Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2024.217

REVIEW ARTICLE

Newcastle Disease Virus as a Viral Vector Platform for Poultry Vaccines: A Review

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ARTICLE HISTORY (24-033)

Received: January 19, 2024
Revised: June 30, 2024
Accepted: July 13, 2024
Published online: August 07, 2024

Key words:
Newcastle disease
Reverse genetics
Recombinant vaccine
NDV-vectored vaccines
viral vector vaccine
Health

A B S T R A C T

Viral vector vaccines are excellent in stimulating a strong immune response to the vaccine antigen. The discovery of reverse genetics has given us an empirical foundation for the use of the Newcastle disease virus (paramyxovirus), as a vaccine vector. It has the potential to be a promising virus vector due to its ability to replicate in the respiratory system, modular nature of transcription, capacity to induce local and systematic immune responses, lower probability of recombination in host cells, high degree of stability to the foreign gene, high titer growth in cell lines, the natural pathogen of poultry, and a proven track record of safety, efficacy, and immunogenicity. Here, we elaborate on the biology of the Newcastle disease virus, important steps in plasmid construct for *in vitro* transcription, rescue of recombinant NDVs, pre-clinical assessment of NDV vectored poultry vaccines, main bottlenecks, and future prospects. By eliminating the primary barrier such as interference of maternally derived antibodies (MDAs), NDV vectored marketable vaccines can reduce vaccinal stress on birds while also relieving economic burden on poultry producers. Furthermore, innovative NDVs can be employed as marker or DIVA vaccines in disease eradication campaigns.

To Cite This Article: Masoud F, Ashraf MA, Usmani MW, Rafique A, and Aslam R, 2024. Newcastle disease virus as a viral vector platform for poultry vaccines: a review. Pak Vet J, 44(3): 539-546. http://dx.doi.org/10.29261/pakvetj/2024.217

INTRODUCTION

The poultry industry is vital in human life, providing primary animal protein through eggs and meat. In 2022, humans consumed an estimated 143 million tons of poultry meat (Hussain *et al*., 2024). As the global demand for food is increasing (Van Dijk *et al*., 2021), the poultry sector is expanding rapidly to cope with the demand. High-density poultry farming has replaced conventional farming to suit market demand. However, this alternative strategy has spread several diseases. Different infectious agents including; bacteria, viruses, and fungi, have been isolated from poultry birds, causing ailments that lead to economic losses (Yehia *et al*., 2023). Viral infections are common in the poultry industry and their Control is essential in reducing the zoonotic risk and limiting new variants in wild birds and their reservoirs.

The virus outbreaks in farmed poultry negatively impact feed intake, feed conversion ratio (FCR), body weight gain, and egg and meat production (Cui *et al*., 2018). The in-practice preventive measures for disease spread include mass vaccination, surveillance, physical separation, and pre-emptive culling of infected birds (Roth and Sandbulte, 2021). Mass vaccination reduces economic losses, prevents inter-species virus spread, and is a primary prophylactic measure recommended by authorities.

Traditionally, vaccines are developed using a modified live virus or a chemically inactivated virus. However, these conventional vaccines have issues with safety, efficacy, cost, and potential reversion to wild pathogens (Abdelaziz *et al*., 2024). Live vaccines also struggle with DIVA (differentiation of infected from vaccinated animals), which is crucial for disease tracking (Ravikumar *et al*., 2022). To address these drawbacks, scientists are developing better vaccines that offer broad-spectrum protection and avoid the limitations of earlier generations vaccines (Jorge and Dellagostin, 2017). This is true for developing vaccines against poultry diseases, where the primary concerns are the financial constraints and the vaccination of a substantial population.

Recent advances in reverse genetics technology (Chen *et al*., 2022), now allow for customized virus

generation in vitro. By introducing multiple genes into viral genome; efficient, safe, and cost-effective viruses can be produced. This approach has enabled researchers to produce viral vector vaccines (Romanutti *et al*., 2020). Viral vector includes a variety of poultry viruses, such as Fowl adenoviruses (FAdVs), Marek's disease virus (MDV), herpes virus of turkey (HVT), Fowlpox virus (FWPV), and Newcastle disease virus (NDV).

This review covers the biology of NDV, earlier history of its in vitro rescue, pre-requisites for the production of recombinant NDV (rNDV) in-vitro, the role of maternally derived antibodies in the vaccine failure, and prior research on the development and evaluation of NDV vectored vaccines.

Biology of Newcastle disease virus: The Newcastle disease virus (NDV) is a member of the genus Avulavirus in the family Paramyxoviridae. The members of this genus are also termed avian paramyxoviruses (AMPV), which are subdivided into fifteen serotypes (APMV-I to APMV-XV) based on serological tests such as hemagglutination inhibition (HI) and Neuraminidase inhibition (NI) tests. Newcastle disease virus belongs to avian paramyxovirus serotype-I (Dharmayanti *et al*., 2024). As determined by the APMV-I fusion protein sequence, the NDV isolates are classified into two divergent classes, I & II. Class I has only one genotype and does not produce disease in birds. Meanwhile, Class II has 21 genotypes, all being pathogenic except genotypes I, II, and X (Goraichuk *et al*., 2023).

The NDV is a negative sense, non-segmented, enveloped RNA virus with a pleomorphic shape having a virion size of 200-300 nm (Figure 1-A). The virus's genome comprises six genes, NP, P, M, F, HN, and L genes, that encode for eight different proteins. The structural proteins include the fusion protein (F), hemagglutinin-neuraminidase protein (HN), matrix protein (M), nucleoprotein (NP), phosphoprotein (P), and large polymerase protein (L). The other two proteins, which are non-structural, 'V' and 'W' proteins, are encoded by the 'p gene' via the RNA editing mechanism (Ganar *et al*., 2014). The F and HN proteins are surface glycoproteins, while the non-glycosylated M protein is beneath the envelope, facilitating the virus's assembly and budding. The NP, P, and L proteins make the ribonucleoprotein complex, which acts as a template for the transcription and replication of the viral genome (Figure 1-B). The NP protein encapsidates the viral genome, L protein acts as a viral polymerase and P protein is its co-factor. The encapsidation of NP into the viral genome is of prime importance at the start of transcription (Kim and Samal, 2016).

The genome lengths of NDV isolates from all over the world are 15,186 nt or 15,192 nt, or 15,198 nt (Dimitrov *et al*., 2019). The typical NDV genome has 55 nucleotides (nt) leader sequence at 3´ end and 114 (nt) trailer sequence at 5´ end. Each gene is marked by the start and stop signals. The gene boundaries of NDV are separated by the intergenic sequence(s) (IGs), one (nt) among the NP, P, and M genes. The IGs of F and HN are 31 nt, while that of the HN and L genes is 47 nts. The increase or decrease of these intergenic sequences affects the attenuation of the NDV isolate, which may be considered an essential tool for developing future vaccines (Yan and Samal, 2008).

The NDV obeys the rule of six, which means its nucleotides must be multiples of six for efficient replication, because the N protein can accommodate six nucleotides simultaneously (Kim and Samal, 2019). The virus has a bipartite promoter with two discontinuous regions: conserved region I (first 18 nucleotides) and conserved region II (nucleotides 73-90) (Marcos *et al*., 2005).

The virus binds by attaching its surface glycoproteins (HN) to sialic acid-linked glycoproteins or gangliosides located in host cells. After the genome entry into the host cell cytoplasm, the NDV negative sense genome transcribes (by viral RNA polymerase; N, P and L proteins) into positive-sense genome, followed by the transcription of the positive sense genome into viral protein by host cell ribosomes. The same positive sense RNA genome is used as a template by host RNA polymerase to produce the negative sense genomic RNA for NDV progeny virus (Kim and Samal, 2016). The transcription starts from 3` leader sequence, and the mRNA from each gene is transcribed from gene start to gene end sequence (Bello *et al*., 2020). The transcription re-initiation of downstream genes is inconsistent (polar gradient transcription), presenting the gradient transcription in the downstream genes (Figure 1-C). The newly formed genomic RNA is wrapped in the N, P, and L proteins to produce nucleocapsid, which is then assembled with the matrix and surface glycoproteins. The M protein facilitates the release from the host cell (Cong *et al*., 2023).

The NDV escapes the host immune system by using its V protein, which inhibits the INF signaling pathway and functioning of MDA5 and RIG1 (PPRs). This inhibits the host's inflammatory and antiviral responses, facilitating NDV replication (Behboudi and sofiani, 2021).

Early history of NDV rescue: The term "reverse genetics" was introduced by Charles weissmann in 1974, describing the generation of viruses from cloned cDNA. This technique has enabled researchers to explore the genome of viruses, and NDV is one of the earliest viruses studied as a viral vector (Chen *et al*., 2022). It all began (Figure 2) with the rescue of Lasota strain of NDV from plasmids by in-vitro transcription (Peeters *et al*.,1999), followed by clone-30 strain (Roomer-Oberdo *et al*., 1999). The recombinant Beaudette C (rBC) strain was first to express the foreign gene (Krishnamurthy et al., 2000), then the heterologous viral gene was expressed by NDV (Nakaya *et al*., 2001; Swayne *et al*., 2003). Later on, the concerns about the recombination of NDV, with host or other viruses were scientifically disproved (Song *et al*., 2011).

Plasmid construction for NDV-vectored vaccines: The infectious NDV can be generated by simultaneous transfection of the full-length (genomic RNA) and helper plasmids (NP, P, and L genes) into the cell lines using appropriate polymerase system (Figure 3) (Kim and Samal, 2016). In NDV, the foreign nucleotide complex is inserted as an independent transcription unit (ITU). The gene start (ACGGGTAGAA) and stop signals

(TTAGAAAAAA) are taken from the NDV, and a Kozak sequence (GCCACC) is added in upstream of the foreign gene ORF to enable the efficient transcription by eukaryotic polymerase (Bello *et al*., 2020). For RNA polymerase II system, the CMV promoter and the SV40 poly-A tail as terminator signals are used. While in the case of the T7 RNA polymerase system, the T7 promoter and terminators can serve the purpose (Molouki and Peeters, 2017). To efficiently attach the heterologous surface protein to the matrix protein of NDV, the transmembrane (TM) and cytoplasmic tail (CT) of the heterologous gene are replaced with the CT of the fusion protein of NDV (Kim and Samal, 2018). After the stop codon, NDV stop signal, and one nucleotide as the intergenic space are added. Therefore, the foreign nucleotide complex should be arranged as follows: NDV start signal --- Kozak sequence --- ORF of foreign gene (without stop codon, cytoplasmic tail, and transmembrane) --- CT sequence of NDV fusion protein --- stop codon --- NDV stop signal --- nucleotide base for intergenic space.

The NDV abides the rule of six, meaning its genome length must be a multiple of six for efficient encapsidation by the N protein, which is required to start the viral genome transcription (Figure 1-D) (Kim and Samal, 2019). Therefore, the rule of six must be considered while designing the foreign nucleotide complex. It can be adjusted by increasing or decreasing the intergenic space before or after the foreign nucleotide complex (Peeters *et al*., 1999).

The exact 5' and 3' ends of genome are also required to replicate recombinant viruses generated by in-vitro transcription. To achieve this, the ribozymes (self-cleaving RNAs) (Avis *et al*., 2012) are placed in the transcription vector to produce the exact ends. In common practice, the hammerhead ribozyme (HamRz), (GCGACTAGTTGTTAAGCGTCTGATGAGTCCGTGA GGACGAAACTATAGGAAAGGAATTCCTATAGTC) (67 bp) is placed after the CMV promoter and before the genome starts to produce the exact 5' end. Meanwhile, hepatitis delta virus ribozyme (HdvRz) (GGGTCGGCAT GGCATCTCCACCTCCTCGCGGTCCGACCTGGGCA TCCGAAGGAGGACGTCGTCCACTCGGATGGCTAA GGGAGAGCTCG) (84 to 89 bp) is added after the trailer sequence of NDV genome before the terminator to produce the accurate 3' end (Li *et al*., 2011).

NDV adopts the gradient transcription mechanism from 3' NP-P-M-F-HN-L 5'. The non-coding region between P and M genes was identified as the best site for heterologous gene expression (Zhao *et al*., 2015). It can accommodate the 4.5 kb length gene, and one NDV vector can express three foreign genes (Kim and Samal, 2019).

The RNA polymerase can be RNA polymerase II (Masoud *et al*., 2022) or T7 RNA polymerase. The T7 RNA polymerase can be provided by modified viruses, plasmids, or cell lines stably expressing the polymerase. Meanwhile, the RNA polymerase II system does not require such arrangement because the cells naturally produce this enzyme (Molouki and Peeters, 2017).

NDV-vectored vaccines for the major poultry pathogens: The Newcastle disease virus is a natural poultry pathogen, and live attenuated vaccines are used globally (Figure 4). The NDV vaccines are primarily derived from Genotype-I (asymptomatic strains), such as V4, I2, and Ulster-2C, or from Genotype II, which includes Hitchner, B1, Clone-30, LaSota, and VG/GA strains (Hu *et al*., 2022). The protective titer of antibodies against ND infection ranges from 3 log 2 to 6 log 2. In poultry birds, the antibodies are the primary mechanism of protection against infections while the cell-mediated immune (CMI) response plays a key role in reducing the shedding of virus. The poultry vaccines are usually judged by the degree of protection against clinical disease, lesion development, decrease in virus shedding, and neutralizing antibodies titer (Rautenschlein and Schat, 2024). The ultimate objective of a vaccination is to reduce the shedding of a particular virus and increase the infectious dose of that virus for future infections. Here, we describe the significance of poultry viral diseases (Hein *et al*., 2021) and their NDV-vectored vaccines (Table 1).

Avian influenza (AI): It is caused by Avian influenza virus (AIV), which has importance due to its zoonotic potential (Subedi *et al*., 2024). Pathotypically; divided into low pathogenic avian influenza (LPAI), i.e., H9N2, and high pathogenic avian influenza (HPAI), i.e., H5N1 and H7N7 (Kim and Samal, 2019). The protection against these infections is mounted by the neutralizing antibodies produced against HA and NA (Yang *et al*., 2023). The HPAI produces infections in humans. So, their control is an ultimate requirement (Peacock *et al*., 2019). While, LPAI particularly H9N2, are considered potential donors for the virulent genes (Bhat *et al*., 2022). Thus, its control is also required for human health security (Charostad *et al*., 2023). Along with conventional vaccines, different virus strains of NDV have been used to express the various pathotypes of AIV.

The LaSota strain has been used to express the hemagglutinin protein of different strains like H5N1 (Lardinois *et al*., 2012) and H5N2 (Veits *et al*., 2006). The protective efficacy of these vaccines has been tested in the SPF birds, showing 100% protection via intramuscular and 80% via oculonasal route (Ma *et al*., 2017). In 2017, china encountered an H7N9 outbreak in poultry; as a counter strategy, the LX strain vectored H7N9 vaccine was deployed, which provided 80% protection (Hu *et al*., 2017).

In many countries, H9N2 vaccination (inactivated vaccines) has been adopted nationally or locally as a preventive measure (Dong *et al*., 2022). Various strains like LaSota, NDV/AI4-TFHN, and rmNA-H9 were used to express the H9N2 hemagglutinin protein. All constructs expressed the foreign gene, but chimeric NDV showed better efficiency than wild NDV (Nagy *et al*., 2016; Liu *et al*., 2018; Xu *et al*., 2019; Masoud *et al*., 2023). All these studies demonstrate the ability of NDV to act as a vaccine vector for avian influenza.

Infectious bursal disease (IBD): IBD also known as, Gumboro disease is caused by Infectious bursal disease virus (IBDV), which replicates in the bursa of fabricius of birds and damages antibody-producing B cells (Hammad *et al*., 2022). As a result, immunosuppression occurs, and birds are susceptible to opportunistic pathogens (Du *et al*., 2023; Barka *et al*., 2023).

Table 1: List of NDV vectored vaccines tested against various poultry birds' infections

Vaccine type	Pathogen	Disease	Antigen expressed	Animal model	Route/Method	Dose	Reference
Live Clone-30	HPAIV H5N1	AI	HA-gene	SPF-Chicken	Oculonasal	$1x10^6$ EID_{50}	Veits *et al.*, 2006
Live LaSota	HPAIV H5N1	AI	HA-gene	SPF-Chicken	Oculonasal vs drinking water	$1x10^6$ EID_{50}	Lardinois *et al.*, 2012
Live LaSota	IBV	IB	S2-gene	SPF-Chicken	Ocular	$1x10^6$ EID_{50}	Toro *et al.*, 2014
Live LaSota	ILTV	ILT	gD-gene	SPF-Chicken & commercial broiler	Intranasal/ Intraocular	$1x10^7$ $TCID_{50}$	Zhao *et al.*, 2014
Live LaSota	ILTV	ILT	gD-gene	SPF-Chicken	Oculonasal	$1x10^6$ $TCID_{50}$	Basavarajappa *et al.*, 2014
Live LaSota	LPAIV H9N2	AI	HA-gene	SPF-Chicken	Oculonasal/Intramuscular	$1x10^7$ FFU	Nagy *et al.*, 2016
Live LaSota	HPAIV H5N2	AI	HA-gene	SPF-Chicken	Coarse sprayer/aerosol	$1x10^6$ EID_{50}	Ma *et al.*, 2017
Live LX strain	HPAIV H7N9	AI	HA-gene	SPF-Chicken	Intranasal	$5x10^6$ EID_{50}	Hu *et al.*, 2017
Live F-Strain	IBDV	IBD	VP2-gene	SPF-Chicken	Intranasal	$1x10^5$ EID_{50}	Dey *et al.*, 2017
Live NDV/AI4-TFHN	LPAIV H9N2	AI	HA-gene	Chicken Immunized with LaSota vaccine	Oculonasal	$1x10^6$ EID_{50}	Liu *et al.*, 2018
Live LaSota	IBV	IB	S-gene	SPF-Chicken	Ocular	$1x10^7$ EID_{50}	Shirvani *et al.*, 2018
Live LaSota	IBV	IB	Codon optimized S-Protein	SPF-Chicken	Oculonasal	$1x10^6$ PFU/100 µL	Abozeid *et al.*, 2019
Live rmNA	LPAIV H9N2	AI	HA-gene	SPF-Chicken	Oculonasal	$1x10^6$ EID_{50}	Xu *et al.*, 2019
Live LaSota	FAdV-4	HPS	Fiber-2	SPF-Chicken	Intramuscular	$1x10^7$ EID_{50}	Tian *et al.*, 2020
Live LaSota	IBDV	IBD	VP2-gene	SPF-Chicken	Oculonasal	$1x10^7$ EID_{50}	Qiao *et al.*, 2021
Live LaSota	LPAIV H9N2	AI	HA-gene	SPF-Chicken	Oculonasal	$3x10^7$ EID_{50}	Masoud *et al.*, 2023

IBD; Infectious bursal disease, IBDV; Infectious bursal disease virus AI: Avian influenza, HPAIV: high pathogenic avian influenza virus, LPAIV: Low pathogenic avian influenza virus IB: Infectious bronchitis, IBV: infectious bronchitis virus, ILT: Infectious laryngeotracheitis, ILTV: Infectious laryngeotracheitis virus HPS: Hydro pericardium syndrome, FAdV-4: Fowl adeno virus type 4, SPF-Chicken: Specific pathogenic free chickens.

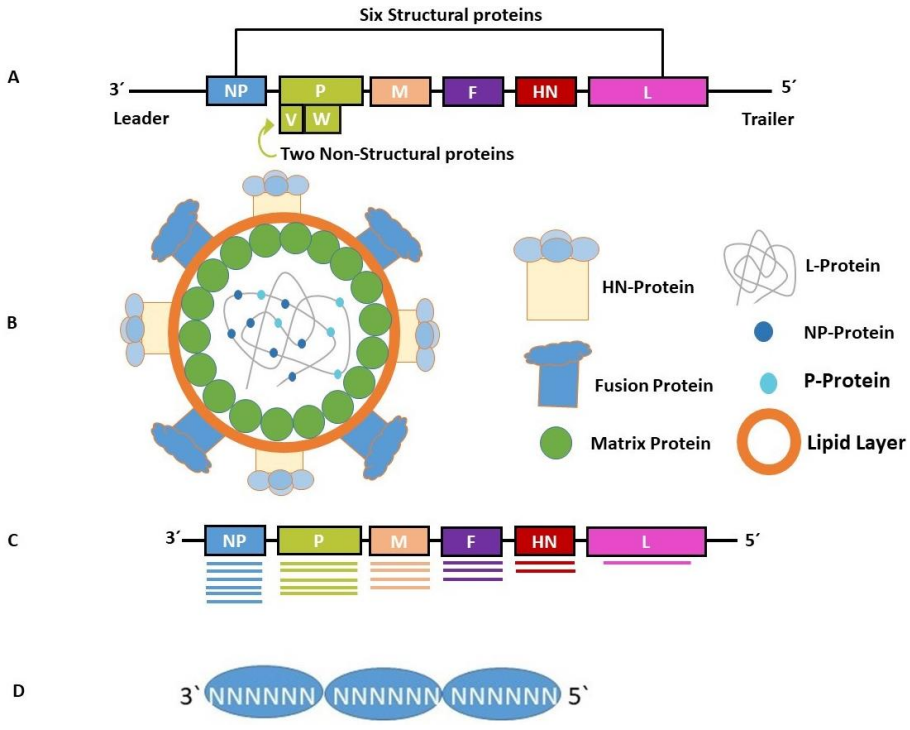

Fig. 1: Schematic diagram of (A) NDV genome (B) Newcastle disease virus (C) Gradient transcription (D) Rule of six (Figure designed by using MS office).

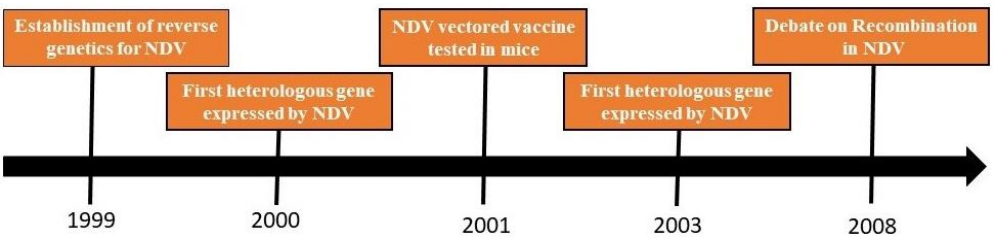

Fig. 2: key milestones in the development of NDV as a vaccine vector (Figure designed by using MS office).

To produce viral vector vaccines, the VP2 gene of IBD was inserted at different positions in NDV strains (LaSota, modified LaSota, and F strain). The immunization of chickens with aforementioned vaccine candidates provided 80% protection against challenge infection, demonstrating their effectiveness (Dey *et al*., 2017; Qiao *et al*., 2021).

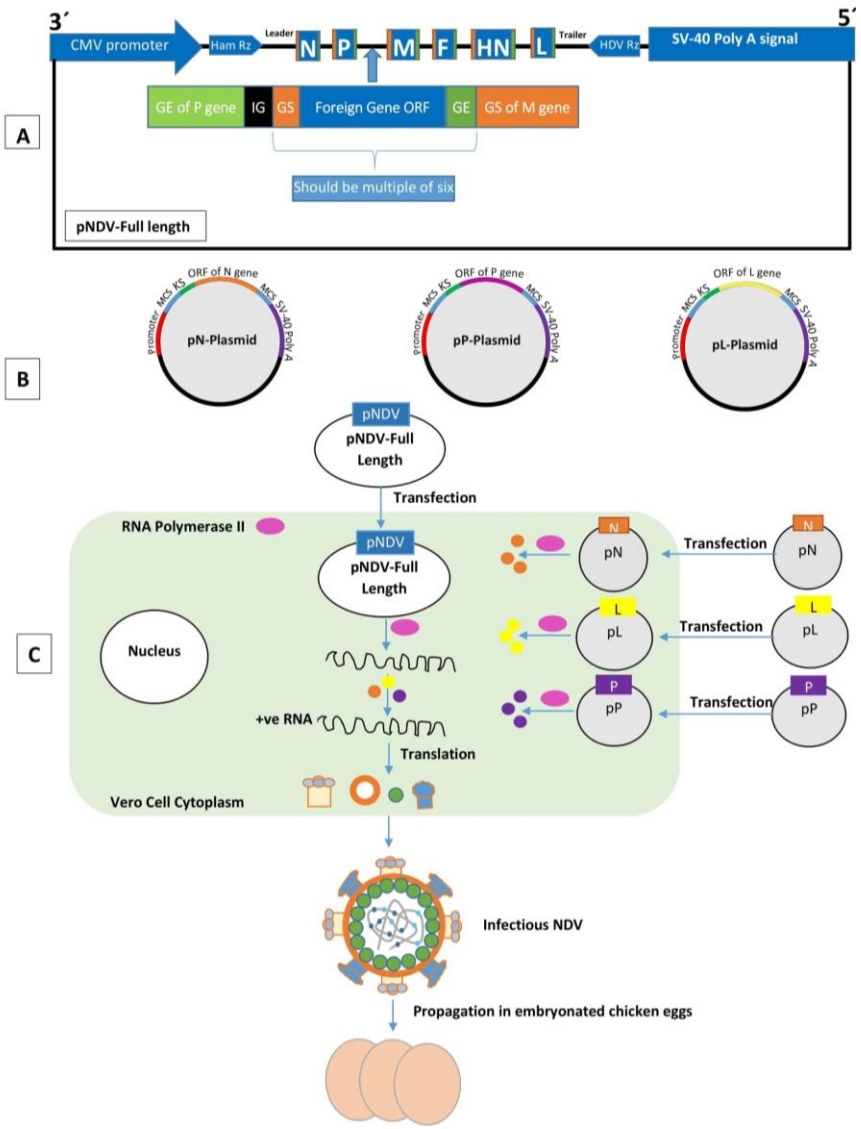

Fig. 3: Important technical points for consideration, when using NDV as a viral vector: A) The full-length NDV genome is sandwiched between the hammer head (Ham Rz) and hepatitis delta virus ribozyme. The foreign gene is in the intergenic space of P and M genes. Each gene of NDV has gene start (GS) (Brown) and gene end (GE) (Green) signals, so the foreign nucleotide complex should contain both of these sequences. The foreign gene should be multiple of six. B) the support plasmids of N, P and L genes are provided, these will make the viral polymerase, which will convert the negative sense RNA to positive sense RNA. In each support plasmid, the ORF of gene is preceded by the kozak sequence under the CMV promoter and SV-40 poly A Tail C) the full length and support plasmids are transfected to cell lines, the RNA polymerase will read all the four construct, as a result three proteins (Phosphoprotein, Nucleoprotein and large polymerase) and negative sense RNA copy of NDV genome are produced. These proteins act as viral polymerase and convert the negative sense RNA to positive sense RNA, which ultimately translated into viral proteins and negative copy of genome. The infectious NDV burst out the cells and then propagated in the embryonated chicken eggs. (Figure designed by using MS office)

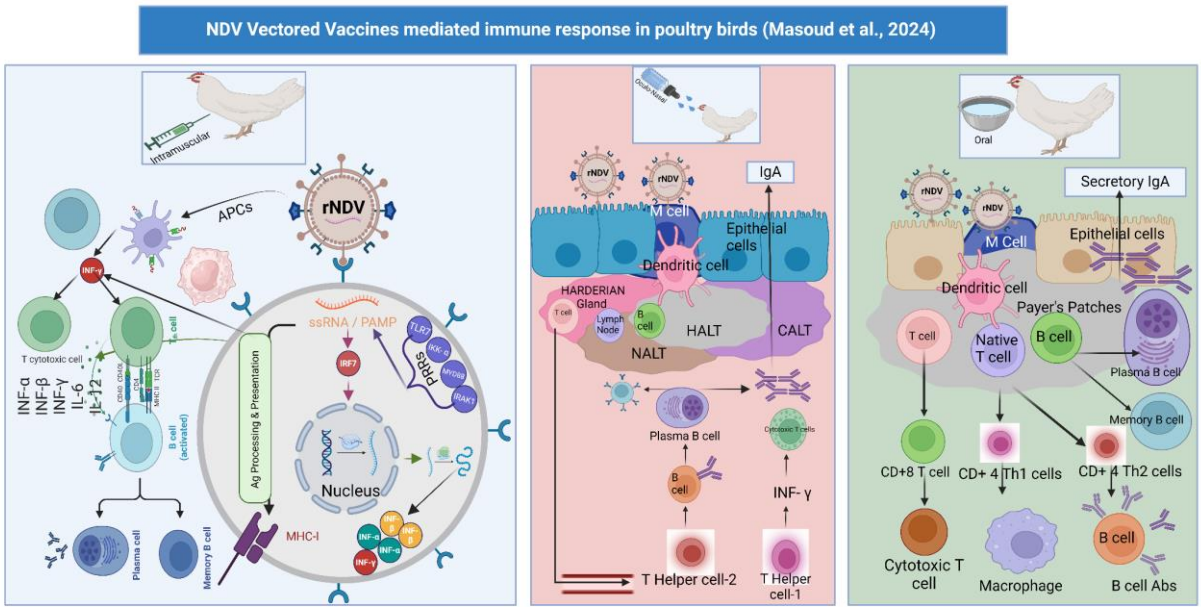

Fig. 4: In this illustration, the NDV is carrying three protective antigens, the two are intrinsic proteins "F" and "HN", while third is depicted here to demonstrate the heterologous antigen of different viruses. Ultimately the NDV transformed to recombinant Newcastle disease virus (rNDV). Intramuscular route: Vaccine antigen is either carried by nucleated cells and represented by MHC-I pathway, or processed and presented by MHC-II, pathway via Antigen presenting cells APCs. PAMPs; pathogen associated molecular pattern, PRRs; Pathogen recognition receptors. Oculo-Nasal route: Nasal associated lymphoid tissue (NALT), Conjunctiva associated lymphoid tissue (CALT), Head associated lymphoid tissue (HALT). Oral route: Payer's patches, M cells. (The figure was designed by using MS office).

Infectious bronchitis (IB): The IB is caused by the infectious bronchitis virus (IBV), which targets the reproductive and urogenital tracts of birds (Abozeid, 2023). For this disease, the different chimera of NDV have been used as a vector to harbor the spike protein (S) of IBV. Later on, the protection level was assessed in the SPF birds and significant protection against challenging infections was observed (Toro *et al*., 2014; Shirvani *et al*., 2018; Abozeid *et al*., 2019).

Infectious laryngotracheitis (ILT): Infectious laryngotracheitis; an important respiratory problem having an impact in egg production of birds (Gowthaman *et al*., 2020). To produce NDV-vectored ILT vaccines, the surface glycoproteins of ILT (gB, gD, and gC) were introduced in the NDV genome. The pre-clinical trials in chickens showed that NDV-expressing gD protein provided complete protection (Basavarajappa *et al*., 2014; Zhao *et al*., 2014).

Hydro-pericardium syndrome (HPS): The hydro-pericardium syndrome (adenovirus infection) is associated with growth retardation and significant mortality in poultry birds (El-Shall *et al*., 2022). In field conditions, the autogenous vaccine prepared from the liver homogenate of infected chicken is employed, whereas inactivated vaccines are administered to breeders. Recently, the NDV-vectored vaccine with FAdv-4 fiber-2 gene was developed. It provides complete protection when administered intramuscularly (Tian *et al*., 2020).

Role of maternally derived antibodies (MDAs) and way forward: The bottleneck for the NDV as a vaccine vector is the interference produced by the maternally derived antibodies (MDAs) (Liu *et al*., 2023), which protects chicks from infection (Hu *et al*., 2020), but also hampers NDV vectored vaccines replication.

Many efforts have been made to reduce the effects of the MDAs on NDV vaccination, one novel approach is the use of chimeric NDV. It is based on the fact that the NDV belongs to APMV-1, and has low cross reactivity with other serotypes. In one study, the fusion and hemagglutinin-neuraminidase proteins of NDV were replaced with the counterpart of AMPV-8 (Steglich *et al*., 2013). In another approach, the ectodomain of fusion and hemagglutinin-neuraminidase proteins of NDV was replaced with the same parts of APMV-2 (Kim *et al*., 2017; Liu *et al*., 2018). Both recombinant bivalent vaccines provided optimal protection, suggesting viable approach to minimize the effects of MDAs.

As mentioned earlier, due to MDAs, the B cell response towards the antigen is reduced, resulting in weak immune response. Thus, the NDV has been engineered to express the granulocyte-macrophage colony-stimulating factor (GM-CSF), showing that NDV replication in the presence of MDAs is comparable with the parenteral virus (Zhang *et al*., 2016). The study suggested that expressing the cytokines along with foreign genes in the NDV can mitigate the effects of MDAs. As the MDAs hijack a considerable amount of antigen, leaving behind a small amount to trigger humoral immune response, using higher antigen concentration ensures more antigen is available to provoke the host immune system (Hu Z and Liu X, 2021).

Conclusion and future perspectives: There is a dire need in developing effective vaccines against the poultry pathogens. The Virus vectors, including NDV have revolutionized vaccinology, (Centlivre and Combadière, 2015). The NDV can act as a suitable vaccine vector because of its ability to express foreign genes as the natural antigen, initiating the humoral, cellular and mucosal immune responses (De Swart and Belov, 2023). The NDV can be the ideal vaccine vector, due to its short modifiable genome, high titer growth in embryonated chicken eggs, cell lines, and respiratory tract of birds, stable expression of foreign gene (4.5 kb), cytoplasmic replication in the host cells and ease of administration via drinking and spray method (Yang *et al*., 2024).

The conventional recombination techniques, such as restriction enzyme digestion and ligation, and bacterial artificial chromosomes (BAC), are used for developing recombinant vaccines, but these are time-consuming and labor-intensive. Efforts should be directed towards the CRISPR/Cas9 technology; preferably HDR (homology-directed reparation pathway), for genome manipulation and viral vector vaccine development (Vilela *et al*., 2020; Bhujbal *et al*., 2022). The Cre/lox system facilitates the insertion of multiple antigens in the virus vector backbone, aiding the development of NDV-based multivalent vaccines (Chang *et al*., 2018).

Adjuvants are essential parts of vaccines as they enhance immune response. Alum or emulsion adjuvants were previously used, but to enhance humoral and cellular immune responses, novel adjuvants are required. Viral vectors possess inherent adjuvant properties, like NDV has the capacity to trigger the alpha and beta interferons production, ultimately upregulating the immune response (Ewer *et al*., 2016). Efforts should be focused on using the molecular adjuvants, either by fusing them with gene of interest or co-expressing in NDV genome (Mahony, 2021).

The research can be directed to understand the impact of MDAs on B cell activation, cytokines role in B cell stimulation, the T cell's immune responses against NDV-vector and its significance in the development of B cells, and how MDAs or other antibodies suppress the vaccine immunization (Hu *et al*., 2020).

The previous work of the past twenty years has proven the NDV as a potential vaccine vector. The vaccines based on this platform can provide a practical strategy for rapid, efficient, and economical immunization of poultry birds. However, further research is required in the utilization of modern techniques for the manipulation of NDV genome, usage of molecular adjuvants and curtailment of MDAs interference for the development of NDV vectored vaccines.

Authors contributions: FM: Conceptualization of idea and original draft writing, MAA and MWU: Data collection and Methodology, AR and RA: Formal analysis and Visualization.

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