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Improving immunoprophylaxis: potential and limitations of biotechnological solutions in Newcastle disease control

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ABSTRACT

Introduction. Newcastle disease continues to inflict significant economic damage on the global poultry industry, dictating the need to revise traditional vaccination strategies. Classical live vaccines, despite their widespread use, have a number of limitations, including interference with maternal antibodies and inability to completely prevent the field virus shedding due mismatch with current genotypes. This paper constitutes an analytical review of the current landscape of genetically engineered vaccines against Newcastle disease. It systematizes the data on key technological platforms: recombinant vector vaccines, reverse genetics-based products, as well as subunit, VLP-, and DNA-vaccines. A comparative analysis of the immunogenicity, safety, and ease of use of these platforms is conducted; particular attention is paid to the possibilities of implementing the DIVA strategy. It is demonstrated that, although vector vaccines have become the industry standard, reverse genetics technologies offer unique potential for controlling viral variability and reducing virus circulation in flocks. The conclusion substantiates the need to integrate various technological approaches to create effective disease eradication programs.

Objective. Formation of an objective picture of the current state of the problem, which is necessary for determining further avenues for improving biosecurity strategies in commercial poultry farming.

Materials and methods. The analytical study was conducted on the basis of domestic and foreign scientific publications on Newcastle disease immunoprophylaxis.

Results. The existing vaccines against Newcastle disease virus have been analyzed and reviewed. Their immunogenicity, ability to overcome maternal antibodies, compatibility with the DIVA strategy, virus shedding control, safety and ease of use have been described. Historical background is also provided. The necessity of transitioning from traditional vaccines to genetically engineered prophylactic products is substantiated.

Conclusion. The development and implementation of subunit and nucleic acid vaccines are the most important evolutionary steps in the field of Newcastle disease immunoprophylaxis, thus enabling the implementation of the virus eradication strategy in poultry flocks.

Keywords: review, Newcastle disease, genetically engineered vaccines, vector vaccines, reverse genetics

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Совершенствование иммунопрофилактики: потенциал и ограничения биотехнологических решений в борьбе с ньюкаслской болезнью

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РЕЗЮМЕ

Введение. Ньюкаслская болезнь продолжает наносить значительный экономический ущерб мировому птицеводству, что диктует необходимость пересмотра традиционных стратегий вакцинации. Классические живые вакцины, несмотря на широкое применение, обладают рядом ограничений, включая интерференцию с материнскими антителами и неспособность полностью предотвратить выделение полевого вируса из-за несоответствия современным генотипам. Настоящая работа представляет собой аналитический обзор современного ландшафта генно-инженерных вакцин против ньюкаслской болезни. Систематизированы данные о ключевых технологических платформах: рекомбинантных векторных вакцинах, препаратах, созданных методами обратной генетики, а также субъединичных, VLP- и ДНК-вакцинах. Проведен сравнительный анализ иммуногенности, безопасности и удобства применения данных платформ; особое внимание уделено возможностям реализации стратегии DIVA. Показано, что, хотя векторные вакцины стали отраслевым стандартом, технологии обратной генетики обладают уникальным потенциалом для контроля вирусной изменчивости и снижения циркуляции вируса в стаде. В заключении обосновывается необходимость интеграции различных технологических подходов для создания эффективных программ элиминации заболевания.

Цель исследования. Формирование объективной картины текущего состояния проблемы вакцинопрофилактики, что необходимо для определения дальнейших путей совершенствования стратегий биозащиты в промышленном птицеводстве.

Материалы и методы. Аналитическое исследование проводилось на базе отечественных и зарубежных научных публикаций, посвященных иммунопрофилактике ньюкаслской болезни.

Результаты. Проведен анализ и обзор существующих вакцин против вируса ньюкаслской болезни, описаны их иммуногенность, способность преодолевать материнские антитела, совместимость со стратегией DIVA, контроль вирусовыделения, безопасность и технологичность применения, приведена историческая ремарка. Обоснована необходимость перехода от традиционных вакцин к генно-инженерным профилактическим препаратам.

Заключение. Разработка и внедрение субъединичных и нуклеиновых вакцин являются важнейшими технологическими этапами в области иммунопрофилактики ньюкаслской болезни, которые позволят на практике применить стратегию элиминации вируса в стаде птиц.

Ключевые слова: обзор, ньюкаслская болезнь, генно-инженерные вакцины, векторные вакцины, обратная генетика

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INTRODUCTION

Newcastle disease (ND), caused by the virus of the species *Orthoavulavirus javaense* (OAVJ), or *Avian orthoavulavirus* type 1 (*Avian orthoavulavirus 1*, AOAV-1), has remained one of the most devastating threats to the global commercial poultry farming for a century [1, 2]. Despite tremendous progress in veterinary virology and biotechnology, this highly contagious infection has been causing billions of dollar economic losses annually since its first discovery in 1926 in Newcastle upon Tyne, England [3]. The impact of the disease is not limited to the direct mortality of the poultry flock, but also entails reduced performance, trade restrictions, costs of quarantine measures and destabilization of food security in the developing countries. The pathogen is characterized by a wide genetic diversity and circulates not only in poultry, but also in wild birds, thus creating a continuous, hard to eradicate natural reservoir of the infection [4, 5].

Historically, the ND control strategy was based on mass immunization using live attenuated (based on lentogenic and mesogenic virus strains) and inactivated vaccines [6]. These products allowed successful control of the epizootics in the 20th century, but currently their limitations are obvious [7]. Conventional live vaccines, while being cost-effective and highly immunogenic, have significant disadvantages, such as post-vaccination adverse reactions, respiratory stress (especially against the background of secondary infections, such as mycoplasmosis, etc.). Inactivated vaccine products are safe in this regard, but they induce a less strong mucosal immunity. Moreover, the vaccines developed on the basis of the virus strains relevant in the 1950s and 1960s and belonging to genotypes I and II [8, 9] often fail to provide protection against currently relevant strains (genotypes V, VII, XIII) [10, 11]. As a result, the vaccinated poultry can be infected with the field strains, thereby maintaining the pathogen circulation.

Given these factors, products derived using recombinant DNA technologies can be viewed as a safe and highly effective alternative. New generation vaccines, i.e. vector constructs (based on fowlpox viruses, turkey herpesvirus, adenoviruses), subunit vaccine products, DNA vaccines, with rational design of transgenes and regulatory elements, can induce robust immunity while reducing the virus shedding [12]. Moreover, such products are compatible with the DIVA strategy (Differentiating Infected from Vaccinated Animals), and are also created with due account of the genetic properties of the relevant field isolates. Yet, they also have some disadvantages: high production cost and the need to control the transgene expression levels [13].

This paper presents an analytical review of the literature on the assessment of the modern landscape of genetically engineered vaccines against ND. The main recombinant DNA-based vaccines are characterized, their advantages over conventional vaccines are critically assessed, and technological barriers to their use are examined.

HISTORICAL BACKGROUND OF THE DEVELOPMENT OF GENETICALLY ENGINEERED VACCINES AGAINST NEWCASTLE DISEASE

The era of classical vaccinology (1950s–1970s). Before the advent of the genetic engineering era, the ND control was based on empirical methods developed in the mid-20th century [14]. The use of lentogenic strains (LaSota, B1) and inactivated vaccines made it possible to contain the outbreak, but by the 1980s, the veterinary community faced the following challenges [15, 16].

1. Maternally derived antibody (MDA) barrier: antibodies transmitted from the hen to the chick can neutralize live vaccine viruses before the chick's own immunity is established (the "window of susceptibility" in the first weeks of life).

2. Safety issues. More immunogenic (mesogenic) strains caused post-vaccination adverse reactions, while safe (lentogenic) strains provided only short-term protection.

3. Differentiation issues (DIVA). Traditional serological tools did not allow differentiating post-vaccination antibodies from field virus antibodies.

Thus, there was a need for new vaccines combining safety of inactivated vaccines and effectiveness of live vaccines, while being able to overcome the maternal immunity.

Progress in molecular biology: gene cloning and target identification (1980s). The first sequencing

of the NDV genome became a prerequisite for new revolutionary solutions. It was demonstrated that the key antigens are surface glycoproteins: fusion protein (F), responsible for the fusion of the virion envelope with the cell membrane, and hemagglutinin-neuraminidase (HN), responsible for the virus attachment to sialic acid receptors. It was the cloning of genes encoding these glycoproteins responsible for the virus infectivity and pathogenicity that formed the basis of technologies for creating the first recombinant constructs [17].

Vector technology pioneers: fowlpox (late 1980–1990s). The first genetically engineered vaccines against NDV were developed based on the fowlpox virus (FPV) as a carrier vector [18]. Due to its extended genome, the FPV allows for the incorporation of foreign genes without losing its own replicative potential, and provides high levels of transgene expression necessary for the formation of the protective antibodies. The historical priority in this area belongs to the research teams led by M. E. Boursnell [19] and J. Taylor [20]: both groups proved that NDV gene *F*-expressing recombinant FPV (rFPV-ND) protects chickens from lethal infection with the virulent strains. It became obvious that protective immunity can be achieved through the use of a gene encoding only one major pathogen's immunogen and delivered by a heterologous virus.

Gold standard: turkey herpesvirus-based vectors (early 1990s). The FPV-based vectors had a significant drawback: if the bird had pre-existing antibodies to the native FPV, vaccination became ineffective [21]. The turkey herpesvirus (HVT), which had previously been used for vaccination against Marek's disease, was adapted for these purposes [22]. The research of scientific groups led by R. W. Morgan [23] and P. J. Sondermeijer [24] resulted in the construction in 1992–1993 of recombinant HVT (rHVT) variants carrying NDV *F* gene. rHVT-ND vaccines demonstrated a unique capacity to overcome maternally derived antibodies, since the herpesvirus spreads from cell to the cell (cell-associated), evading humoral immunity factors [25]. This also paved the way for mass vaccination in hatcheries (*in ovo*) [26].

Reverse genetics. In parallel, another trend was developing – the direct modification of the NDV genome [27]. For a long time, this was impossible, since the NDV is a negative-sense RNA virus and conventional genetic engineering tools are not applicable to it. In 1999, two European laboratories, B. P. Peeters group in the Netherlands [28] and A. Römer-Oberdörfer group in Germany [29], developed reverse genetics systems for the NDV, and generated, for the first time, an infectious virus from cloned cDNA. This allowed changing the F protein cleavage site to reduce the virulence, insert marker genes, or replace genes encoding surface proteins with genes encoding proteins of the relevant NDV field strains [30].

Current stage: platform diversification (2000s – present). In the 21st century, progress in the field of development follows the path of platform expansion [12].

1. Development of subunit vaccines through protein expression using baculoviruses in insect cells with the aim of achieving maximum safety.

2. Development of DNA vaccines (based on *F* gene-encoding plasmids) that have shown effectiveness but have encountered delivery-related problems.

3. Development of plant-based vaccines (based on potatoes, corn), started in the 2000s as an attempt to create low-cost products for oral administration.

The history of the development of genetically engineered ND vaccines has gone from the first laboratory experiments on gene cloning in the 1980s to the creation of commercially successful vector platforms (HVT) in the 1990s and full control over the viral genome through reverse genetics at the turn of the century.

RECOMBINANT VECTOR VACCINES (rVVs) – MARKET LEADERS

The vector vaccines are currently among the most successful biotechnological products in poultry farming. The operating principle of the platform is based on using a heterologous virus (vector) as a means to deliver a transgene – typically a gene encoding the F protein or, more rarely, the HN protein [31]. Viruses that are not pathogenic to poultry are used as a vector, thereby reducing the risks of heterologous infection.

Turkey herpesvirus (rHVT-ND)-based vectors. Recombinant constructs based on rHVT (recombinant Marek's disease virus serotype 3) are the gold standard of modern ND vaccine prevention and occupy the main market share of genetically engineered vaccines. HVT is a vector unique in its properties: it persistently (often life-long) and asymptomatically infects poultry during vaccination [32]. After administration (*in ovo* or subcutaneously to day-old chicks), the virus replicates in lymphocytes and epithelial cells, continuously expressing the *F* gene of the NDV. This creates an effect of constant stimulation of the immune system by ensuring prolonged antigen presentation in the body [33]. The main competitive advantages of the platform include the following: overcoming MDA (due to rHVT ability to escape the circulating neutralizing antibodies via intercellular bridges), which allows effective vaccination in hatchery even at high MDA titres in chicks; bivalent protection (vaccination provides immunity against both Marek's disease and ND); absence of post-vaccination reactions (vector vaccines do not affect the tracheal epithelium and do not provoke the development of respiratory syndrome, which is important in case of high bacterial load of *Mycoplasma* spp., *Escherichia coli*). An additional advantage is the compliance of this class of vaccines with the DIVA strategy: vaccinated birds produce antibodies only to the F protein insert, but not to other viral proteins. This allows differentiation of vaccinated poultry from those infected with the field virus using discriminating ELISA (enzyme-linked immunosorbent assay) [34].

A limitation of rHVT-ND vaccines is the delayed formation of the immune response: it takes up to four weeks to reach the protective immunity, so strict biosafety measures must be followed to prevent infection during this period, including additional administration of the live vaccines. Administration of two recombinant vaccines developed using the same rHVT platform (for example, rHVT-ND and rHVT-IBD against Gumboro disease) is also impossible due to the competition of the viruses for the replication sites. rHVT-ND vaccines do not provide sterile immunity; moreover, they are cell-associated, which implies their storage in liquid nitrogen and administration within an hour after thawing [31].

Fowlpox virus-based vectors (rFPV-ND). rFPV-ND were historically the first genetically engineered vaccines against NDV. Today, they retain their niche mainly in the regions where protection against fowlpox is relevant.

The mechanism of action of rFPV-ND vaccines is that the extended genome of the vector allows the insertion of large inserts, enabling high-level expression of *F* and *HN* genes in the cellular cytoplasm. rFPV is stable and safe, capable of stimulating a strong T-cell immune response, which is important for viral clearance [35]. Unlike rHVT vaccines, rFPV vaccines are ineffective if poultry is seropositive to the FPV [36].

The vector vaccines as a class of products, and primarily the rHVT platform, have revolutionized the ND prevention by solving the problem of MDA interference and automating the vaccination process. Nevertheless, delayed immunity formation and impossibility of simultaneous use of several rHVT vaccines open up the prospects for further developments and combined vaccination schemes. According to the recommendations of the World Organization for Animal Health (WOAH), the strategy for ND control in highly enzootic areas should be based on a combination of two approaches: the use of vector vaccines to form long-lasting cellular immunity and the use of conventional live vaccines to cover the "window of susceptibility" in the first weeks of life [37].

REVERSE GENETICS TECHNOLOGIES: RESPONDING TO GENETIC VARIABILITY

Genetic and antigenic variability of the NDV are the key reasons why conventional vaccination programs often provide only clinical protection, not always blocking the field virus replication and shedding. In this regard, reverse genetics technologies have become a novel stage in improving the immunoprophylaxis tools: they have enabled the transition from selecting effective native attenuated strains to the rational design of viral genomes with predictable phenotypic properties [38]. The reverse genetics approaches for the ND pathogen are based on generating the infectious virus from the cloned full-length cDNA complementary to the genome of the negative-sense RNA virus. In practice, this means the possibility of replacing protective antigens, individual virulence determinants, introducing marker inserts, and creating chimeric variants as close as possible to the relevant field isolates.

Selective pressure on the NDV is maintained by a high flock density in commercial poultry farming, mass vaccination with live vaccines, and the virus circulation in wild avifauna. As a result, vaccines based on classical NDV genotypes (often I, II), while providing partial or complete clinical protection, are unable to control the replication of current virulent genotypes (V, VII, etc.) [38, 39]. Reverse genetics methods make it possible to construct genotype-matched vaccine strains that reduce the risks of field virus spread [40].

From a methodological point of view, the NDV reverse genetics is based on the following manipulations [27]:

1) construction of full-length cDNA of NDV genome in a plasmid vector;

2) synthesis of the viral RNA and formation of a ribonucleoprotein complex with helper replicative proteins (NP/P/L);

3) replication of the infectious virus in cell cultures or chicken embryos;

4) phenotypic and genetic validation: assessment of stability, degree of attenuation, immunogenicity and ability to inhibit field virus replication and shedding.

Genotype-matched and chimeric vaccines. Another significant area that has been developed based on reverse genetics methods is vaccines in which the insert (F and/or HN) matches the circulating field genotype [40]. An approach is being widely implemented in practice, when the genomic backbone is a safe vaccine or a pre-attenuated strain/construct, and the *F* and *HN* genes in it are replaced with those of an epizootically relevant genotype. The advantages of this approach are optimization of the vaccine antigenic composition, more stringent control of the virus shedding, and the possibility of rapid modernization of the construct: when the dominant genotype is changed, a "reassembly" of the strain is possible [41]. However, such vaccines are often classified as genetically modified live viruses, which complicates registration, logistics, and implementation in different jurisdictions. Although there is no risk of classical reassortment with respect to the NDV, issues of genetic stability, possible accumulation of mutations during large-scale use, and production control remain understudied. Several NDV variants or genotypes may circulate on farms as well, which reduces the effectiveness of the genotype-matched concept for all scenarios [36].

Marker vaccines and DIVA strategy in the framework of reverse genetics. A promising application of reverse genetics technologies is the creation of marker (DIVA-compatible) vaccines, as this makes it possible to diagnostically distinguish the vaccinated poultry from those infected with a field virus by a panel of antibodies (for example, to NP or other internal proteins) or by field virus-unique PCR targets [42]. DIVA-compatible vaccines are a key tool for ND eradication programs, as they allow maintaining an evidence base for the disease freedom. The DIVA strategy requires the simultaneous availability of validated diagnostic tests, regulatory recognition and a sustainable monitoring system and is not only a biotechnological, but also an organizational and economic challenge [43].

SUBUNIT AND VLP VACCINES

Subunit vaccines and vaccines based on virus-like particles (VLP) represent a trend of genetic engineering vaccinology, the key principle of which is the absence of a replication competent virus in the product composition. In the ND context, this indicates a rejection of "live" vaccine infection as a mechanism for inducing immunity and an attempt to replace it with antigenic stimulation based on the use of protective viral antigens [44]. For NDV, surface glycoproteins F and HN remain the most significant antigenic targets in the development of subunit vaccines, with protein F generally considered to be the primary inducer of neutralizing antibodies [45]. Unlike vector and live vaccines, the efficacy of subunit and VLP products depends on the structural and conformational state of the antigen, the post-translational modifications it has undergone, and the manner in which its presentation is organized [46].

Subunit vaccines are a type of vaccines, in which specific purified recombinant antigens (subunits) of the pathogen are used to stimulate the immune response. They do not contain any replicating components, have high stability, and significantly reduce the risk of adverse reactions compared to conventional vaccines. Such vaccines require the presence of adjuvants in the immunogenic composition, specific administration schemes (double immunization) and, in some cases, additional delivery systems (liposomes, nanoparticles) [47]. VLP vaccines are highly immunogenic, replication-defective, self-assembling structures that

mimic the morphology of the native pathogen. Composed of self-assembling viral structural proteins, they do not contain any genetic material, which makes them incapable of replication or causing disease. NDV VLPs are formed through the co-expression of the structural proteins F, HN, and M, with the matrix protein (M) typically serving as the scaffold for assembly [48]. The concept of subunit and VLP vaccines is attractive due to the combination of high safety profile and possibility of integration into the DIVA strategy.

Expression systems and their significance for NDV antigens. The efficacy of subunit and VLP vaccines is determined not only by the choice of antigenic target but also by the choice of the expression system, on which the post-translational modifications of the final product depend. To date, successful application of the following expression systems for NDV antigen biosynthesis has been reported:

– *baculovirus system (Sf9/Hi5 cells)* is often considered optimal for producing viral glycoproteins and VLPs; it provides the necessary post-translational modifications and high yields of the target protein. It is technologically compatible with VLP assembly as well [49, 50];

– *yeasts and E. coli* are convenient and cheap, but when F and HN proteins are expressed, they can synthesize a product with an altered conformation or with incorrect glycosylation, which reduces the proportion of functional epitopes [50, 51];

– *mammalian cells* provide the most correct conformation of glycoproteins, but significantly increase the cost and production requirements, which is critical for large-scale use [52];

– *plant platforms* (transient expression or stable transformants) are attractive for their scalability and potential low cost, but face challenges related to dose standardization, expression variability, and regulatory control [53].

The analysis of the subunit and VLP vaccines pros and cons is demonstrated in Table 1.

DNA VACCINES AND NEW DELIVERY SYSTEMS

The most radical departure from traditional vaccinology methods in ND control has been the development of nucleic acid (DNA) vaccines. In this case, the bird's body does not play the role of a recipient of the finished antigen, but it is a bioreactor that independently synthesizes protective antigens. The principle of the technology is based on the introduction of genetic constructs – plasmid vectors encoding transgenes (F/HN); this ensures simulation of the natural infectious process *in vivo* without the involvement of the live pathogen [13]. The transgenes in the composition of the genetic constructs are under the control of a strong promoter (often cytomegalovirus – CMV). In the cell nucleus, such constructs trigger the transcription and translation of viral proteins, which are subsequently presented on the cell surface. This presentation mechanism is the main immunological advantage of the DNA vaccines: they are able to induce both the T-cell immune response and the production of virus-neutralizing antibodies [21]. A further advantage is the speed with which DNA products can be developed. In the event of the emergence of a new NDV genovariant, a plasmid construct can be created or updated within a short timeframe, facilitating a rapid response to epizootic challenges [15].

In practice, attempts to realize the potential of DNA vaccines in poultry farming face serious obstacles. Thus, "naked" plasmid DNA is weakly immunogenic when administered by standard routes, mainly due to low cellular uptake *in vivo* and degradation by nucleases. As a result, achieving immunogenicity requires the administration of high doses of DNA and the use of complex delivery methods (electroporation), which is economically impractical in commercial poultry farming [54].

New delivery systems. The understanding that the effectiveness of nucleic acid vaccines is determined not only by the quality of the antigen, but also by the method of its delivery, has led to the active development of supporting technologies. Early experimental methods, such as biolistic particle delivery system ("gene gun") or electroporation, have shown high efficiency and ability to induce a strong immune response even with small DNA doses [55]. However, this requires the use of sophisticated equipment and individual administration, which again is not applicable for large-scale vaccination. Researchers see the solution to this problem in the use of chemical and nanotechnology carriers. Incorporation of genetic material into the structure of liposomes, cationic polymers, or chitosan and PLGA (polylactide-co-glycolide)-based nanoparticles allows it to be protected from degradation and enhances the transfection efficiency [56, 57]. The use of mucoadhesive polymers (for example, chitosan) opens up prospects for the design of mucosal DNA vaccines for aerosol or oral administration [58]. Nevertheless, despite active laboratory research, scaling up of the nanovaccine production and ensuring their stability and uniformity in industrial volumes still pose complex technological challenges.

Thus, nucleic acid vaccines represent a technologically advanced segment, which has not yet been adapted to the realities of mass poultry farming. Their undeniable advantages – speed of development, stimulation of both cellular and humoral immunity – are offset by low cost-effectiveness and unsuitability for mass vaccination purposes. The future of this platform depends on progress in the development of affordable nanosystems for delivery.

FINAL ANALYSIS

To systematize the data, we conducted a comparative analysis of the main types of ND vaccines (conventional live vaccines [38, 59, 60], rVVs [31, 36], vaccines developed on the basis of reverse genetics technologies [36, 41, 61], subunit and VLP vaccines [48, 62], DNA vaccines [63, 64]) according to six parameters critical for commercial poultry farming: immunogenicity (including mucosal immunity), ability to overcome maternally derived antibodies, compatibility with the DIVA strategy, control of virus shedding, safety, and practicality of administration. The results of this analysis are summarized in Table 2, which is presented electronically in the *Supplementary files* section at https://doi.org/10.29326/2304-196X-2026-15-2-123-130.

CONCLUSION

The conducted analytical literature review indicates a fundamental global reassessment of Newcastle disease control strategies. The transition from the use of conventional attenuated strains to the rational design of genetically engineered products is a natural technological response to the evolutionary variability

Table 1
Advantages and limitations of subunit and VLP vaccines

Vaccines	Advantages	Limitations
Subunit (F/HN)	*Biosafety:* absence of replication eliminates the risk of reversion to virulence and vaccine virus spread (a significant parameter for high-biosafety facilities and for monitoring programs). *Antigenic composition control:* epitopic composition may be changed in accordance with the current genotypes of the circulating field viruses. *DIVA compatibility:* Subunit vaccines contain 1–2 major antigens. Discriminating tests for other antigens, such as NP, can be used to differentiate infected and vaccinated poultry. *No "vector" limitations:* there is no problem of interference between several vector vaccines based on the same platform (as with rHVT vectors), and it is also possible to combine antigens of different pathogens in one product (polyvalent subunit vaccines)	*Low mucosal immunity and limited replication control.* Since the NDV infection gateways are the mucous membranes, live and vector vaccines are effective due to local replication. The subunit vaccine provides a predominantly systemic humoral response that blocks the ND clinical manifestations, but may not be sufficient to completely inhibit the virus replication. *Dependence on adjuvants and immunization schedule.* Formation of the protective immunity requires adjuvants and repeated administrations, which increases the cost and labor intensity. *Large-scale use limitations.* Individual injections and the need for multiple doses limit their application in the broiler production with a short production cycle. *Challenges of post-translational modifications.* Incorrect folding or glycosylation of proteins can reduce the effectiveness of the immune response formation
VLP	*Increased immunogenicity:* F/HN presentation on the VLP surface facilitates B-cell activation and high-affinity antibody production. *Preservation of conformational epitopes:* essential for glycoprotein F, since neutralizing antibodies often specifically recognize conformational structures. *Potential to reduce replication:* induce a stronger immune response, especially when delivered correctly (including mucosal variants), may bring efficacy closer to live vaccines while maintaining biosafety	*Production complexity and cost.* Unlike subunit proteins, VLPs are multicomponent structures. Stable expression cell lines, assembly and purification control systems are required, which increases the cost of a dose. *Standardization and quality control.* It is necessary to confirm the size, composition, and density of the F/HN exposure. *Large-scale use limitations.* Even if VLPs are immunogenic, their commercial success is determined by the possibility of large-scale and economically justified administration (aerosol, water, *in ovo*, injection). Optimal schedules are still unknown for many VLP candidates

of the pathogen and the intensification of commercial poultry farming.

Today it can be stated that recombinant vector vaccines, primarily those based on turkey herpesvirus (rHVT-ND), have taken a dominant position in the hatchery vaccination segment. Their ability to overcome the maternally derived antibody barriers and induce a lifelong basic protection solved one of the main problems of the 20th century veterinary immunology. However, as the analysis demonstrates, none of the existing technological platforms is optimal: vector vaccines are unable to rapidly induce the mucosal immunity necessary to block the "infection gateways", while subunit, VLP and DNA vaccines, having an optimal safety profile, face economic and logistical barriers to mass use.

A special place in future disease control is given to reverse genetics technologies. The possibility of creating genotype-associated vaccines opens the way to solving the problem of the virus shedding. It is the reduction of field virus circulation in the vaccinated flock, rather than merely preventing clinical manifestations, that is becoming the new criterion for the efficacy of the vaccine products. Furthermore, the widespread implementation of DIVA-compatible vaccines (both vector-based and those marked using reverse genetics) is a prerequisite for the transition from a disease containment strategy to an eradication strategy in endemic regions.

Summarizing the above, it can be stated that the future of specific ND prevention lies in the area of combined strategies. Most likely, the optimal protection scheme will be based on a combined approach: the use of vector vaccines *in ovo* to establish fundamental systemic immunity in combination with mucosal stimulators (based on reverse genetics or next-generation vectors). Further progress in this area will likely be associated with the improvement of antigen delivery systems and the adjustment of regulatory standards for veterinary medicinal products obtained using recombinant DNA technologies.

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