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Review Article

Epidemiological Evolution, Vaccine Development, and Control Strategies of Highly Pathogenic Avian Influenza H5N1 Virus

Song Yating, Zhou Meifang, Huang Weizhong, Luo Yuewen, Deng Genggao, Zhang Chunhong, Ding Li, Yang Yalin, Qiu Maoguo, Zhao Xin, Li Xiangyang

Zhaoqing Center for Animal Disease Prevention and Control, Zhaoqing 526000, China

***Corresponding Author: Li Xiangyang**

Abstract:
Since its initial identification in Guangdong, China, in 1996, the highly pathogenic avian influenza virus (HPAIV) H5N1 subtype has evolved into multiple genetic clades, with clade 2.3.4.4b emerging as the dominant strain. This clade demonstrates significant antigenic variability and continues to cause widespread outbreaks in poultry, as well as sporadic infections in humans worldwide. This review comprehensively outlines the epidemiological progression of H5N1, recent advancements in vaccine development, and comprehensive control measures. Viral evolution is primarily influenced by antigenic drift and reassortment, with specific amino acid mutations facilitating adaptation to mammalian hosts. Clade 2.3.4.4b, currently prevalent in Asia, Europe, and the Americas, exhibits considerable genetic diversity. Various vaccine platforms have been utilized, encompassing whole inactivated virus vaccines, live attenuated and vectored vaccines, virus-like particles (VLP) vaccines, subunit vaccines, and nucleic acid-based vaccines. However, widespread vaccine distribution encounters obstacles such as immune evasion, potential viral shedding, and differing international immunization regulations. Looking ahead, effective pandemic preparedness necessitates innovative vaccine strategies, strengthened global surveillance, improved biosecurity measures, and interdisciplinary cooperation to efficiently control transmission and mitigate pandemic threats.

Key words: H5N1; whole inactivated virus vaccine; live attenuated and vectored vaccine; virus-like particles (VLP) vaccine; nucleic acid vaccine; broad-spectrum protection

1. Introduction

Since its initial identification in domestic waterfowl in Guangdong, China, in 1996, the highly pathogenic avian influenza virus (HPAIV) H5N1 has evolved into multiple genetic clades, including 2.3.4.4b, resulting in substantial antigenic diversity and causing persistent poultry outbreaks alongside sporadic human infections worldwide[1-3]. The virus not only imposes significant economic losses on the poultry industry but also represents a potential pandemic threat due to its capacity for cross-species transmission, as exemplified by recent outbreaks in U.S. dairy cattle[4,5]. Although sustained human-to-human transmission has not been observed, the virus circulating in multiple host species and the potential for reassortment with seasonal influenza strains present significant public health challenges. Vaccination remains a critical component of the control strategy. Current vaccine platforms include whole inactivated virus vaccines, live attenuated and vectored vaccines, virus-like particles (VLP), subunit vaccines, and nucleic acid-based vaccines[2,6]. Emerging platforms, such as mRNA and VLP vaccines, demonstrate advantages in flexibility and breadth of protection, making them particularly suitable for outbreak response and pandemic preparedness[7,8]. Nevertheless, large-scale vaccine deployment faces challenges including immune escape mutations, potential viral shedding, and divergent international immunization policies—notably the coexistence of culling and vaccination strategies[2]. In light of these considerations, this review systematically analyzes the epidemiological progression of H5N1 and recent advancements in vaccine development. The objective is to guide the development of broad-spectrum vaccines and integrated control strategies to bolster global endeavors in containing avian influenza outbreaks.

2. Epidemiology of Highly Pathogenic Avian Influenza H5N1 Virus

Avian influenza refers to a range of infections and disease syndromes caused by avian influenza virus (AIV). In poultry, AIV infection results in diverse clinical outcomes, including asymptomatic infection, respiratory disease, reduced egg production, and systemic severe illness with mortality rates reaching 100%[9]. AIV is classified within the genus *Alphainfluenzavirus* of the family *Orthomyxoviridae*, and its genome comprises eight segments of single-stranded, negative-sense RNA. These segments may be designated in descending order of electrophoretic mobility as vRNA1 to vRNA8, or named according to their encoded proteins: PB2, PB1, PA, HA, NP, NA, M, and NS[10]. It is currently known that influenza A viruses encode at least 10 essential proteins (PB2, PB1, PA, HA, NP, NA, M1, M2, NS1, and NEP/NS2) and 7 accessory proteins (such as PB1-F2 and PA-X), most of which contribute to viral host adaptation and immune modulation[10-15]. AIVs are classified into 19 hemagglutinin (HA) and 11 neuraminidase (NA) subtypes based on antigenic differences in these surface glycoproteins[16]. Among these, H17N10 and H18N11 have been identified exclusively in bats, while H1-H16, H19, and N1-N9 are capable of infecting diverse avian and mammalian species, including humans[17,18]. According to their pathogenicity in experimentally infected chickens, AIVs are categorized as highly pathogenic (HPAIV) or low pathogenic (LPAIV). Unlike LPAIVs, HPAIVs contain multiple basic amino acids at the HA cleavage site, a feature strongly correlated with increased tissue tropism and virulence[19-21]. Influenza A viruses evolve through antigenic drift (minor changes due to point mutations) and antigenic shift (major changes resulting from genetic reassortment), enabling them to cross species barriers and establish transmission in new populations[22]. Their segmented RNA genome enables genetic reassortment during co-infection of a single host cell with different influenza viruses, promoting the emergence of novel reassortants. This mechanism has played a pivotal role in influenza pandemics in humans; three of the four pandemics since the 20th century—1957, 1958, and 2009—were caused by reassortant viruses[23-25].

The HPAIV H5N1 was first identified in domestic waterfowl in Guangdong, China, in 1996, and was designated as A/goose/Guangdong/1/1996 (Gs/GD/96)[26]. Although other lineages, such as H5N2 HPAIV originating in Mexico in the mid-1990s, were co-circulating at the time, the Gs/GD/96 lineage rapidly became dominant[27]. This strain first caused outbreaks in poultry in mainland China and Hong Kong in 1997, resulting in 18 human infections and six fatalities, marking the first confirmed avian-to-human transmission of H5N1[28-30]. Following the closure of live poultry markets, the virus was not detected in poultry again until 2002, when outbreaks occurred on farms, ultimately leading to large-scale epidemics and additional human fatalities[31,32]. In 2005, H5N1 was detected in wild migratory birds at Qinghai Lake, China, and has since spread persistently among avian species worldwide[33-35]. The Gs/GD/96-derived H5 viruses have undergone rapid evolution, forming more than 30 distinct genetic clades exhibiting substantial antigenic diversity[2]. From 2005 to 2014, clades 2.1, 2.2, and 2.3.1.2a-c were the predominant circulating variants. Clade 2.2, in particular, spread to poultry in Africa, the Middle East, and Europe, where reassortment with local wild bird or poultry viruses resulted in the emergence of new subtypes, including H5N6 and H5N8[33,36-38]. After 2014, clade 2.3.4.4 became dominant across Asia, Europe, and North America, with various genotypes (e.g., H5N2, H5N6, H5N8, collectively referred to as H5Nx) circulating widely[39-41]. By the end of 2015, earlier H5N1 lineages had been largely replaced by clade 2.3.4.4 H5Nx viruses[42]. Between 1997 and 2021, a total of 896 human H5N1 cases were reported across 23 countries, with 888 cases occurring after 2003. Most cases presented as severe pneumonia, with a case fatality rate exceeding 50%. Notably, approximately half of the characterized viruses carried mammalian-adaptive mutations—including E627K, D701N, and T271A in the PB2 protein—which are associated with enhanced virulence and/or replication efficiency in mammals, although no significant alteration in HA receptor binding specificity was observed[43]. Clade 2.3.4.4 is currently divided into eight subclades (2.3.4.4a-h), with 2.3.4.4b being the predominant subclade[44,45]. H5N8 viruses within this subclade continued to circulate in wild birds until 2020, when reassortment with other avian influenza viruses led to the emergence of novel H5N1 strains[46]. Subsequently, clade 2.3.4.4b H5N1 viruses spread rapidly across Europe, Asia, and Africa in the autumn of 2021, becoming the dominant global subtype. The virus was introduced to North America via migratory birds in early 2022 and had been detected in Europe, Southeast Asia, and Antarctica by autumn 2023[47-51]. Currently, HPAIV clade 2.3.4.4b H5N1 continues to cause outbreaks in wild and domestic birds globally and has expanded its host range to include dairy cattle, poultry, and other animal populations across multiple U.S. states (Figure1 A). More than 100 reassortant H5N1 genotypes have been identified in North America, indicating substantial genetic diversity[52]. Since April 2024, the U.S. Centers for Disease Control and Prevention (CDC) has confirmed 70 human infections through genomic sequencing. In three cases, an S247N substitution in NA was identified; in vitro studies indicate that this mutation may confer a slight reduction in susceptibility to NA inhibitors, including oseltamivir (Figure1 B). To elucidate H5 evolutionary dynamics, Xie et al.[2] analyzed approximately 10,000 HA sequences from the GISAID database (1997–2024), selected 782 representative sequences for phylogenetics. Bayesian evolutionary tree analysis reveals that clade 2.3.4.4b H5N1 viruses have been detected throughout Asia, Europe, North America, and South America. North and South American strains are closely related and share a direct common ancestor with Eurasian lineages, while Asian strains form a distinct cluster closely related to European viruses, suggesting Asia as the probable origin of this subclade. Nucleotide and amino acid homologies of the HA gene ranged from 96.3% to 99.5% and 95.1% to 100%, respectively, indicating that the HA protein remains relatively conserved despite active viral evolution. The expanded geographic distribution and host range of clade 2.3.4.4b H5N1 infections highlight the crucial role of reassortment in internal and NA genes, along with adaptive mutations, in facilitating host adaptation[40,53,54].

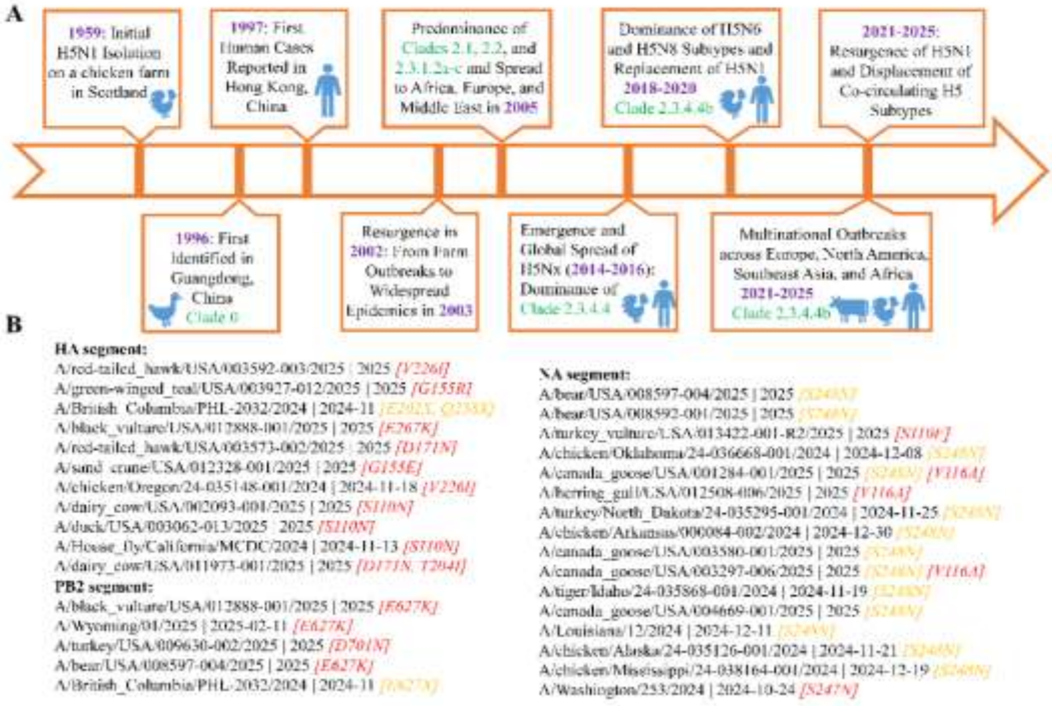


Figure1 Evolutionary timeline of HPAIV H5N1 and key mutations in circulating clade 2.3.4.4b viruses in the U.S.. (A) This timeline illustrates major evolutionary milestones and key clade transitions of HPAIV H5N1. (B) Functionally significant mutations in HA, NA, and PB2 among circulating U.S. clade 2.3.4.4b H5N1 strains identified via subsampled phylogenetic analysis.

3. Advances in Vaccine Development for HPAIV H5N1

3.1 Whole Inactivated Virus Vaccines

The whole inactivated virus (WIV) vaccine platform represents one of the cornerstone strategies for controlling HPAIV infections, with a well-established development and manufacturing pipeline (Figure 2A). Conventionally, WIV vaccines are produced by propagating virus in 9-11-day-old specific-pathogen-free (SPF) embryonated chicken eggs, followed by chemical inactivation using agents such as formaldehyde, β-propiolactone, or binary ethylenimine, and subsequent concentration and purification. Tian et al.[55] constructed an attenuated H5N1/PR8 reassortant strain using reverse genetics and developed a formaldehyde-inactivated oil-emulsion vaccine. This vaccine induced hemagglutination inhibition (HI) antibody responses lasting up to 43 weeks in SPF chickens and provided complete protection in chickens, ducks, and geese, demonstrating long-term immunity in poultry. To address the currently circulating clade 2.3.4.4b H5N1 virus, Song et al.[56] developed an antigen-optimized inactivated whole-virus vaccine strain, designated 22W_KY, by introducing an R90K mutation and an avian-origin PB2 segment, followed by inactivation with binary ethylenimine (BEI). Intranasal immunization in mice significantly boosted systemic IgG, mucosal IgA, and pulmonary T-cell responses, resulting in broad protection against heterologous and heterosubtypic viral challenges. Park et al.[57] developed a multivalent inactivated vaccine comprising four subtypes (H1N9, H3N8, H5N1, and H7N3), which induced cross-subtype protection in both mice and ferrets, substantially reducing viral loads and lung pathology, thereby supporting the utility of multivalent designs in broadening immune coverage. Serological analyses further corroborate the cross-protective potential of inactivated vaccines. Huang et al.[58] reported that after immunization with an existing H5N1 inactivated vaccine (NCT00535665), a pseudovirus neutralization antibody titer ≥1:980 correlated with over 60% cross-protection against contemporary strains, suggesting that high antibody levels are crucial for broad neutralization. Long-term immune memory is another advantage of inactivated vaccines. Nakayama et al.[59] demonstrated that cynomolgus macaques maintained high levels of neutralizing antibodies five years post-vaccination with an H5N1 whole-virus particle vaccine, and upon challenge, mounted a recall response against multiple clades including 2.3.4.4b, effectively suppressing viral replication and cytokine activation. Kilany et al.[60] developed a multivalent inactivated vaccine targeting strains prevalent in Egypt and the Middle East. A single dose achieved complete seroconversion in SPF chickens and provided 100% protection against H5N1 and H5N8 challenges, while significantly reducing oropharyngeal virus shedding. In summary, both monovalent and multivalent inactivated whole-virus vaccines exhibit strong immunogenicity, sustained protection, and substantial cross-protective potential, making them essential tools for controlling H5 avian influenza outbreaks—particularly in poultry vaccination programs and as part of human prepandemic vaccine stockpiling strategies.

3.2 Live-Attenuated and Vectored Vaccines

Live attenuated influenza vaccines (LAIVs) have garnered significant attention due to their ability to mimic natural infection and induce concurrent humoral, cellular, and mucosal IgA immune responses (Figure 2B). Compared to inactivated vaccines, LAIVs induce broader cross-reactive immunity and longer-lasting protection, accompanied by a favorable safety profile with fewer adverse reactions[61]. Notably, a live-attenuated vaccine against equine influenza virus (EIV), based on the cold-adapted and temperature-sensitive strain A/eq/Kentucky/1/91 (H3N8), has been approved for use in horses in the U.S. and has proven both safe and effective in field trials[62]. In parallel, viral vectored vaccines—such as those utilizing Newcastle disease virus (NDV) or herpesvirus of turkeys (HVT)—represent a non-replicating vaccine strategy that eliminates the risk of viral recombination. When administered intranasally, these vectors simulate natural infection and facilitate native antigen presentation, leading to robust and specific antibody responses.

Numerous studies have demonstrated the protective efficacy and application potential of LAIVs and viral vectored vaccines across various host species. Liu et al.[63] developed an intranasal vaccine based on a deleted-NS1 live attenuated influenza viral vector (DelNS1 LAIV) system targeting bovine-origin H5N1 and related clade 2.3.4.4b strains. A single immunization in mice and hamsters elicited sustained neutralizing antibodies, mucosal IgA, and T-cell responses for up to two months, and provided complete protection against both bovine- and mink-origin H5N1 viruses, highlighting its potential against emerging variants. The NDV vector represents another important platform. Kim et al.[64] noted that NDV-vectored live attenuated vaccines expressing H5 HA can confer simultaneous protection against highly pathogenic avian influenza and Newcastle disease, and have been licensed for poultry use in China and Mexico. The another group developed an NDV-vectored vaccine, rK148/22-H5, expressing the HA of clade 2.3.4.4b H5N1, which conferred complete protection against lethal H5N1 and NDV challenge in SPF chickens and significantly reduced viral shedding[65]. This vaccine also showed robust efficacy in broilers and ducks in the presence of maternal antibodies, while complying with the Differentiating Infected from Vaccinated Animals (DIVA) strategy. Furthermore, recombinant vectored vaccines based on the HVT have demonstrated broad cross-protection. Nassif et al.[66] evaluated a commercial HVT-H5 vaccine against antigenically drifted Egyptian strains, reporting 80-90% clinical protection rates and a reduction in viral shedding by 2.8-3.8 log units, confirming its efficacy against antigenically divergent viruses. To address vaccination challenges in waterfowl, Zhao et al.[67] constructed a trivalent vaccine using duck enteritis virus (DEV) as a vector, designated rDEV-dH5/H7. The recombinant virus stably maintained and expressed two H5 and one H7 HA genes, offering complete protection against DEV challenge while inducing durable hemagglutination inhibition antibodies and cross-clade protection against heterologous H5 and H7 viruses. In summary, live attenuated and vectored vaccine platforms—including DelNS1, NDV, DEV, and HVT vectors—exhibit notable advantages in rapid response to emerging strains, enabling multivalent immunization, overcoming pre-existing immunity, and reducing viral transmission. These platforms represent a promising direction for the development and application of avian influenza vaccines.

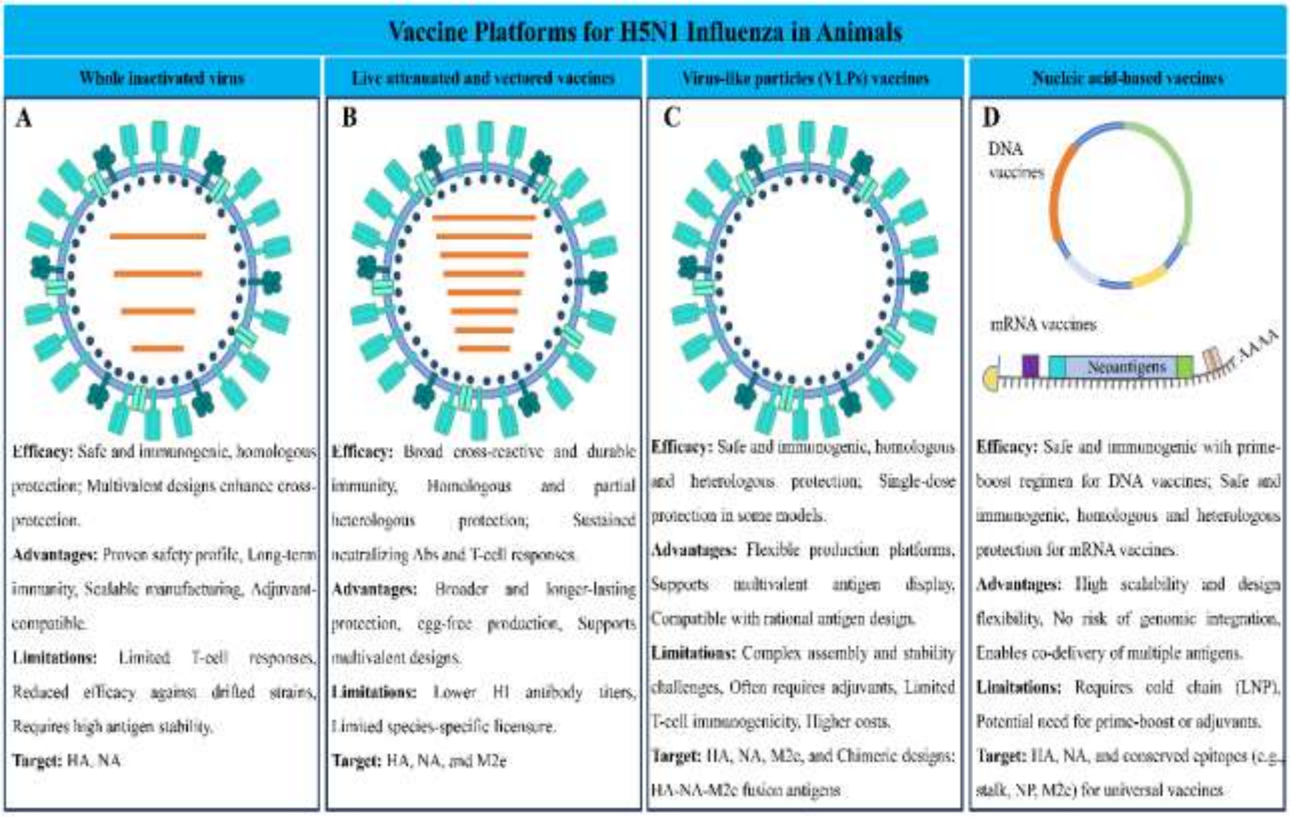
3.3 VLP and Subunit Vaccine

Virus-like particle VLP vaccines have demonstrated considerable flexibility and scalability in the development of vaccines against multiple influenza subtypes, including H5, H6, H7, and H9 (Figure 2C). They have been successfully produced in various expression systems such as insect cell-baculovirus, plant cell, and mammalian cell platforms[68-72]. Lacking infectivity while retaining the structural and antigenic properties of native virions, VLPs can be delivered via multiple routes to induce broad immune responses and provide cross-protection against both homologous and heterologous viral strains, establishing them as a promising alternative to conventional inactivated vaccines[73]. Typical influenza VLPs are composed of HA and matrix protein (M1), with HA serving as the key antigen for eliciting serum immune responses and being widely utilized in subunit vaccine development[74]. Kong et al.[8] developed an H5N1 VLP vaccine using the insect cell-baculovirus system; a single immunization induced high levels of HI antibodies. Formulation with ISA 71 adjuvant further enhanced Th1 and Th2 immune responses and conferred superior cross-protection against antigenically distinct H5N1 viruses compared to ISA 201 adjuvant. Importantly, increasing the vaccine dose improved cross-protective efficacy and enabled sterilizing immunity against heterologous H5N1 challenge, mediated largely by neutralizing antibodies. Plant expression systems also offer a rapid and low-cost production method for VLPs. For instance, Elbohy et al.[75] expressed H5/H9 HA-VLPs in tobacco plants, which induced specific antibodies in both mice and chickens. Notably, the VLP platform shows great potential for developing universal influenza vaccines. Kim et al.[73] designed a VLP displaying consensus NA subtypes tandemly linked with the M2 ectodomain (m-cNA-M2e VLP). Immunization of mice with this construct elicited broad neuraminidase-inhibiting (NAI) antibodies, M2e-specific antibodies, and interferon-gamma-secreting T-cell responses, protecting against diverse influenza A viruses and influenza B virus, including in aged mice. Chen et al.[76] enhanced VLP immunogenicity by fusing B-cell activating factor (BAFF/BLyS) and a proliferation-inducing ligand (APRIL) to the transmembrane domain of HA, improving interaction with cognate receptors. To strengthen M2e immunogenicity, Fatimah et al.[77,78] generated chimeric proteins comprising human and avian influenza M2e epitopes fused to the C-terminal end of Macrobrachium rosenbergii nodavirus capsid protein (NvC), which self-assembled into VLPs. These VLPs induced broad IgG responses, balanced Th1/Th2 immunity, and increased CD8+/CD4+ T-cell ratios in BALB/c mice. Even without adjuvant, they provided complete protection against a 4×LD_{50} challenge of H1N1 or H3N2 virus, significantly reducing lung pathology and viral load. Ramirez et al.[79] developed a multicomponent vaccine, H5-FliCc-CpG-E2, based on an E2 protein nanoparticle co-delivering H5 HA, a TLR5 agonist (FliCc), and a TLR9 agonist (CpG). This vaccine induced higher IgG levels and broader H5 cross-reactivity, with the coupling mode of adjuvants enabling precise modulation of Th1/Th2 bias: FliCc promoted IgG1 (Th2), CpG favored IgG2c (Th1), and co-conjugation achieved a balanced response. Vaccinated animals showed 100% survival following H5N1 challenge, underscoring the ability of nanocarrier-based co-delivery to tailor the strength, breadth, and polarity of anti-influenza immunity. In the realm of subunit vaccines, Tjärnhage et al.[80] designed an antigen-targeting subunit vaccine directed against MHC class II molecules or chemokine receptors on antigen-presenting cells (APCs). A single immunization elicited robust antibody and T-cell responses, protecting mice against H1N1, H5N1, and H7N1 challenges with immunogenicity comparable to licensed vaccines. In summary, both VLP and subunit vaccine platforms—through rational antigen design, adjuvant integration, and targeted delivery strategies—can significantly broaden immune protection and efficacy. They represent a pivotal direction for the development of universal vaccines against diverse influenza subtypes.

3.4 Nucleic Acid Vaccine

Nucleic acid-based vaccines represent a transformative approach in influenza vaccine development by enabling in vivo expression of target antigens, thereby eliciting broad cellular and humoral immune responses without exposure to live virus[61] (Figure 2D). This technology offers a rapid, stable, and scalable manufacturing pathway that is independent of egg-based production systems, enhancing both biosafety and production flexibility. DNA vaccines often yield suboptimal humoral immunity despite HA targeting. Enhancement strategies include molecular adjuvants, improved prime-boost regimens, and advanced delivery systems. In contrast, mRNA vaccines provide enhanced safety through minimal genetic design, cytoplasmic restriction, and non-integrating mechanisms.

In contrast to traditional vaccine platforms—such as inactivated and live attenuated vaccines—which face limitations including extended development timelines, complex production processes, and challenges in rapid large-scale manufacturing during outbreaks, nucleic acid vaccines offer high adaptability and scalability. Leonard et al.[81] developed DNA and mRNA-LNP nucleic acid vaccines targeting the human-origin H5N1 strain A/Texas/37/2024. Both vaccines utilized a single open reading frame (ORF) design to co-deliver HA and NA antigens within a single nucleic acid molecule. This configuration facilitated correct protein expression: the DNA vaccine conferred complete protection in mice, while the mRNA-LNP vaccine induced neutralizing antibodies and provided full protection against lethal H5N1 challenge, underscoring the potential of nucleic acid platforms in combating zoonotic influenza viruses. Rudometov et al.[82] demonstrated that a DNA vaccine encoding a modified HA from H5N8 (pVAX-H5), administered at a 100 μg dose, elicited peak antibody titers and cross-neutralizing antibodies against multiple clade 2.3.4.4b strains, effectively protecting animals from lethal infection. Furthermore, a ferret model study[83] showed that a multivalent DNA vaccine expressing HA, NA, NP, M1, and M2 proteins conferred simultaneous protection against homologous 2009 pandemic H1N1 and heterologous H5N1 challenges. Vaccination restricted viral replication to the upper respiratory tract, shortened the duration of viral shedding, and consistently improved survival rates while reducing clinical symptoms. In the context of mRNA-LNP vaccines, the Hensley group[7] developed a candidate based on the HA protein of a clade 2.3.4.4b H5 isolate. This vaccine induced high levels of neutralizing antibodies and anti-HA stalk antibodies in mice, accompanied by strong T-cell responses. It also effectively prevented morbidity and mortality in ferrets, exhibiting immunogenicity comparable to that of inactivated vaccines. Collectively, these findings indicate that nucleic acid vaccine platforms not only enable rapid response to emerging strains but also facilitate broad-spectrum immune protection through rational antigen design—such as co-expression of HA and NA or targeting conserved epitopes—offering a promising strategy for controlling current H5N1 outbreaks and future pandemic threats.



Efficacy: Safe and immunogenic, homologous protection; Multivalent designs enhance cross-protection.

Advantages: Proven safety profile, Long-term immunity, Scalable manufacturing, Adjuvant-compatible.

Limitations: Limited T-cell responses, Reduced efficacy against drifted strains, Requires high antigen stability.

Target: HA, NA

Efficacy: Broad cross-reactive and durable immunity, Homologous and partial heterologous protection; Sustained neutralizing Abs and T-cell responses.

Advantages: Broader and longer-lasting protection, egg-free production, Supports multivalent designs.

Limitations: Lower HI antibody titers, Limited species-specific licensure.

Target: HA, NA, and M2e

Efficacy: Safe and immunogenic, homologous and heterologous protection; Single-dose protection in some models.

Advantages: Flexible production platforms, Supports multivalent antigen display, Compatible with rational antigen design.

Limitations: Complex assembly and stability challenges, Often requires adjuvants, Limited T-cell immunogenicity, Higher costs.

Target: HA, NA, M2e, and Chimeric designs: HA-NA-M2e fusion antigens

Efficacy: Safe and immunogenic with prime-boost regimen for DNA vaccines; Safe and immunogenic, homologous and heterologous protection for mRNA vaccines.

Advantages: High scalability and design flexibility, No risk of genomic integration, Enables co-delivery of multiple antigens.

Limitations: Requires cold chain (LNP), Potential need for prime-boost or adjuvants.

Target: HA, NA, and conserved epitopes (e.g., stalk, NP, M2e) for universal vaccines

Figure 2 A comparative review of vaccine platforms for H5N1 in animal species.

4 Practical Challenges in Vaccine Application

Global avian influenza outbreaks have intensified over the past two decades, leading to significant economic losses in the poultry industry. Between 2005 and 2020, approximately 246 million birds died or were culled worldwide due to outbreaks and control measures[84]. Since 2022 alone, more than 170 million birds have been affected in the U.S.[52], while over 17,000 outbreaks were reported in Europe between 2020 and 2025[85]. Common control strategies adopted globally include enhanced biosecurity, quarantine, compensatory culling, and regional vaccination[86]. Countries like China, the U.S., and Germany offer economic compensation equivalent to up to 50% of the bird's value to producers. However, this compensation is often inadequate to ensure full compliance with culling measures. These challenges have promoted widespread adoption of vaccination in developing countries and motivated nations traditionally reliant on culling-only policies to explore emergency vaccination as part of their outbreak control strategies[87].

Controlling the spread of HPAIV H5N1 in poultry remains a substantial challenge, requiring integrated strategies including enhanced biosecurity, surveillance, culling, and vaccination. Although large-scale culling remains the most commonly employed control measure in Europe and the U.S.[40], its long-term sustainability is limited by economic constraints. Vaccines for avian influenza are developed and stockpiled for outbreak control, prevention, emergency response, or routine immunization; however, their application remains contentious and has not been widely adopted in most countries. Major barriers to vaccination include the reliance on immunologically naïve poultry as "sentinels" for detecting emerging strains, trade restrictions imposed by importing countries due to difficulties in DIVA, concerns over incomplete protection against variant viruses, and the high costs and operational complexity of large-scale immunization[88]. Since 2004, China has implemented a core strategy combining culling with mass vaccination[89]. Although the U.S. has not adopted routine avian influenza vaccination, it has stockpiled over 200 million doses of vaccines based on various platforms, including HVT-vectored vaccines, H5 reverse genetics-based vaccines, RNA particle vaccines using alphavirus RPH5, and modified DNA/plasmid DNA vaccines[90]. While eradication of H5N1 from natural reservoirs is currently unfeasible, proactive preparedness for its pandemic potential remains critical. Of concern, some strains within clade 2.3.4.4b H5N1 have acquired antiviral resistance mutations[91], underscoring the need for multi-target antiviral strategies to mitigate the risk of resistant reassortants. Numerous vaccine candidates have shown promising results in preclinical studies, including induction of potent neutralizing antibodies, mucosal IgA, and T-cell responses[63]; complete protection in ferrets by mRNA vaccines[92]; and the capacity for rapid scale-up of clade 2.3.4.4b-specific vaccines using mRNA technology[7]. Intranasal administration of DelNS1-LAIV vaccines has been shown to elicit durable protection in mouse and hamster models[63]. Current research is advancing the development of broadly protective and rapidly deployable H5N1 vaccine strategies to counter emerging variants and zoonotic threats.

5 Conclusion and Perspectives

The HPAI H5N1 virus, particularly clade 2.3.4.4b, is evolving rapidly through continuous antigenic drift and reassortment, posing a substantial threat not only to the poultry industry but also highlighting its pandemic potential through cross-species transmission events, such as recent outbreaks in U.S. dairy cattle[4,5]. Enhanced mammalian adaptation—evidenced by widespread emergence of key mutations including E627K, D701N, Q591K, and M631L in the PB2 protein, as well as combinatorial mutations in HA that may improve both environmental stability and human-type receptor binding—calls for a renewed assessment of its public health risk[5]. In terms of vaccine development, although current technological platforms have made significant progress, several challenges remain. The large-scale deployment of vaccines still faces obstacles: vaccination does not completely prevent viral shedding and may drive antigenic escape mutations, and divergent immunization policies—such as the reliance on culling in Europe and the U.S. versus more extensive vaccine use in Asia and Africa—pose challenges to sustainability[2]. Looking forward, breakthroughs in avian influenza vaccine development and application will require progress in several key areas: First, the integration of deep mutational scanning with artificial intelligence (AI)-assisted modeling to improve prediction of high-risk mutations and emergent strains (Figure 3A). Second, fostering interdisciplinary collaboration across structural biology, virology, immunology, and public health to translate basic research into field applications (Figure 3B). Third, a fundamental reevaluation of the human-animal-environment interface is essential to prevent future outbreaks through improved farming practices and reduced ecological disruption, thereby mitigating spillover risk at the source (Figure 3C). Fourth, pursuing broad-spectrum vaccine designs that engage both humoral and cellular immunity, alongside novel antiviral strategies targeting viral and host factors (Figure 3D). Only through globally coordinated efforts, multidimensional interventions, and sustained scientific innovation can we effectively curb the spread of H5N1 avian influenza and reduce its potential impacts on public health, economies, and societies.

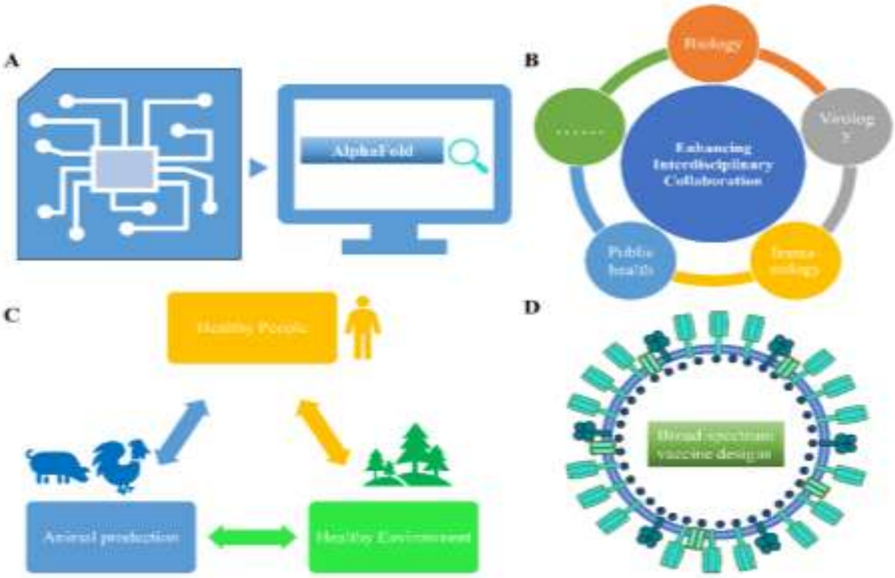


Figure 3 Key future directions for advanced avian influenza vaccine development and outbreak prevention. (A) AI-enhanced predictive modeling. (B) Interdisciplinary translation. (C) One health risk mitigation. (D) Broad-spectrum immunization strategies.

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Conflict of Interest Statement

The author declares no competing financial, professional, or personal interests that could influence this study.

Author contributions

Conceptualization: Song Yating, Zhao Xin, Li Xiangyang; Data curation: Song Yating, Zhou Meifang, Huang Weizhong; Formal analysis: Song Yating, Zhou Meifang, Deng Genggao, Zhang Chunhong; Investigation: Song Yating, Ding Li, Yang Yalin, Qiu Maoguo; Software: Song Yating, Luo Yuewen; Supervision: Song Yating, Luo Yuewen; Visualization: Song Yating, Zhao Xin; Writing – original draft: Song Yating; Writing – review & editing: Song Yating, Zhao Xin, Luo Yuewen, Li Xiangyang.

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