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Review Article

Avian influenza vaccination strategies: challenges and innovations within a One Health framework

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Avian influenza viruses remain a major global threat to poultry, wildlife, and human health. Their ongoing evolution, especially in wild birds and aquatic ecosystems, jeopardizes poultry health and food security worldwide. Avian influenza virus has demonstrated high mutability and pandemic potential, as evidenced by the global spread of highly pathogenic avian influenza H5N1. Its complex ecology, supported by avian reservoirs and aquatic ecosystems, facilitates cross-species transmission, including marine mammals, making control measures more challenging. This review highlights the limitations in managing avian influenza virus, emphasizing that vaccination alone is insufficient. It underscores the critical need for integrated One Health strategies, combining surveillance, biosecurity, and interdisciplinary collaboration to mitigate spread and prevent future outbreaks.

Keywords: avian influenza; vaccination; One Health.

Introduction

Avian influenza (AI) is a highly contagious viral disease affecting domestic and wild birds, caused by avian influenza virus (AIV). The virus is classified into low pathogenic avian influenza (LPAI) and highly pathogenic avian influenza (HPAI) based on its virulence in poultry. While LPAI strains typically cause mild symptoms or subclinical infections, HPAI strains, such as H5 and H7 subtypes, can lead to severe outbreaks with high mortality rates.(1)

The first documented cases of AI date back to 1878 when it was referred to as "fowl plague" in Italy.(2) Since then, AIV has been a persistent threat to the poultry industry and public health due to its potential for zoonotic transmission. The emergence of HPAI H5N1 in 1997 in Hong Kong marked a turning point, leading to widespread outbreaks in birds and sporadic human infections with high fatality rates.(3) The virus has continued to evolve through antigenic drift and reassortment, resulting in the emergence of novel strains, including H5N6, H5N8, and H9N2, which have caused significant economic losses and public health concerns worldwide.(4)

Wild waterfowl and migratory birds serve as natural reservoirs of AIV, playing a critical role in its transmission across geographic regions.(5) The virus can spread through direct contact with infected birds, contaminated environments, and water sources, highlighting the importance of biosecurity measures in poultry farming.(6) Additionally, recent reports of AIV infections in mammals, including marine species and domestic animals, raise concerns about potential cross-species transmission and the risk of future pandemics.(7)

Given the continuous evolution of AIV and its ongoing threat to animal and human health, this review evaluates the current challenges and recent advances in vaccination strategies against AIV, specifically focusing on the implications of viral persistence in aquatic environments and the necessary complexity integration of a One Health approach to mitigate zoonotic and pandemic risks for achieving effective control.

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Avian influenza as a global hazard

AIV is classified among the most contagious notifiable diseases by the World Organization for Animal Health (WOAH). It's devastating effects on poultry production, socioeconomic stability, international trade, and wildlife conservation have elevated it to a critical priority on the global agenda. These concerns are further intensified by the persistent risk of zoonotic transmission, particularly the emergence of HPAI H5N1 strains with pandemic potential.[8]. In response, a tripartite coalition of the Food and Agriculture Organization (FAO), the World Health Organization (WHO), and WOAH advocates a unified One Health approach, underscoring the importance of cross-sectoral collaboration to mitigate risks, safeguard animal health, and protect global public security.

Causative agent: influenza A virus

AI is caused by influenza A viruses, members of the *Orthomyxoviridae* family. These are enveloped, segmented, negative-sense single-stranded RNA (-ssRNA) viruses, typically 80-120 nm in diameter. The family *Orthomyxoviridae* currently comprises six recognized genera: *Influenza A, B, C, Isavirus, Thogotovirus,* and *Quaranfilvirus.*[9] Among them, influenza A viruses are the most widespread, infecting a broad range of avian and mammalian species and serving as the primary cause of AI. In addition, two novel orthomyxoviruses have been proposed as new genera: influenza D virus, linked to respiratory disease in swine and cattle, and Wellfleet Bay virus, associated with mortality in eiders in North America.[10,11] IAVs are further classified into subtypes based on the antigenic properties of their surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). To date, 16 HA (H1–H16) and 9 NA (N1–N9) subtypes have been isolated from birds, yielding a wide diversity of strains with variable pathogenicity.[9]

Virulence of avian influenza viruses: pathogenesis and pandemic potential

AIV are classified into highly pathogenic and low pathogenic forms based on their virulence in poultry. HPAI viruses, particularly the H5 and H7 subtypes, cause rapid systemic infection, severe tissue damage, and mortality rates that can approach 100 % in poultry. The H5N1 subtype, notably clade 2.3.4.4b, has raised major global concern, driving spillover into wild birds and even terrestrial and marine mammals, with devastating economic consequences.[1] The pathogenicity of HPAI, as outlined by Tourky et al.,[12] consists of the virus entering via the respiratory and intestinal tracts, binding to α-2,3 sialic acid receptors, followed by systemic dissemination. A critical molecular determinant of virulence is the presence of polybasic amino acids (arginine, lysine) at the HA cleavage site, enabling widespread replication. This triggers an exaggerated host immune response, including interferon induction and pro-inflammatory cytokines like IL-6 and TNF-α, leading to sever damage to respiratory and digestive systems, manifesting in clinical signs such as sneezing, nasal discharge, diarrhea, coughing, discoloration of body parts, swelling, and neurological signs.[12,13] Furthermore, HPAI viruses such as H5N1 are also environmentally resilient, persisting for prolonged periods in feces, surfaces, and tissues, particularly under cold conditions.[14]

In contrast, LPAI viruses generally cause mild or asymptomatic disease, limited to the respiratory and intestinal tracts. This reduced pathogenicity is due to a monobasic amino acid at the HA cleavage site, which restricts replication. Consequently, mortality is low, and these infections have historically been considered of limited concern.[1] However, certain LPAI strains, such as H7N9, present a recognized zoonotic threat.[15] Importantly, circulating low-pathogenic H5 and H7 viruses in poultry and wild waterfowl pose a persistent pandemic risk, as mutations or recombination events at the HA cleavage site can transform them into novel HPAI strains.[16]

The role of wild birds in the spread of avian influenza virus

Wild birds, particularly migratory waterfowl, act as natural reservoirs of diverse AIV subtypes and often carry the virus asymptomatically, thereby posing a persistent threat to poultry health. Frequent contact between wild and domestic birds facilitates viral transmission, especially in production systems where domestic ducks forage in wetlands during the day and return to poultry flocks at night. This practice, common in Southeast Asia, has been strongly linked to the introduction and dissemination of AIV. Moreover, the long-distance migration of wild birds contributes to the transboundary spread of AIV, underscoring their critical role in the global epidemiology of the disease.[17,18]

Transmission dynamics of avian influenza virus in poultry-aquaculture farming systems

AIV transmission among waterfowl occurs mainly via the fecal-oral route and water-related behaviors such as preening contaminated feathers and cloacal drinking.[19] Water thus plays a central role as a reservoir and vehicle for viral spread. Integrated farming systems, where ducks and geese are raised in rice paddies alongside fish production, enhance resource utilization by improving pest control and soil fertilization.[20,21] However, these systems also create significant biosecurity challenges, as shared water environments facilitate viral persistence and increase opportunities for cross-species transmission, thereby amplifying the risk of AIV outbreaks.

Biosecurity risks in integrated poultry-aquaculture systems

The use of poultry manure as fertilizer in aquaculture ponds can enhance plankton growth for fish production, but simultaneously creates a pathway for pathogen transmission to humans through aquatic organisms exposed to contaminated water. In addition, the improper disposal of dead poultry into water bodies facilitates interspecies disease transmission and has been implicated in the spread of H5N1.[22] Historical evidence further supports these risks: during 2004-2005, outbreaks of H5N1 in China, Romania, and Croatia were linked to integrated farming practices, prompting global organizations to investigate manure-fed ponds as potential reservoirs of AIV.[18]

Unexpected spread of avian influenza virus to aquatic species and marine mammals

The pathogenicity of AIV is no longer confined to waterfowl, but has expanded to include non-traditional hosts, thereby widening the epidemiological risk. In Egypt, where H5N1 is considered endemic, multiple factors have been implicated in sustaining viral circulation, including the use of substandard vaccines, weak biosecurity measures, inadequate eradication strategies, migratory bird flyways, and poor carcass disposal practices. Aquatic species also play a role in viral maintenance. Eissa et al.,[22] demonstrated that bottom filter feeders such as the red swamp crayfish (*Procambarus clarkii*) could harbor H5N1 particles in their hemolymph after ingesting benthic material contaminated with poultry droppings. Similarly, the scavenger-like sharp-toothed catfish (*Clarias gariepinus*) was shown to carry the virus in its bloodstream, while untreated poultry manure used to fertilize aquaculture ponds tested positive for H5N1. These findings highlight the potential for aquaculture systems to serve as secondary reservoirs of infection.

Beyond aquatic environments, AIV has also been detected in marine mammals. Transmission occurs through direct or indirect contact with infected waterfowl, with reported cases of H7N7 in seals and H13N2/H13N9 in whales.[23,24,25] In 2022, Peru reported mass mortalities in pelicans and sea lions linked to a reassortant H5N1 strain derived from Eurasian and American lineages. Affected marine mammals exhibited respiratory, digestive, and neurological symptoms, with histopathology revealing severe pneumonia and emphysema.[26,27] Viral adaptation to both α2–3 and α2–6 sialic acid receptors raises further concern, as this dual receptor binding could facilitate transmission among mammals and increase the likelihood of spillback into avian populations, thereby amplifying pandemic risk.

Challenges in developing avian influenza vaccines

The development of effective vaccines against AIV is hampered by multiple scientific, practical, ecological, and economic barriers.

<u>Scientific challenges</u>

The major obstacle is the virus's rapid evolution. Antigenic drift in the HA protein reduces vaccine efficacy against newly emerging strains, while antigenic shift through reassortment can generate novel subtypes with little or no cross-protection.[9] With 16

HA and 9 NA subtypes in circulation, and only a subset (H5, H7, H9) posing major risks to poultry, vaccines typically target single subtypes. Protection across divergent subtypes or between clades (e.g., H5N1 clade 2.3.2.1a vs. 2.3.4.4b) is often limited, emphasizing the need for continuous molecular surveillance and frequent updates of vaccine strains.[28]

Practical challenges

Large-scale deployment is constrained by limited global production capacity, cold-chain requirements, and the labor-intensive nature of vaccination, since most vaccines require individual administration by injection. These factors increase costs and complicate mass immunization, particularly in resource-limited settings.[29]

Ecological challenges

AIV persistence in aquatic environments is influenced by temperature, salinity, and pH, with prolonged survival in cold freshwater facilitating recurrent spillover to wild and domestic birds.[30] Moreover, wild aquatic birds serve as natural reservoirs that continuously reseed infections, limiting the effectiveness of poultry vaccination alone. Effective control therefore requires integration of vaccination with enhanced farm-level biosecurity and active surveillance of both wild and domestic populations.[31]

Economic and regulatory challenges

Despite recognition by the WOAH that vaccination can be a legitimate control tool under strict surveillance, many trading partners restrict imports from vaccinating countries due to Differentiating infected from vaccinated animals (DIVA) concerns.[32].This creates a major economic disincentive and restricts vaccination mainly to emergency outbreak situations. In addition, vaccine registration and approval processes are lengthy and costly. By the time a vaccine is licensed, circulating strains may have drifted, requiring reformulation and re-approval, which delays outbreak response and reduces effectiveness.

Vaccination strategies

Vaccination remains a cornerstone of AI control, though substantial challenges persist. The ideal vaccine should be safe, cost-effective, broadly protective across avian species, compatible with DIVA strategies, and capable of overcoming interference from maternal antibodies.[33]

The effectiveness of AI vaccines is evaluated using both direct and indirect methods. Direct challenge studies remain the gold standard, as they provide definitive evidence of protection against clinical disease and virus transmission. However, these trials are costly and complex. For routine quality control, indirect serological methods are widely used, including virus neutralization assays, hemagglutination inhibition titers, and HA antigen quantification.[34]

Vaccine technology has evolved from traditional inactivated formulations to recombinant vectors, DNA vaccines, and novel mRNA-based platforms. These innovations aim to address the dual challenges of rapid viral evolution and DIVA compliance. The development of broad-spectrum, durable vaccines is critical not only for sustainable disease control, but also for protecting international trade and strengthening integrated One Health biosecurity frameworks. Table 1 provides a comparative overview of the main avian influenza vaccine types, highlighting their mechanisms of action, advantages, and limitations.

Implementing a comprehensive One Health framework to mitigate avian influenza threats

Effective control of AIV cannot rely on vaccination alone, but requires a comprehensive One Health approach. Key components include:[39]

- Development of next-generation vaccines with broader and more durable protection.
- Strengthening laboratory diagnostic capacity and integrating animal and human health surveillance systems.
- Continuous monitoring of virus evolution in poultry, wild birds, and the environment.
- Risk mitigation strategies for wild birds, including research on migration and ecological drivers of virus spread.
- Implementation of stringent biosecurity practices across production systems.

Table 1. Comparative overview of the main avian influenza vaccine types, including their mechanisms of action, advantages, and limitations.

Vaccine type	Mechanism of action	Advantages	Disadvantages	References
Inactivated vaccines	Prepared in embryonated chicken eggs, used as oil emulsions, and administered intramuscularly or subcutaneously.	Primarily stimulate humoral immune antibodies.	- Vaccinated birds can become infected and shed the virus without showing clinical signs, creating a silent spread scenario. - Require multiple doses.	(35)
Vector recombinant vaccines	Inserting the HA gene into the fowl poxvirus vaccine strain or herpesvirus turkey (HVT) vectors.	-Induction of both cell-mediated and humoral immune responses. - Differentiating infected from vaccinated birds (DIVA).	These birds exposed to the fowl poxvirus will not develop antibodies toward AIV.	(36,37)
mRNA platforms vaccines	mRNA encapsulated in lipid nanoparticles (LNPs) enters host cells and translated into viral antigen. This stimulates both adaptive immunity and innate immunity.	- This platform has induced strong, balanced immune responses. - Self-adjuvating.	Excessive activation of the innate immune response can trigger a negative feedback loop, reducing mRNA stability and translation efficacy.	(38)
DNA vaccines	Administration of plasmid DNA-based vaccines encoding the HA gene leads to its transcription and translation within host cells, producing HA. protein. This endogenously synthesized antigen is presented via both MHC I and MHC II pathways, eliciting a comprehensive immune response, encompassing cytotoxic T-cells and neutralizing antibodies, mimicking a live virus infection.	- Boost broad immune response. - Flexible administration routs. - Enhanced by avian promoters.	- High production costs. - Require multiple doses to achieve effective immunity.	(29)

HA: hemagglutinin.

- International collaboration to harmonize vaccine strategies and trade policies, ensuring safe and sustainable commerce.
- Partnership with Quadripartite organizations (FAO, UNEP, WHO, and WOAH) to coordinate efforts against HPAI, safeguarding both animal and public health.
- Adopting this integrated strategy is essential to prevent future outbreaks, protect global food security, and reduce the threat of emerging and re-emerging zoonotic epidemics and pandemics at the human-animal-environment interface.

Conclusions

AIV remains a persistent and evolving threat with profound implications for animal health, public safety, and environmental sustainability. Its long-term stability in aquatic environments, capacity to cross species barriers, and potential to disrupt food systems underscore the urgent need for integrated One Health–based control strategies. Effective mitigation will require not only strengthened biosecurity and sustained surveillance, but also continuous innovation in vaccines and diagnostics. Above all, enhanced collaboration among international organizations, scientists, and policymakers is critical to controlling AIV, safeguarding global food security, and reducing the risk of future pandemics.

Conflict of interest

The authors declare that there is no conflict of interest.

Author's contributions

Ola Y. Abido: conceptualization, literature search, data analysis and critically revision of the work.

Esteer Ragheb-Fouad: literature search, data analysis, written of the first draft of the manuscript and critically revision of the work.

All authors reviewed and approved the final version of this manuscript for publication.

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Estrategias de vacunación contra la gripe aviar: retos e innovaciones en el marco de "Una Sola Salud"

Resumen

Los virus de la gripe aviar siguen representando una grave amenaza mundial para las aves de corral, la fauna silvestre y la salud humana. Su continua evolución, especialmente en las aves silvestres y los ecosistemas acuáticos, pone en peligro la salud de las aves de corral y la seguridad alimentaria en todo el mundo. El virus de la gripe aviar ha demostrado una alta mutabilidad y potencial pandémico, como lo demuestra la propagación mundial de la gripe aviar H5N1 altamente patógena. Su compleja ecología, sustentada por reservorios aviares y ecosistemas acuáticos, facilita la transmisión entre especies, incluidos los mamíferos marinos, lo que hace que las medidas de control sean más difíciles. Esta revisión destaca las limitaciones en la gestión del virus de la gripe aviar, haciendo hincapié en que la vacunación por sí sola es insuficiente. Subraya la necesidad crítica de estrategias integradas de "Una Sola Salud", que combinen la vigilancia, la bioseguridad y la colaboración interdisciplinaria para mitigar la propagación y prevenir futuros brotes.

Palabras clave: influenza aviar; vacunación; Una Sola Salud.

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