

Articles

Genetic Diversity and Evolutionary Insights into Newcastle Disease Virus Circulating in Bangladesh

Farah Zereen^{1,2}, Md. Abdur Rahman^{1,3}, Md. Golzar Hossain¹, Jahangir Alam⁴, Masaru Shimada⁴, Md. Tanvir Rahman¹, Muhammad Munir⁵ and Sukumar Saha¹

¹Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh; ²Department of Microbiology, Gono Bishwabidyalay, Dhaka, Bangladesh; ³Department of Animal Production, Gono Bishwabidyalay, Dhaka, Bangladesh; ⁴Department of Molecular Biodefense Research, Graduate School of Medicine, Yokohama City University, Yokohama 236-0004, Japan; ⁵Division of Biomedical and Life Sciences, Faculty of Health and Medicine, Lancaster University, Lancaster, UK.

Abstract | Newcastle disease, caused by avian orthoavulavirus serotype 1, is a highly contagious and economically devastating viral infection affecting both wild and domestic birds. The disease is characterized by severe respiratory, gastrointestinal, and neurological symptoms, often leading to high mortality and substantial production losses, particularly in commercial poultry. In Bangladesh, multiple virulent and avirulent ND virus (NDV) genotypes and subgenotypes continue to circulate despite ongoing vaccination programs. This review presents a comprehensive analysis of the genetic landscape of prevalent NDV strains in Bangladesh between 2010 and 2024 using full-length coding sequences of the fusion (F) gene. Specifically, we focused on genotypes, subgenotypes, host species, and annual distribution patterns in the country. We further investigated phylogenetic relationships, evolutionary divergence, and amino acid substitutions in the full-length F protein. Comparative analyses of amino acid variability and sequence similarity between currently used vaccine strains and prevalent Bangladeshi field isolates were also scrutinized. We have unraveled considerable genetic divergence, and highlighted mismatches in genotypic coverage. These findings underscore the urgent need to develop genotype-matched NDV vaccines to improve disease control strategies in Bangladesh's poultry sector.

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Introduction

Newcastle disease (ND) is a highly fatal and economically important viral infection of birds, particularly poultry, caused by Newcastle disease virus (NDV), a prototype member of the orthoavulavirus serotype 1 [1]. The NDV infection is not only limited to commercial poultry, it can also infect a wide range of wild bird species [2]. First reported in 1926 in Indonesia and then travelled to England and Sri Lanka [3, 4], triggering a global panzootic [5]. Subsequent panzootics have occurred: a second in the late 1960s driven by increasing international trade and air travel [6]; a third in the early 1980s that initially affected racing pigeons and later spread to chickens [7–9]; and a fourth beginning in Southeast Asia in the 1980s, which has since extended to the Middle East, Europe, the Americas, and Africa [10–14]. While Bangladesh first reported ND outbreaks in 1981, the infection remains enzootic in many regions worldwide [15]. Clinical manifestations include neurological signs, gastrointestinal disorders, respiratory illness, and reduced egg production, while severe cases often lead to sudden death [16].

NDV belongs to the genus *Orthoavulavirus* in the subfamily *Avulavirinae*, family *Paramyxoviridae* [17]. It is a non-segmented, negative-sense, single-stranded RNA virus with a genome of approximately 15,000 nucleotides that encodes six structural proteins: hemagglutinin-neuraminidase (HN), RNA-dependent RNA polymerase (L), matrix (M), fusion (F), phosphoprotein (P), and nucleocapsid protein (NP) [18,19]. The NP and P proteins encapsulate viral RNA [20], while M facilitates virus assembly and budding [21]. F and HN mediate viral entry into host cells [22], and L directs transcription and replication of the viral genome [23].

The NDV genome exhibits marked genetic diversity, with strains varying in pathogenicity [24]. The NDV is classified into three pathotypes based on virulence: velogenic, mesogenic, and lentogenic [25]. Velogenic strains cause severe systemic disease and high mortality [26]; mesogenic strains primarily affect the respiratory and nervous systems with moderate mortality [27,28];

and lentogenic strains generally cause mild or asymptomatic infections, particularly in the intestines [29].

Phylogenetic analysis based on F and HN genes provide high resolution clustering patterns and complement whole-genome analyses. However, the complete F gene sequence is the preferred target for comprehensive NDV studies, including virulence evaluation, pathotyping, genotyping, and phylogenetic analysis, as recommended by WOAHA [25]. Based on full-length F gene sequences, NDV is divided into two major classes: class I and class II [30]. Class I strains are generally avirulent and mostly detected in wild aquatic birds and live bird markets [31, 32]. In contrast, Class II strains are typically virulent and are frequently recovered from domestic poultry as well as wild birds [33, 34]. Class I consists of a single genotype with three sub-genotypes, whereas Class II encompasses at least 21 genotypes (I–XXI), each containing several sub-genotypes [35]. NDV has a long history in Southeast Asia, where genotypes II, IV, VII, and XIII continue to circulate simultaneously [36]. Preventive vaccination is the main strategy for controlling ND in commercial poultry; yet outbreaks continue to occur in vaccinated flocks due to circulating virulent strains of NDV, causing losses from death, poor growth, and reduced egg production.

A key determinant of NDV virulence is the amino acid motif at the F protein cleavage site (residues 112–117) [37]. Lentogenic strains typically harbor a monobasic motif (112G-R/K-Q-G-R↓L117), whereas mesogenic and velogenic strains carry a multibasic motif (112R/G/K-R-Q/K-K/R-R↓F117), which enables efficient cleavage and viral entry [38]. Characterizing the F protein cleavage site is critical for assessing virulence, guiding vaccine design, and understanding NDV evolution [39]. The high genetic variability of NDV in poultry populations highlights the need for continuous molecular surveillance [35] using the complete F or HN gene, or ideally, whole-genome sequences.

Therefore, this review investigates the genetic diversity of NDV isolates in Bangladesh from 2010 to 2024 using full-length coding sequences of the fusion (F) gene available in the NCBI GenBank database, focusing on

phylogenetic classification, evolutionary divergence, and genotype mismatches between vaccine strains and circulating viruses.

Materials and Methods

Genetic ecology of NDVs in Bangladesh

Complete coding sequences of the F gene from NDV strains circulating in Bangladesh between 2010 and 2024 were retrieved from the NCBI GenBank database. These sequences were analyzed to determine prevalent genotypes, subgenotypes, host species, and annual distribution of isolates, based on the updated NDV classification system [35].

Evolutionary relationships of complete F gene sequences

Complete F gene sequences of Bangladeshi NDV isolates, along with their translated protein sequences, were retrieved from the NCBI GenBank database. Phylogenetic relationships were inferred using MEGA 11 software [40], supported by BLAST searches in GenBank. The phylogenetic tree was generated using the Maximum Likelihood method with the Poisson correction model [41], and statistical support was evaluated with 1,000 bootstrap replicates [42]. Initial trees for heuristic searches were automatically generated using Neighbor-Joining and BioNJ algorithms applied to pairwise distance matrices estimated by the Poisson model. The tree with the highest log-likelihood value was selected. Evolutionary rate variation among sites was modeled with a discrete Gamma distribution (two categories, +G, with estimated parameters). The final tree was drawn to scale, with branch lengths representing the number of substitutions per site.

Evolutionary divergence of F gene sequences

Evolutionary divergence among NDV isolates within and between genotypes/subgenotypes was assessed using amino acid sequences of the complete F gene, applying the Poisson correction model [41]. Ambiguous positions were removed using the pairwise deletion option. All analyses were performed in MEGA 11 [40].

Amino acid variability in F gene sequences

Amino acid variability among Bangladeshi NDV isolates was examined by comparing complete F gene sequences

across genotypes and subgenotypes. Variability in Bangladeshi isolates was also compared with commonly used NDV vaccine strains (LaSota, B1, and Mukteswar). Multiple sequence alignments were conducted using MEGA 11 [40].

Sequence similarity between vaccine strains and field isolates

Sequence similarity analyses were performed between the complete F gene sequences of vaccine strains (LaSota, B1, and Mukteswar) and circulating Bangladeshi NDV isolates across genotypes and subgenotypes. Vaccine strain sequences were retrieved from the NCBI GenBank database, and analyses were carried out in MEGA 11 [40].

Results

Genetic ecology of NDVs in Bangladesh

A total of 26 complete fusion (F) gene sequences of NDV isolates from Bangladesh were analyzed, revealing genetically diverse strains circulating among multiple bird species. All isolates belonged to class II and were classified into several genotypes and subgenotypes. Specifically, two isolates were assigned to genotype II, one to genotype VI (subgenotype VI.1.1, formerly VI.b), four to genotype VII (subgenotype VII.2), sixteen to genotype XIII (eleven in subgenotype XIII.2, four in XIII.2.3, and one unclassified), and three to genotype XXI (subgenotype XXI.1.2). Isolates from subgenotypes VI.1.1 and XXI.1.2 were recovered from pigeons, whereas isolates from genotypes II, VII.2, XIII.2, and XIII.2.3 originated from chickens.

evolutionary rate differences among sites (2 categories (+G, parameter = 0.0500)). This analysis involved 26 amino acid sequences. There were total of 553 positions in the final dataset. Evolutionary analyses were conducted in MEGA11. Only bootstrap values above 70 are shown.

All isolates were predicted to be virulent based on the presence of multibasic amino acid motifs such as “RRQKRF,” “RRKKRF,” or “RTKKRF” at the F protein cleavage site, except for genotype II isolates, which contained the monobasic motif “GRQGRL” characteristic of lentogenic strains. Notably, no

complete F gene sequences of Bangladeshi NDV isolates were available in the NCBI GenBank database for the years 2011, 2013, 2014, 2015, 2019, and 2022 (Table 1).

Table 3. Amino acid variability in complete F gene sequences of circulating Bangladeshi NDVs within each genotype/subgenotype

Isolates under Genotype / Subgenotype	Cleavage site	Variable amino acid position	Variables in neutralizing epitope of F protein
Genotype II	GRQGRL	17, 25, 264, 429, 441, 486, 520, 549, 550	Conserved
Subgenotype VII.2	RRKKRF	19, 24, 25, 31, 78, 97, 113, 170, 186, 190, 193, 235, 402, 442	Epitope 1 (at 78 th) and epitope 2 (at 170 th)
Subgenotype XIII.2	RRQKRF	4, 5, 6, 8, 10-14, 18, 19, 24-26, 28, 29, 43, 49, 52, 66, 79, 97, 104, 107, 108, 110, 163, 170, 200, 208, 255, 270, 279, 286, 312, 321, 325, 328, 330, 338, 342, 364, 385, 395, 402, 442, 465, 479, 485, 486, 489, 492, 494, 496, 514, 516, 517, 520, 522, 530, 545	Epitope 1 (at 79 th) and epitope 2 (at 163 th and 170 th)
Subgenotype XIII.2.3	RRQKRF	4, 13, 63	Conserved
Subgenotype XXI.1.2	RRQKRF	16, 17, 19, 21, 150, 235, 294, 425, 470, 479, 487, 530, 537	Conserved

Phylogenetic analysis of Bangladeshi NDV isolates

Phylogenetic analysis revealed close evolutionary relationships among isolates within genotype II, subgenotype VII.2, XIII.2.3, and XXI.1.2. By contrast, subgenotype XIII.2 isolates were distributed across separate subclades, indicating greater divergence within this group. Distinct clustering patterns were observed between genotypes and subgenotypes (Figure 1). BLAST analysis further showed that Bangladeshi isolates shared high sequence similarity (93.98%–99.96%) with isolates from India, Pakistan, Japan, Indonesia, and Jordan (Table 2).

Evolutionary divergence of NDVs in Bangladesh

Analysis of 26 amino acid sequences of Bangladeshi NDV isolates, with a total alignment length of 553

positions, showed the following pairwise mean evolutionary distances: genotype II (0.02), subgenotype VII.2 (0.00–0.03), subgenotype XIII.2 (0.00–0.07), subgenotype XIII.2.3 (0.00–0.01), and subgenotype XXI.1.2 (0.00–0.02) (Supplementary Tables 1.a–1.e). The overall mean evolutionary distances within each group were 0.02 (II), 0.02 (VII.2), 0.04 (XIII.2), 0.00 (XIII.2.3), and 0.02 (XXI.1.2).

Amino acid variability in F gene sequences of Bangladeshi NDV isolates

Amino acid variability was examined across complete F gene sequences within each genotype and subgenotype. Genotype II isolates exhibited nine substitutions while retaining the conserved lentogenic cleavage site “GRQGRL.” Subgenotype VII.2 isolates, featuring “RRKKRF” and “RTKKRF” motifs, showed 14 variations. Subgenotype XIII.2 isolates carrying the virulent “RRQKRF” motif displayed 61 variable sites, whereas XIII.2.3 isolates showed only three substitutions. Subgenotype XXI.1.2 isolates, which also carried the “RRQKRF” motif, exhibited 13 variations.

Notably, substitutions were detected in neutralizing epitopes 1 and 2 of subgenotypes VII.2 and XIII.2, whereas epitope 3 remained conserved across all isolates (Table 3). Further comparison with vaccine strains (LaSota, B1, and Mukteswar) confirmed substitutions at epitope 1 in VII.2, XIII.2, and XIII.2.3. Compared with LaSota and B1, substitutions at epitope 2 were observed in VII.2, XIII.2, and XIII.2.3; while compared with Mukteswar, substitutions at epitope 2 were present in all circulating genotypes/subgenotypes in Bangladesh. Epitope 3 remained conserved across all isolates (Figure 2). Additionally, Sequence Demarcation Tool (SDT) based analysis identified similarities across all strains isolated from the country (Figure 3). This highlight the genetic diversity and percussion this might have on the vaccine efficacies in the poultry sector.

Sequence similarity between vaccine strains and Bangladeshi NDV isolates

Comparative sequence similarity analysis revealed a considerable genetic mismatch between circulating NDV isolates and commonly used vaccine strains.

LaSota and B1 showed high sequence similarity (98–100%) only with lentogenic strains of genotype II and moderate similarity (86–89%) with virulent strains of genotypes VI.1.1, VII.2, XIII.2, XIII.2.3, and XXI.1.2. By contrast, Mukteswar exhibited 88–92% sequence similarity with all circulating genotypes and subgenotypes in Bangladesh (Figure 4).

Discussion

NDV infects more than 200 avian species, however, chickens are the most susceptible, followed by turkeys [19], while other vulnerable species include pheasants, guinea fowl, partridges, and quail. Waterfowl and wild birds often serve as reservoirs [43], and pigeons are also frequently affected [44]. In the present study, NDV was detected only in chickens and pigeons in Bangladesh. Pigeons, as natural reservoirs of lentogenic and mesogenic strains, typically harbor the virus asymptomatically and contribute to genotype diversity through genetic drift, mutations, recombination, and geographic spread. Migratory behavior further facilitates the introduction of new genotypes into naïve regions or the reintroduction of strains into previously controlled areas [45]. Our findings showed that class II NDV strains circulating in Bangladesh include lentogenic genotype II and virulent genotypes VI (VI.1.1), VII (VII.2), XIII (XIII.2 and XIII.2.3), and XXI (XXI.1.2) [35, 46, 47].

The F protein cleavage site remains a critical molecular marker of NDV virulence [48]. In this study, “RRQKRF” was the most prevalent virulent motif, while alternative motifs “RRKKRF” and “RTKKRF” were identified in subgenotype VII.2. Previous studies have reported that the presence of glutamine (Q) at the third position of the cleavage site enhances viral cell-to-cell spread [49, 50], suggesting a possible role in viral evolution.

Phylogenetic and BLAST analyses provided insights into viral transmission across species and regions. Phylogenetically, Bangladeshi isolates clustered closely within most genotypes, except for genotype XIII.2 isolates. BLAST searches showed that genotype II isolates from Bangladesh shared strong homology (up to

99.96%) with chicken-derived isolates from Jordan and India. The subgenotype VI.1.1 isolate closely matched (99.4%) a pigeon-derived isolate from Pakistan. Subgenotype VII.2 isolates showed 98% similarity to strains identified in barn owls and eagles from India and Indonesia, respectively. Genotype VII remains of global concern due to its role in multiple zoonotics [51, 52]. Subgenotype XIII.2 isolates displayed 93–97% similarity to isolates from India and Japan, while XIII.2.3 isolates shared 94.3% similarity with a Japanese chicken strain. Subgenotype XXI.1.2 isolates showed 98% similarity to Pakistani pigeon-derived strains. Together with divergence analysis, these findings support intra-genotypic evolution of NDVs in Bangladesh.

Divergence analysis revealed low pairwise and overall mean evolutionary distances (0.00–0.03) among genotypes II, VII.2, XIII.2.3, and XXI.1.2, suggesting limited diversity. By contrast, genotype XIII.2 showed moderate divergence (>0.03), indicating genetic heterogeneity. The overall mean distance of 0.08 across all 26 isolates suggests relatively low similarity among genotypes, highlighting the mutational potential of NDVs.

Several classic experimental studies have investigated single amino acid substitutions in the F protein neutralization epitopes [53–55]. Amino acid variation within neutralizing epitope 1 (residues 72, 74, 75, 78, 79), epitope 2 (residues 157–171), and epitope 3 (residues 343–353) of the F gene further supports ongoing viral evolution [56]. Substitutions at these sites can alter antigenic structure by changing side-chain size, polarity, or flexibility, thereby modifying the local 3D conformation of epitopes and their recognition by antibodies. Such mutations may also change surface charge distribution, reducing complementarity with antibody binding sites and lowering binding affinity [57]. Additionally, substitutions can create N-X-S/T motifs, introducing N-linked glycosylation sites that add glycans capable of shielding epitopes from immune recognition [58].

In our study, substitutional analysis across Bangladeshi NDV isolates revealed changes at residue 78 in VII.2 and residue 79 in XIII.2 within epitope 1, and at residue

170 in VII.2 and residues 163 and 170 in XIII.2 within epitope 2. Epitope 3 remained conserved across all isolates. Such variation in the F protein can alter B-cell and T-cell epitope presentation and reduce the efficacy of neutralizing antibodies, which often target conformational epitopes on the F protein. Consequently, antigenic drift resulting from these amino acid substitutions may reduce vaccine effectiveness, contributing to breakthrough infections when mismatches occur between vaccine strains and circulating viruses [39, 48, 54, 59-61].

Traditional NDV vaccines derived from lentogenic or mesogenic strains of genotypes I, II, III, IV, V, VIII, and XV [62-64] are still widely used. In Bangladesh, LaSota (GenBank accession no. AF077761) and B1 (GenBank accession no. JN872151), both under genotype II, are administered to chicks, whereas the mesogenic Mukteswar strain (GenBank accession no. EF201805) under genotype III is used in older birds [65, 66]. In our study, analysis of amino acid variability in complete F gene sequences of Bangladeshi NDV isolates compared with vaccine strains (LaSota, B1, and Mukteswar) revealed substitutions in epitope 1 at residues E74K (XIII.2.3), K78G (VII.2), and A79T/G (XIII.2). For epitope 2, compared with LaSota and B1, substitutions were detected at residues E163D (XIII.2), D170G (VII.2), D170T (XIII.2), and D170N (XIII.2.3). Compared with Mukteswar, substitutions included E163D (XIII.2), G170D (II, VI.1.1, VII.2, XXI.1.2), G170D/T (XIII.2), and G170T/N (XIII.2.3). These variations may compromise immune recognition, which could help explain why NDV remains enzootic and continues to cause significant economic losses despite routine vaccination [56, 67].

Sequence similarity analysis showed that LaSota and B1 exhibited the highest similarity (100% and 98%) with genotype II field isolates ON713864 and ON713865, respectively. However, similarity decreased markedly against other genotypes: 80–89% with VI.1.1, VII.2, XIII.2, XIII.2.3, and XXI.1.2. Mukteswar showed 88–92% similarity with all circulating genotypes. Such genotypic mismatch likely reduces vaccine-induced immunity due to antigenic variation in surface proteins,

particularly the F protein [66]. In addition, biosecurity weaknesses such as inadequate wild bird control, poor farm hygiene, mixed-species farming and vaccination issues, including incomplete flock coverage, improper administration, irregular booster schedules, and use of genotype II vaccines against genotype VII/XIII strains, create ecological and immunological pressures that allow the virus to evolve, adapt, and persist in poultry populations [68].

Recent advances addressing epitope variability in the F protein include the development of genotype-matched antigens, such as genotype VII-matched live/chimeric vaccines [69], DNA vaccines with IL-28b adjuvant [70], thermostable recombinant GVII live vaccines [71], locally matched inactivated GVII.2 vaccines [72], non-GVII lentogenic strain-based vaccines [73], and viral-vector-based vaccines [74]. Nevertheless, older genotype II-based vaccines remain vulnerable to epitope drift [75]. Genotype-matched vaccines have consistently shown superior protection. For example, in China, where ND is endemic, genotype-matched vaccines combined with improved biosecurity have significantly reduced outbreaks [64, 76]. In India, an inactivated GVII strain vaccine provided 100% protection against GVII challenge in chickens, outperforming the commercial GII LaSota vaccine, which gave 80% protection [77]. In Bangladesh, A study tested three booster vaccines—live GII LaSota, inactivated GII LaSota, and inactivated GXIII.2 against the local virulent GXIII.2 BD-C161/2010 strain in chickens primed with live LaSota. All boosters prevented death, but the inactivated LaSota outperformed the live version, and the GXIII.2 vaccine gave full clinical protection and significantly reduced virus shedding [66]. In another experiment, the dominant GVII.2 strain isolated from diseased chickens in Bangladesh was used to develop an inactivated vaccine, which provided nearly 100% protection against the homologous strain. This result contrasted with the only 57% protection against GVII.2 observed in chickens vaccinated with the commercial live GII LaSota strain [72]. Collectively, these findings emphasize the urgent need for updated, genotype-

specific NDV vaccines for Bangladesh.

Future research in Bangladesh should prioritize the development of genotype-matched vaccines using cutting-edge technologies, targeting circulating strains VII and XIII, supported by continuous molecular surveillance. Policymakers should also evaluate the cost-effectiveness of genotype-matched vaccines and enhanced biosecurity strategies to achieve sustainable NDV control in the poultry sector. Full-length F gene sequences were analyzed due to the limited availability of HN or whole-genome data for Bangladeshi NDV strains in the NCBI GenBank database.

Conclusion

The genetic diversity of NDV genotypes and subgenotypes circulating in Bangladesh between 2010 and 2024 reveals the co-circulation of multiple strains, including novel and virulent variants. Subgenotype VII.2, associated with the fifth panzootic, and the recently detected a novel sub-genotype XIII.2.3 pose significant threats to poultry health and production. The ongoing evolution and emergence of new variants complicate control measures based on genotype II and III vaccines, which no longer fully protect against circulating field strains. The reduced effectiveness of NDV vaccines is due to genetic differences between vaccine and circulating strains, which lower cross-protection. To minimize future outbreaks and economic losses, it is essential to develop high-quality, genotype-matched NDV vaccines tailored to the predominant strains (particularly VII and XIII) circulating in Bangladesh.

Development of such genotype-matched vaccines employing advanced technologies, coupled with continuous molecular surveillance, optimized vaccination strategies, and strengthened biosecurity, represents the most effective pathway to sustainable ND control in Bangladesh.

Data Availability Statement

Data sharing does not apply to this article as no new data were generated in this study.

Conflict of Interest

The authors declare that they have no financial or other competing interests with any organization or company, nor among the authors and co-authors.

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Authors' Contribution

Farah Zehreen: Methodology, data curation, software, formal analysis, and writing-original draft; Md. Abdur Rahman: Methodology, data curation, software, formal analysis, and writing-original draft; Md. Golzar Hossain: Writing-reviewing & editing, funding acquisition; Masaru Shimada: Writing-reviewing & editing; Sukumar Saha: Conceptualization, supervision, project administration, writing-reviewing & editing, funding acquisition.

Figures and Figure Legends:

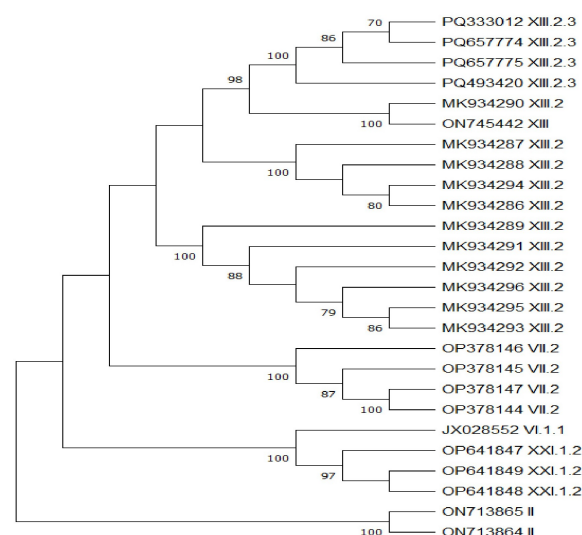


Figure 1: Phylogenetic tree of Bangladeshi NDV isolates, in which the evolutionary history was inferred by using the Maximum Likelihood method and Poisson correction model with 1000 bootstrap replicates. The tree with the highest log likelihood (-3585.91) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the

branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbour-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Poisson model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model

NDV accession	Epitope 1 (72-79)										Epitope 2 (157-171)										Epitope 3 (343-353)										
Name	D	K	E	A	C	A	R	A	S	I	A	A	T	N	E	A	V	H	E	V	T	G	L	D	L	C	T	R	I	V	F
1. EF201805 Mukteswar III																															
2. AF077761 LaSota B																															
3. JN872151 Hatchner II																															
4. ON713805 B																															
5. ON713864 B																															
6. JX028552 V1.1.1																															
7. CP378147 V1.2																															
8. CP378146 V1.2																															
9. CP378143 V1.2																															
10. CP378144 V1.2																															
11. MK934296 XIII.2																															
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20. MK934287 XIII.2																															
21. MK934286 XIII.2																															
22. ON745442 XIII																															
23. PQ333012 XIII.2.3																															
24. PQ3403420 XIII.2.3																															
25. PQ357774 XIII.2.3																															
26. PQ357775 XIII.2.3																															
27. OP461849 XXX1.1.2																															
28. OP461848 XXX1.1.2																															
29. OP461847 XXX1.1.2																															

Figure 2: Amino acid variability at antigenic epitopes in F gene sequences of Bangladeshi NDVs compared to NDV vaccine strains used in Bangladesh in which antigenic variation in epitope 1 and epitope 2 were distinguished but epitope 3 remain conserved.

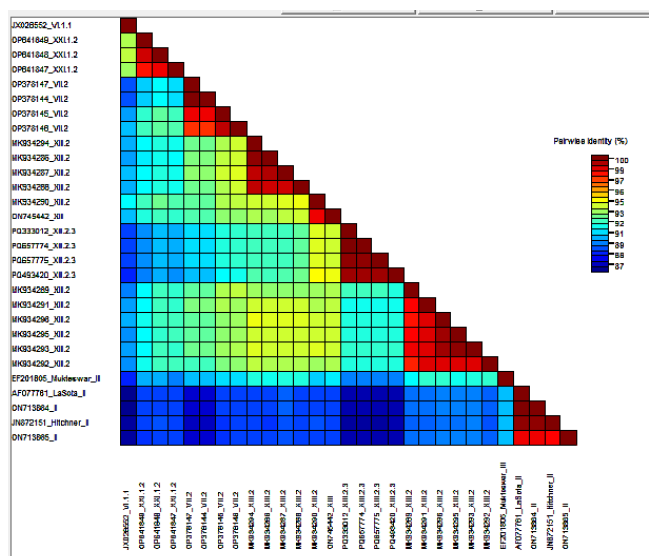


Figure 3: The pairwise identities-based plot of F protein sequences aligned by MAFFT and displayed by Sequence Demarcation Tool (SDT) software.

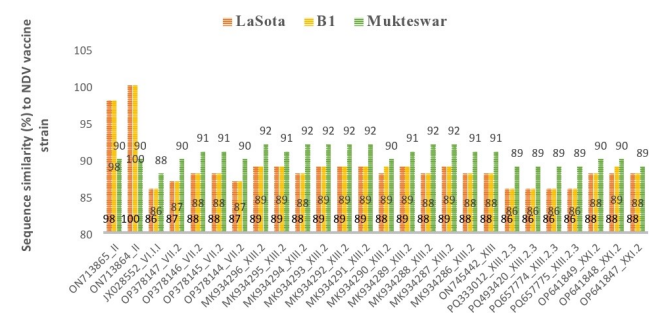


Figure 4: Sequence similarity (%) of NDV vaccine strains used in Bangladesh (LaSota, B1 and Mukteswar) comparing to complete F gene sequences across different genotypes and subgenotypes of circulating Bangladeshi ND V isolates in which orange color

indicates LaSota vaccine strain and it's similarity (%) is labelled at inside base; gold color indicates B1 vaccine strain and it's similarity (%) is labelled at centre; and green color indicates Mukteswar vaccine strain and it's similarity (%) is labelled at outside end.

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- Supplementary Table 1.a Evolutionary Divergence between genotype II isolates
- Supplementary table 1.b Evolutionary Divergence among sub-genotype VII.2 isolates
- Supplementary table 1.c Evolutionary Divergence among sub-genotype XIII.2 isolates
- Supplementary table 1.d Evolutionary Divergence among sub-genotype XIII.2.3 isolates
- Supplementary table 1.e Evolutionary Divergence among sub-genotype XXI.1.2 isolates