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Bangladesh's Silent Threat: Persistence and Reassortment of Avian Influenza in A Poultry-Dense Ecosystem-A Comprehensive Review of Knowledge Gaps and Future Perspectives

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ABSTRACT

Avian influenza in Bangladesh presents a continuous and challenging threat due to the sustained co-circulation of highly pathogenic H5N1 and low pathogenic H9N2 avian influenza viruses within a densely populated poultry ecosystem. This study analyses the persistence and genetic reassortment dynamics of these viruses, emphasizing the significance of genetic exchange in sustaining viral virulence and adaptability. The research highlights highly pathogenic H5N1 strains possessing the H9N2-PB1 gene, illustrating that genetic reassortment promotes the virus's persistence and evolution. It reinforces the enzootic character of LPAI H9N2, present in 55.8% of chickens, and reveals defects in the culling compensatory mechanism. Despite extensive control efforts over the decades, systemic challenges, like insufficient culling strategies, vaccine inconsistencies and the shedding of critical virus reservoirs, such as domestic ducks and backyard poultry, have impeded successful management. The work explores the difficulties presented by the endemicity of LPAI H9N2, its function as a genetic donor for H5N1, and the consequent "silent threat" that hinders surveillance and control efforts. Proposed future options include enhanced surveillance through whole-genome sequencing, the expansion of immunization initiatives, and the implementation of sustainable financial support schemes for farmers. The government and poultry industry should emphasize the necessity for a coordinated, multifaceted strategy to limit the risks of avian influenza in Bangladesh.

Keywords: avian influenza, genetic reassortment, silent threat, vaccination, whole genome sequencing

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Introduction

Avian influenza, which is caused by type A influenza viruses, is one of the most significant global hazards to the health security of the poultry and the preparedness for pandemics. When avian influenza viruses repeatedly infiltrate and persist in circulating in domestic poultry, they are exceedingly dangerous, despite their historical presence in wild bird reservoirs. The primary reason for the threat is that new strains of the virus have the potential to trigger catastrophic human pandemics.

The global avian influenza ecosystem is currently characterized by the explosive panzootic transmission of highly pathogenic H5N1 and the established, long-term circulation of low pathogenic H9N2 (Monne et al., 2013). The evolutionary threat posed by the avian influenza virus originates in its segmented RNA genome, which enables antigenic shift, or reassortment. The emergence of new genotypes is primarily caused by the process of gene segment exchange between co-infecting viruses, as

confirmed by the discovery of naturally occurring reassorting HPAI H5N1 viruses with the H9N2-PB1 gene in Bangladesh poultry (Monne et al., 2013a;Hossain et al., 2021 ;Carnegie et al., 2023). The dynamics of avian influenza virus are particularly unstable in Bangladesh, a country with one of the highest chicken densities in the world, fragmented agricultural systems, a significant amount of trading through live bird markets, and 71% of rural families raising backyard poultry (Monne et al., 2013a). The persistent and simultaneous co-circulation of HPAI H5N1 and LPAI H9N2 in this location suggests that avian influenza has become endemic (Hossain et al., 2021). The development of H5N1 is accelerated by LPAI H9N2, which acts as an active "gene pool". This contributes to a "silent spread", in which passive surveillance can easily overlook subclinical infection (both H9N2 and hidden HPAI in vaccinated flocks), thereby maintaining the endemic danger (Carnegie et al., 2023).

Control is persistently undermined by systemic failures, despite decades of national intervention, which included the adoption of a vaccination strategy in 2012 (Monne et al., 2013b). These include the structural exclusion of key reservoirs, ducks, and backyard poultry from the control program; a persistent vaccine mismatch against endemic strains (e.g., Clade 2.2 vaccines versus Clade 2.3.2.1a); and the collapse of the culling compensation policy (violating OIE principles) (Monne et al., 2013ba). Consequently, the primary goal of this comprehensive review is to underscore the current state of knowledge regarding the reassortment and persistence dynamics of H5N1 and H9N2 in the poultry-rich ecosystem of Bangladesh. The review conducts a critical analysis of the epidemiological, virological, and socio-economic challenges, identifies the critical knowledge gaps that prevent effective control, and suggests a foundation for future perspectives in sustainable disease mitigation (Carnegie et al., 2023).

Virology and pathogenesis of avian influenza

Avian influenza, also referred to as avian flu, is a highly contagious disease of birds that is caused by type A influenza viruses (genus Alphainfluenzavirus) that are members of the Orthomyxoviridae family (Alexander, 2007a, Djurdjević et al.,2023). These viruses are naturally present in untamed aquatic birds, including swans, geese, and ducks, which typically exhibit no signs of illness (Alexander, 2007b). The classification of avian influenza viruses into two primary categories is based on their capacity to induce severe disease in poultry (WHO, 2023). LPAI infections are generally mild or even subclinical, producing no clear clinical signs or only minor manifestations such as decreased egg production and ruffled feathers. Globally, H9N2 is the most widespread LPAI subtype (Peiris, 2009). Severe systemic disease in poultry is frequently the result of Highly Pathogenic Avian Influenza (HPAI), which frequently results in the sudden onset of illness, rapid deterioration, and mortality rates that approach 100% (WHO, 2023). The HPAI H5N1 virus of the Guang/Goose lineage is the primary pandemic threat (Webster et al., 2007), with the H5 and H7 subtypes being the most significant HPAI strains (WHO, 2023; Djurdjević et al., 2023).

Influenza viruses are negative-sense RNA viruses, characterized by their single-stranded structure and a segmented genome. This segmented structure promotes their fast evolution (Taubenberger & Kash, 2010). The subtype of the virus (e.g., H5N1) is defined by the two principal surface proteins, hemagglutinin (HA) and neuraminidase (NA) (Taubenberger & Kash, 2010). In a co-infection, two separate avian influenza viruses (AIVs) can exchange complete gene segments, a phenomenon referred to as antigenic shift or reassortment, owing to their segmented structure (Peiris et al. 2009; Hossain et al., 2021). This approach constitutes a significant risk factor in densely populated poultry environments like Bangladesh, as it generates novel strains with distinct virulence or transmission attributes (Hossain et al., 2021).

The persistent enzootic co-circulation of many subtypes in Bangladesh is the distinguishing characteristic that underscores the threat posed by the Avian Influenza Virus. This environment poses a significant danger for ongoing genetic evolution and reassortment (Hossain et al., 2021; Monne et al., 2013a). LPAI, a virus of low virulence, has been enzootic since 2006 and is reported annually (Hossain et al., 2021; Monne et al., 2013a). Although it typically induces a milder disease in poultry, its persistent, high-prevalence circulation in commercial and backyard chickens is crucial because it sustains a high baseline viral burden throughout the ecosystem (Hossain et al., 2021). This highly pathogenic virus became entrenched and continues to circulate following the emergence of HPAI in 2007 and is represented by the predominant local strain, Clade

2.3.2.1a (Monne et al., 2013b; Djurdjević et al., 2023). Nevertheless, the volatile environment has also led to the emergence of Clade 2.3.4.4b and Clade 2.3.4.4h variants. The emergence of novel variants with unknown epizootic and zoonotic potential is facilitated by the essential conditions for genetic reassortment (antigenic shift) that are created by the co-circulation of these distinct subtypes (Monne et al., 2013b; Hossain et al., 2021) recognize the prevalent LPAI H9N2 as a critical "gene pool donor". It frequently contributes its internal gene segments to the HPAI strains, which modulate viral replication and host interaction (Monne et al., 2013b). This genetic mixing is not solely theoretical; a natural reassortant HPAI H5N1 virus containing an H9N2-PB1 gene was identified in Bangladeshi poultry (Monne et al., 2013b). The PB1 gene is essential for viral replication, and the pathogenicity assessment confirmed that the virus's Intravenous Pathogenicity Index (IVPI) of 3.0 was not diminished by the incorporation of the H9N2 segment (Monne et al., 2013a; Monne et al., 2013b). This verified reassortment illustrates a mechanism by which HPAI can preserve lethality while potentially gaining benefits from the endemic H9N2 pool (Monne et al., 2013b). The efficacy of vaccines is continually challenged by the continuous diversification that results from the immense selective pressure exerted by viral circulation in partially immune commercial flocks, which accelerates antigenic drift in H5N1 strains (Monne et al., 2013b; Djurdjević et al.,2023)

The highly complex and efficient transmission pathways in Bangladesh contribute to the endemic status of the Avian Influenza Virus (Hossain et al., 2021; Gupta et al.,2021a). A. Bird-to-Bird Transmission: The virus is excreted in high concentrations in the faeces of infected birds, resulting in the contamination of the environment, food, and water (Pervin et al., 2020a). The virus is effectively transmitted through personnel who travel between farms and live bird markets, as well as contaminated equipment, vehicles, feed sacks, and egg trays (Pervin et al., 2020a). The primary reservoir is migratory waterfowl, which introduce the virus into domestic colonies through direct contact with backyard poultry or contaminated water sources (Pervin et al., 2020a). B. Animal-to-Human Transmission: Human infections are sporadic and associated with prolonged exposure to contaminated poultry environments (Gupta et al., 2021a; ECDC, 2024;). Direct, unprotected exposure to sick or dead birds, their excrement, or contaminated bodily fluids (e.g., during slaughter or defeathering) constitutes the principal transmission route (ECDC, 2024; Gupta et al., 2021b,). The principal concern regarding a future pandemic is prolonged person-to-person transmission, which remains infrequent (ECDC, 2024; Gupta et al., 2021b,).

The pathogenicity of the avian influenza virus is a crucial determinant of disease severity in animals and poses a risk to humans. (WOAH, 2023). The HPAI virus can infect and obliterate cells across many organ systems, leading to a systemic infection (WOAH, 2023). LPAI is generally marked by decreased egg production or respiratory and intestinal manifestations (WOAH, 2023). The infection is generally confined to the cells lining the respiratory and gastrointestinal systems (WOAH, 2023; James et al., 2024).

The clinical manifestations of Avian Influenza Virus based on the pathogenicity of the predominant strain. (WOAH, 2023). Clinical indications frequently serve as the initial indicator of an outbreak; however, they may be non-specific, especially in the case of LPAI (Swayne, 2018). Highly Pathogenic Avian Influenza (HPAI) (e.g., H5N1) results in abrupt and exceedingly elevated mortality rates (often 90–100%), along with pronounced depression and anorexia (failure to eat or drink). Oedema of the head, eyelids, comb, and wattles, frequently accompanied by bluish or cyanotic discoloration, and petechial hemorrhages on the shanks and feet are typically indicative signs. Severe diarrhea, frequently characterized by a watery and greenish appearance (Figure.1). Indicators for stress include tremors, impaired coordination, and torticollis (twisted neck) (Swayne, 2018). Low Pathogenicity Avian Influenza (LPAI), such as H9N2, is generally mild, subclinical, or indistinguishable from other prevalent poultry infections (WOAH, 2023). The disease typically causes depression and reduces feed intake. Mortality is often minimal until subsequent bacterial infections occur. Sneezing, coughing, and wheezing can range from moderate to mild. A notable indicator is a substantial decline in egg production (ranging from 10–40%) and inferior eggshell quality in layers. (WOAH, 2023) The necropsy findings in poultry infected with avian influenza differ markedly according to the strain's pathogenicity, classified as low pathogenicity avian influenza (LPAI) or highly pathogenic avian influenza

Figure 1. Gross lesions of H5 HPAIV a. Sudden death, b. Edema & cyanosis, c. Shank hemorrhage, d. Coughing, Sneezing &Lacrimation e. Cyanosis & Edema of head f. Proventricular hemorrhage (*Ayuti et al.,2024; Islam et al.,2023*)

(HPAI) (Swayne, 2013; Kash et al,2010). Highly Pathogenic Avian Influenza (HPAI) typically manifests as a severe, acute, systemic illness characterized by distinctive lesions resulting from extensive vascular injury and necrosis (Zec et al., 2023; Kash et al,2010). Petechial hemorrhages, characterized by pinpoint bleeding, are often observable on the unfathered skin of the legs and feet (Swayne, 2013; Kash et al,2010). Significant periorbital and facial oedema may be observed (Rahman et al., 2015). Extensive petechial and ecchymotic hemorrhages are significant observations, frequently found on serosal surfaces, the epicardial adipose tissue of the heart, the mucosa of the gizzard and proventriculus, and inside the abdominal adipose tissue (Swayne, 2013; Zec et al., 2023). Subepicardial hemorrhages are a common occurrence (Zec et al., 2023; Kash et al,2010). Necrosis, or tissue death, is a significant lesion typically seen as multifocal to consolidating pale or white discoloration in the pancreas and spleen (Lean et al., 2022; Zec et al., 2023). The lungs exhibit congestion, oedema, and frequently hemorrhage, occasionally presenting as dark red and solidified (Rahman et al., 2015; Lean et al., 2022). The kidneys may exhibit congestion, oedema, and urate deposits (Rahman et al., 2015). Hydropericardium (excess fluid in the pericardial sac) and congestion accompanied by petechial hemorrhages on the cardiac surface may be observed (Lean et al., 2022). Catarrhal enteritis and hemorrhages in the intestinal lymphoid tissue may be detected (Swayne, 2013).

A necropsy may disclose moderate inflammation of the sinuses, trachea (tracheitis), and bronchi, frequently associated with a serous or mucoid discharge (Swayne, 2013; Hy-Line International, 2024). Cloudy or thickened air sacs (air sacculitis) may be noticed (Swayne, 2013). A significant decline in egg production in laying hens is indicated by misshapen, congested, or involuted (regressed) ovarian follicles (Swayne, 2013). Yolk debris in the abdominal cavity (egg yolk peritonitis) resulting from internal laying is a significant observation associated with some LPAI strains (Kohnle et al., 2024; Hy-Line International, 2024).

Epidemiology and prevalence of avian influenza in poultry

The Avian Influenza Virus is not merely a sporadic hazard but a persistent, enzootic issue in Bangladesh, sustained by the continuous co-circulation of HPAI H5N1 and LPAI H9N2 (Hossain et al., 2021; Nasreen et al., 2015). The elevated prevalence is influenced by

intricate host-species interactions, seasonal environmental variables, and structural deficiencies within the poultry value chain. Avian influenza has been present in Bangladesh since the emergence of HPAI H5N1 in 2007, while LPAI H9N2 has been enzootic since 2006, with annual reports confirming its role as a permanent reservoir (Hossain et al., 2021). The primary reason for persistent prevalence is this cohabitation, which provides continuous opportunities for genetic reassortment, facilitating the emergence of novel virus strains (Monne et al., 2013a). Environmental monitoring reveals that 28.3% of documented H9N2 infections originated from environmental samples, typically associated with waste in live bird markets, hence corroborating the virus's persistence (Hossain et al. 2021). Approximately 71% of rural families rear backyard poultry, resulting in a substantial, unmanaged susceptible population that complicates management measures and sustains a high endemic risk (Monne et al., 2013b). Commercial and backyard poultry constitute the predominant hosts, representing over fifty-five percent (55.8%) of documented H9N2 cases (Hossain et al., 2021). Chickens serve as a crucial reservoir, demonstrating a greater frequency of viral transmission to ducks and quails than the reverse (Hossain et al., 2021). This transmission continues despite immunization in the commercial sector, indicating subclinical circulation and vaccine evasion (Hossain et al., 2021 &Nasreen et al., 2015). Domestic ducks are recognized as a potential primary reservoir or source of HPAI H5N1 spillover to chicken populations and house crows (Hossain et al. 2021& Nasreen et al., 2015). Ducks frequently excrete HPAI asymptomatically, considerably adding to the "silent threat" (Hossain et al., 2021). Quails frequently serve as hosts for H9N2 epizootics, primarily instigated by spillover events from chickens (Hossain et al., 2021). Moreover, live bird market, responsible for 95% of poultry retail, serve as contamination locations where the Avian Influenza Virus is consistently identified in the environment (Monne et al., 2013b).

The prevalence of the Avian Influenza Virus exhibits distinct seasonal patterns associated with climate and host behavior, affecting transmission intensity (Hossain et al., 2021). The circulation of H9N2 varies considerably by sector; commercial farms exhibit the highest detection rate at 70% of cases during the winter season, while live bird markets (LBMs) experience peaks of 47.9% during the monsoon and 40% in the winter season (Hossain et al., 2021). Backyard farms exhibit a detection rate of 58.6% throughout the summer months (Hossain et al., 2021). Annual epizootic waves of HPAI H5N1 and H5N6 in house crows occur in winter, frequently serving as sentinel indicators of elevated HPAI activity during the colder months. This seasonality requires climate-aware and timely actions to optimize resource efficacy (Nasreen et al., 2015).

The interface of zoonosis of avian influenza

The widespread, enzootic presence of Avian Influenza Virus in Bangladesh's densely populated poultry ecosystem creates a persistent and intricate zoonotic connection, wherein the danger of animal-to-human transmission is constant. This interface is primarily characterized by direct human occupational exposure, the incidence of verified human cases, and the ongoing risk of genetic reassortment. The degree of human-poultry interaction across the supply chain is the principal driver of zoonotic spillover. Live bird markets are identified as significant zoonotic interfaces, marked by elevated environmental pollution, inadequate biosecurity, and substantial human traffic. Employees at LBMs—who manage live avians, conduct slaughtering, and handle contaminated waste—face heightened risk. Surveillance in Dhaka LBMs has identified Avian Influenza Virus RNA in nasopharyngeal swabs (4.5%) and hand swabs (18.5%) of poultry workers, including asymptomatic individuals, indicating frequent, unnoticed occupational exposure to the virus. Notably, three of the previously documented human H5N1 infections in Bangladesh involved live bird market workers. The prevalent low-biosecurity practices among 71% of rural households rearing backyard poultry facilitate ongoing contact and exposure risks, especially for children and smallholder farmers (Monne et al. 2013; Monne et al., 2013b).

Bangladesh has documented human infections linked to HPAI H5N1, including a fatality involving a two-year-old child reported in April 2013. Monne et al. (2013) documented eight human cases of H5N1 between 2008 and 2013. The endemic LPAI H9N2 virus has been confirmed to sporadically infect humans, usually resulting in lesser illness ((Monne et al. 2013; Monne et al., 2013b).

The most significant future public health issue is the possibility of antigenic shifts at the human-animal

interface, where the virulence of H5 may merge with the increased transmissibility of other prevalent human strains. The discovery of natural reassortant HPAI H5N1 strains with the H9N2-PB1 gene in Bangladeshi poultry validates the possibility of this genetic recombination (Monne et al. 2013a). This reassortment establishes the requisite conditions for the generation of novel variations with uncertain epizootic and zoonotic potential, which may amalgamate the lethality of H5N1 with improved human adaptation, thus instigating a pandemic (Monne et al.,2013b; Vergne et al., 2021).

Diagnosis of avian influenza in poultry in Bangladesh

Veterinary monitoring includes the acquisition of cloacal swabs, tracheal swabs, swabs from freshly deposited faeces, and pooled environmental swabs (notably from live bird markets) from several avian species, including commercial chickens, backyard chickens, domestic ducks, and waterfowl. Although active surveillance (systematic sampling in high-risk areas) has commenced, the overall system predominantly depends on passive surveillance, which identifies outbreaks only when farmers report observable poultry mortality (Suarez et al., 2007). This methodological deficiency frequently overlooks the preliminary, subclinical phases of HPAI and the widespread prevalence of LPAI H9N2 (Suarez et al., 2007). Samples are examined by institutions including the Central Disease Investigation Laboratory (CDIL) of the Department of Livestock Services (DLS) and the National Reference Laboratory for Avian Influenza (NRL-AL) at the Bangladesh Livestock Research Institute (BLRI).

The primary diagnostic strategy employs fast and accurate molecular techniques to ensure timely intervention. The real-time reverse transcription PCR (RT-PCR) assay is the standard for rapid detection. Samples are first analysed for the presence of the influenza A virus utilising universal matrix-gene-specific primers (Fereidouni et al., 2014). This method produces outcomes in a couple of hours. Positive samples are quickly subtyped using suitable primers (Table.1 and 2) to identify circulating strains, specifically H5 and N1 (for HPAI) and H9 (for LPAI) (Fereidouni et al., 2014). To obtain definitive confirmation and characterization, rRT-PCR-positive samples require further analysis.

Samples are injected into embryonated chicken eggs to confirm the existence of a live virus. Whole-genome sequencing is crucial for epidemiological monitoring. Whole Genome Sequencing (WGS) allows laboratories to perform high-resolution phylogenetic analyses to determine the precise clade of circulating viruses (e.g., 2.3.2.1a or 2.3.4.4b) and to identify critical reassortment events (e.g., H5N1

Table 1. Avian Influenza H5N1 RT-PCR primer

Target	Primer	Sequence (5'–3')	Amplicon Size (bp)	Reference
H5 (HA)	R	AGCATGGGCAAAAAGCAACC	200	WHO, 2011
	F	GCCTTTTTCAGAAATTGTTGC		
N1 (NA)	R	GCAGAAATTATGTTCGATTGGAC	270	Hoffmann et al., 2007
	F	GTGAAAGTATCTGGTCTCCAT		
H5 (HA)	R	CCARTRGGKGCKATAAAYTC	249	WHO, 2024
	F	GTCTGCAGCRTAYCCACTYC		
H5 (HA)	R	GTGACGAATTCATCAATGTRCCG	424	WHO, 2024
	F	CTCTGGTTTAGTGTTGATGTYCCAA		
H5 (HA)	R	GACCRATCCTGTCACCTCTGAC	219	Tsukamoto et al., 2010
	F	AGACCAGCTAYCATGATTGC		
M(Matrix, Universal Influenza A)	R	AGATGAGTCTTCTAACCGAGGTCG	244	Spackman et al., 2002
	F	TGCAAAAACATCTTCAAGTCTCTG		
H5 Probe-79 FAM	R	ACATGCCCAAGACATACTGGAA	130	Qinghai/1/2005 (H5N1); Chenet et al.,2007
	F	CACACAACGGGAAGCTCT-GCGATCT-TAMRA		

Table 2. Avian Influenza H9N2 RT-PCR primer

Target	Primer	Sequence (5'–3')	Amplicon Size (bp)	Reference
H9 (HA)	R F	ATCGGCTGTTAATGGAATGTGTT TGGGCGTCTTGAATAGGGTAA	221	Rashid *et al*., 2019
N2 (NA)	R F	TTAGATGTGTTTGCAGGGCAC GGTTCTAAAATTGCGAAAGCC	560	Vatandou *et al*., 2011
H9 (HA)	R F	AACCAGGAGTGGAGAATTTC GGTGGTGTAGTTGCTTCTCC	182	Lee *et a*l., 2010
NP	R F	GAATGCCCTCTTCTCCGAAG GCTGCAAAAGCTCTTCCATC	189	Sun *et al*., 2014
H9 (qRT-PCR specific)	R F	GGGCACATTGTTGTTGTTGA TCCACCTGTCCTTGACTGAG	138	Monne *et a*l., 2008
M(Matrix, Universal Influenza A)	R F	AGATGAGTCTTCTAACCGAGGTCG TGCAAAAACATCTTCAAGTCTCTG	244	Spackman *et a*l., 2002
N2 (NA)	R F	GCAGAAATTATGTTCGATTGGAC GTGAAAGTATCTGGTCTCCAT	270	Hoffmann *et al*., 2007
p2fP	R F	AGCAAAAGCAGGGGAACTCC-3 CCATACCATGGGGCAATTAG-5	808	Akhter *et a*l. (2017) (H9N2)

acquiring the H9N2-PB1 gene) that indicate increased zoonotic or epizootic risk (Monne et al., 2013a). Serology is used for the retrospective assessment of population exposure and vaccine efficacy. The Haemagglutination Inhibition (HI) Assay identifies antibodies targeting H5 or H9 in serum derived from domestic poultry, including chickens and ducks (Comin et al., 2013). It aids in evaluating population immunity levels and the effectiveness of vaccination programs (Comin et al., 2013).

Vaccination program for avian influenza and obstacles to effectiveness

In Bangladesh, vaccination against avian influenza serves as a crucial strategic instrument for disease control, primarily focused on diminishing clinical sickness, decreasing viral shedding, and alleviating the risk of zoonotic spillover (Monne et al., 2013b). After acknowledging that a stringent eradication campaign was "uneconomical and unfeasible" for endemic illness, the Government of Bangladesh (GoB) opted to initiate vaccination in 2012 (Monne et al., 2013b; Hossain et al., 2021) (Table.3). The Drug Administration Authority officially permitted the limited use of H5N1 vaccinations for commercial poultry beginning in 2014 (Ansari et al.,2016) (Table:4).

Here are the common vaccine names available in the market:

1.H9N2 subtype vaccines (commonly used)

The "silent killer" strain that impairs egg development and production is usually prevented by these inactivated ("killed") vaccinations. GuardFlu vet (Incepta vaccination): an H9N2-specific inactivated vaccination. GuardFlu Plus vet (Incepta Vaccine): A combination vaccine against Newcastle disease (ND) and H9N2. Square Pharmaceuticals' Gallimune H9+ND is an inactivated combination vaccination for Newcastle disease and H9N2. A killed vaccination that offers protection against H9N2 and Newcastle disease is called 'Cevac New Flu H9 K' (ACI Animal Health/ CEVA).

2. H5N1 subtype vaccines (restricted/regulated)

Vaccination against H5N1 is more carefully regulated by the Bangladesh government. Historically, the following were used or registered: Vectormune HVT AIV (ACI Animal Health / CEVA) is a vector vaccination designed to provide long-term protection against

Table 3. Types of vaccines and strains used

Technical specification	HPAI H5N1 vaccine technology	LPAI H9N2 vaccine technology	Citation
Types of vaccines	Recombinant Vector (HVT-AI): Incorporates the H5 gene into the Herpesvirus of Turkey (HVT) genome (Monne et al., 2013a)	Inactivated Whole-Virus: Often formulated as a bivalent product combined with Newcastle Disease Virus (NDV) (*Incepta ,2024).	(Monne *et al.*, 2013a, (*Incepta ,2024)
Vaccine Strains	Clade 2.2 (e.g., Vectormune HVT-AI, based on A/swan/Hungary/4999/2006) and Clade 2.3.2.1 (e.g., Inactivated Re-6, based on A/dk/GD/S1322/2010) (Monne et al., 2013a)	H9N2 strains (G1 lineage) (Hossain *et al*,. 2021a).	(Monne *et al.*, 2013a); Hossain *et al,*. 2021)
Route	Recombinant: Subcutaneous (SQ) injection, typically administered to day-old chicks (DOCs) at the hatchery [(Monne et al., 2013a) Inactivated: Intramuscular (IM) or Subcutaneous (SQ) injection for older birds(Monne et al., 2013a)	Intramuscular (IM) or Subcutaneous (SQ) injection, with a dosage of 0.25 ml for chicks and 0.5 ml for older birds (*Incepta 2024)	(Monne *et al*., 2013a); (Mim, 2024)

* Incepta 2024: Incepta Pharmaceuticals discovered a inactivated low pathogenic Vaccine (H9N2)

Table 4. Schedule and target population

Target population	Vaccine	Dose schedule	Citation
Commercial day-old chicks (DOCs)	HVT-AI Recombinant	Single dose at Day 1 at the hatchery level (Monne *et al.,* 2013a).	(Monne *et al.,* 2013a)
Commercial Layers/ Breeders (H5)	Inactivated H5	1st dose at 10-12 weeks; 2nd dose at 45 weeks (Hossain *et al,*. 2021).	(Hossain *et al,*. 2021)
Intensified schedule (If HVT missed)	Inactivated H5	1/2 dose at 3 weeks; full dose at 10-12 weeks; 3rd dose at 45 weeks(Hossain *et al,*. 2021).	(Hossain *et al,*. 2021)
Layers/Breeders (H9)	Inactivated H9N2 and NVD	1st dose at 7-14 days; 2nd dose at 6-10 weeks; 3rd dose at 14-15 weeks (pre-lay) (*Incepta, 2024).	(*Incepta, 2024).
Commercial Sonali chickens	Inactivated H9N2 (Alternative)	1/2 dose at 3 weeks; 1/2 dose at 6 weeks (Hossain *et al,*. 2021).	(Hossain *et al,*. 2021)

H5N1. Sinder Fluvac (Imported): A popular name in technical circles, this is an inactivated H5 vaccine (strains such as Re-6, Re-8, or newer) that is occasionally imported for commercial usage (Hossain et al. 2021).

Avian influenza control by OIE guideline and Bangladesh experience

The World Organization for Animal Health (OIE, now WOAH) instituted the international control framework for HPAI H5N1, requiring a synthesis of rigorous biosecurity measures, prompt culling policies, and transparent compensation protocols to effectively eliminate the disease (Table.5). Nevertheless, these global standards are unsustainable due to considerable operational and regulatory shortcomings, as evidenced by the prolonged experience of Bangladesh, an environment where HPAI H5N1 and LPAI H9N2 co-circulate endemically (Monne et al., 2013b; Hossain et al., 2021). The OIE's policy, as delineated in the OIE Terrestrial Animal Health Code and the Terrestrial

Manual, emphasizes the eradication of Highly Pathogenic Avian Influenza (HPAI), the reduction of viral load, and the mitigation of zoonotic risk.

Since 2007, Bangladesh has seen Highly Pathogenic Avian Influenza, with both H5N1 and Low Pathogenic Avian Influenza becoming endemic (Paul et al., 2016). Local circumstances obstruct the execution of control measures, which adhere to OIE principles. The prevalent practice of backyard poultry is increasing, and the cohabitation of various poultry species (chickens, ducks) in densely populated rural areas complicates the enforcement of OIE-standard biosecurity measures (Alam et al., 2022; Høg et al., 2019). Low-income farmers frequently encounter financial or logistical barriers that hinder adherence to recommended practices regarding culling, biosecurity improvements, or movement limitations, resulting in low compliance despite compensation initiatives (Swayne, 2018; Alam et al., 2022; Høg et al., 2019). The ongoing circulation of viruses in live bird markets, marked by inadequate cleanliness and rapid turnover of both ill and healthy birds, undermines the efficacy of control measures within the poultry supply chain (Paul et al., 2016; Thomas et al., 2021; WOAH 2023).

Table 5. Key control elements of OIE

Core OIE recommendation	Strategy detail	Reference
Notification and transparency	Mandatory reporting of all HPAI and H5/H7 LPAI outbreaks to the OIE through the World Animal Health Information System (WAHIS) [OIE Terrestrial Animal Health Code, Chapter 10.4].	OIE (WOAH)
Early detection and surveillance	Requires immediate investigation, active and passive surveillance (including in high-risk areas like Live Bird Markets), and rapid, accurate diagnosis to facilitate early warning systems [OIE Terrestrial Animal Health Code, Article 10.4.29].	OIE (WOAH)
Disease control measures	Stamping Out (culling) of infected and contact flocks is the preferred eradication method. This is coupled with movement restrictions and the establishment of restricted zones [OIE Terrestrial Animal Health Code, Chapter 10.4].	OIE (WOAH)
Biosecurity	Implementation of strict hygiene and biosecurity protocols at all levels of production to prevent virus introduction and dissemination, notably separating domestic poultry from wild birds [OIE Terrestrial Animal Health Code, Chapter 3.4.7].	OIE (WOAH)
Vaccination	A complementary tool, particularly in endemic settings, used as part of a comprehensive control strategy. Vaccines must be antigenically matched to circulating strains and accompanied by surveillance (Vaccination to Live - VTL) [OIE Terrestrial Animal Health Code, Chapter 10.4.32].	OIE (WOAH)

Socio-economic impact of avian influenza in Bangladesh

The ongoing endemic situation creates a sustained financial burden due to the requirement for substantial and ongoing expenditures by both the government and farmers on improved biosecurity measures, constant monitoring, and the acquisition of potentially ineffective vaccines (Pervin et al., 2020). The initial outbreak of avian influenza resulted in anticipated financial losses for the poultry industry in 2007 and 2008 that surpassed 38,580 million Bangladeshi Taka (BDT) (Alam et al., 2010).

The risk of zoonotic spillover and the consequent public health alarm frequently result in extensive market volatility. Reports of human or animal instances provoke instant consumer panic, resulting in a significant and rapid decline in demand for poultry meat and eggs (Unesi, 2008). In the 2007-2008 downturn, chicken prices declined by approximately 28%, while egg prices decreased by 26.5%, as a substantial number of consumers reduced consumption, leading many farm owners to bankruptcy (Alam et al., 2010). This market disruption exceeds the farm gate, engendering adverse consequences throughout other industries, including feed mills, veterinary services, and transportation, resulting in job losses and diminished revenue across the value chain. The classification of avian influenza as endemic leads to global trade obstacles or stringent limitations on poultry exports, hindering Bangladesh's access to international markets and constraining potential foreign exchange revenues [WOAH, 2023].

Poultry farming in Bangladesh is predominantly organized within the smallholder and backyard sectors, serving as a vital source of income and economic empowerment, especially for rural women (Thomas et al., 2025). The loss of flocks due to sickness or culling depletes the household's capital, increasing the poverty of vulnerable families (Thomas et al., 2025). The concept of forced culling is sometimes compromised by farmers' unwillingness to report outbreaks, primarily due to insufficient or delayed reimbursement (Ripa et al., 2021). This behavior maintains the "silent threat" and undermines government control efforts. The diminished availability and consumer reluctance concerning poultry products adversely affect food security and nutrition, as meat and eggs serve as vital and economical sources of high-quality protein, directly influencing the nutritional status of children and the broader population (Hossain et al., 2021; Gaide et al.,2022; Thomas et al., 2025;).

Research gaps and future directions

A significant gap exists in comprehending the conditions for gene segment interchange between HPAI H5N1 and H9N2. The existing surveillance system is deficient in centralised longitudinal whole-genome sequencing to identify high-risk areas for these genetic exchanges.

The research underscores the importance of measuring the impact of new gene combinations on viral tropism and virulence across different animal models. It highlights the lack of reverse genetics and in vivo pathogenicity assessments of local Bangladeshi virus strains to determine if novel H5N1 clades or H5N1/H9N2 reassortants exhibit increased human transmissibility or adaptability.

The frequency and transmission of subclinically infected poultry have been overlooked owing to insufficient passive surveillance. The identification of HPAI in vaccinated or asymptomatic flocks indicates that the covert transmission of LPAI H9N2 and undetected HPAI is inadequately recorded. Thorough serological investigations are essential to determine the actual duration and prevalence of infection in primary reservoirs such as domestic ducks and backyard poultry.

The existing immunisation initiative is impeded by two primary challenges: a discrepancy between outdated Clade 2.2 vaccines and the prevalent Clade 2.3.2.1a, as well as the omission of essential hosts. Current vaccinations against emerging reassortant viruses provide insufficient field efficacy data to evaluate the protection levels and duration of immunity. Moreover, high-risk backyard poultry and domestic ducks, comprising 71% of rural households, are not mandated to receive vaccinations, leading to unregulated susceptible populations that perpetuate the endemic cycle.

The principal problem is the inadequate culling compensation process, exacerbated by the withdrawal of external funds, which renders the strategy impracticable. Moreover, there is an absence of economic modelling to assess the true costs of disease loss compared to the compensation required to ensure farmer compliance and avert the illegal transfer of sick birds.

Live bird markets function as viral amplification hubs; nevertheless, there is a significant deficiency in effective and cost-efficient biosecurity measures suited to the socio-economic circumstances of market workers. The sanitation in these marketplaces is insufficient, and there is a lack of research in behavioural science to formulate and execute straightforward, sustainable biosecurity measures that can mitigate opposition from traders and smallholders.To bridge these gaps, priority areas include:

1.Advancing molecular surveillance and risk prediction

- Real-Time Reassortment Mapping: Implement regular whole-genome sequencing surveillance in high-risk areas like Live Bird Markets and backyard poultry to track gene exchange, aiding targeted prevention efforts.
- Climate-Integrated Risk Modeling: Create transmission models that factor in subclinical infections and seasonal epidemiology to accurately forecast outbreak risks, enabling proactive resource deployment before peak seasons.
- Pathogenicity Assessment: Focus on reverse genetics and in vivo pathogenicity studies to evaluate the impact of new gene combinations on virulence, assisting in the assessment of public health risks.

2. Strategic optimization of vaccination

- Regulatory policy should mandate the immediate replacement or supplementation of outdated vaccine strains (e.g., Clade 2.2) with those

exhibiting high antigenic congruence to dominant Clade 2.3.2.1a and emerging variants (e.g., 2.3.4.4b).
- Strategically extend vaccine coverage to under-vaccinated domestic ducks and backyard chickens with single-dose formulations to enhance compliance and suppress viral load.
- Urgent field efficacy trials are essential to assess the protection levels and duration of immunity of current vaccines against circulating reassortant viruses, facilitating a quality control feedback mechanism.

3. Sustainable policy and infrastructure reform

- Conduct socio-economic studies to assess the true cost of disease loss compared to compensation.
- Develop and pilot economic models for sustainable, state-funded compensation that ensure timely and transparent payments.
- Incentivize farmer compliance and promote immediate reporting of outbreaks to align with OIE principles.
- Shift policy towards mandating and funding structural reforms in live bird markets to improve sanitation.
- Integrate behavioral science and social network analysis to co-design biosecurity practices in collaboration with market traders and workers.

Conclusions

A continuous "silent threat," the Avian Influenza Virus in Bangladesh is primarily caused by high-density co-circulation of HPAI H5N1 and H9N2 strains, as well as systemic policy and infrastructural deficiencies. These flaws enable the virus to genetically reassort, creating new strains that evade control strategies. Farmers underreport as a result of inefficient culling compensation systems, and the faulty vaccination program overlooks important reservoirs, thereby sustaining the endemic problem. Restoring financial compensation, improving predictive surveillance, and updating vaccine coverage to cover all important hosts are only a few of the strategic changes needed for sustainable control. To protect the poultry industry and public health, these deficiencies must be filled in order to move from reactive to resilient management.

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