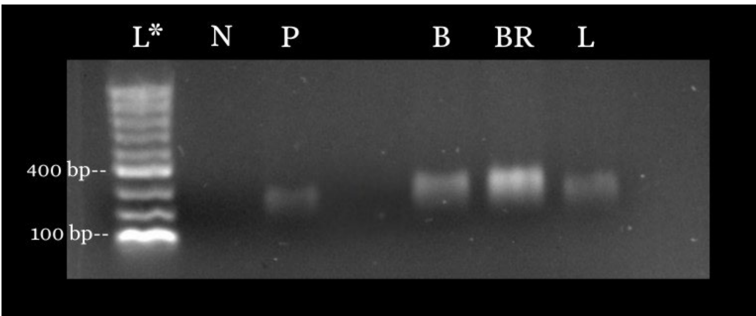

Figure 2. Partial F gene RT-PCR amplicons gel electrophoresis run. L*: ladder, N: negative control-Allantoic fluid from embryonated eggs inoculated with sterile saline; P: positive control-Allantoic fluid from embryonated eggs inoculated with velogenic NDV; B, BR, L: Allantoic fluid from eggs inoculated with lung tissue homogenates of backyard, broiler, and layer chickens, respectively.

3.3. Phylogenetic Analysis

Analysis of the fusion protein cleavage site revealed that all three Lebanese Newcastle disease virus (NDV) isolates were identical at the nucleotide level, with no sequence differences detected among them. They all possessed the polybasic Fusion protein cleavage site motif ^112RRQKR^117, which is characteristic of virulent NDV strains. The three isolates Phylogenetic analysis based on partial F-gene sequencing placed the Lebanese isolates within Genotype VII, where they clustered with reference strains from multiple geographic regions (**Figure 3**). The Lebanese sequences exhibited 100% nucleotide identity with an Indonesian isolate included in the dataset. In the phylogenetic tree, they grouped in close proximity to genotype VII viruses from the Middle East (Israel, Turkey), North Africa (Libya, Tunisia), South Asia (Pakistan), and Southeast Asia (Indonesia, Malaysia). Although BLAST searches of short F-gene fragments returned high similarity to some isolates designated as Genotype VI from Israel, the overall F-gene phylogeny consistently put the Lebanese isolates within Genotype VII. European isolates included in the analysis were positioned on more distant branches, reflecting greater sequence differences.

4. Discussion

Newcastle Disease Virus (NDV) remains one of the most significant pathogens affecting poultry worldwide, with its virulence largely determined by the sequence and activation of the fusion (F) protein cleavage site. The 112^RRQKR^117 motif identified in all three NDV isolates recovered from Lebanon is a well-characterized sequence associated with virulent strains, including both mesogenic and velogenic types [17]. The presence of this motif highlights the potential for

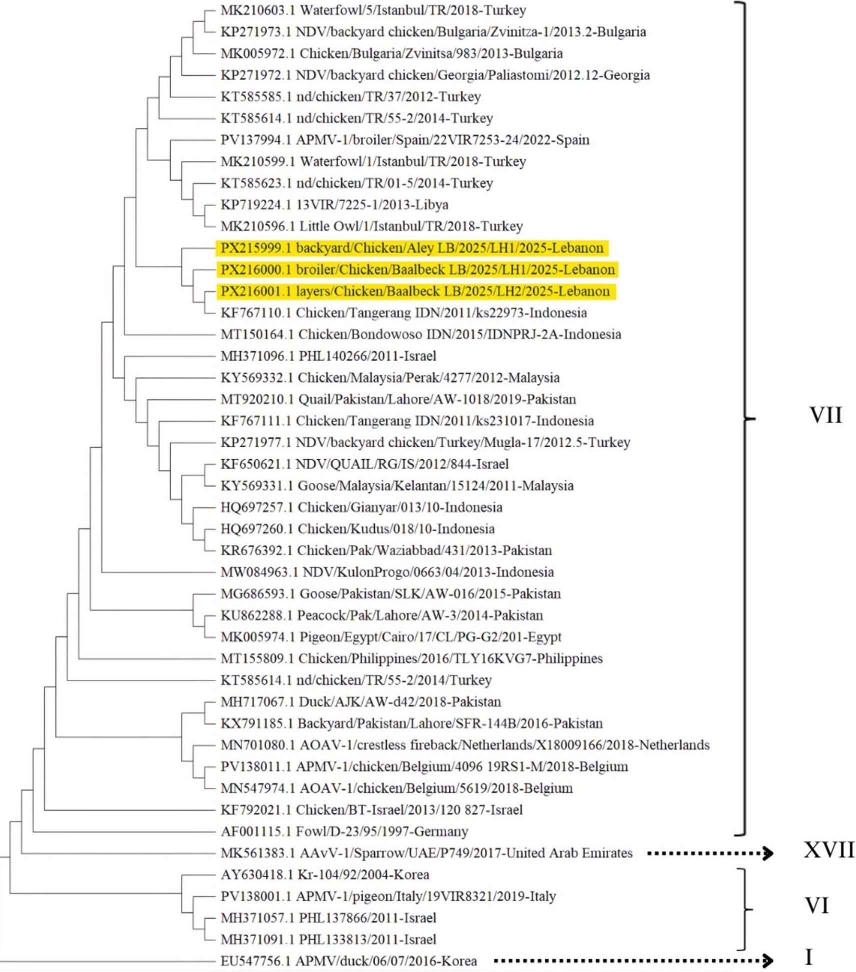

Figure 3. Phylogenetic tree based on the Fusion (F) protein gene sequence of *Avian paramyxovirus* 1. The phylogenetic tree was constructed using 26 partial F gene sequences (*including the ones identified in this study highlighted in yellow*), 10 complete F gene sequences, 6 complete genomes, and 3 partial genome sequences. Roman numerals indicate the genotype of each isolate.

pathogenicity, yet it is important to emphasize that the RRQKR cleavage site is not genotype-specific. This motif is widely reported across a range of virulent NDV strains of different genotypes, including genotype VI and genotype VII, which are the predominant circulating strains worldwide [17] [18]. Interestingly, the previously isolated Lebanese genotype VI strain in 2013 (Accession number KC425723) harbored the fusion cleavage motif RRQRR, whereas our more recent genotype VII isolates display the RRQKR motif. While both motifs are characteristic of virulent NDV strains, this difference likely reflects the circulation of distinct viral genotypes rather than the local evolution of one into the other. In fact, genotype VI and genotype VII represent genetically different lineages that have been co-circulating globally, with genotype VI historically associated with pigeon-adapted strains and genotype VII becoming the dominant lineage in poultry outbreaks in the region [7] [19] [20]. The detection of genotype VI in Lebanon more

than a decade ago, followed by the identification of genotype VII in the present study, suggests a possible genotype shift in the local poultry population, potentially due to new introductions from neighboring countries or broader regional spread [7]. This observation emphasizes the importance of molecular surveillance in tracking not only mutations within a genotype but also the replacement of one genotype by another or the coexistence of multiple genotypes together.

In the current study, preliminary sequence analysis based on a partial F-gene fragment revealed a high level of sequence similarity with NDV isolates from genotype VII, mostly, and a few isolates from genotype VI, reflecting the limitations of relying on short genomic fragments for genotype assignment. This observation highlights the challenges associated with fragment-based analyses, where the homogeneity of certain genomic regions can produce unreliable results, particularly in the absence of comprehensive whole-genome sequencing [21].

To address these limitations, we conducted a phylogenetic analysis of partial F-gene sequences using a reference tree obtained from NCBI. After selecting sequences highly similar to those of our isolates, our partial-F-gene phylogenetic analysis placed the Lebanese isolates within genotype VII. Although the unified classification system relies on the complete F gene coding sequence and includes additional criteria [22], our results are consistent with common NDV genotype classifications. This robust classification provides a more precise understanding of the relationship between the Lebanese isolates and other NDV strains globally. Moreover, the phylogenetic analysis confirmed that the Lebanese isolates shared 100% nucleotide identity with a previously identified strain from Indonesia, emphasizing the genetic conservation of circulating genotype VII viruses across geographically distant regions.

This high level of conservation may reflect the transboundary spread of genotype VII NDV through multiple pathways, including the international trade of live poultry and poultry products, the movement of contaminated equipment or feed, and the potential role of migratory birds as natural carriers. While the exact route of introduction into Lebanon cannot be determined from this study, these findings underscore the capacity of genotype VII strains to disseminate globally and highlight the importance of coordinated international surveillance and biosecurity measures to limit virus spread.

The close clustering of the Lebanese isolates with genotype VII strains from countries such as Turkey, Israel, Libya, Tunisia, Pakistan, and Southeast Asia further confirms the extensive transboundary circulation of this lineage [4] [7] [23]. This wide geographical spread suggests that genotype VII has become a globally dominant lineage actively circulating across continents. The Lebanese isolates also clustered more distantly from European strains, highlighting the genetic divergence that exists between viruses circulating in Europe and those in the Middle East and Asia. This distinction reflects the regional variability and genetic adaptability of NDV strains, which can exhibit considerable variation in pathogenicity depending on their geographic origin [7]. Remarkably, the Lebanese isolates de-

rived from different poultry populations- backyard chickens (non-vaccinated), commercial layers, and broilers (with routine vaccination programs)-exhibited similar genomic characteristics. These results show that, despite different vaccination approaches, the isolates share a common genetic background, which may impact vaccine performance and control strategies. The observed difference in mortality rates, namely 12.5% in unvaccinated backyard chickens compared to less than 10% in routinely vaccinated commercial flocks, sheds light on the protective role of vaccination in reducing the clinical impact of genotype VII NDV infections. The higher mortality observed in the unvaccinated backyard flock highlights the role of such flocks as potential reservoirs for NDV. These unvaccinated populations may serve as sources of spillover infections to vaccinated commercial poultry, emphasizing the need for targeted surveillance across both backyard and commercial sectors to limit viral circulation. In Lebanon, routine immunization in commercial farms primarily relies on genotype II live vaccines, including LaSota, B1, and Clone 30, often complemented with inactivated vaccines produced mainly from LaSota seed strains. While most killed vaccines are based on genotype II, some use genotype VII strains to better match circulating viruses. Phylogenetically, these commercially available NDV vaccines, belonging mostly to genotype II, exhibit a genetic divergence of at least 10% from genotype VII isolates, based on pairwise comparisons of their F and HN gene nucleotide sequences [24]-[26]. Nevertheless, previous reports explicitly showed that vaccination, particularly with live vaccines and inactivated formulations, can mitigate disease severity even when the virus is not fully matched to the vaccine strain [27]. This protective effect may be explained not only by vaccine-induced humoral immunity and the production of specific neutralizing antibodies but also by additional mechanisms, including competitive exclusion through early colonization of the upper respiratory tract by vaccine viruses, stimulation of innate immune responses, and activation of cell-mediated immunity such as cytotoxic T lymphocytes [28]. These complementary immune pathways likely contribute to cross-protection against divergent genotypes, including genotypes VI and VII. Moreover, the high genetic similarity between our isolates and strains circulating in various regions highlights the importance of continuous molecular surveillance and routine evaluation of vaccine strains to ensure continued effectiveness against evolving NDV genotypes.

Monitoring the genetic evolution of NDV will allow for the timely detection of emerging strains that may pose new challenges to poultry health. Furthermore, the close phylogenetic relationship between Lebanese isolates and those from neighboring countries highlights the possibility of cross-border transmission of NDV and suggests the need for regional cooperation in disease control efforts.

In conclusion, the Lebanese NDV isolates are genetically related to globally circulating genotype VII strains, which are responsible for ongoing outbreaks in poultry populations across Asia, the Middle East, and Africa. The significant genetic similarity of our isolates to strains from multiple countries emphasizes the

global mobility of NDV and its potential for transboundary spread. These findings support the continued implementation of molecular surveillance and the use of proper vaccines to control the spread of NDV. Further research into the pathogenicity and epidemiology of genotype VII strains, alongside vaccine efficacy studies, will be crucial for mitigating the impact of NDV outbreaks on poultry industries in Lebanon and beyond.

Ethics Statement

The authors confirm that all procedures involving animals were conducted in accordance with the ethical standards and guidelines of the American University of Beirut (AUB), Beirut, Lebanon. This study was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at AUB under approval number 25-07-660.

Disclosure

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

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Afonso, C.L. (2021) Virulence during Newcastle Disease Viruses Cross Species Adaptation. *Viruses*, **13**, Article 110. https://doi.org/10.3390/v13010110

[2] Swayne, D.E. (2020) Diseases of Poultry. 14th Edition, Wiley-Blackwell.

[3] Wang, Y., Yu, W., Huo, N., Wang, W., Guo, Y., Wei, Q., *et al.* (2017) Comprehensive Analysis of Amino Acid Sequence Diversity at the F Protein Cleavage Site of Newcastle Disease Virus in Fusogenic Activity. *PLOS ONE*, **12**, e0183923. https://doi.org/10.1371/journal.pone.0183923

[4] Eid, A.A.M., Hussein, A., Hassanin, O., Elbakrey, R.M., Daines, R., Sadeyen, J., *et al.* (2022) Newcastle Disease Genotype VII Prevalence in Poultry and Wild Birds in Egypt. *Viruses*, **14**, Article 2244. https://doi.org/10.3390/v14102244

[5] Orabi, A., Hussein, A., Saleh, A.A., El-Magd, M.A. and Munir, M. (2017) Evolutionary Insights into the Fusion Protein of Newcastle Disease Virus Isolated from Vaccinated Chickens in 2016 in Egypt. *Archives of Virology*, **162**, 3069-3079. https://doi.org/10.1007/s00705-017-3483-1

[6] Ghalyanchilangeroudi, A., Hosseini, H., Jabbarifakhr, M., Fallah Mehrabadi, M.H., Najafi, H., Ghafouri, S.A., *et al.* (2018) Emergence of a Virulent Genotype VIII of Newcastle Disease Virus in Iran. *Avian Pathology*, **47**, 509-519. https://doi.org/10.1080/03079457.2018.1495313

[7] Miller, P.J., Haddas, R., Simanov, L., Lublin, A., Rehmani, S.F., Wajid, A., *et al.* (2015) Identification of New Sub-Genotypes of Virulent Newcastle Disease Virus with Po-

tential Panzootic Features. *Infection*, *Genetics and Evolution*, **29**, 216-229. https://doi.org/10.1016/j.meegid.2014.10.032

[8] Al-Shammari, A.M., Hamad, M.A., AL-Mudhafar, M.A., Raad, K. and Ahmed, A. (2020) Clinical, Molecular and Cytopathological Characterization of a Newcastle Disease Virus from an Outbreak in Baghdad, Iraq. *Veterinary Medicine and Science*, **6**, 477-484. https://doi.org/10.1002/vms3.262

[9] Mayahi, V. and Esmaelizad, M. (2017) Molecular Evolution and Epidemiological Links Study of Newcastle Disease Virus Isolates from 1995 to 2016 in Iran. *Archives of Virology*, **162**, 3727-3743. https://doi.org/10.1007/s00705-017-3536-5

[10] Naguib, M.M., Höper, D., Elkady, M.F., Afifi, M.A., Erfan, A., Abozeid, H.H., *et al.* (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. *Transboundary and Emerging Diseases*, **69**, 849-863. https://doi.org/10.1111/tbed.14121

[11] Yang, H., Zhao, J., Xue, J., Yang, Y. and Zhang, G. (2017) Antigenic Variation of Lasota and Genotype VII Newcastle Disease Virus (NDV) and Their Efficacy against Challenge with Velogenic NDV. *Vaccine*, **35**, 27-32. https://doi.org/10.1016/j.vaccine.2016.11.048

[12] Roohani, K., Tan, S.W., Yeap, S.K., Ideris, A., Bejo, M.H. and Omar, A.R. (2015) Characterisation of Genotype VII Newcastle Disease Virus (NDV) Isolated from NDV Vaccinated Chickens, and the Efficacy of Lasota and Recombinant Genotype VII Vaccines against Challenge with Velogenic NDV. *Journal of Veterinary Science*, **16**, 447-457. https://doi.org/10.4142/jvs.2015.16.4.447

[13] Abdelsabour, M.A., Helal, A.M., Nagar, E.M.S.E., El-fatah, W.A., Abodalal, S.E.S.A., Madbouly, Y.M., Arafa, A.A., Ibrahim, H.M. and Hussein, A. (2024) Efficacy of a Locally Prepared Live Clone Vaccine against Newcastle Disease Virus Genotype IV and Genotype VIID in Egypt: Clone 30 Vaccine. *Journal of Advanced Veterinary Research*, **14**, 639-643. https://advetresearch.com/index.php/AVR/article/view/1746

[14] Barbour, E. (2017) Protection by Classical and Vectored Vaccines against Endemic Velogenic Newcastle Disease in Broiler Farms and Evaluation of a Developed Autogenous Vaccine against Predominant Genotype VI. *International Journal of Vaccines & Vaccination*, **4**, Article ID: 00075. https://doi.org/10.15406/ijvv.2017.04.00075

[15] Dufour-Zavala, L. and American Association of Avian Pathologists (2008) A Laboratory Manual for the Isolation, Identification and Characterization of Avian Pathogens. 5th Edition, American Association of Avian Pathologists.

[16] Kant, A., Koch, G., Van Roozelaar, D.J., Balk, F. and Huurne, A.T. (1997) Differentiation of Virulent and Non-Virulent Strains of Newcastle Disease Virus within 24 Hours by Polymerase Chain Reaction. *Avian Pathology*, **26**, 837-849. https://doi.org/10.1080/03079459708419257

[17] Patel, S.S., Chauhan, H.C., Kumar Sharma, K., Patel, A.C., Bulbule, N.R., Raval, S.H., *et al.* (2024) Genetic Evolution of Newcastle Disease Virus Sub-Genotype VII.2 Isolates, Diagnosed from Vaccinated Poultry Farms of Gujarat, India. *Gene*, **930**, Article ID: 148859. https://doi.org/10.1016/j.gene.2024.148859

[18] Dharmayanti, N.I., Nurjanah, D., Nuradji, H., Suyatno, T. and Indriani, R. (2024) Newcastle Disease Virus: The Past and Current Situation in Indonesia. *Journal of Veterinary Science*, **25**, e3. https://doi.org/10.4142/jvs.23022

[19] He, Y., Taylor, T.L., Dimitrov, K.M., Butt, S.L., Stanton, J.B., Goraichuk, I.V., *et al.* (2018) Whole-Genome Sequencing of Genotype VI Newcastle Disease Viruses from Formalin-Fixed Paraffin-Embedded Tissues from Wild Pigeons Reveals Continuous

Evolution and Previously Unrecognized Genetic Diversity in the U.S. *Virology Journal*, **15**, Article No. 9. https://doi.org/10.1186/s12985-017-0914-2

[20] Radwan, M.M., Darwish, S.F., El-Sabagh, I.M., El-Sanousi, A.A. and 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**, 311-316. https://doi.org/10.1007/s11262-013-0950-y

[21] Bello, M.B., Yusoff, K., Ideris, A., Hair-Bejo, M., Peeters, B.P.H. and Omar, A.R. (2018) Diagnostic and Vaccination Approaches for Newcastle Disease Virus in Poultry: The Current and Emerging Perspectives. *BioMed Research International*, **2018**, Article ID: 7278459. https://doi.org/10.1155/2018/7278459

[22] Dimitrov, K.M., Lee, D., Williams-Coplin, D., Olivier, T.L., Miller, P.J. and 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**, 1228-1235. https://doi.org/10.1128/jcm.03044-15

[23] Sultan, H.A., Elfeil, W.K., Nour, A.A., Tantawy, L., Kamel, E.G., Eed, E.M., *et al.* (2021) Efficacy of the Newcastle Disease Virus Genotype VII.1.1-Matched Vaccines in Commercial Broilers. *Vaccines*, **10**, Article 29. https://doi.org/10.3390/vaccines10010029

[24] Mahamud, S.N.A., Bello, M.B., Ideris, A. and Omar, A.R. (2022) Efficacy of Genotype-Matched Newcastle Disease Virus Vaccine Formulated in Carboxymethyl Sago Starch Acid Hydrogel in Chickens Vaccinated via Different Routes. *Journal of Veterinary Science*, **23**, e25. https://doi.org/10.4142/jvs.21242

[25] Bello, M.B., Mahamud, S.N.A., Yusoff, K., Ideris, A., Hair-Bejo, M., Peeters, B.P.H., *et al.* (2020) Development of an Effective and Stable Genotype-Matched Live Attenuated Newcastle Disease Virus Vaccine Based on a Novel Naturally Recombinant Malaysian Isolate Using Reverse Genetics. *Vaccines*, **8**, Article 270. https://doi.org/10.3390/vaccines8020270

[26] Otiang, E., Thumbi, S.M., Campbell, Z.A., Njagi, L.W., Nyaga, P.N. and Palmer, G.H. (2021) Impact of Routine Newcastle Disease Vaccination on Chicken Flock Size in Smallholder Farms in Western Kenya. *PLOS ONE*, **16**, e0248596. https://doi.org/10.1371/journal.pone.0248596

[27] Liu, H., de Almeida, R.S., Gil, P., Majó, N., Nofrarías, M., Briand, F., *et al.* (2018) Can Genotype Mismatch Really Affect the Level of Protection Conferred by Newcastle Disease Vaccines against Heterologous Virulent Strains? *Vaccine*, **36**, 3917-3925. https://doi.org/10.1016/j.vaccine.2018.05.074

[28] Kapczynski, D.R., Afonso, C.L. and Miller, P.J. (2013) Immune Responses of Poultry to Newcastle Disease Virus. *Developmental & Comparative Immunology*, **41**, 447-453. https://doi.org/10.1016/j.dci.2013.04.012