Advantages of the NDmolog*R7* Vaccine over Existing Vaccines

The NDmolog*R7* vaccine has been specifically engineered to replace the *F* and *HN* genes with highly homologous counterparts from NDV *genotype* VII. This high degree of antigenic matching ensures a strong and durable antibody response capable of effectively neutralizing circulating field strains. By leveraging dual antigenic homology, NDmolig*R7* provides optimal protection by preventing viral shedding and transmission while mitigating declines in egg production. Strengthening poultry resistance to NDV, combined with strict biosecurity measures and continuous field surveillance, could ultimately support efforts to eradicate NDV *genotype* VII.

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NDmoLog*R7*®

NDmolog*R7* Targeted Protection: The Smart Choice for Newcastle Disease/*gen.* VII

BIOVAC

Head Office: Ha'ILAN St. 30 | P.O.B. 300 | Or-Akiva 3060000 Israel
Tel: +972-4-6266772 | vireo@biovac.co.il | www.biovac.co.il

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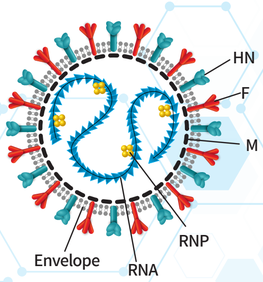
NDmolog*R7*: a Targeted Protection

Introduction

Newcastle disease, caused by virulent strains of Newcastle disease virus (NDV) is one of the major threats to the global poultry industry, that results in significant economic losses. Due to its substantial impact on animal health and trade, the disease is regulated by the World Organization for Animal Health (WOAH), necessitating mandatory reporting and the enforcement of trade restrictions on affected regions.

NDVs are enveloped, single-stranded RNA viruses that belong to the genus Orthoavulavirus *(Family: Paramyxoviridae)*. The NDV genome encodes for six major structural proteins, including the envelope glycoproteins Hemagglutinin-Neuraminidase (HN) and Fusion (F) that are essential for viral entry. The HN protein facilitates viral attachment to host cells while the F protein mediates virus-target membrane fusion (Fig. 1). These envelope glycoproteins are also primary antigens, that are pivotal to host immune response. Neutralizing antibodies directed against these proteins form the cornerstone of protective immunity following vaccination (1).

Fig. 1: Schematic diagram of the NDV particle



The Hemagglutinin-Neuraminidase (HN) Protein

The HN protein, embedded in the NDV envelope, has several essential functions. It:

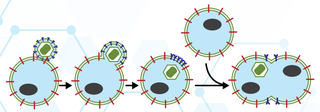
1. Mediates viral attachment by binding to sialic acid receptors on the target cell surface
2. Activates the F protein, facilitating membrane fusion and enabling viral entry into the host cell
3. Exhibits neuraminidase activity, that cleaves sialic acid residues to promote the release of newly assembled virions from the infected cell surface

Beyond these, the HN protein is a key immunogenic antigen that elicits protective immune responses (2,3).

The Fusion (F) Protein

The F protein, that also resides on the envelope, is critical in mediating viral fusion and entry into host cells. Moreover, upon viral entry, the infected cell expresses the F protein on its membrane, promoting its adhesion and fusion with adjacent cells. This leads to syncytia (giant fused cells) formation, facilitating direct cell-to-cell spread of the virus while evading host neutralizing antibodies (Fig. 2).

Fig. 2: The F protein mode of action



A key feature of the F protein is its mandatory cleavage-activation mechanism of action. The amino acid sequence of the F cleavage site serves as a primary determinant of virulence, distinguishing between virulent and non-virulent strains (4). The F protein also plays a pivotal role in eliciting neutralizing antibodies, that are essential for protective immunity (2).

Newcastle Disease Virus *genotype* VII

Since the early 2000s, *genotype* VII has emerged as the dominant NDV strain, spreading extensively across multiple regions of the globe, *e.g.*, Africa, Asia, the Middle East, South America and Europe (5,6).

Challenges in Disease Control

Despite the implementation of stringent vaccination programs utilizing both live and inactivated vaccines, alongside rigorous monitoring and biosecurity measures, Newcastle disease continues to inflict substantial economic losses. The primary impact is observed in reduced egg production and compromised egg quality, even in flocks with comprehensive vaccination coverage.

The Role of Homology in Vaccine Efficacy

Currently available Newcastle disease vaccines, both live and inactivated, offer protection against morbidity and mortality in poultry. However, they do not effectively prevent viral infection, nor do they eliminate subsequent viral shedding and transmission. As a result, vaccinated birds may still become infected, remain asymptomatic and serve as reservoirs for viral dissemination.

Research has demonstrated that vaccination with a homologous vaccine strain – one that antigenically matches the circulating field strain – significantly reduces viral shedding and transmission compared to vaccination with heterologous vaccines (*i.e.*, that differ from the circulating field strain) (10). Biovac LTD has experimentally validated this notion in a comparative trial that proved complete prevention of egg production decline and markedly decreased viral shedding in a challenge model that involved laying hens (homo-/hetero-logously vaccinated) (7).

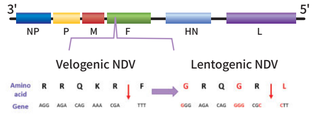
Importance of Antigenic Matching (Homology) of Both Envelope Proteins to Field Viruses

The antigenic envelope proteins F and HN play essential and complementary roles in the multi-stage pathogenesis of NDV. Consequently, a high degree of homology between the vaccine strain and circulating field strains significantly enhances the simultaneous induction of a neutralizing immune response against both proteins. This dual-targeted approach provides a synergistic protective effect compared to vaccines that elicit immunity against one of these proteins (8,9). Additionally, the combined antigenic stimulation and complementary antibody response increase the likelihood of robust and long-lasting immunity, particularly in the context of ongoing antigenic drift in circulating NDV field strains.

Development of a Homologous Vaccine

Combining the above findings with additional data (10), Biovac LTD has generated the recombinant *r*ND-LS/"F-HN" *gen.* VII vaccine strain. This strain relies on a LaSota vaccine backbone, wherein the *F* and *HN* genes, that encode for the relative antigenic envelope antigens, were replaced by those of NDV/*gen.* VII. To attenuate its inherent virulence, the cleavage site of this *gen.* VII F protein was modified to a non-virulent, *gen.* II-like form, prior to its insertion (from 112RRQKRF117 to 112GRQGRL117) (Fig. 3).

Fig. 3: Attenuation of the F (VII) protein into a non-virulent (II-like) form



R= Arginine; Q= Glutamine; K= Lysine; F= Phenylalanine; G= Glycine; L= Leucine;

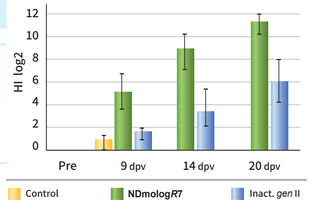
Intracerebral Pathogenicity Index (ICPI)

The intracerebral pathogenicity test is a standard assay for NDV virulence assessment. It involves the intracerebral inoculation of one-day-old specific pathogen-free (SPF) chicks with the viral strain (at question) and their downstream clinical follow-up and scoring. According to the ICPI scoring system, a value ≥ 0.7 indicates a virulent (velogenic) NDV strain (7). NDmolog*R7* vaccine strain's ICPI value is 0.2, categorizing it as lentogenic (non-virulent) and highlighting its safety.

Vaccine Performance

A single administration of the vaccine to 12-day-old SPF chickens induces a protective antibody response within a few days, provoking a rapid immune activation (Fig. 4).

Fig. 4: NDmolog*R7* Induces a rapid protective antibody responce



Moreover, qPCR analysis of challenge virus shedding, based on cloacal and tracheal swab samplings at 2, 5, 8 and 14 days post-challenge *(dpc)*, demonstrated that SPF chickens vaccinated once at 12 days-of-age (challenge at 29 *dpv*) completely eliminated challenge virus shedding in any of the tested time points. In contrast, 40% and 20% of the *gen.* II-vaccinated birds, that were seemingly healthy, excreted the challenge virus at 5 and 8 *dpc*, respectively (Fig. 5). Hence, NDmolog*R7* provokes a fast and potent immune response that entirely prevents *gen.* VII/NDV dissemination, thus efficiently blocking the viral infectious cycle.

Fig. 5: NDmolog*R7* abolishes challenge strain excretion