Review

Bolstering Poultry Health: The Urgency of Updating Newcastle Disease Virus (NDV) Vaccines in India

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Bolstering Poultry Health: The Urgency of Updating Newcastle Disease Virus (NDV) Vaccines in India

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Abstract: Newcastle disease is a global disease, spreading quickly during outbreaks in poultry and leading to significant economic loss due to high morbidity, mortality, trade restrictions and embargoes. This disease has been present in India for the past 96 years, since 1928. Antigenic differences among Newcastle disease virus (NDV) strains are resulting in massive outbreaks in vaccinated and unvaccinated poultry flocks around the globe. Synanthropic bird reservoirs contribute significantly to the extensive genetic diversity of the virus. This diversity, along with the vulnerability of many avian species, has led to diagnostic and vaccination failures. The genetic attributes of the circulating strains in India, meanwhile, remain mostly undisclosed. Ongoing evolution of spectrum of NDV genotypes in India, this review paper underscores the need for vigilant monitoring and adaptation of vaccination strategies to address emerging variants.

Keywords: newcastle disease virus; chicken; vaccines

1. Introduction

Poultry represents one of the most commonly available, cheapest and acceptable sources of protein that accounts for more than 40% among the livestock [1]. In recent decades, the poultry industry has experienced remarkable expansion due to increasing demand for eggs and meat. United States and China are the leading global producers in the poultry meat and egg segments respectively. Since 1990, there has been a remarkable increase of over 100 percent in global egg production [2]. Poultry production in India has witnessed a radical transition from handful rearing of native chickens in backyard to high-capacity farms with environmental controlled settings boasting annual turnover of more than 10 billion USD [3]. In India, the broiler and layer sectors are steadily growing, ranking third and fourth globally by producing 138.38 billion eggs [4] and 4.3 MMT of poultry meat [5], respectively. On the other hand, diversified poultry accounts more than 1.43% of total poultry population. However, this rapid expansion brings challenges like antimicrobial resistance, residues, and frequent disease outbreaks [6]. Repeated outbreaks of several infectious diseases specially of viral origin are hampering its growth rate with Newcastle Disease (ND) & Avian Influenza (AI) are the most concerned diseases. Globally, a total of 2606 lakh cases were recorded in poultry with 40.22% death rate (Table 1). The probability of NDV among laying chickens during first phase of egg production is around 10% with resultant 15% production loss [7]. The economic impact because of NDV outbreaks in US and Bangladesh poultry flocks was 162 and 288 million dollars respectively [8,9].

Newcastle disease (ND) is one of the most infectious avian diseases caused by avian paramyxovirus type1 (APMV-1) with an ability to infect over 250 species of poultry [10,11] and claiming loss of millions of dollars to the avian industry. World Organization for Animal Health [12] terrestrial animal health code defines the Newcastle Disease as an infection of birds caused APMV–

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1 that either having intracerebral pathogenicity index (ICPI) of 0.7 or greater in day-old chicks (*Gallus gallus*) or multiple basic amino acids at the C-terminus of the F2 protein and phenylalanine at residue 117, which is the N-terminus of the F1 protein. In poultry, NDV is reflected in four disease forms such as Velogenic (viscerotropic and neurotropic), Mesogenic, Lentogenic and Asymptomatic enteric form.

Newcastle disease Infection in chickens is characterized by high mortality, torticollis, greenish diarrhoea, gasping, watery albumens and affecting mainly trachea, proventriculus, intestinal segments and caecal tonsils (Figure 1). Along with Highly Pathogenic Avian Influenza, this disease is recognized worldwide as one of the two most destructive and dreaded diseases causing up to 100% deaths in unvaccinated poultry flocks [13]. Although, all species are susceptible, the disease progression and mortality vary among species and breed.

Nervous signs (torticollis)

Nervous signs (torticollis) with flaccid paralysis of legs

Trachea characterized by hemorrhages with exduate

Pin-point to diffuse hemorrhages on tips of proventricular gland

Multi-focal necrosis on capsular surface of spleen

Mucosal hemorrhages on the ileal intestinal segment

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Hemorrhages in caecal tonsils

Figure 1. Velogenic Newcastle Disease: Typical clinical signs and post-mortem lesions in vaccinated flocks of Rhode Island Red and White Leghorn grower chickens.

2. Newcastle Disease Virus (NDV) and Genotypes

2.1. NDV Genotypes: A Global Update

Newcastle Disease is the second most widespread disease globally, affecting many countries after rabies [14] with significant prevalence. High number of outbreaks have been recorded in Asian followed by African region with least impact in Europe and United States (Figure 2). Functional fusion (F) protein is the primary determinant of APMV-1 pathogenesis which is generated by its precursor F0 within the fusion protein cleavage site (FPCS) [15]. Based on FPCS, ND viruses have been categorized into two classes wherein the class-I represents 9 genotypes with genome size of 15,198 nucleotides and class II as 11 genotypes [16,17]. Class I genotypes exhibit a global distribution among wild birds and are often non-pathogenic to chickens but still could be traced in live bird markets. The class II encompasses a majority of highly pathogenic viruses, as well as certain non-pathogenic and vaccine strains [18] with worldwide circulation [19]. The lower genetic diversity of class I viruses than class II may be due to their circulation primarily among wild birds having no history of ND vaccination, resulting in reduced immune pressure on these viruses [20].

In 1926, ND was first documented in Java of Indonesia [21,22]. Subsequently, in 1927, the virus was isolated by Doyle in Newcastle upon Tyne, England, and designated as NDV. Following this discovery, numerous outbreaks of virulent NDV affecting various avian species were reported across continents, including Asia (China, Korea, India, Japan, Sri Lanka, Saudi Arabia, Kazakhstan, Philippines), Africa (Kenya), Australia, Europe (France, Italy, England, Scotland, Spain, and Russia) and North America (United States of America, Canada, and Costa Rica) [23,24]. Ever since the first emergence in 1926, there have been five global ND pandemics, each originated by distinct genotypes. The inaugural pandemic, spanning from 1920 to 1960, emerged concurrently in Southeast Asia and Europe, requiring approximately three decades to proliferate globally. This pandemic was driven by NDV variants possessing genotypes I, II, III, and IV [25]. Notably, chickens, waterfowl, and various bird species were primary targets of infection, with regional variations in disease manifestation [26]. During the 1960s and 1970s, a second pandemic potentially originated in the Middle East. This was driven by the intensified commercialization of the global poultry industry and increased international trade of parrots. This phase primarily featured NDV genotypes V and VI, posing a threat to ornamental and caged bird populations [27,28]. The third pandemic, attributed to genotype VIb isolates, emerged in the late 1970s initially among racing pigeons but swiftly spread globally, hampered by challenges in implementing rigorous husbandry practices within racing pigeon communities [19]. Presently, the fourth ongoing pandemic, believed to have commenced in the late 1980s, is linked to genotypes V, VI, VIII and more specifically VII.1. This pandemic has inflicted significant economic loss to the poultry industry across Southeast Asia, Africa, America, Middle East and Europe, [29–31].

Currently, fifth panzootic was originated in Asia in late 2000 with genotype VII.2 prominently. Given its worldwide prevalence in well-entrenched epidemic form, this disease poses a permanent threat to both small-scale and industrial poultry rearing. Across the regions, Asia followed by Africa, America, Europe and Oceania revealed to have highest number of NDV cases and associated deaths

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with several genotypes reported (Table 1). Since 2005, 131 countries have notified the NDV outbreaks in poultry. Iran is the country with highest burden of NDV outbreaks followed by Vietnam, China, India and Afghanistan. Genotype VII has been emerged as a dominant version in Iran since 2011 affecting various poultry species [32]. Recent outbreaks despite rigorous vaccination in Middle East, Asia, Europe, Africa & South America were due to Genotype VII.1.1 and Latin America was associated with genotypes V, VI, VII, XII, XVI [33]. Australia officially declared NDV free zone in 2002 and no outbreaks were detected till 2011 in commercial poultry populations [34,35].

Early genotypes (pre-1960s) have a genome length of 15,186-nt, while contemporary genotypes (post-1960s) have 15,192-nt. G124S and K192N alterations in the F gene of ancient genotypes III and IV viruses created genotype I viruses, which evolved into genotype II viruses by adding L69M and D82E [36]. Research relevant to Genotype VII revealed 273 publications since 2004, while Genotype XIII was reported since 2012 with 38 publications indicating the emerging scenario of Genotype XIII (Figure 3).

Genotype VII has played a significant role in the most recent fourth pandemic of Newcastle Disease Virus (NDV), becoming the dominant strain globally. This genotype exhibits a complex diversity, with sub-genotype VIIa primarily impacting countries in Asia and Europe, whereas genotype VIIb is more prevalent in South Africa. Furthermore, sub-genotypes VIIc, d, e, f, g, h, and i have been identified in isolates from China, Kazakhstan, and South Africa [19,37]. The newly characterized sub-genotype VIIi exhibits genetic similarity to strains identified in Indonesia, as well as those collected in Pakistan and Israel in 2013 [38]. This sub-genotype has been responsible for Newcastle Disease (ND) outbreaks in Pakistan since 2012 [37] and is deemed enzootic due to its widespread distribution, thus contributing to the ongoing fifth ND panzootic. In Israel, there has been a transition from the previously dominant sub-genotypes VIId and VIIb to VIIi since 2012 [39].

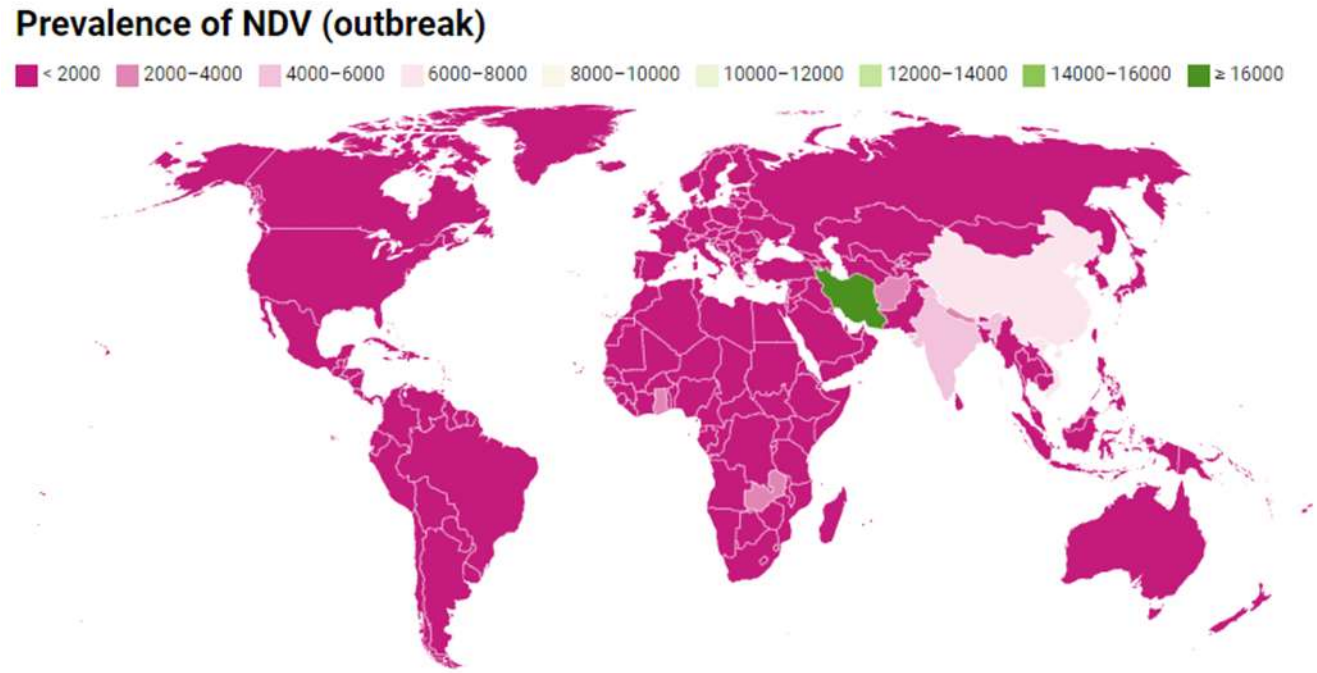

Figure 2. The NDV outbreaks (in numbers) across the countries during 2005-2024 [12].

Table 1. Global occurrence of NDV genotypes, cases and deaths reported from 2005-2024 in different regions [12].

Region	Total cases (in lakhs)	Deaths (in lakhs)	% Deaths	Genotype	References
Asia	2135.32	828.61	38.80	VIId	[40]
				VII	[41]
				VIIi	[42]

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				VIII XIII VII.1.1 XIII.2.1 VII.2 XIII.2	[43] [44] [45] [46] [47] [48]
Africa	411.33	203.33	49.43	NT	-
America	46.40	10.35	22.31	VII VIIa, VIId	[49,50]
Europe	13.01	5.83	44.81	VIIa VII.2	[51] [52,53]
Oceania	0.00017	0.00017	100.00	NT	No genotypes reported

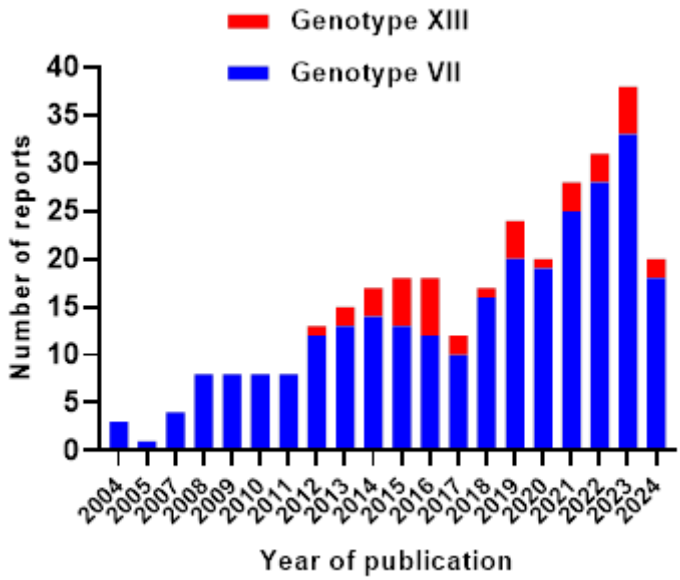

Figure 3. Number of publications (year wise) reporting the Genotypes VII and XIII in poultry across the globe (Source: PubMed, 2024 (https://pubmed.ncbi.nlm.nih.gov/) Search strings: Search query: Newcastle disease genotypes VII in poultry and Search query: Newcastle disease genotypes XIII in poultry.

2.2. NDV Genotypes: An Indian Update

Newcastle disease in India was first witnessed in Ranikhet of Uttarakhand [Edwards, 1928, 54] followed by Tamil Nadu region [55] and continued to pose a threat to both commercial and backyard poultry productions. Currently, India is under endemic situation for ND resulting in substantial economic losses. Recurring outbreaks affecting many avian species are still occurring in Southern, Northern, Central and Northeastern parts as detailed in Tables 2 and 3. In India, virulent NDV strains were reported from diverse avian species viz. pigeons, Mynahs, Emu, Owl, peafowl and quails besides domesticated chickens. The relative risk ratio of Newcastle disease is higher for intensive production systems with respect to semi-intensive (6.7) and backyard or free range (2.1). Similarly, the risk ratio for broilers is of 4.5 and 4.9 with respect to commercial layers and backyard birds [56]. Khorajiya et al. [57] reported economic loss of 45,000 USD in vaccinated layer flocks of Gujarat, India due to GXIII NDV induced infection. Mixed poultry production systems, live and wet markets, porous borders, high density farms, open transportation of live birds supports the frequent NDV outbreaks [58].

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Ever since from 2005, a total of 5071 outbreaks with 3.9 million attacks and 0.22 million deaths were noticed in India till 2023 [12] with a death rate of 5.96% in different classes of poultry (Figure 4; Tables 2 and 3). Higher outbreak intensity was noticed in North-Eastern, Punjab and Tamil Nadu regions, while least outbreaks were recorded in Telangana, Madhya Pradesh, and Himachal Pradesh (Figure 5). Three genotypes (IV, VII and XIII) belonging to 5 different classes have been recorded in India till date [59–61]. Genotype XIIIb (KX372709-KX372711) was first reported in 2006 in commercial chickens at Nagpur [62]; while Genotype VII existence is dated back to 1989 in chickens at Tamil Nadu region [59]. In chickens, Genotype XIIIb (Acc. no. KX372709-KX372711) was first reported in 2006 in commercial chickens in Nagpur [Morla et al., 2016, 62], while the existence of Genotype VII dates back to 1989 in the Tamil Nadu region [59].

The study on NDV isolates from pigeons, using monoclonal antibodies for antigenic analysis, found evidence of atypical antigenic types of virus circulation in India, which may contribute to vaccine inefficacy [63]. The occurrence of NDV outbreaks in vaccinated poultry flocks with Genotype XIII such as Pandu-KR072665 from Ranchi, KX345397 from Kamrupa, and XIII from Bareilly) in India suggests ongoing virus evolution. Further, the isolation of Genotype XIIIb from vaccinated layer and broiler flocks from 2006-2014 from Central India with up to 93% mortality suggesting the panzootic potential in the country [62]. Velogenic NDV strains of Genotype-IV isolated from Indian chicken and pigeon in 1997 and 2000 respectively provides the strong evidence of its circulation in Indian sub-continent with an origin indigenous in nature [59].

In mixed viral infections, especially during bird-to-bird transmission NDV strain sequences may change. In India, Genotypes II, III, VI, VIII, XIIIa are reported from 1989-2013 in wild and domesticated poultry of which Genotypes II, III, VI are genetically distinct [64]. Jakhesara and colleagues [65] identified the circulation of Genotypes II, IV and XIII in vaccinated chicken flocks from different parts of India. ND viruses recovered from peafowl indicated the persistence of genotype II in non-domesticated avian host [66]. Higher mortality of 79.4% in layer and broiler flocks of Northern India revealed to caused by an isolate of Genotype XIIIa [60]. Phylogenetic and evolutionary distance analyses of NDV revealed a circulation of novel sub genotype of VIIIe in vaccinated chicken flocks of Tamil Nadu [67]. Exacerbation of clinical signs upon co-infection of NDV Genotype XIII and XIIIe with Low pathogenic avian influenza was reported in Southern region of India [68]. Very recently, whole genome sequencing of NDV isolated from commercial layer farms of Chhattisgarh region revealed the presence of genotype VII.2 caused outbreaks in 2023 [61]. In addition, emergence of novel genotypes XXII.1 and XXII.2 from North-Eastern states are being witnessed in the country [69]. A recent outbreak among commercial chickens in Kashmir was caused by Genotype VIIi and linked to the NDV strain Chicken/Israel/2011/1115 818 [70].

Different genotypes of NDV reported in various regions across the country was illustrated in Table 2. To understand the dynamics of vNDV genotypes across various geographical locations of India, the universally available nucleotide sequences submitted during various outbreaks were downloaded and analyzed. Recent analysis of nucleotide sequences reveals distinct geographic variations with GXIII prevalent in South and GVII in Northern parts of India (Figure 5a). This regional differentiation suggests that nucleotides are somewhat unique to specific districts or regions, indicating localized evolutionary pressures. Furthermore, recent strains from 2022 and 2023 showed a strong nucleotide similarity, suggesting a common ancestor closely related to Genotype VII (Figure 5b). These findings highlight the potential influence of geographic and environmental factors on strain evolution and distribution. Comparatively higher death rates in chickens and other poultry were observed to be associated with Genotype XIII than Genotype VII (Table 3).

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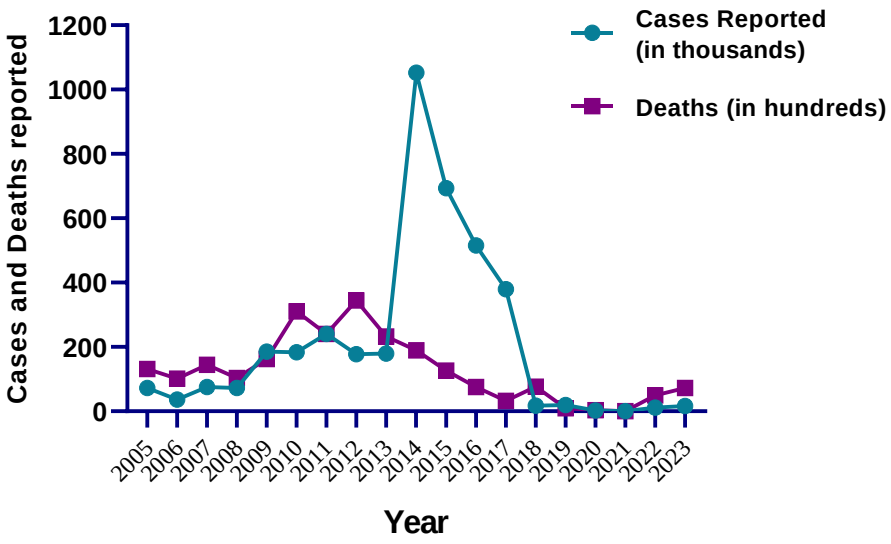

Figure 4. Year wise velogenic NDV cases reported and deaths occurred in India during different years.

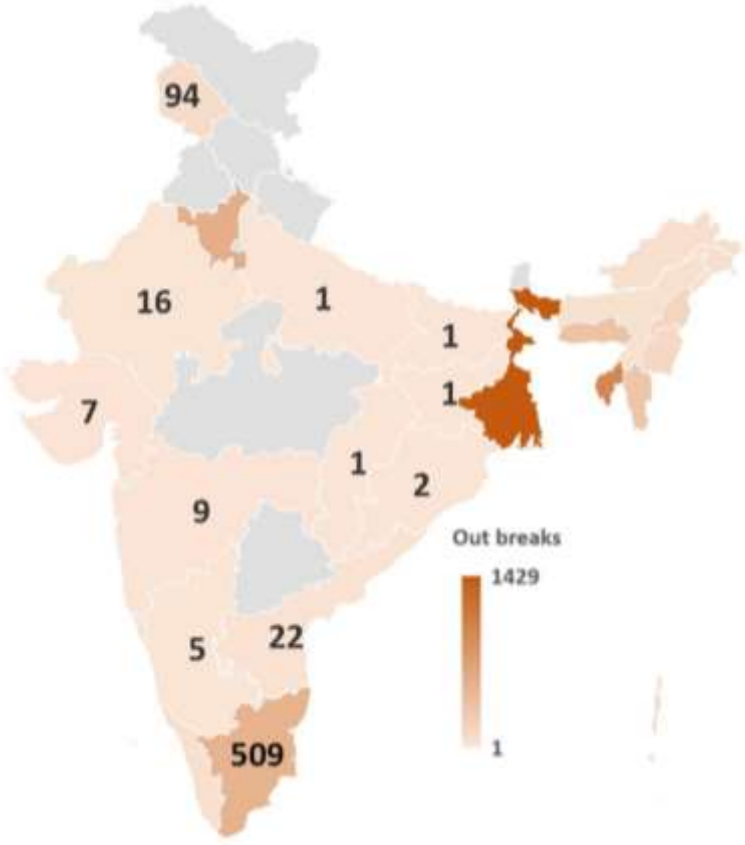

Figure 5. Infection pressure of NDV in different regions of India as indicated by number of outbreaks. Light intensity colour indicates lower number.

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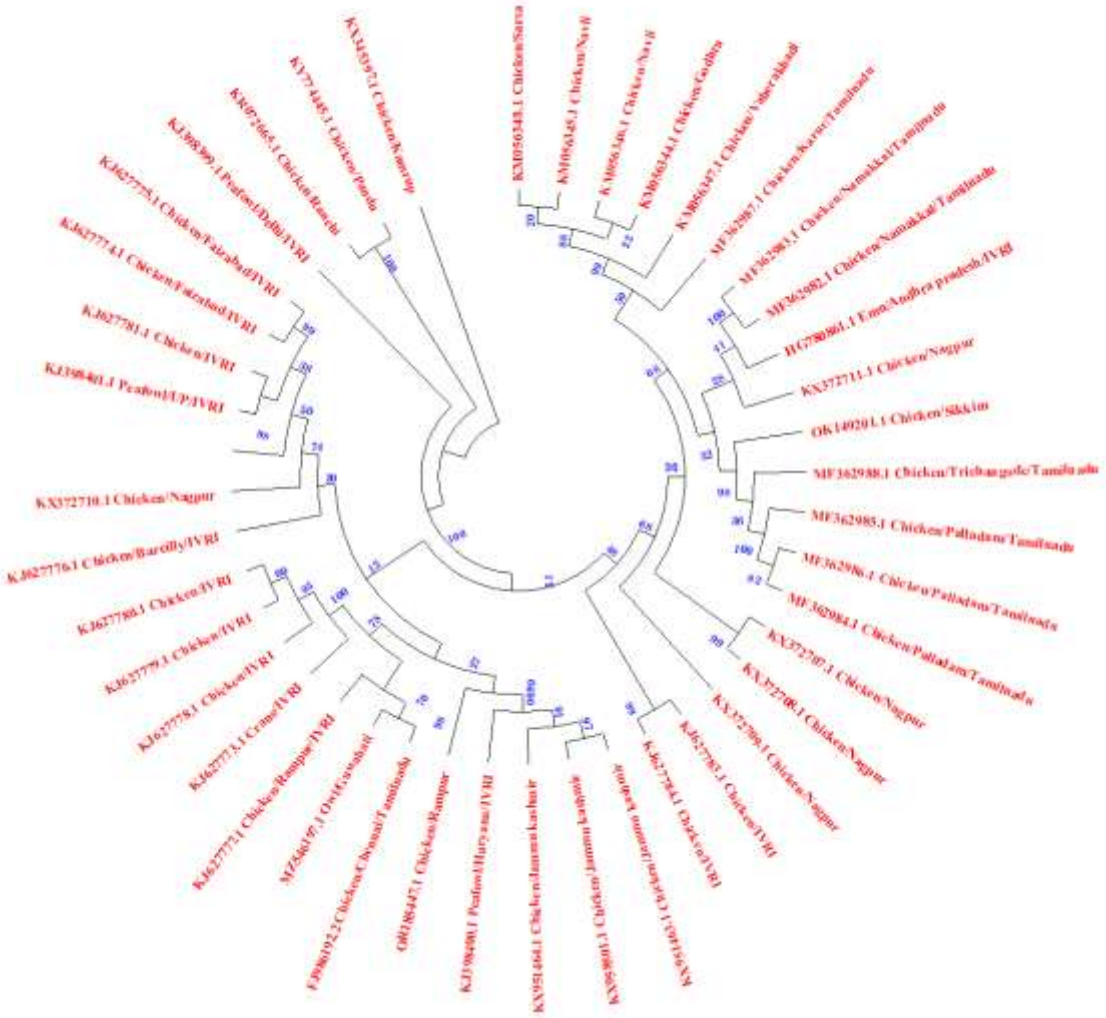

(a)

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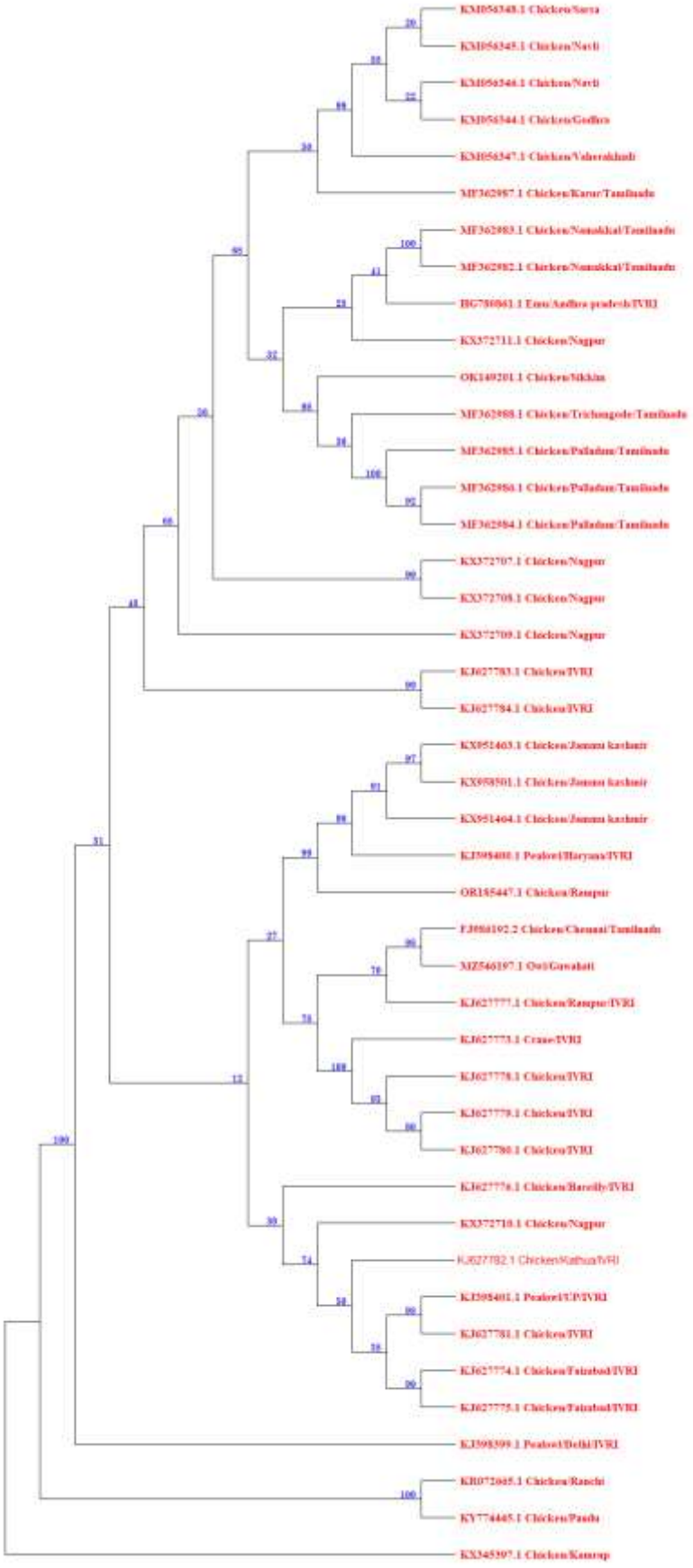

(b)

Figure 6. (**a**) Phylogenetic tree (traditional/branch style) illustrating the evolutionary and distance relationships between various Newcastle Disease Virus (NDV) strains and genotypes circulating in

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India. The tree is constructed based on nucleotide sequences from previous reports and shows the distinct clustering of strains according to geographic regions. (**b**) The phylogenetic analysis (Circle tree/branch style) of Genotype VII and XIII NDV strains-based nucleotide sequences of F gene in the highly variable region including F gene cleavage site.

The evolutionary history of Genotype VII and XIII strains was inferred by using the Maximum Likelihood method and Tamura-Nei model [71]. A bootstrap consensus tree, derived from 500 replicates [72], represents the evolutionary history of the analyzed taxa. Branches with less than 50% bootstrap support were collapsed. Initial trees for the heuristic search were automatically generated using the Neighbor-Join and BioNJ algorithms on a pairwise distance matrix estimated with the Tamura-Nei model, selecting the topology with the highest log likelihood value. This analysis included 43 nucleotide sequences, with a total of 479 positions in the final dataset. Evolutionary analyses were performed using MEGA software version 11 [73].

Table 2. NDV Genotypes isolated from different poultry species across India (2005-2023).

Year	Region	Species	Genotype	References
2006-2012	Central India	Chicken	XIIIb	[62]
2016-2020	North-Eastern India	Chicken	XXII.1, XXII.2 & XIII	[69]
2010-2012	Southern India	Emu	XIII	[74]
2011-2013	Northern India	Pea fowl	VIIi & XIII	[75]
2014	Southern and Northern India	Chicken	XIII	[76]
2015	North-Eastern India	Chicken	XIII & XIIIc	[77]
2015-2016	Northern India	Chicken	XIIIa	[60]
2015-2016	Southern India	Chicken	XIII & XIIIe	[68,78]
2019	Eastern India	Commercial and backyard poultry	XIII	[79]
2023	Northern India	Chicken	VII.2	[61]

Table 3. Details of cases reported, deaths and genotypes identified in different locations of India.

Region	Cases	Deaths	% Deaths	Genotype	References
Andhra Pradesh	2941	1581	53.76	XIII	[65]
Andaman and Nicobar	26843	2437	9.08		
Arunachal Pradesh	1889	762	40.34		
Assam	20496	5996	29.25	XIII & XIIIc	[77]
Bihar	35	15	42.86		
Chhattisgarh	4869	1213	24.91		
Daman & Diu	83	4	48.19		
Goa	24	4	16.67		
Gujarat	3773	442	11.71	XIII	[57,80]
Haryana	2624586	59284	2.26	VIIi	[64,75]
				XIII	[65]
Jammu & Kashmir	30773	4642	15.08		
Jharkhand	32	0	0.00		
Karnataka	5720	4709	82.32		
Kerala	55936	3701	6.62		
Lakshadweep	26473	11735	44.33		
Maharashtra	13496	12768	94.61	XIIIb	[62]
Manipur	73240	3516	4.80		
Meghalaya	23847	7801	32.71		

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Mizoram	26694	9115	34.30		
Nagaland	25934	5439	20.97		
Orissa	205	25	12.20		
Pondicherry	9552	2932	30.70		
Rajasthan	13140	3130	23.82		
Tamil Nadu	684845	17540	2.56	XIII &XIIIe	[65,68,78]
Tripura	144320	34160	23.67		
Uttar Pradesh	12	9	75.00	XIIIa XIII, VIIi	[60] [64]
West Bengal	95642	40390	42.23	XIII	[65]

3. Newcastle Disease: Vaccines and Vaccination

Vaccination is the most viable and practically feasible strategy for prevention of control of NDV outbreaks in endemic countries [81]. All the countries share common practices, including the use of commercial live or inactivated vaccines (prepared from genotype II) and similar vaccination regimes (number of doses, route of administration, combination of live and inactivated options). The seroprevalence rate of ND in India is as high as 83% [82]. In India, nearly all farmers carryout ND vaccination [83] with variable periodicity. Currently, Genotypes I and II are the most used in NDV vaccines worldwide including India. Several lentogenic and mesogenic strains have been in use for decades with varying ICPI efficacies (Figure 7) in different formats (Figure 8). Among lentogenic strains, cloned versions have lower ICPI and V4 and Ulster 2C HAS '0' ICPI indicating their least pathogenic effects on chicks. Further cloned strains possess low virulence with better efficacy. For disease control, immunization using lentogenic and mesogenic strains in both live and inactivated/killed vaccine formats is currently practiced at different stages, depending on the birds' production status.

Owing to the immediate immune spiking, live vaccines having lentogenic virus are commonly employed to prime the immune system of bird followed by vaccination with mesogenic strain and inactivated vaccines. However, the vaccination is tailored depending upon infection pressure and type of chicken production (Table 4). For example, administration of ND killed (concentrated) vaccines is common in commercial broilers upon hatch to ensure lasting immunity in addition to traditional live vaccines. In India, vaccines are available in various formats and combinations (Table 5), with some being imported and others manufactured locally.

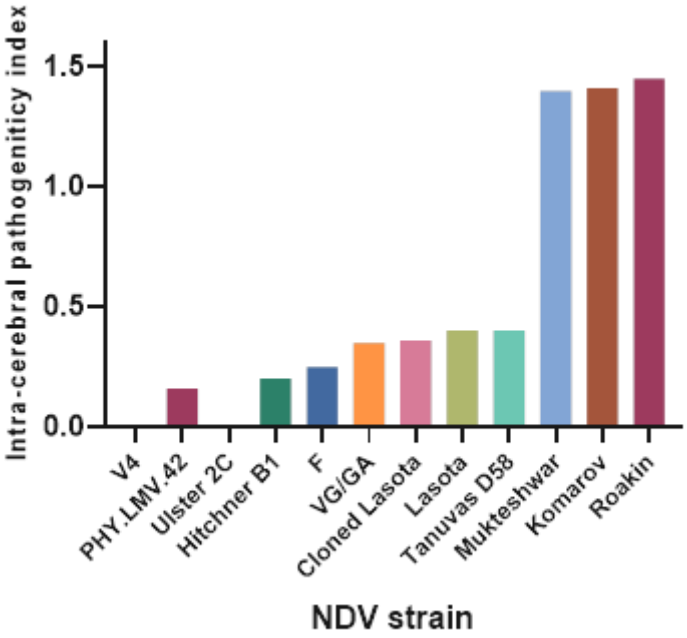

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Figure 7. Different vaccine strains of NDV being used in poultry and their residual virulence from 0 in asymptomatic enteric to 1.4 in mesogenic strains.

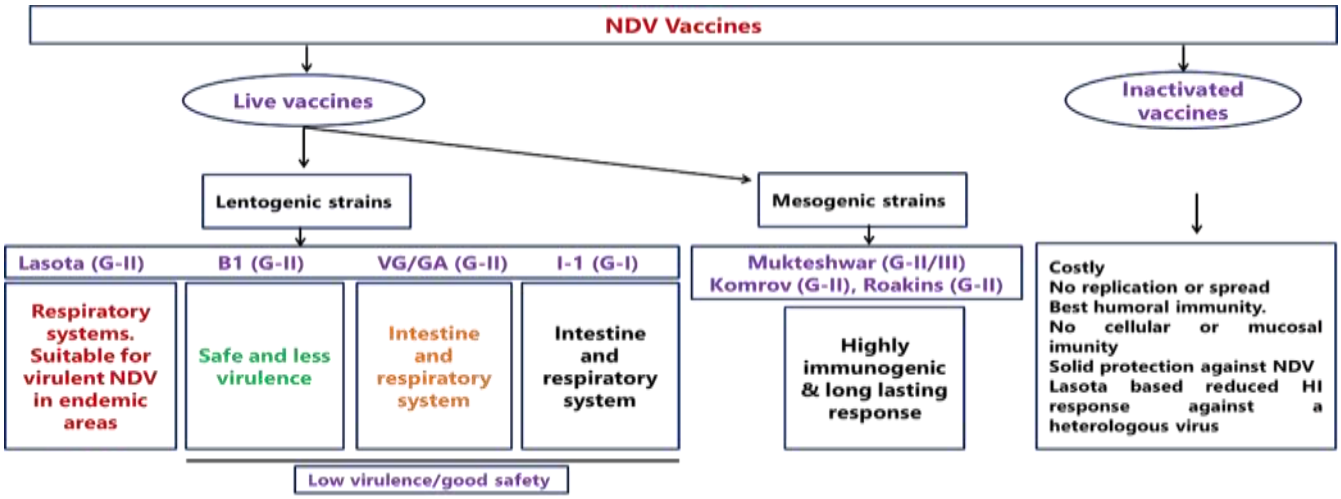

Figure 8. The makeup of ND vaccines being used in the poultry industry.

Table 4. Consensus vaccination regime in birds of varied production potentials in India [84].

Type of bird	Vaccination regime
Commercial layers (BV300, IB)	5-6 days ND Lasota, + ND Killed, 11 wk R2B/RDVK, 16 wk ND Killed
Commercial broilers (Cobb 430Y, Ross)	DOH: ND Killed, 5-6 days ND Lasota, 10 days ND Killed, 28 days ND Live Booster
Dual-purpose and coloured birds	0 day: ND D58, 21 days: D58, 56 days: R2B, 14 wk: D58 clone, 16 wk: ND Killed

Table 5. Commercially available vaccine strains (Genotype II) in different formats in India.

Vaccine strains		Combination (if any)
Live	**Killed**	
F/B1/Lasota/D58/ D26+FC126/Nobilis ND clone 30/VH clone/R2B/CH-80 cloned/CL-79	Lasota/VH	ND+IB ND+IB+IBD

R2B (Mukteshwar), a mesogenic ND vaccine strain, is widely utilized in the Indian subcontinent. It has demonstrated excellent efficacy in older birds (6–8 weeks) by providing long-lasting immunity; however, it has been shown to be pathogenic for young chicks. Phylogenetic analyses of vaccine strains in India, including R2B, based on F, HN, and whole genome sequences, categorize them within Genotype II [85]. This viral strain originated from the passage of one of three Indian field isolates at the Indian Veterinary Research Institute in 1945 and has been employed as a vaccine candidate for booster immunization since then [86]. R2B was integrated into vaccination programs within the Indian poultry industry during the 1980s and is currently produced by major Indian poultry vaccine manufacturers such as Indovax Private Limited (Haryana), Hester Biosciences (Gujarat), and Ventri Biologicals (Maharashtra), with billions of doses administered to date. The inclusion of this strain in vaccination protocols for commercial laying stocks has markedly increased egg production from 3 billion in 1961 to 139 billion by 2023 (Figure 9). This enhancement is attributed to a substantial reduction in Newcastle Disease (ND) incidence to below critical levels (Figure 10).

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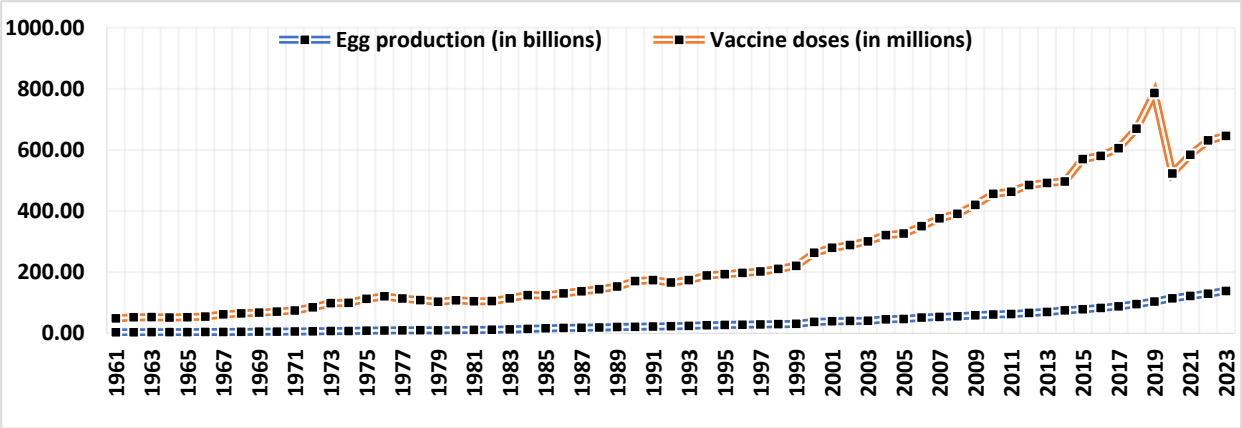

Figure 9. Year wise deployment of ND vaccine (R2B/Mukteshwar strain of Genotype-II) doses and egg production trend in India (Calculated based on FAOSTAT, 2024 database).

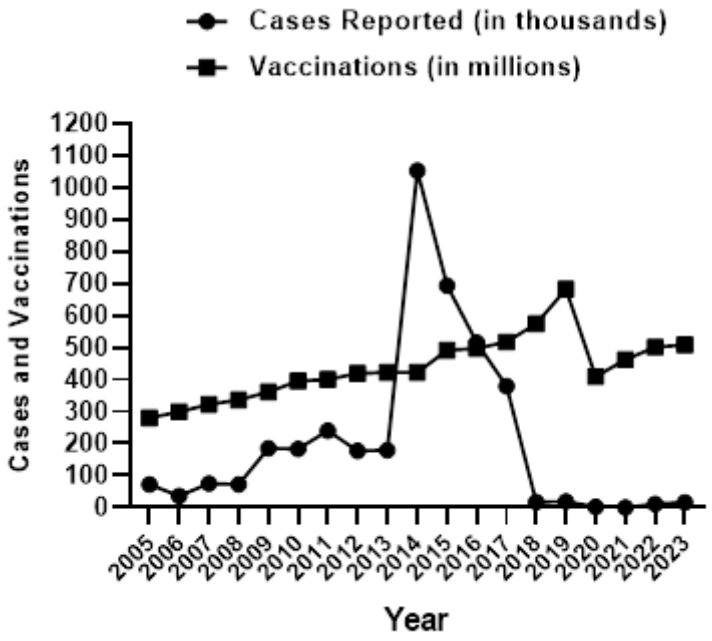

Figure 10. Trends in ND vaccination with Mukteshwar strain and NDV cases reported (Calculated based on FAOSTAT, 2024 database).

4. Does Heterologous Genotype-Based Vaccines Offer Better Protection than Homologous?

Regardless of vaccination regimes that depend on conventional heterologous vaccines for the circulating NDV, frequent outbreaks of ND or non-optimum control occur, with considerable virus shedding and severe economic loss (mortalities and/or drop in egg production). The examination of all the Indian isolates of Genotype II, VII, XIII and other documented viruses revealed a cleavage site (RRQKRF in velogenic and GRQGRL in lentogenic) indicating the high pathogenic potential in these circulating virulent strains (Figure 11). Amino acid sequences of vaccine Genotype II with Genotype VII and XIII showed great diversity at 4, 8, 9, 13, 16, 20, 22, 29, 30, 82, 112-117, 121, 124, 145, 192, 195, 203, 231, 232, 272, 288, 396, 421, 482, 552 and 553 (Table 6). Owing to 3.93-17.31% nucleotide (Table 7) and 10.14-14.96% amino acid (Table 8) variability among vaccine and circulating virulent field strains (Genotypes VII & XIII), the protection levels are questionable. Although vaccination with Genotype-II for ND control has been widely accepted strategy globally, its efficacy is variable concerning types and genotypes. Recent instance of 73.68% disease incidence in completely ND-vaccinated layer flocks of India [87] provides further evidence of the inefficacy of conventional genotype vaccines.

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Figure 11. Protein sequences of different genotypes of Newcastle disease isolated in India indicating differentiation of Fusion protein clevage site (FPCS) (1-9).

Genotype VII NDV homologous vaccine induced higher haemagglutination titers with reduced clinical disease and mortality in birds challenged with genotype VII NDV strain [88]. The integration of recombinant genotype VII-based NDV inactivated vaccines, such as NDV IBS002 and NDV AF2240-I, alongside the existing commercial immunization regimen, provides comprehensive protection with reduced virus shedding against corresponding virulent strains. This approach addresses the limitations of the LaSota strain, which has shown reduced efficacy in certain contexts [89]. Efficacy studies of commercially available live and killed Genotype VII based vaccines with a prime-boost vaccine regime against Genotype VII1.1 challenge in broilers demonstrated 93-100% survivability with the virus shedding continued till 10 days post-challenge (dpc) [90]. Vaccination with Genotype XIII.2 (killed BD-C161/2010) homologous to infection outbreak in chickens resulted in sustained liveability (100%) with no clinical illness, reduced viral shedding till 7 dpc [48]. Similarly, immunization of virulent NDV genotype VII infected broilers with recombinant live and autogenous inactivated GVII 1.1 based vaccine reduced mortality, clinical symptoms, mean severity index of internal organ lesions and reduced viral shedding of tracheal and cloacal swabs [91].

Homologous ND vaccines often reduce viral shedding in oro-pharyngeal swabs and decrease the number of birds shedding virus [92–94]. Further, this also achieves sterile immunity which is otherwise not possible with mismatched genotypes due to induction of suboptimal immunity [95]. Administration of a booster dose of inactivated genotype VII followed by Lasota live vaccine (genotype II) in layers under the Genotype VII experimental infection module witnessed a sustained egg production of up to 87.5% [96]. Genotype VII-based ND vaccine, when administered with montanide to birds infected with genotype VII NDV induced 100% protection with circulating antibodies, lasting 12 weeks post-vaccination and gradually declines at 16 weeks [97]. In a study by Wang et al. [98], chickens infected with the virulent DHN3 strain, when vaccinated with the inactivated form of genotype VII-based rDHN3-mF, exhibited a significantly stronger anti-DHN3 antibody response. This vaccination provided superior (100%) protection and faster viral clearance compared to Lasota strains. When, genotype VII-based NDV vaccine loaded with poly-lactic-co-glycolic (PLGA) nanoparticles afforded 100% protection to the chickens infected with genotype VII virulent NDV [99]. The adjuvant potential on induction of Th1 cell mediate and humoral immunity was also superior with enhanced IFN-γ expression.

Recombinant vaccines (Vectormune ND) with Genotype I based D-26 strain showed better protection rates of up to 100% to different genotypes i.e., IV, V VIIa, VIIb, VIId and VIII with significant reduction of virus titers (Ceva Animal Health study). Genotype II based vaccines in different strain formats yielded varied results to velogenic genotype VII challenge in chickens. Lasota, Clone, 12IR and B1 vaccines resulted in 90, 80 and 60% protection rate with no viral shedding noticed in any of the vaccinated groups [81]. Vaccination with LaSota strains accompanied by Toll-like Receptor adjuvants (Imiquimoid & OD-1826) increased antibody titers and reduced viral shedding in birds challenged with genotype VI velogenic NDV [100]. Although, heterologous/mismatch vaccines were advocated against NDV challenge their efficacy was reported with limited success

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restricting to genotype VII. Further, the success of genotype II based vaccines against genotype XIII strains is questionable. Matching genotype-based vaccines results in virus spill overs with limited protection (Table 9).

Table 6. Identities of amino acid sequences at different regions of NDV sequence between vaccine genotypes (G-II) and velogenic genotypes (G-VII, G-XIII).

Strains	F1 region										Fusion protein clevage site						F2 region														
	4	8	9	13	16	20	22	29	30	82	112	113	114	115	116	117	121	124	145	192	195	203	231	232	272	288	396	421	482	552	553
AEL75045.1 (LaSota)	R	K	N	M	T	A	V	A	N	D	G	R	Q	G	R	L	I	G	K	K	Q	A	N	K	N	T	M	K	E	K	M
AHN09749.1 (F)	R	K	N	M	T	A	V	A	N	D	G	R	Q	G	R	L	I	G	K	K	Q	A	N	K	N	T	M	K	E	K	M
AFX98109.1 (R2B)	R	K	N	M	T	A	V	A	N	D	R	R	Q	K	R	F	I	G	K	K	Q	A	N	K	N	T	M	K	E	K	M
UQW17861.1 (Genotype 7)	K	R	I	L	I	M	I	T	S	E	K	R	Q	K	R	F	V	S	N	N	R	T	T	Q	Y	N	I	R	A	R	A
AHG26218.1 (Genotype VII)	K	R	I	L	I	M	I	T	S	E	R	R	Q	K	R	F	V	S	N	N	R	T	T	Q	Y	N	I	R	A	R	A
ATU83336.1 (Genotype XIII)	K	R	I	L	I	M	I	T	S	E	R	R	Q	K	R	F	V	S	N	N	R	T	T	Q	Y	N	I	R	A	R	A
AIQ78308.1 (Genotype XIII)	K	R	I	L	I	M	I	T	S	E	R	R	Q	K	R	F	V	S	N	N	R	T	T	Q	Y	N	I	R	A	R	A

Table 7. Percentage identity between genotypes of vaccine and velogenic NDV strains in India based on nucleotide sequences.

Strain	JF950510.1_Lasota	KC987036.1_F	JX316216.1_R2B
JF950510.1_Lasota	-	96.07	93.616
KC987036.1_F	96.07	-	93.04
JX316216.1_R2b	93.62	93.04	-
KF740478.1_ Genotype VII	84.58	84.05	84.73
MZ546197.1_Genotype VII	83.05	84.96	82.69
KY774445.1_Genotype XIII	83.22	82.88	83.81
KM056347.1_Genotype XIII	83.20	85.43	83.56

Table 8. Percentage identity between genotypes of vaccine and velogenic NDV strains in India based on amino acid sequences.

F gene	AEL75045.1_Lasota	AHN09749.1 _F	AFX98109.1_ R2B
AEL75045.1 _Lasota	-	98.37	94.03
AHN09749.1_F	98.37	-	94.94
AFX98109.1_R2B	94.03	94.94	-
AHG26218.1_Genotype VII	89.67	89.67	89.86
UQW17861.1_Genotype VII	88.77	88.77	88.77
ATU83336.1 _Genotype XIII	88.77	88.59	88.95
AIQ78308.1_Genotype XIII	88.77	88.77	88.95
HN gene	**AEL75044.1_ Lasota**	**AHN09747.1_ F**	**AFX98110.1_ R2B**
AEL75044.1_Lasota	-	96.19	92.55

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AHN09747.1_F	96.19	-	90.99
AFX98110.1_R2B	92.55	90.99	-
AHG26219.1_Genotype VII	86.97	86.27	88.01
UQW17859.1_Genotype VII	87.52	86.29	88.05
ATU83337.1_Genotype XIII	88.25	87.19	87.72
AIQ78309.1_Genotype XIII	85.92	85.04	86.60

Table 9. Overview of traditional and newer genotype-based vaccines for control of NDV in poultry.

Features	**Vaccine strains**	
	Traditional genotypes	**Newer genotypes**
	GI, GII across the globe	GV (America), GVI (Pigeons), GVII (Asia and Africa)
Protection from morbidity & mortality	++	+++
Reduction in virus shedding	+	+++
Field virus silent dissemination & spill over	+++	-

5. Strategies for Updating Vaccine Genotype to Mitigate Dominant Field Virus Strains

The occurrence of recombination in NDV strains has been a subject of controversy. Nevertheless, experimental evidence supporting the occurrence of recombination in NDV is currently lacking. However, it is necessary to adopt a comprehensive strategy to assess ND virulence. This involves characterizing novel strains using the protocols established by the World Organization for Animal Health (WOAH). Currently, it may be deemed daunting to assert that the utilization of reverse genetics has the potential to be harnessed in the development of a secure recombinant attenuated vaccination for poultry. Some of the plausible strategies for generating homologous vaccines from virulent strains in the field have been discussed under this section.

5.1. Classical Inactivation of Field Viruses

A killed or inactivated vaccine is one of the primary methods used to control and prevent the spread of this disease. The development of a ND killed vaccine involves virus isolation, inactivation, adjuvant addition, formulation and testing. A virulent strain of the NDV strain can be isolated from the infected birds and further propagation can be done by increasing the titre by inoculating in embryonated chicken eggs. The most common inactivated reagents that are available for inactivating the conventional killed vaccines are formaldehyde and β-propiolactone [101]. These reagents inactivate the virus ensuring that it should not replicate but retain its ability to induce an immune response. To boost the immune response, an adjuvant is added to the inactivated virus which will improve the vaccine's effectiveness by prolonging the presence of antigen in the body. The commonly used adjuvants are aluminium hydroxide [102] or montanide [103]. Recently, to activate aluminium adjuvants to induce cell-mediated immunity the composite nano adjuvant N-2-HACC-Al NPs were synthesized by the N -2 Hydroxypropyl trimethyl ammonium chloride chitosan (N-2-HACC) and aluminum sulfate (Al2(SO4)3). It showed higher vaccine efficacy than those of the commercially combined vaccine [103]. Furthermore, encapsulation of inactivated NDV with PLGA particles induced strong immune responses compared to the commercial oil-based adjuvanted NDV-killed vaccine [98]. So, further formulation can be done by the addition of adjuvants and stabilizers. The use of killed vaccines in controlling ND in poultry is highly significant because it does not contain live virus making it safer as live vaccines might cause mild reactions and lead to complications. It can be formulated in such a way as to cover multiple strains, offering broader protection against various field strains as well as it will provide long-lasting immunity with adjuvants.

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5.2. Reverse Genetics Approach

Reverse genetics technology has revolutionized the field of virology, particularly in the development of vaccines for avian diseases such as the Newcastle Disease Virus (NDV), which causes severe economic losses in the poultry industry. This technology allows scientists to manipulate the genome of RNA viruses, including NDV, facilitating the creation of improved, targeted vaccines. The application of reverse genetics in NDV vaccine development has led to significant breakthroughs in the production of more effective, safer, and targeted vaccines for poultry. Reverse genetics technology first emerged in the 1990s and was a major leap forward in molecular biology. For negative-sense RNA viruses like NDV, it was difficult to manipulate viral genomes because their RNA genome is not infectious on its own. However, in 1996, Palese., P [105] and his co-workers pioneered the reverse genetics technique for negative-strand RNA viruses, allowing recombinant viruses to be generated from cloned cDNA. In the case of NDV, reverse genetics was successfully applied in 1999 when recombinant NDV was recovered using this technology [106]. This opened new possibilities for studying and manipulating NDV's genome for vaccine development.

Traditional live-attenuated vaccines for NDV have been effective, but they sometimes pose a risk of reverting to virulence [107]. Reverse genetics has addressed this issue by enabling the targeted attenuation of the virus, ensuring that the vaccine strains remain stable and do not revert to virulence. By introducing specific mutations in key genes such as the "F" the pathogenicity of NDV can be reduced while maintaining its ability to stimulate a strong immune response. Vectored vaccines use NDV as a vector to express foreign antigens from other pathogens [94]. This emerging NDV vector can be utilized for both bivalent and multivalent vaccine preparations, targeting not only NDV but also other important poultry viral diseases. NDV's ability to stably accommodate foreign genes, combined with its capacity to induce mucosal, humoral, and cellular immunity, makes it an ideal candidate for such vaccines. A variety of recombinant poultry viral vaccines have been developed using NDV as a vector, offering protection against multiple pathogens. Notable examples include vaccines targeting Avian Influenza (AI) virus [108], Infectious Bursal Disease Virus (IBDV) [109], Infectious Laryngotracheitis Virus (ILTV) [110] Reovirus [111], Infectious Bronchitis Virus (IBV) [112], and Chicken Infectious Anemia (CIA) [113]. Chimeric recombinant based Genotype VII.1.1 vaccines have gained commercial success with '0' ICPI and higher protection levels (up to 93%) in ND challenged birds. These vaccines involved Lasota and VG/GA strain as backbones into which the F and HN genes of KBNP-C4152R2L and CK/ME/19 strains were inserted [89]. These advancements highlight the versatility of NDV-vectored vaccines in offering broad-spectrum protection against multiple poultry diseases.

Reverse genetics has also been instrumental in the development of DIVA vaccines, which allow for the differentiation between vaccinated animals and those naturally infected with NDV. This is done by modifying the NDV genome to delete or modify certain antigenic genes, providing a unique marker for distinguishing between vaccine-induced immunity and natural infection. This advancement is crucial for disease surveillance and control in poultry populations. The future of reverse genetics in NDV vaccine development is promising. Advances in gene-editing technologies, such as CRISPR, could further refine the manipulation of the NDV genome. Additionally, the development of more sophisticated vaccine delivery systems, such as in-ovo vaccination combined with reverse genetics-designed vaccines, could provide even greater efficiency and protection for poultry [114].

5.3. CRISPR-Based Gene Editing Vaccines

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and the associated Cas9 protein represent a powerful third generation of programmable genome editing tools. When a virus invades a cell, CRISPR binds to viral RNA, and Cas9 induces double-strand breaks in targeted DNA sequences. A key advantage of the CRISPR/Cas9 system is that its expression plasmids transiently deliver the Cas9 enzyme and gRNA, allowing for gene disruption without integrating transgenes into the host genome [115]. This versatile system has been successfully applied in various avian species, including chickens and quail, to manipulate somatic cells [116], create eggs with modified

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biochemical compositions [117,118], produce myostatin knockout chickens [119], and study resistance to avian influenza [120]. CRISPR also plays a crucial role in understanding virus-host interactions, viral editing, and the functionality of virulent viral factors. With CRISPR, researchers can easily and precisely knock-in, knock-out, or knock-down genes [121].

In the field of vaccinology, CRISPR is becoming recognized as a precise, cost-effective, and efficient technology that is redefining the traditional recombinant vaccines. This innovative approach allows for the simultaneous insertion of larger gene fragments containing multiple antigens, facilitating the development of multi-valent vaccines that offer extended immunity [121]. Unlike conventional vaccines, CRISPR edited vaccines requires no plaque purification, attenuation and inactivation [122]. Herpes virus turkey (HVT) was developed as a vaccine vector with high immunogenic potential by targeted gene editing of gB, gI, gE [123] for generation of vaccines against Infectious bursal disease [124] Infectious laryngotracheitis (ILT) and avian influenza (HPAI) [125]. Development of homologous vaccines using Genotype VII and XIII for ND control using CRISPR tool mainly includes knock out of FPCS followed by knock-in of suitable genes from Lasota, F, B1 or other genotypes II for the creation of recombinant mutants. Direct editing of negative and positive senses single-stranded viruses can be possible with NHEJ-CRISPR/Cas 9 enzyme complex Cpf1 derived from Prevotella and Francisella [126]. Successful integration of NDV F gene into ILTV viral backbone vector by non- homologous end joining was reported by Atasoy et al. [127] using CRISPR/Cas9 and Cre-Lox system. This probably the first attempt to develop CRISPR mediated multi-valent vaccine. However, selection of protospacer adjacent motifs, off-target effects, delivery methods etc must be given due consideration for CRISPR edited vaccines

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6. Conclusion

Newcastle disease threat to poultry and non-poultry existed for decades and continues in the globe. Its existence is endemic in India with complex genotype circulation in poultry flocks. Since the virus is a single serotype with great antigenic & genetic variations, mismatching of current live (G-I, II, V) and inactivated vaccines with dominant field strains (G-VII, VIII, XIII) is possible. Although, the conventional genotype-based vaccines provide clinical protection against different velogenic strains especially GVII and GXIII, their inability to block the viral shedding causes increased viral persistence in the environment. A genotype-matched NDV vaccination for India would be intriguing. A genotype XIII vaccination may improve immunity and protect birds from aggressive NDV epidemics. Administration of live vaccines to homologous field strains decreases viral shedding in the flock and spillage into environments besides eliciting higher hemagglutination titers. Mutations in live vaccines with F & L proteins from field variants guarantees safety. Reverse genetics of virulent strains; Modified live vaccines with F, HN proteins from prevalent field strain in lentogenic backbone and Recombinant vaccine technologies (rNDV_G-VIII) and Mutations in AA of F clevage site (A-VIII) are some of the strategies for the development of homologous NDV vaccines with matched genotype to circulating field viruses. Live recombinant NDV vaccines (HIMMVAC, RINNOVAC EL7, GENOVAC N5) are available globally for Genotype VII alone with its usage restricted to certain regions in the country. Commercial preparation and availability for Genotypes VII, VIII and XIII vaccines are lacking with their safety and immunogenicity features questionable. Updating and optimization of vaccines with homologous genotypes matching with dominant field strains (Genotype VII, VIII, XIII) or importing from other countries may offer sustainable solution for Indian poultry industry. CRISPR based gene edited vaccines offer promising solution for mitigating the infection pressure caused by diverse genotypes.

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