Biologicals 91 (2025) 101840

Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

Vaccination and surveillance for high pathogenicity avian influenza in poultry—current situation and perspectives

Nancy C. Sajjadi [a], Celia Abolnik [b], Francesca Baldinelli [c], Ian Brown [d], Angus Cameron [e], Sjaak de Wit [f], Madhur Dhingra [g], Olivier Espeisse [h], Jean Luc Guerrin [i], Timm Harder [j], Jeremy Ho [k], Tze-Hoong Chua [l], Khaled Hussein [m], Nicholas Lyons [g], Isabella Monne [n], Yukitake Okamuro [o], Damian Tago Pacheco [g], Gounalan Pavade [p], Nicolas Poncon [q], Teguh Yodiantara Prajitno [r], Jose Gonzales Rojas [s], David Swayne [t], Arjan Stegeman [u,*]

[a] *Rapporteur, Sajjadi Consulting, Fairfax, VA, USA*
[b] *University of Pretoria, South Africa*
[c] *European Food Safety Authority (EFSA), Biological Hazard & Animal Health and Welfare Unit (BIOHAW), Parma, Italy*
[d] *Pirbright Institute, United Kingdom*
[e] *EpiMundi, France*
[f] *Royal GD (Deventer) and Faculty of Veterinary Medicine, Utrecht University, Netherlands*
[g] *Food and Agricultural Organization of the United Nations, Italy*
[h] *IABS Chair, France*
[i] *Ecole Nationale Vétérinaire de Toulouse, INRAE, University of Toulouse, France*
[j] *Friedrich-Loeffler Institute Isle of Riems, Germany*
[k] *Agriculture, Fisheries and Conservation Department, Hong Kong, China*
[l] *Veterinary Health Division, National Parks Board, Singapore*
[m] *Poultry Veterinarian, United Kingdom*
[n] *Istituto Zooprofilattico Sperimentale Delle Venezie (IZSVe), Padua, Italy*
[o] *Animal Health Division, Food Safety and Consumer Affairs Bureau Ministry of Agriculture, Forestry and Fisheries, Japan*
[p] *World Organization of Animal Health, France*
[q] *Ministry of Agriculture and Food Sovereignty, France*
[r] *Japfa Comfeed Indonesia Tbk, Indonesia*
[s] *Wageningen Bioveterinary Research (WBVR), Netherlands*
[t] *Birdflu Veterinarian LLC, USA*
[u] *Utrecht University, Department of Population Health Sciences, Yalelaan 7, 3584 CL, Utrecht, Netherlands*

ARTICLE INFO

Keywords:
High pathogenicity avian influenza (HPAI)
H5N1
Bird flu
Vaccination
Surveillance
Trade

ABSTRACT

The International Alliance for Biological Standardization (IABS), in collaboration with the World Organization for Animal Health (WOAH) convened a hybrid meeting on 22–23 October 2024 at the WOAH Headquarters (HQ) in Paris, France to discuss the global state of vaccination and surveillance for high pathogenicity avian influenza (HPAI) in poultry. The primary objective of the meeting was to advance vaccination acceptance to both control virus spread and reduce disease. Vaccination is increasingly recognized as a tool to complement biosecurity, movement controls and stamping-out of infected flocks. However, concerns persist regarding the risk of undetected, sustained transmission (silent infection) in vaccinated flocks as a result of inadequate surveillance. This has contributed to both vaccination hesitancy and trade barriers. The meeting aimed to assess the current state of the art regarding HPAI surveillance programs in vaccinated populations and their effectiveness. Representatives of multiple stakeholders were invited to share their experiences and perspectives on the use of vaccination and accompanying surveillance to control the growing H5N1 panzootic and its global impact. Several conclusions and recommendations emerged as essential to advancing the acceptance of vaccination strategies. These included (1) the utility of quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) as a sensitive, specific and economical tool to detect virus in vaccinated populations, (2) regular testing of dead birds within a flock as a highly effective method for early detection of outbreaks in vaccinated flocks and demonstrating freedom from

* Corresponding author
E-mail address: j.a.stegeman@uu.nl (A. Stegeman).

https://doi.org/10.1016/j.biologicals.2025.101840
Received 18 April 2025; Accepted 3 May 2025
Available online 13 May 2025

infection and, (3) the importance of collecting information on circulating field strains in the selection of candidate vaccine antigens to ensure adequate efficacy. Testing sentinel birds was deemed less effective for surveillance and serological testing of vaccinated birds was considered more useful for assessing immunity levels than for determining the infection status of a flock. There was broad agreement on the need to standardize surveillance outcomes in terms of accepted confidence levels to promote safe and fair trade. However, it was acknowledged that context and pragmatic considerations will shape the development of situation specific plans, which must be statistically valid, scientifically sound, economically feasible and operationally sustainable for both governments and industry. Concomitantly, it was recommended that trade policies tied to vaccination and surveillance should be based solely on science and risks. To this end, enforcement of existing international rules and resolution of disputes are considered a shared responsibility. Peer reviewed publications were proposed as a central mechanism for developing the stronger guidelines needed to facilitate fair trade agreements and enable implementation of global vaccination programs. Rapid dissemination of information, consistent messaging and exchange of virus isolates were also seen as critical for coordinating an effective global response to controlling HPAI.

1. Introduction

1.1. HPAI virus biology and disease: high level overview

Avian influenza viruses (AIVs) are single-stranded, negative-sense RNA (ss (−) RNA) viruses categorized as high pathogenicity (HPAIVs) or low pathogenicity (LPAIVs) based on their ability to cause disease and mortality in chickens after intravenous administration in a laboratory setting [1]. AIVs can also efficiently infect other types of domestic poultry (ducks, turkey, pheasants, geese, quail and guinea fowl), wild birds, but only sporadically spill over to humans and other mammals. The ability of an AIV to infect an animal, the severity of resultant disease, and mortality rates are dependent on the specific viral strain(s), the host species infected, the challenge dose and the environmental conditions. HPAIVs evolve from LPAIV strains of subtypes H5 or H7 during infection of terrestrial poultry, mainly chicken and turkeys, through multiple genetic mechanisms including mutation and reassortment [2]. The main reservoir for LPAIVs is water birds [3]. The A/goose/-Guangdong/1/96 strain of HPAI that arose in Southern China in 1996 is the progenitor of the H5Nx Gs/GD lineage from which all the different clades currently causing concern have descended.

1.2. Vaccination strategies to prevent and control HPAIV spread: first Conference and significant Developments since October 2022

A rise in HPAI outbreaks and a shift from seasonal to year-round epidemics caused by AIV strains in 2021 led to an IABS meeting at WOAH HQ in October 2022. It focused on removing unnecessary barriers to expanding vaccine use. The conclusions and recommendations were made publicly available [4]. Primary trade concerns were tied to the anticipated failure of surveillance programs to detect silent infection. Two critical action items emerged:

- Demonstrate vaccine effectiveness in multiple field trials
- Evaluate the appropriateness of surveillance strategies.

To address the strategic challenges associated with increased adoption of vaccination, including trade barriers, WOAH recognized by the World Trade Organization (WTO) through its sanitary and phytosanitary (SPS) agreement as the international standard setting organization for animal health and zoonoses adopted Resolution No.28 [5] in May 2023. Importantly, it states that:

> "In accordance with WOAH international standards, the use of vaccination will not affect the status of a country or zone free from high pathogenicity avian influenza if its surveillance program supports the absence of infection."

During the past two years, the scientific literature continued to document the threats posed by HPAIV spread and the evaluation of control measures including vaccination [6–8]. Unfortunately, HPAI has since spread to Antarctica and the sub-Antarctic regions of South Georgia and the Falkland Islands (Malvinas), likely introduced by birds migrating from South America [9]. Significant spillovers and spillback events have led to the transmission of HPAI into many mammal species including wild animals, dairy cattle and humans [10–13].

The clade 2.3.4.4b H5N1 is currently regarded as the most widely spread HPAIV strain and there is rising concern that it, or another H5Nx strain arising from this clade, could develop into a pandemic [14,15]. While vaccination has been implemented in several places for more than a decade (e.g., China including Hong Kong Special Administration Region (SAR), Indonesia), it has not been used as a preventive measure in North America or Europe where epidemiological-driven approaches to surveillance of vaccinated poultry flocks are being developed to enable an increase in vaccine use [16].

1.3. Vaccination and surveillance for HPAI in poultry October 2024 conference agenda

In response to the unprecedented HPAI panzootic, IABS convened a second hybrid workshop at WOAH HQ in October 2024. The meeting was co-chaired by Dr. Arjan Stegeman (Utrecht University of Netherlands) and Dr. Gounalan Pavade (WOAH). To better understand current attitudes toward vaccination, WOAH polled 133 member states in advance asking, "In the last five years, has vaccination (Terrestrial Animal Health Code Article 4.18.2) been used as one of the control measures for LPAI or HPAI?". In response 81 % of all members said no. Responses to another question revealed that half of the respondents are not considering the use of vaccination as a HPAI complementary control tool citing its impact on surveillance, international trade, and silent infections.

Participants were also asked in advance what they considered to be the most important outcome(s) of surveillance, specifically in vaccinated populations. Of primary importance to all stakeholder types—university, private sector, non-governmental organization (NGO), and government—was monitoring the circulation of AIVs. Interest in this topic was followed by assessing vaccination effectiveness, protecting public health and informing AIV control measures (albeit with differing priority levels across stakeholders). The least important factors identified by all attendees were supporting trade and prioritizing future animal health investments. Furthermore, they identified monitoring the circulation of AIVs with a focus on harmonization and improvement of methods for surveillance specifically in vaccinated populations, as of primary importance. The purposes of surveillance were articulated during the opening of the meeting:

- Surveillance should ensure safe trade to minimize viral spread by poultry and poultry products to importing countries
- Surveillance should ensure fair trade to prevent unjustified barriers for poultry and poultry products to exporting countries

The meeting agenda was developed to identify and address the implications of HPAI viruses' evolution and shifts for surveillance and vaccination. To gain a wide perspective, speakers representing multiple stakeholders were organized into the following sessions conducted over two days:

- Introduction, including objectives and expected outcomes
- Tools for monitoring and surveillance in vaccinated poultry populations
- Field experiences on surveillance in vaccinated populations
- Surveillance and trade: Risk assessment
- Surveillance and trade: Risk management

The overarching goal was to address the following questions:

- What can we learn from recent in country experiences related to vaccination?
- Which surveillance strategies are the most suitable in vaccinated populations?
- Which vaccines are now available and how do they compare?
- How do we overcome trade barriers related to vaccination?

Panel discussions concluded each session and provided opportunities to gather input from in-person and online participants representing additional international stakeholders. This report summarizes information presented and opinions expressed during the conference. Specifics are not ascribed to individual speakers. Select citations provided in presentations are included as references. Conclusions and recommendations collected from session moderators were reviewed in the closing session. They reflect current thinking, identify ongoing challenges and provide a roadmap for future action and are listed in Sections 10.0 and 11.0 of this report.

2. Vaccination and surveillance: overview of objectives and limitations

The panel discussion sessions provided participants and presenters an opportunity to further engage on topics of high interest. More importantly, they helped crystallize the objectives of vaccination and surveillance and the technical, economic, cultural and logistic challenges that must be overcome to achieve success in preventing disease spread. The following highlights are being provided upfront to assist readers in their review and consideration of the content provided in subsequent sections.

The goal of vaccination is to both control virus spread and reduce disease. It is designed to induce herd immunity that hampers virus incursion, limits virus transmission and clinically fully protects a vaccinated flock. Nevertheless, certain circumstances (e.g., inadequate vaccination, concomitant infections) may result in an insufficient immune response or coverage. The selective pressure created by the development of insufficient or patchy herd immunity in responses to vaccination increases the potential for HPAI immune escape variants to emerge upon natural infection. Therefore, antigenic changes in the virus must be monitored to ensure the use of vaccines antigenically matched to circulating strains thereby preventing vaccine failures and suboptimal protection in the vaccinated population. International consensus standards for correlates of protection are not yet available but are expected to facilitate expanded use of vaccination.

Standards for surveillance won't be prescriptive in terms of sample type, frequency or size but instead should represent agreement on acceptable outcomes of vaccination. Surveillance strategies must be statistically sound and scientifically valid to quantify freedom from infection with high confidence. Development of a surveillance strategy and sample testing plans will need to be tailored to specific situations such as emergency surveillance, demonstration of freedom or assessment of vaccination effectiveness. They must also be cost-effective and feasible for governments and the private sector to implement. Leveraging from experience with other diseases such as Salmonellosis or Newcastle disease may be helpful. Sensitivity will be paramount in easing trade restrictions and RT-qPCR testing was identified as a critical tool. Historical approaches to surveillance developed prior to the availability of molecular testing must be re-evaluated.

For example, sentinel birds are now considered not only impractical but, in contrast, may pose significant risks for increasing transmission. Unvaccinated birds are easily infected by environmental exposure, which not only allows for amplification of viruses, but the potential to generate new strains through genetic mixing with circulating LPAI strains, making sentinels a source of new infection to vaccinated animals within the flock. Serological testing as an isolated surveillance approach also has its limitations.

The design of vaccines that allow differentiation of infected from vaccinated animals (DIVA) led to the development of companion serological methods for measuring immune responses. However, such DIVA test methods are not necessarily practical for monitoring a vaccinated flock for subsequent infection. Depending on the test performance characteristics, application might result in false positive results and the lack of confirmatory tests would lead to expensive and unnecessary investigations. RT-qPCR testing is an appropriate test to use in surveillance for infection in vaccinated flocks.

Inducing sterilizing immunity by vaccination is unrealistic and vaccinated flocks will remain susceptible to infection, albeit at a markedly lower level compared to unvaccinated flocks. Therefore, upon virus incursion, limited HPAIV replication and excretion is expected but does not necessarily indicate sustained transmission. The choice of what to sample, the frequency of sampling and the sample size are critical to achieving overall sensitivity in surveillance and evaluation of transmission risk. Demonstrating absolute freedom from disease (i.e. proving complete absence in the population) is impossible in most situations. Instead, expressing design prevalence as a maximum acceptable prevalence that should be detected with specified statistical confidence must be seen as integral to successful approaches.

Many stakeholders warned that failure to increase vaccination uptake could eventually unleash a pandemic and create conditions that could be catastrophic for human health, the poultry industry, biodiversity and the global economy. While scientific data are subject to interpretation and there can be differences in opinion in terms of risk tolerance, a distinction must be drawn between skepticism that is integral to good science and its use, potentially, to justify protectionist practices. As such, there was a plea for greater specificity in identifying any outstanding scientific issues that must be overcome to increase vaccine acceptance and reduce trade restrictions. Building stronger consensus will be essential to collectively managing the threat of HPAI through ethical and cost-effective means thus benefiting humans and animals globally.

3. IABS, WOAH, and the food and Agriculture Organization (FAO): overarching aims and roles

IABS is a non-profit organization that provides forums for scientists, regulators and other stakeholders to discuss the development of biological products with a focus on regulatory science aspects. WOAH addresses issues of animal health standards and trade pertaining to infectious disease generally, and HPAI in particular. They establish standards for the improvement of animal health and welfare and veterinary public health worldwide, including the prevention of disease spread through international trade of animals and animal products. They publish the Terrestrial Animal Health Code (10.4 covers avian influenza [AI]) and the Manual of Diagnostic Tests and Vaccines (Chapter 3.4.1 covers AI).

The Food and Agriculture Organization (FAO) is a specialized agency of the United Nations that leads international efforts to achieve food security for all, through efficient, inclusive, resilient and sustainable

agrifood systems for better production, better nutrition, a better environment, and a better life, leaving no one behind. FAO contributes to improving animal health to make livestock production more productive and sustainable while achieving food security and optimal health for all at the human-animal-environment interface. FAO also supports its members in prevention, preparedness and rapid response to influenzas through their country teams.

FAO and WOAH jointly have spearheaded the 'Global Framework for the Progressive Control of Transboundary Animal Diseases' (GF-TADs) to achieve the prevention, detection and control of transboundary animal diseases (TADs) and in particular to address their regional and global dimensions. The initiative combines the strengths of both international organizations to achieve agreed common objectives. Recently FAO and WOAH have launched the Global strategy for the prevention and control of high pathogenicity avian influenza (2024–2033) with the aim to achieve sustainable, resilient poultry production systems. As part of their joint efforts, FAO is committed to global risk monitoring of avian influenza and to development of innovative vaccines and surveillance technology to reduce the burden of the diseases. OFFLU is the joint WOAH-FAO global network of expertise on animal influenza viruses. OFFLU aims to monitor the global animal influenza risk, share information and biological materials to reduce the impacts of animal influenza viruses. As part of Quadripartite efforts with World Health Organization (WHO), OFFLU regularly shares information on animal influenza viruses to contribute to preparedness for influenza human vaccines.

A link to details of the program and regional technical meetings was provided [17] and the objective of the OFFLU AI matching (AIM) program was cited:

> "To provide up-to date information to the poultry sector, governments, and poultry vaccine manufacturers on antigenic characteristics of circulating avian influenza viruses including comparisons with vaccine antigens. This information will facilitate selection of appropriate vaccines for poultry and updating of poultry vaccine antigens in places where vaccines are being used".

WOAH noted that the outcomes of this IABS workshop will provide valuable input for its General Assembly meeting in 2025 where delegates will debate the ongoing technical and political challenges associated with worldwide avian influenza vaccination.

4. The risk of keeping the status quo: urgent reasons to advance vaccination in poultry

Many presentations mentioned the disastrous outcomes caused by the growing H5N1 panzootic. A summary of losses and concerns related to HPAI spread is provided to emphasize the urgency expressed by many stakeholders (Table 1). While there are acknowledged risks and costs associated with vaccination, there are also significant risks associated with not increasing vaccine use as an additional layer of intervention.

5. The origins, evolution and expansion of the existing panzootic

AIVs are broadly classified by their 18 different hemagglutinin (HA) surface protein subtypes (H1–H18) and 11 different neuraminidase (NA) subtypes (N1–11). To date 16 HA subtypes and 9 NA subtypes have been described in birds. HPAIVs have arisen from LPAI viruses through changes in the HA proteolytic cleavage site. All naturally occurring HPAIV strains isolated to date have been either of the H5 or H7 subtype. Presentations including the keynote address reviewed the origin and evolution of the HPAI causing the current panzootic.

The first HPAI (H5N1) virus (although not belonging to the Gs/GD lineage) was isolated in 1959 following an outbreak in chickens in Scotland. Up until October 2022, there had been an additional 43 HPAI outbreaks based on distinct lineages. Of these, 41 were eliminated but two remain entrenched (H5Nx Gs/GD, especially clade 2.3.4.4b, and H7N3 N. American). Between 2005 and 2022 there were five transcontinental waves of H5 Gs/GD HPAIVs and from 2016 to 2022 they all were of the 2.3.4.4b clade. Since 2022, HPAI has spread to Central and South America [9]. Changes in virus behavior indicate that H5Nx HPAI has evolved high fitness traits in avian species. While these viruses are more dangerous, the upside from a vaccine development standpoint, is that clade 2.3.4.4b is moderately stable antigenically.

Table 1
Overview of HPAI threats under current control measures.

Who/What Impacted	Types of Losses	Additional Concerns
Ecosystem/ Wildlife	• Biodiversity, especially endangered species, through high mortality disease spread	• Larger reservoirs of circulating virus in animals creating spillover/spillback
Poultry Industry	• Mass mortality due to disease and stamping out interventions • Inability to feed people who rely heavily on poultry derived protein productions for nutrition. • Loss of profits due to trade restrictions • Increased costs associated with interventions and monitoring	• Adverse impacts for bird welfare • Disease and disposal of carcasses lead to increased outbreaks
Governments	• Increased costs associated with interventions and monitoring • Compensation to farmers • Short- and long-term negative effects on human health due to disease and insufficient nutrition	• Silent infections introduced through imported products
Citizens and Consumers	• Death due to zoonotic events • Job loss and food insecurity created by shortages and high prices due to possible pandemics, shutdowns etc. • Reduced quality of life/ wellbeing due to psychological stress and socioeconomic outcomes	• Evolution of more virulent strains at human to animal interface contributing to spillover and spill back events • Evolution of efficient human-to-human transmission

As a transglobal disease predominantly caused by H5N1 [18], there are multiple epicenters from which new strains can readily emerge and spread [19]. Although threats of more outbreaks are ever present, there has been a declining HPAI trend in reports to WOAH of HPAI in poultry and wild birds from Oct 2022–Sep 2023 to Oct 2023–Sep 2024. This has been attributed to increased awareness, leading to improved biosecurity measures, as well as the natural immunity being built up in wild birds that recover. Correspondingly, there have been reductions in the number of poultry killed and the number of dead wild birds, but caution was given; stakeholders should not "rest on their laurels" but rather view this as an opportunity to apply additional layers of intervention to drive down the disease.

The capacity of some species of birds to fly East to West and spread HPAI clade 2.3.4.4b to the Americas demonstrates the degree to which this virus has developed selective advantages [20,21]. The degree to which wild birds become infected, shed virus and succumb to disease and death, creates a complicated story for transmission which has now been detected in over 300 species and more than 25 different orders. The Convention on the Conservation of Migratory Species of Wild Animals (CMS) and the FAO Co-Convened Scientific Task Force on Avian Influenza and Wild Birds, released a statement in 2023 detailing the devastating effects [22].

With this increasing spread in wild and domestic animals and transmission to humans, the risk of human-to-human transmission conversions is growing. The precise mechanism of transmission from

animals to humans is not always clear, but the infection of dairy farm workers in the USA and the transmission through dairy cattle are presumed to occur by contact with raw milk and milking equipment. The H5N1 virus replication in mammary glands leads to high titers in milk. The virus causes mastitis in cows and thus far, mostly conjunctivitis in humans. As of October 2024, there had not been reports of infection in the upper airways so H5N1 in humans and cows is not yet considered a respiratory or systemic disease. At this point, the H5N1 virus is still highly adapted to avian hosts [23] although it replicates well in bovine mammary tissues and initial infection does not require much virus.

The ongoing process of genetic mixing and mutation used by influenza viruses produces many genotypes. Iterative reassortment appears to explain how the current strain arose and spread so rapidly. Specifically, immunity that builds in the wild bird population makes them less susceptible to infection, so they shed lower amounts of virus. Smaller amounts of virus in the environment means reduced chances of strains infecting farms. At the same time, patchy wild bird population immunity may create a selection pressure leading to the evolution of immune escape mutants and the potential for a new epidemic. Sub-optimal vaccination is expected to create the same kind of immune response pressures in poultry flocks. Ongoing monitoring to assess any changes in viral genetics in wild birds, poultry and mammals is necessary to make appropriate antigenic matches as needed to update vaccines and to identify genetic changes that may alter virulence and/or transmissibility impacting the effectiveness of vaccination.

6. Tools for monitoring and surveillance in vaccinated poultry populations

One of the central trade barriers is the concern that poultry vaccinated in exporting countries will not be fully protected from infection with circulating field strains. Specifically, the absence of clinical disease coupled with low level virus shedding means that infected vaccinated animals may not be detected during surveillance and their export making importing countries vulnerable to disease transmission. The pathogenesis of HPAI in poultry, the susceptibility of other species to infection, and the ability to detect infection and virus shedding in vaccinated animals, each factor into the risk-assessment tied to silent infections. Recall that the stated realistic goal of vaccination is to reduce susceptibility, transmission and disease but not necessarily prevent infection entirely. A robust immune response is intended to dramatically reduce susceptibility and virus replication upon infection, shorten the shedding period, and decrease virus shedding to levels that prevent sustained transmission. In the absence of sustained transmission, infections are expected to fade out and outbreaks averted. Therefore, the monitoring and surveillance of vaccinated poultry populations takes several forms and serves multiple purposes (Fig. 1: Overview of Surveillance Objectives and **Strategies**).

6.1. *Measuring immune responses to vaccines*

Monitoring vaccinated flocks for evidence of vaccine induced protection is the first step to demonstrating vaccine effectiveness. To be considered fit for purpose, traditional companion diagnostic tests must differentiate an infected animal from a vaccinated animal (DIVA). Similarly, for the purposes of exporting poultry products from countries employing vaccination, surveillance tests need to be able to differentiate "vaccinated only" from "vaccinated and then infected" birds with sufficient sensitivity and specificity to assure importing countries that products are free of risks regarding HPAIV.

It is known that antibody responses to HA protein that inhibit virus-receptor binding provide the primary protection against sustained transmission. There is little cross protection between HA subtypes; H5 vaccines only protect against H5 virus strains. The matching of vaccine to circulating strains is critical because there is reduced cross-protection even between strains and lineages within a single subtype. There is also little to no protection from antibodies to other viral proteins (non-neutralizing antibodies). Antigenic drifts of H5Nx viruses are predominantly driven by substitutions near the receptor binding site (RBS) [24] and change in N-glycosylation pattern [25], as these glycans have been proposed to shield antigenic sites on the HA protein. This enables H5Nx viruses to escape natural or vaccine-induced immunity and leads to vaccine failure in the field. Thus, these substitutions are clearly associated with the evolutionary advantage of antigenic escape. Cell mediated immunity can provide additional protective value particularly for vectored vaccine but there are no standard tests for evaluation.

Serological tests that can be used to detect immune responses for any influenza virus include the agar gel immunodiffusion test (AGID) and

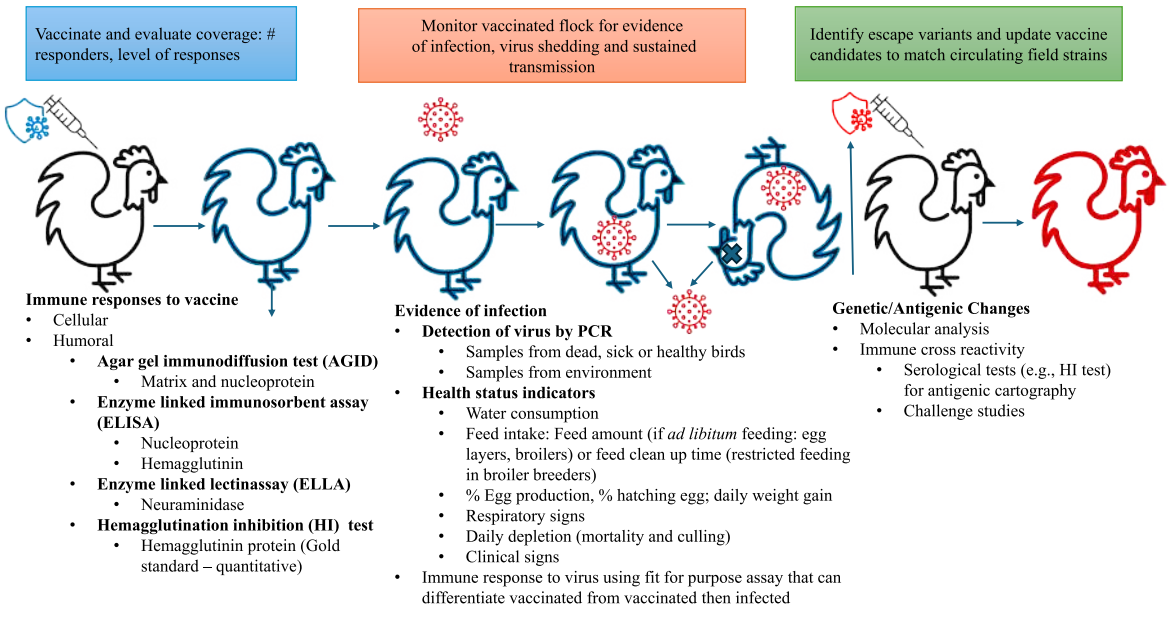

Fig. 1. Overview of surveillance objectives and strategies.

generic commercial enzyme linked immunosorbent assay kits (ELISAs). Subtype specific tests include the hemagglutination inhibition (HI) test which targets the HA protein, several HA-subtype-specific commercial ELISAs and the NA inhibition enzyme linked lectin (ELLA) assay targeting the NA enzyme. Currently, the HI test, as a surrogate of detecting neutralizing antibodies, is the gold standard for evaluating levels of immune response although correlation with levels of protection is not well defined. Because agglutination of red blood cells in the assay is mediated by the HA protein, the choice of antigen is critical in evaluating protection conferred by the anti-vaccine antibodies. Selecting circulating field strains as the source of antigen may provide the most relevant data in terms of cross-protective capability.

6.2. Detecting evidence of infection in vaccinated flocks

Historically, unvaccinated and individually marked sentinel birds were dispersed throughout the house or farm and treated like vaccinated birds. They can be monitored for evidence of clinical disease and sampled routinely for laboratory testing. As discussed further in later sections, the use of sentinel birds is now discouraged and vaccinated birds are monitored for infection using serological and molecular tests [26].

Detection of NP antibodies to field virus using commercially available ELISA tests are only fit for use with vaccines not containing the NP protein (e.g., HA subunit or vector vaccines). Detection of antibodies to NA is not an option for routine surveillance. Direct detection of virus using molecular tests provides an alternative strategy for monitoring vaccinated flocks for silent infection. Research has shown that in birds infected with LPAI or in birds vaccinated with subunit vaccines and challenged, virus levels peak around three days after infection. Virus is detectable by RT-qPCR from days 1–8 after challenge. Because molecular testing allows producers to move products more rapidly than with serological tests, RT-qPCR remains is generally accepted as the primary option for testing birds or products for viruses at the time of processing.

6.3. Evaluation of transmission risk: limitations of serological methods and advantages of molecular tests

The risk of sustained transmission in vaccinated flocks infected with HPAI is a function of the vaccine coverage (number of birds that received immunization), the level and duration of the immune response to vaccine, and the efficacy of protection. The latter, always with a target of achieving a reproduction number (R) of significantly less than one in a vaccinated population, could be evaluated at the level clinical disease, evidence of HPAIV infection and levels of HPAIV shedding. These factors make it difficult defining a serological threshold for protection which would necessarily vary with poultry species, vaccine and virus strains in circulation.

However, there is a continuum of correlation between immune response levels and clinical disease that exists between two extreme poles: (1) birds with no measurable immunity that are fully susceptible to disease and (2) birds with responses that provide sterilizing immunity. The latter is considered unachievable in practice, and there is no intermediate threshold for serological tests that will guarantee adequate protection. Data excerpted from a recent publication [7] demonstrated that using the HI test, there is an overlap of HI titers between flocks with R status <1 than and those >1. Although higher HI titers generally confer higher levels of protection, it is at best seen as a general proxy for protection and not a measure that can be used to indicate the status of individual birds or at flock level.

It was emphasized that serological tests used in the frame of a DIVA vaccination approach do not provide evidence of current field virus infection in a flock but rather indicate past exposure. Antibody responses to the most abundant and highly antigenic viral protein, NP, can take up to 14 days to develop. When DIVA vaccines are used, antibodies produced against the NP protein expressed by a field virus, a protein which will be missing in the vaccine, become a marker of field virus infection. The utility of this approach to monitor post vaccination infections depends on the sensitivity and the specificity of the test, which in turn necessitates determining an appropriate sample size (e.g., using European rules, at least 20 per unit) and a pre-defined exclusion prevalence that can be agreed upon as acceptable to enable free and safe trade.

Low sensitivity of a serological assay means true NP positives are missed (false-negative results obtained) whereas low specificity means true NP negative results are falsely identified as positive. It was noted that even with a high specificity testing scheme, false positives can be expected to occur at a frequency that has practical consequences. For example, at 99.8 % specificity, there is a probability of two false positives in 1000 tests [8]. There are several explanations for the emergence of such false positive serological responses (e.g., infection of a bird that was inadvertently missed or improperly vaccinated, anti-NP antibodies from prior immunization or cross reactivity to LPAIVs or a technical limitation of the test). To distinguish in such cases whether active infection is ongoing and what kind of field virus is causing it, active swab monitoring using RT-qPCR is required. These scenarios highlighted that using sero-surveillance to monitor vaccinated flocks for evidence of infection has limitations.

The time delay until incursions become detectable is a problem as is the current restriction of testing to only antibody measurements performed using high-throughput antibody assays. Also, the associated costs (blood sampling, material [ELISA] and working time [HI]) can be prohibitive. That said, sero-surveillance is useful for retrospectively confirming freedom from infection in non-vaccinated or DIVA-vaccinated flocks and for estimating population immunity. These approaches could be enhanced by improved ELISAs, new (surrogate) targets and careful integration of sero-surveillance approaches into broader surveillance concepts based ideally on passive surveillance using RT-qPCR. This idea was further explored in a presentation on France's recent field experience with vaccination.

As an EU member, France shared their experience with post vaccination surveillance based on the application of EU rules and their compliance with them in the preventive vaccination of duck flocks. All duck flocks were vaccinated and per the EU requirements, each flock was inspected every 30 days by an official veterinarian and each time 60 birds were randomly selected for swabbing and RT-qPCR testing. In the meantime, dead birds (as few as one, if more samples were not available) were subjected to RT-qPCR testing every week. At the last veterinarian visit, samples were also taken from at least 20 birds per unit for serological testing by NP ELISA. The extensive cost of this program was noted and ultimately factored into proposed adjustments to the current EU test plans in terms of more cost-effective sampling strategies. The data from France suggested that enhanced passive and environmental sampling by RT-q PCR are more sensitive and efficient tools than active surveillance of healthy birds. Data from studies conducted in unvaccinated and vaccinated birds showed the dramatic difference in the dynamics of infection between aquatic birds and poultry species [8].

Unvaccinated ducks reached higher proportions of infected birds and at earlier timepoints than unvaccinated layer chickens. There were fewer deaths in the ducks, and those peaked prior to the peak in proportion of infectious birds whereas in layers, there were more deaths with fewer proportion of infectious birds. In vaccinated birds, when vaccination did not provide protection against sustained transmission, the immune response led to almost complete clinical protection and a dramatic decrease in infection levels in ducks whereas for layers, infection levels were not significantly impacted although deaths were reduced considerably.

Based on simulations derived from data on poultry species that do not show early signs of clinical infection, the probability of an infection not being detected based on sampling five dead birds by RT-qPCR was modeled as a function of sampling timing post outbreak initiation. Delays in sampling from seven to 14 or 30 days negatively impacted the

lowest level achievable whereas reductions in the probability of escaping detection can be gained by increasing the sample size. Detecting virus by surveillance conducted on pools of dead birds with specified sample size taken at defined intervals is referred to as "bucket sampling", this type of sampling at regular intervals is referred to as "enhanced passive surveillance". The use of environmental monitoring for signs of infection was also explored.

Analysis of aerosol samples and dust collected by wipes on walls and feeders on poultry farms in France during 2020–2021 suggested that dust contributes to viral dispersal and environmental sampling could be a valuable surveillance tool to complement tracheal swabs [27]. Detection of outbreaks occurred as early as two days after incursions using environmental testing. Although dust and other environmental sample matrices may contain substances that can inhibit RT-qPCR reactions, studies have shown that the addition of 1 % bovine serum albumin (BSA) to the RT-qPCR reaction mix improves the sensitivity [28].

Based on data from the duck vaccination program and related research, the opinion from France was that a combination of testing would likely be the most cost effective with targeted sampling of sick and dead birds being primary and complemented by environmental sampling and random sampling of birds. Adoption of this approach as an official plan would require additional validation of environmental sampling procedures and achieving global consensus.

It is also very important to detect escape-variant strains as they are likely to give rise to the next epidemic. There are two primary mechanisms by which influenza viruses evolve changes to the HA and NA proteins that allow them to evade neutralization by the immune system. Antigenic drift occurs by the accumulation of mutations introduced by errors during replication. Antigenic shifts occur when the entire HA and/or NA genes are replaced by the corresponding genes from another strain. For HPAI, most changes occur through antigenic drift.

A combination of replication speed and lack of proof-reading by polymerases allows for rapid accumulation of mutational changes as evidenced by the multitude of clades of the Gs/GD strain that have arisen since 1996 [29]. These molecular mutations have also been reflected in antigenic changes for HA and NA. Possible pressures driving the evolution of HPAI include silent infection (particularly in domestic ducks with resultant immunity in the population), the mode of transmission (possibly be related to the high density and close contact of birds kept under conditions of the poultry industry), the genetic homogeneity of inbred high performance poultry breeds, the ability of the virus to infect other species including mammals, and geographical segregation. Each of these conditions can accelerate the evolution of HPAI.

Additionally, it has been shown that the evolution of a viral population can be influenced by the immune pressure exerted by vaccination. Based on studies in chickens in which vaccination was performed with vaccines that yielded either 100 % protection and minimal shedding or 70 % protection with higher level of shedding, the monitoring of the evolution of the viral population revealed more diversity after challenge in the sub-optimally vaccinated group [30]. Some of the minority variants were tied to antigenic sites and receptor binding domains which specifically drive spread. Therefore, it is critical to monitor HPAIV evolution within vaccinated poultry populations but also in wild bird populations having access to vaccination regions.

Screening for changes in putative antigenic sites is preferable to scanning the entire HA1 target for changes [31]. Effective vaccination requires selection of antigens appropriately matched to field strains.

Antigenic matching can be evaluated using cross HI testing of sera from vaccinated birds in the field in combination with an antigen/virus obtained from field isolates. However, the gold standard for proving cross protection of vaccinated birds against circulating field strains is a challenge study conducted using an appropriate challenge strain and dose level under high level biosecurity conditions. Most importantly, data and viral isolates derived from antigenic monitoring must be made available to others through international initiatives (e.g., OFFLU's AIM program) reaching also the medical field in a One Health approach (e.g. WHO VCM).

Anonymous reporting facilitates geographical representation of the current situation and can provide information on vaccination breakdown which must be anticipated. The process for being able to update marketed vaccines in response to antigenic changes in circulating field strains in the EU was explained and a suggestion made to adopt an efficient process already in place for rapidly updating equine influenza vaccines.

6.4. Monitoring the antigenic evolution of H5Nx to measure and enhance the efficacy of H5 vaccines

Amino acid substitutions near the receptor binding site and in antigenic sites in the globular domain of the HA protein were deemed to be positively selected to reduce antibody binding and therefore were supposed to be responsible for vaccine failures. Since HA and NA continuously change in Influenza viruses and results in antigenic drift, human influenza vaccines require frequent updates. To facilitate decision making, antigenic cartography has been developed introduced by global influenza surveillance network, organized by the World Health Organization (WHO) [32]. Antigenic cartography is a powerful method to calculate and visualize antigenic distances between vaccine strains and circulating H5Nx viruses and plays a very important role in vaccine updating decisions. More importantly, antigenic cartography can be used as a surveillance tool to measure the evolution and epidemiology of influenza viruses.

Antigenic cartography to monitor antigenic variations in H5N1 viruses have been introduced in the project "Monitoring AI virus variants in Indonesian poultry and defining an effective and sustainable vaccination strategy" from Oct 2007–September 2008 (OSRO/INS/703/USA-AUL). The project revealed antigenic differences between circulating H5N1 viruses of clade 2.1.3 and the H5 vaccines used in the Indonesian poultry industry. The findings have important implication for H5 vaccine efficacy and control of H5Nx by vaccination.

7. Field experiences on surveillance in vaccinated populations — case studies from six countries/territories

Table 2 provides a summary of the commercially vaccines used by three countries. In general, all vaccines tested were considered to be safe and efficacious. European regulators noted that vaccination programs in EU member states do not require the use of commercially licensed vaccines. However, plans to use any form of vaccine including marketed products, requires regulatory review before implementation of a vaccination program.

7.1. France

Due to unprecedented outbreaks of HPAI H5 clade 2.3.4.4b since 2016 that led to the culling of over 40 million poultry, France made the decision to vaccinate all duck flocks (Table 2) with the vaccination campaign launched in October 2023. Over 95 % duck farmers committed to the vaccination campaign with at least one dose of vaccination reported. Surveillance of the vaccinated flocks was performed in compliance with EU regulation 2023/361 [33] and included enhanced passive surveillance conducted by farmer or technician under supervision of veterinarian (bucket sampling; weekly RT-qPCR testing for M gene on 5 dead ducks per epidemiological unit), active monitoring conducted by appointed veterinarian (random sampling of 60 birds every 30 days per epidemiology unit) and serological monitoring for evidence of effectiveness also conducted by appointed veterinarian (NP ELISA on 20 birds per batch at the end of the production). A network of approved and recognized laboratories throughout France conducted the surveillance testing. The cost of vaccination and surveillance was shared between government (85 %) and farmers (15 %).

Modifications had been made to the approved vaccination and

Table 2
Commercially available vaccines used in case studies.

Country	Vaccines Used	Species vaccinated	Notes
France	Volvac BEST AI + ND -Boehringer Ingelheim (baculovirus recombinant HA)	Pekin ducks	• Vaccination of breeding ducks is not mandatory. Ducklings hatched from vaccinated flocks are excluded from export.
	Response AI H5 - Ceva (mRNA for HA)	Mule ducks	• DIVA surveillance for infection using NP ELISA serology; subcutaneous and intramuscular injections used
	Authorized for use by ANMV French Agency for Animal Medicinal Products	Muscovy ducks	• No live virus vaccines allowed even if attenuated.
The Netherlands	Vectormune AI (rHVT) Ceva	Layer flocks	• Eggs destroyed
	VAXXITEK HVT + IBD + H5 (Boehringer Ingelheim)		• Vaccines selected based on protection achieved in prior challenge/transmission challenge experiments
	Volvac BEST AI + ND -Boehringer Ingelheim		• Challenges conducted under high containment
Uruguay	Vectormune AI Ceva	Broilers	•A private-public scientific committee was assembled to evaluate ten commercially available vaccines in making their selections
	Fowlpox H5 (BIAH)	Layers	
	Boost with heat inactivated whole vaccine H5N8	Breeders	

surveillance plan after the campaign began for improvement. Based on 11-week challenge results and in response to surveillance data, which indicated a decrease of immunity against HPAI after 11 weeks of age, a third dose was added for flocks in high density farm areas during high-risk periods. A change to the passive surveillance protocol (weekly sampling of 5 dead or morbid ducks with samples to be stored at 4 °C or −20 °C, or if not possible, dispatch to a laboratory at 4 °C) was also implemented as a result to address issues related to sample availability, sample transport and storage condition.

By late September 2024, a total of 60.5 million ducks at 2295 facilities have received at least one dose of HPAI vaccination. Overall, the farmers commitment to vaccination compliance with the vaccination protocol were high and results of vaccination have been very positive. Only 10 HPAI outbreaks have been reported in the first 6 months since the vaccination campaign launched in October 2023. Seven of the HPAI outbreaks were reported in other poultry, but not in ducks. In January 2024, three HPAI outbreaks were reported in ducks, of which two of them were reported in vaccinated ducks and one was reported in unvaccinated breeder ducks. The two HPAI outbreaks in vaccinated ducks occurred in the same epidemiology unit and were related to decreased vaccine protection in ducks with low and heterogeneous level of immunity at 10 weeks of age, having as well as incomplete vaccine protection (infection of ducklings with only single dose of vaccination before completing the protective vaccination protocol). Under the AI surveillance program, nearly one million samples have been tested in the past year. While there has been some LPAIV detected in the vaccinated population, no H5 or H7 HPAIV had been detected which indicated the effectiveness of the vaccination campaign.

7.2. The Netherlands

The Netherlands do not vaccinate poultry because there is currently no endemic LPAI of any subtype and no HPAI. They have a national surveillance program to monitor every commercial poultry farm and screening of healthy wild birds is performed by academic laboratories. Suspicious deaths of birds and mammals are investigated by the Wageningen Bio Veterinary Research (WBVR). Farmers pay for the testing required under the national AIV surveillance program. Serological screening is performed for indoor poultry, pullet flocks, free range/organic birds (every quarter), turkeys (every flock) and ducks totaling at least 30 birds per sampling with a minimum of 5 samples taken per house for each type of farm.

They have a strong passive surveillance program for commercial poultry with notification mandatory under specified circumstances for each type of production primarily based on levels of mortality. Data collected over 20 years were used to establish mortality levels for chickens, ducks, turkeys and other bird flu susceptible animals, that are considered early indicators of an outbreak and warrant obligatory reporting. If there are other signs of disease, farmers are obliged to consult a veterinarian immediately. Under this science-based system, 90 % of infected flocks are culled within 24 h of notification. The farmers would get 100 % compensation for healthy birds, 50 % for sick birds and no compensation for dead birds, which encourages early reporting by the farmers.

Although they do not vaccinate against any AIV subtype, they are conducting a vaccination/challenge study using two small layer flocks vaccinated against H5 (Table 2). The aims of the study are: (1) to measure the efficacy of vaccines under field conditions (noting that prior studies in 2003 conducted in the field did not compare to experimental conditions), (2) monitor the duration of protection as measured by health, virus shedding and transmission, (3) identify duration and correlates of protection, and (4) evaluate the performance of the diagnostics. The study was started in September 2023 using three vaccinated groups and a control group at each of two small scale farms as follows:

- Group 1: Vectormune AI (rHVT) Ceva (Day 0)
- Group 2: VAXXITEK HVT + IBD + H5 Boehringer Ingelheim (Day 0)
- Group 3: VAXXITEK HVT + IBD + H5 (Day 0) + VOLVAC BEST AI + ND (Subunit) boost at 12 weeks
- Control group (unvaccinated)

The birds were tested prior to challenge by weekly RT-qPCR testing and monthly serological testing (60 samples for NP ELISA; 120 samples for H5 HI-test using homologous vaccine-like antigens). Challenges with H5 2.3.4.4b virus are scheduled for weeks (W) 8, 24, 52 and 83. Data from W8 challenge showed results similar to experimental studies and W24 data will become publicly available soon. The challenge at W52 was recently performed.

Surveillance results to date show no evidence of infection in all four groups by weekly RT-qPCR testing and ELISA results. Serological testing showed 99.76 % negative NP ELISA results for vaccinated birds, consistent with 99.79 % negative in negative control groups which indicated that the specificity of the NP ELISA test is not affected by vaccination. Despite the 0.2 % false positivity rate for the NP ELISA is very low, this will result in on average 2.9 suspicious samples per vaccinated layer flock which is not sustainable. Evaluation of antibody response to the vaccination by the HI test using H5 that is homologous to the vaccine showed that >99 % of sera were positive but using the H5 2.3.4.4b antigen, HI titers were much lower.

Rough estimates for surveillance labor costs associated with testing and farm visits (not including investigation of suspicious results) are 7500–15,000 Euros per flock per year for each farmer. With 2200 farms, surveillance would also require 32-73 additional veterinarians who are not available as there is already a shortage. Thus, especially for small farms, surveillance requirements are a barrier to vaccination uptake.

7.3. Uruguay

The Uruguayan poultry sector is characterized by a strong orientation towards the domestic market and a concentration of production within a 30 km radius of Montevideo, the capital. There are many different kinds of establishments in this area (e.g., layer farms, breeder farms, and broiler farms) with heterogenous biosecurity levels. This epidemiological situation poses a challenge for disease prevention. Outbreaks have occurred in wild birds and backyard poultry, totaling fourteen from February 2023 to March 2024. In these cases, authorities acted within 24 h to eliminate carcasses and the affected populations. The government financially compensated the producers affected by these outbreaks. Outbreaks in wildlife in a reserve including birds, terrestrial mammals as well as sea lions far from farms, have been rapidly managed by the relevant competent authorities. In response to these outbreaks, the government adopted a resolution to implement a vaccination plan. The aims were to:

- increase resistance in poultry to avian influenza viruses
- reduce the viral replication in respiratory and gastrointestinal tract of infected birds to reduce virus shedding
- prevent clinical diseases and death in poultry
- provide a complement to biosecurity measures

To determine the most appropriate vaccines for the country's reality, a public-private scientific committee was established to evaluate the available options. The evaluation mainly took into account of the similarity of vaccine strain to circulating strains, scientific information on the vaccine efficacy and capability of DIVA. Tests were also performed to determine the level of vaccine protection in the animals. A heat-inactivated H5N8 clade 2.3.4.4 whole vaccine was selected to vaccinate birds from ages 8–12 weeks (either subcutaneously in the neck or intramuscularly in the chest). A boost was required 21–28 days after the first dose. For breeders, a rHVT or an Avian pox vaccine was administered on the day of hatch (or upon arrival for imported birds). While the vaccines were free for producers at government cost, vaccination was mandatory for both light and heavy breeders (and for licensed and unlicensed commercial layer farms) and needed to be performed under supervision by trained, accredited veterinarians. Vaccination started in May 2023 and boosters were given during the rearing, growing and production phases. Considerations for continuing vaccination included the epidemiological situation, the ability to export poultry products, the availability of ongoing government support, and the appearance of antigenic variants.

To date, vaccine coverage has included all breeder and hatchery farms, and a large majority of layer farms. Active surveillance has been carried out on farms, including sampling of live birds (60 birds in pools of 10), bucket sampling (up to 10 dead birds of the day) and serological testing (11 serum samples per house) to determine the level of vaccine protection. Results of active surveillance show no evidence of HPAIV and, except for some farms, adequate levels (>1/32) of immunity in most farms by HI testing. HI titers increased over time and across all vaccination protocols.

7.4. Indonesia

HPAI poses a threat to food security in Indonesia; poultry is a major source of protein for its population. Indonesia has experienced three introductions of H5N1 viruses into the country: 2003 clade 2.1.3, 2012 clade 2.3.2.1c and 2022 clade 2.3.4.4b. By 2004 Indonesia has officially adopted vaccination against H5N1 as one of nine elements ratified as national HPAI control strategy:

1. Biosecurity improvement
2. Vaccination in endemic areas
3. Depopulation in endemic areas
4. Re-shape the poultry marketing system
5. Surveillance (tracing)
6. Restocking
7. Stamping out in newly infected area
8. Public awareness and education
9. Monitoring and evaluation

From 2003 through 2011 approved heterologous vaccines were used to control H5N1, but it was evident that vaccination using heterologous vaccination with antigenically distant vaccine seeds fail to protect. More importantly, heavily vaccinated long live poultry, such as breeders and layers, produced outlier strains in flocks that had been infected and escaped vaccine protection. This was the driver for high antigenic drift that cause vaccine escapes. Consequently, the poultry industry suffered from significant losses from 2006 to 2010. Also, infected broilers made their way into collector yards and live bird markets causing the rise in human infection and human fatalities in the cities.

The OFFLU project "Monitoring AI virus variants in Indonesian poultry and defining an effective and sustainable vaccination strategy" project introduced the antigenic cartography as a tool to select vaccine and challenge strain candidates. The project documented that the use of non-homologous vaccines in long-lived birds, such as egg-layers and breeders provided suboptimal protection and have caused a faster antigenic drift of the AIV, when experiencing concurrent challenge with the enzootic virus. OFFLU presented by 2010 the final recommendation to the Indonesia government that vaccine using Indonesian viruses, representing antigenically most AI viruses in Indonesia (antigenic match) and equipped with a high antigen content (antigenic mass) should be considered. Surveillance of circulating virus using DIVA vaccines and sentinel birds has been identified as less useful in Indonesia, as the virus was endemic and the target of vaccination using antigenically matching vaccines in combination with stringent biosecurity is the reduction of H5N1 virus load in the field.

From 2012-till now Indonesia faced the introduction of new clades of H5N1 (clade 2.3.2.1c and clade 2.3.4.4b), and in both cases matching vaccine solutions were deployed to help the country to control Avian Influenza.

After 2007, they also began collaborating in a public-private partnership working with the Indonesian government on HPAI control. In 2008 the government issued a decree for implementation of AI-free compartments requiring all major producers to, among other things, implement strict biosecurity measures, perform routine independent surveillance and monitoring of poultry for clinical signs of disease, and use of a licensed AI vaccine in the vaccination program. Surveillance monitoring data are submitted monthly into a centralized data capture system. The company's poultry health network has 200 veterinarians to perform testing and interpret the serological, molecular and antigenic cartography data.

Surveillance initially included the use of sentinel birds, but the practice was discontinued because it was not practical and posed a risk of amplifying infections. If AIV is present in the environment, unvaccinated birds can become infected more easily than vaccinated ones, serving as carriers or amplifiers of the disease. The presence of unvaccinated birds can put selective pressure on the AIV, encouraging the evolution of strains that can bypass the immunity provided by the vaccine. Also, progeny from unvaccinated sentinel birds also do not have maternal antibodies against AIV which creates problems for broiler farms since all broilers are unvaccinated in Indonesia (only breeders and layers are vaccinated) and these farms have low biosecurity. Diseased birds are culled and sold to live bird markets thereby increasing the risk of human disease.

Active surveillance is performed using the HI test with H5 and H9 antigen. Samples are taken from 30 birds per house (around 8000 birds) every five weeks. Titer results are tracked over time after vaccination from pullet phase to egg laying phase. RT-qPCR testing is also performed on the same 30 birds every five weeks. They also conduct passive

surveillance, based on depletion (mortality and culling; >0.3 % per day) and decrease in egg production and quality (>3 consecutive days; showing significant deviation from performance objectives) observed, by performing necropsies, rapid tests (lateral flow assay (LFA)) and RT-qPCR using portable PCR technology. For AIV, they perform antigenic cartography twice a year for updating the HPAI vaccine as they produce their own vaccine. As a result of their current vaccination and surveillance strategy the number of outbreaks during the rainy season has declined since 2011.

7.5. China

Slides and recordings of the presentation by China were not made available in preparation of this report. However, several references cited in the presentation were captured [34–37]. The vaccines used were developed and produced in China.

7.6. Hong Kong special administrative region, China

Hong Kong currently has 29 licensed chicken farms with varying capacity and a total maximum holding of ~1,300,000 broiler chickens, which are solely supplied for local consumption with no involvement of international trade. Sporadic outbreaks have occurred at the farm level since 1997 leading to culling of over 3.4 million birds. In 2003, a compulsory preventive HPAI vaccination campaign was implemented and since then, there has only been a single outbreak at the farm level in 2008. When the vaccination campaign was first introduced, an H5N2 European LPAIV vaccine strain was selected. Due to antigenic mismatch with the field strain of HPAIV, it was later decided in 2012 to switch to the recombinant HPAI vaccine developed by Harbin Veterinary Research Institute of China for better antigenic match with the circulating strain. Since then, a total of five vaccines developed and produced in China have been used with updates introduced once every few years as the strains have evolved and the corresponding vaccines have been progressed from monovalent to trivalent forms. The general HPAI vaccination strategy for chicken farms in Hong Kong involves the first dose at 8–10 day-old with booster at 4 weeks after the first dose. For chickens aged 120 day-old or above (e.g. breeders), a booster dose is required once every 6 months.

Surveillance is conducted at local poultry farms, in poultry markets and at other locations. Additionally, pet birds, park birds and wild birds are monitored. Dead wild birds are monitored for circulating HPAI virus strains. From 2018 to 2023, with a total of over 23,000 dead wild birds tested (representing ~30 % of surveillance testing overall), only 13 birds have been tested positive for H5 or H7 AIV. Poultry farms make up ~40 % of the surveillance testing program. It includes random sampling of 30 vaccinated birds (oropharyngeal and cloacal swabs) from each pre-sale chicken batch for H5 and H7 AIV RT-qPCR test and 30 blood samples from each batch of vaccinated birds taken at four weeks after the second vaccination for HI testing with H5 and H7 subtypes. Random blood samples are also taken from vaccinated breeders and evaluated by HI testing. Environmental sampling and testing by RT-qPCR are performed on a regular basis. Passive surveillance is performed on dead chickens during regular farm inspections. Market sales of chickens are only approved if the AI test results meet the government requirements (e.g., all samples are negative for H5 and H7 AIV by RT-qPCR and ≥70 % of blood samples from each batch of vaccinated chickens show H5 and H7 HI titers ≥1:16). Failing to meet the minimum serological titer requirement leads to revaccination of the batch.

Sentinel birds were also part of mandatory surveillance testing up until 2022 when it was abandoned. With 20 years of experience, they have determined that silent infection is unlikely to occur in well-vaccinated flocks. High titer antibody responses to well-matched antigens from circulating field strains are considered adequately protective. Limited virus shedding, if any, is very unlikely to result in sustained transmission. However, HPAIV can be introduced into unvaccinated sentinel birds creating a transmission risk, which was one of the identified risk factors that may have contributed to an HPAI outbreak occurring at the farm level in 2008 after outbreak investigation. Monitoring sentinel chickens also led to multiple false positive serological test results causing unnecessary suspension of trade and economic hardship for farmers. None of these serology-positive birds tested positive for HPAI virus by RT-qPCR.

Overall, Hong Kong has demonstrated vaccination to be an effective control measure for HPAI outbreaks and transmission in poultry. HPAI vaccination with ongoing antigenic updates will continue in Hong Kong for the foreseeable future; currently there is no exit strategy. Noted during discussion afterwards was that defining an exit strategy prior to implementation of a vaccine program has no scientific basis. Decisions to discontinue vaccination should be data driven and risk based.

8. Surveillance and trade: risk assessment

8.1. Science and statistics

Risk-based surveillance uses information about the probability of occurrence and the magnitude of biological and economic impact of health hazards to inform design of sampling strategies. In epidemiology, surveillance can be classified in terms of its purpose—determining whether disease is present or absent in a population [38]. In classic surveys, if the goal is to determine the level of disease present, then ideally the estimates of occurrence in the sample would be identical to the true proportion of occurrence in the population. Whereas in evaluating disease eradication, the goal is to discover every case of disease. In contrast, if the purpose is to determine that a disease is absent, then the goals become either to demonstrate freedom from disease (find at least one case and show not free) or to detect disease early (find the first case). The objective drives the type of analysis and sample size calculations. For surveillance of HPAI in vaccinated populations, the expected outcome is that disease is absent.

8.1.1. Surveillance for disease absence: looking for cases of infection

Demonstrating absolute freedom from disease, that is proving complete absence in the population, is impractical in most situations. Such proof could only be made possible by testing every animal using a perfect test (no false positives or negatives) and testing all animals simultaneously. Given this immutable reality a probabilistic approach compares two theoretical populations: one which is assumed to be infected at a defined hypothetical prevalence (i.e., the design prevalence, P* which can never be zero) and one that is not infected. Surveillance is then statistically powered to have a high probability of detecting positives at P*. If there are no positives found, then any disease present is presumed to be below P*.

The higher the P* value, the smaller the sample sizes needed to detect it. Conversely, if P* is low, then a large sample size is required to increase the sensitivity of the surveillance. More importantly, the P* value must be standardized to allow for comparisons of surveillance strategies and plans across studies and countries. Thus, for harmonization of outcomes to promote safe and fair trade, there must be agreement on a P* value that represents an acceptable level of infection.

Assessing freedom of disease can be done by comparing the probability distributions of the number of expected positive results in two hypothetical populations, one free from disease (prevalence equals zero) and the other with disease present at the design prevalence (P*). A cut-point number of positive results is used to assess surveillance sensitivity (the proportion of positive results from a positive population that are above the cut-point) and specificity (the proportion of negative results from a negative population that are below the cut-point).

When the specificity of our diagnostic test is 100 %, the number of positives from a disease free population will be zero, and a cut-point of 1 can be used. For a population with disease at P*, if the sample size is too small, there is a risk of not detecting it, because there is not a high

chance of including at least one of the infected animals in the sample. Moreover, if the test has imperfect sensitivity (false negative results) the likelihood of disease detection is further reduced.

For example, assuming 5 % of the population is infected (P*), the probability of observing zero positive test results using a sample size of 100 and a test with 95 % sensitivity can be calculated. The frequency of negative samples with P* for this sample size can be computed assuming a binomial distribution yielding a surveillance sensitivity of 99.23 % in the above example. This surveillance sensitivity increases to almost 100 % (99.995) if P* is assumed 10 % and other assumptions are kept constant. As the test is assumed to have perfect specificity, there is no chance of obtaining a false positive, giving a surveillance specificity of 100 %.

However, as the P* drops below 5 %, the probability of getting zero positives (while keeping sample size constant) increases and the surveillance sensitivity drops significantly (e.g., 61.5 % for P* = 1 %). The surveillance sensitivity is similarly impacted by making other changes to the assumptions in the model. Test sensitivity does not have a huge impact on surveillance sensitivity, but sample size does. For example, to increase the surveillance sensitivity of 61.5 % for P* = 1–97.8 % requires a sample size increase from 100 to 400.

These examples have assumed that the individual test has perfect specificity (100 %; no false positives). However, in practice false positives present a problem. One approach to managing them is to use confirmatory testing. By conducting independent tests in series and considering that the sample is only positive if each of the tests is positive, the combined test specificity can be greatly increased. For example, three independent tests, each with a specificity of 95 %, yield a combined specificity of 99.99 %. The tradeoff is a decrease in sensitivity. In this example, if the sensitivity of each test was also 95 %, the combined sensitivity would be 85.7 %.

An alternative approach is to recognize that false positives are inevitable when using a test with imperfect specificity, and to increase the cut point. To do this, the expected false positive rate for the test is determined and a cut point is established. By shifting away from 100 % test specificity, the theoretical disease-free population will now have less than 100 % surveillance specificity creating overlapping distributions between theoretically negative and P* infected populations. The cut-point is a chosen value above which the rate of positives is considered indicative of disease. Unfortunately, the cut point approach is rarely used in practice, as it is difficult to explain the probabilistic interpretation of freedom in the face of positive test results.

Surveillance strategies to demonstrate freedom from disease must be affordable and there needs to be increased focus on greater efficiency. Samples sizes based on representative sampling (as proposed in some regulatory frameworks) may be too large. The efficiency of surveillance to demonstrate freedom from disease can be improved using risk-based sampling, accounting for small populations, combining evidence from multiple surveillance components (while accounting for lack of independence between components) and incorporating the value of historical evidence. These advanced topics were mentioned in a presentation but not explored further. Applying the concepts of surveillance sensitivity and specificity to the monitoring of vaccinated populations to demonstrate freedom from disease must consider the fact that specificities may be low for some tests and that the prevalence of disease in a vaccinated population will be lower than in a naïve population. However, absence of disease can also be evaluated using early detection.

8.1.2. Surveillance for disease absence: seeking early detection

Early detection can be defined in different ways for different applications [39]. Disease can be presumed to be present and the metric for early detection is an increase in cases or outbreaks. For surveillance of vaccinated populations, it means finding cases at the individual level (first bird in a barn or first barn in a district flock/herd level) or first case into a country of a new strain globally. In this scenario, early must be defined at both the unit level (first wild bird, first domestic bird, first barn etc.) and based on the pathogenesis (when infected, when viremic, when sero-converted, when clinical disease, when dead). Pragmatically this means finding evidence of the disease when there is still time to prevent or minimize adverse consequences using an affordable approach.

Early detection strategies can also be designed to target a given sensitivity. This means defining a timeframe for "early" (e.g., must find evidence of disease within one week) and what proportion of incursions need to be successfully detected. As to the latter, the expectation is always "100 %" for trade to be considered acceptable yet this is virtually unachievable. Early detection surveillance sensitivity (SSe) in terms of finding the first case can be calculated:

Equation <1> SSe=CP X CT X SeD

Where CP is the percentage of population under surveillance, CT is the frequency of observations related to target time to detection (e.g. for within one week target but annual testing, the value is 1/52–0.02) and SeD is the detection sensitivity of the test.

The SSe increases with greater coverage, more frequent testing and with higher sensitivity tests. Perfect surveillance sensitivity would require the entire population to be under constant surveillance using a test that has no false positives and no false negatives. Ways to improve early detection surveillance include risk-based targeting, reduced stringency in defining early (i.e. not first case but when prevalence > P*; e.g. not first bird but first barn with 5 % prevalence) and modeling of disease dynamics. Examples of these can be found in the literature addressing early detection and early warning for African swine fever (ASF) [39] and AI [40].

Clinical and production related observations have lower sensitivity but can be used to increase efficiency.

Gains need to be balanced against costs and logistics. If testing is inexpensive then surveillance can be statistically structured to achieve sensitivity with larger sample sizes. Expensive high sensitivity tests are only practical for small sample sizes, and they don't dramatically increase overall surveillance sensitivity. More frequent testing and large sample sizes have the greatest impact on disease detection.

8.1.3. Outcome based surveillance: design considerations for emergency vs. preventive vaccination

Output based surveillance is based on the purpose of vaccination. In the EU vaccinations are categorized as preventive and emergency (further subcategorized as suppressive or protective). For emergency vaccination the aim is early detection before farm-to-farm transmission. For preventative vaccination, the aim is to maintain and prove freedom from disease. Several key questions must be addressed in designing a surveillance strategy:

- Who to sample?
- What to sample?
- How many samples?
- When to sample and how often?

Data from transmission experiments using unvaccinated and vaccinated ducks in France or chickens in the Netherlands, where vaccination did not prevent disease but reduced mortality, shed light on these questions [7]. For example, the proportions, over time, of infected, recovered and dead ducks in vaccinated flocks were compared and informs the "who and what to sample" question. For swabs taken from infected and dead ducks, RT-qPCR testing is performed and for recovered or seroconverting ducks, serological testing is done. To address the question of "how many and when", a hypothesis and assumptions need to be defined. These tie to the intended outcomes of vaccination.

For preventive, a design prevalence, P*, is used to calculate sample size as described previously. For emergency vaccination, a design reproduction number (Rh) is required. If surveillance detects infection

before transmission, then the Rh will be reduced. The aim is to reach Rh < 1 meaning the epidemic will eventually cease. Given the outbreak dynamics in ducks and the monitoring of 10 dead ducks at either weekly or bi-weekly (every two weeks), the frequency that infection is not detected, can be calculated. With weekly measuring, the Rh < 1 whereas for biweekly it is above 1. The sample size also influences this detection. As shown in Equation <1> increasing sample size can further increase the probability of detection. Confidence in detection can then be calculated:

Equation <2> Confidence = 1- [probability of escaping detection].

Modeling the data for vaccinated ducks and surveillance with five dead birds, the probability of escaping detection was shown for 7,14- and 30-day frequency. After 20 days of monitoring, the confidence in detection for 7-day frequency is nearly 100 %. That level is not reached for 14-day monitoring frequency until more than 30 days have elapsed, and not at all for 30-day sampling interval (maximum ~90 % confidence reached). All strategies achieve the same outcome (Rh < 0.4) so it is important when making decisions to consider what will be the most cost-effective means of achieving them.

For preventive vaccination, the aim is to maintain and demonstrate freedom from infection. Unlike emergency vaccination, which is used to control epidemics, in preventive vaccination, it is not known how and when infection will occur. Two papers provide details on using scenario trees to achieve confidence in freedom from infection [39,41].

From a United States Department of Agriculture (USDA) perspective, there are several considerations factored into addressing the questions surrounding vaccination surveillance as listed above. Active and passive surveillance are both still needed for vaccinated and unvaccinated birds. Active surveillance will need to be higher in vaccinated birds because with good coverage there will be fewer clinical signs and deaths. Surveillance is valuable for detecting outbreaks and providing freedom from disease but with vaccination, disease may be more difficult to identify. Geographic and industry specific vaccine usage will also inform surveillance strategies including targeting of the most at-risk groups.

Surveillance design is based on its objectives as well as the vaccine type selected (e.g., will determine whether a DIVA approach is needed), the species being vaccinated, the purpose of production and costs (test type, personnel, laboratory supplies and capacity). In the US, the National Poultry Improvement Plan (NPIP) disease surveillance program which includes AI, provides ongoing surveillance in commercial poultry and breeding facilities in all 50 states and Puerto Rico. All surveillance plans have sampling strategies statistically powered to detect 10 % prevalence with a 95 % confidence level.

Models to look at within flock transmission to predict potential HPAI in vaccinated flocks with partial immunity have been developed by USDA Veterinary Services Center for Epidemiology and Animal Health in collaboration with some universities. Applying the model to rHVT vaccines in chickens, the predicted prevalence of infection in a 100,000-bird layer flock can be simulated for sub-optimum vaccine conditions. Transmission rates were based on previously published data [42] and three groups of immunity levels:

- very poor immunity
- properly vaccinated but only partially immune
- fully protected

Preliminary results from studies at Southeastern Poultry Research Laboratories (SEPRL) suggest that virus is slower to spread and longer to detect in vaccinated flocks. This justifies targeted sampling of dead birds, and live birds with clinical signs, as a priority with 6 pooled samples (11 bird pools) tested using RT-qPCR. Results from one of the scenarios showed the fraction detected varies but increases over time after flock exposure. Additional transmissions are needed to improve the ability of the models to predict transmissibility to other flocks.

The European Commission (EC) also evaluated surveillance strategies. It asked the European Food Safety Authority (EFSA) to perform an assessment of available vaccines against HPAI in poultry. In collaboration with the European Medical Agency (EMEA) and European Reference Laboratory they published official opinions [7,8]. Evaluations were performed within the framework of the EU Regulation (2023/361) [33] and rules for both emergency and preventive vaccination. They also took into consideration WOAH resolution No.28 [5] and the requirement for surveillance to demonstrate freedom from HPAI virus circulation in all vaccinated flocks with sampling at a frequency that is proportionate to risk of HPAI.

For emergency vaccination, the first step was to define the objectives of surveillance:

- HPAIV early detection
- Assessment of vaccination effectiveness
- Demonstrating freedom from HPAIV in vaccinated establishment (to authorize movement)
- Demonstrating freedom from HPAIV in vaccinated zone

Simulations were used to quantify the reduction in infectiousness under given surveillance scenarios and to estimate the probability for an outbreak escaping detection. R_0 values were estimated to allow comparison of different surveillance strategies. A strategy was considered effective if the probability of escape detection was less than 0.01 (1 %) for more than 95 % of the outbreak simulations and the resultant R_0 value < 1.

For layers, in flocks larger than 3000, sampling dead birds ($n = 5$) at a 7-day interval was shown to be effective for early detection. Longer sampling intervals can be supported by larger sample sizes. For ducks, in flocks larger than 6000, dead birds ($n = 5$) at a 7-day sampling interval were also shown to be effective. Molecular and serological testing of live bird samples was effective for a random sample size of 60 drawn every two weeks but not recommended based on concerns associated with handling and restraint of animals. Taken together, these results led to a recommendation that for emergency vaccination molecular testing of dead birds with repeated sampling in time is appropriate.

In the context of preventive vaccination, HPAIV early detection was defined as the probability of "at least one infected establishment detected" by the surveillance. Assuming perfect specificity, freedom from HPAIV means the probability that the population is free from HPAI given that the surveillance did not detect any infected establishment. Scenario tree models without using temporal discounting were used to estimate the sensitivity of the surveillance system.

For active surveillance, the assumptions were that up to 15 dead birds would be taken from all vaccinated flocks and tested by RT-qPCR at 30-day intervals; passive surveillance performed on unvaccinated flocks served as the base line. The results showed confidence in freedom to be >99 % for three species in high-risk areas and a probability of early detection ranging from 74 to 93 %. Scenarios with variations in sampling interval and proportions of vaccinated flocks were also assessed. With sampling less than 100 %, and testing every two weeks or weekly, confidence in freedom remained above 98 % but had an impact on the early detection sensitivity.

These data resulted in a recommendation to test up to 15 dead birds every 30 days in vaccinated flocks to achieve >99 % confidence in disease freedom within high-risk zones for HPAIV infection. If the aim is to increase the early detection, then the sampling interval should be reduced; passive surveillance should be always in place and enhance the overall surveillance sensitivity. Risk mitigation measures to enable safe movement of birds were also assessed. To do this, the recommendation is to demonstrate freedom from infection by sampling up to 15 dead birds not less than 72 h prior to movement.

8.2. Economics

The objectives of surveillance not only drive the scientific and statistical design elements, but also the economic evaluation of strategies.

Two of the most widely used methods are cost benefit analysis (CBA) and cost effectiveness analysis (CEA). CBA compares both costs and benefits in purely monetary terms which allows for aggregating different types of benefits such as human lives being saved versus biodiversity. However, assigning monetary value to certain types of benefits (e.g., value of a human life) can be controversial and may need to rely on modeling (e.g., what would happen if infected birds left the market?). CEA uses effectiveness units such as disability adjusted life years (DALYs), lives saved, and infected animals prevented, to measure benefits. When applied to surveillance, examples of units include US dollars (USD) per animal health death averted, USD per virus mutation detected, and USD per infected birds leaving the market. These are used to compare one intervention to another by estimating how much it costs to gain a unit of outcome. Although CEA is less comprehensive than CBA since benefits of different natures cannot be aggregated, it is easier to conduct and can provide good information for decision making.

In 2017, a CEA was performed for early detection in live bird markets in Asia. At that time there were outbreaks of HPAI H7N9 that caused a high number of human deaths and a high case fatality rate (CFR) in China. The details and data have been published [43] and were briefly reviewed. Using portable RT-qPCR, earlier detections would mean closing live markets earlier and thus fewer infected birds would be leaving the market.

In the case presented, results showed that although increasing frequency of testing from twice a week to daily would yield 11 fewer infected birds leaving the market, the weekly cost of surveillance would increase by $1260 USD. This translates to a cost-effectiveness ratio (CER) of $114/per infected bird averted. For this information to be useful for decision making, a cost-effectiveness threshold needs to be established.

It was noted that trade concerns around surveillance are high income centric. For many countries around the world, the focus is on public health, food security and nutrition and budget constraints need to be factored into the design of surveillance. Otherwise, scientifically sound plans that are too expensive are simply reduced to practical levels (e.g., 60 birds in EU plans). Correspondingly, surveillance for vaccine monitoring must be integrated into vaccination plans and costed accordingly. OFFLU is in the process of establishing a guiding group on socioeconomics, but engaging experts from the private sector with such profile has been challenging.

It is clear that vaccination can improve animal health and welfare, food security and provide indirect benefits to humans by preventing exposure to HPAI (5). However, because poultry vaccines do not provide sterilizing immunity, there is always a chance for subclinical infections, virus excretion, risk of zoonoses, and the chance of onward to spread to other unvaccinated animals including humans. Human cases of H5 viruses have declined in China, Indonesia and Egypt which may be due to HPAI control in poultry. However, China continues to have H5N6 cases even with vaccination. Data from studies show that when vaccines are not properly matched to strains then virus shedding from challenged vaccinated animals persists [44]. Also, work from Indonesia done during vaccination campaigns suggest there was vaccine induced antigenic drift. However, there were also geo temporal patterns of antigenic shift [24].

Recently completed studies investigating antigenic diversity of H5 viruses globally showed a very high diversity. This research was undertaken to inform design of vaccines as part of pandemic preparedness to protect humans. WHO has selected 44 vaccine candidates over 25 years for emergency use in humans. National Influenza Centers are providing data to WHO collaborating offices and filtering to WHO. There is a need to integrate systems for poultry vaccination surveillance into this decision-making process. The idea is to link post vaccination surveillance in animals (e.g., systematic random sampling on vaccinated farms) to the WHO GISRS system to update candidate virus vaccines.

9. Surveillance and trade: risk management

9.1. Processes and objectives explained

Responses to the current HPAI global situation and ongoing discussions on interventions must not only address scientific aspects but also include risk management. A risk manager must be assigned to identify and ask risk questions, to mandate risk questions and formulate the risk question, and to select appropriate measures and procedures to mitigate risk within existing legal frameworks. In carrying out these tasks, the risk manager must consider factors beyond science such as the economic impacts of taking (or not taking) measures, legal aspects, cultural and political dimensions influencing various stakeholder acceptance, ecological factors and implications for trade.

For member countries of the WTO, a set of rules exists to manage trade. The SPS agreement defined the conditions under which trade may be restricted– only to protect human, animal or plant health and based on science (using WOAH standards or scientific principles and risk assessment). No arbitrary or unjustified restrictions can be imposed. Temporary restrictions can be implemented in emergency situations. In cases of disagreement, parties can use the WTO dispute process.

Good risk management rapidly yields some observable impact using interventions that are easy to understand and sustainable over time. One presenter summarized research suggesting that higher acceptance and compliance for all stakeholders is observed when:

- risks are visible (e.g., mortality)
- measures taken are not considered partial or unjustified
- decision makers respect vulnerable sub-populations
- decisions were reached by a transparent process
- alternative options clearly have been weighed

Pathways to effective HPAI control must ultimately manifest in preparedness. Countries are encouraged to use international standards (e.g., WOAH, EFSA opinions), develop and reference national surveillance and emergency response plans, and establish trusting relationships that support communication and collaboration. It is also important to be knowledgeable about industry structures and concerns, to build on capacity and competencies of the affected sector and to synergise capabilities of veterinary services across regions and administrative levels. It is critical to anticipate and plan for up-scaling capacities during an event and stay flexible. The role of science in managing risks is in “peace time” is different than during emergencies. Leveraging opportunities for research, collaboration and relationship building during peacetime can help mitigate the impacts of a crisis. Achieving equivalence of outcomes will ultimately require bilateral/multilateral engagement and involve subject matter experts across different disciplines. It was noted that “Democracy cannot dominate every domain-that would destroy expertise—and expertise cannot dominate every domain—that would destroy democracy” [45].

9.2. Compartments- british government experience

The United Kingdom (UK) is both an importing and exporting country for poultry and poultry products. They have a diverse poultry industry so managing risks efficiently and with equity is important. Geographically, the UK is part of the flyway with birds returning from eastern Europe and central Asia after the winter and there is a high risk for wild bird incursions. Over the last 20 years, there have been many outbreaks of HPAI, LPAI and two cases of Newcastle disease virus (NDV). To provide trade partners with the necessary assurances that they are disease free, there needs to be adequate biosecurity that can be consistently maintained. The UK government and industry worked together to implement a pragmatic compartment scheme in 2011. Compartmentalization is defined by WOAH as:

> " an animal subpopulation contained in one or more establishments, separated from other susceptible populations by a common biosecurity management system, and with a specific animal health status with respect to one or more infections or infestations for which the necessary surveillance, biosecurity and control measures have been applied for the purposes of international trade or disease prevention and control in a country or zone."

This requires setting up a sub-population which is in operation 24 h a day, seven days a week, 365 days a year. This is distinct from zoning. A zone is defined by WOAH as:

> ".a part of a country defined by the Veterinary Authority, containing an animal population or subpopulation with a specific animal health status with respect to an infection or infestation for the purposes of international trade or disease prevention or control."

So far, the compartment strategy has only been applied to poultry although other countries have considered compartments for swine (e.g., African Swine Fever). The UK program started small with a few businesses and has grown over time. Details have been documented in a publicly available report [46]. The standards for compartments are high and they are regularly checked through audits. The scheme was effective until outbreaks in 2022 and 2023 led to loss of status for both established compartments.

Failure investigations showed that in 2023, a single shed on an individual farm was responsible for introducing disease into the compartment. Severe weather, extreme infection pressure and people mediated factors contributed to the lapse. The infection was quickly controlled but compartment approvals were revoked. A strengthened compartment scheme has been implemented, and further approvals are pending. Getting acceptance of compartments from trading partners and achieving reputational recovery after failures can be time consuming so having recovery plans that are agreed upon in advance, improving understanding of international standards, and raising biosecurity awareness can mitigate the risk of compartment failures.

9.3. Compartments- US private sector experience

A representative from a global leader in the poultry industry that supplies broiler breeder stock to more than 100 countries presented its successes, challenges and weaknesses surrounding compartmentalization. Establishing compartments requires both robust biosecurity measures and extensive surveillance programs to effectively detect and prevent disease and ensure continuity of trade. Compartments are built to address all of the potential risks across the entire supply chain and the whole unit and thereby excellent tools to support the goal of global shipment of breeding stock. Comprehensive and routine auditing by USDA-trained auditors ensures compliance to established requirements. However, companies and regulators can have difficulty in understanding and applying the concepts of compartmentalization and regionalization.

Although both compartmentalization and regionalization aim to control disease spread and maintain trade, they differ in focus. Compartmentalization allows specific disease-free areas or facilities within a country to continue trading during disease outbreaks due to strict internal controls whereas regionalization (also called zoning) applies to geographic areas. In zoning, if a disease occurs in one part of the country, unaffected regions can still trade as long as they are geographically separated. Compartments contain an animal subpopulation with a distinct health status that is maintained through strict surveillance, control and biosecurity measures. In contrast, zones of a country are characterized by the absence of specific diseases that are clearly separated by natural, artificial and/or legal boundaries. They operate under a common control policy for specific diseases.

For primary breeders, their business is trade and thus they require uninterrupted movement of breeding stock around the world. They also have a huge responsibility to mitigate the risks to the poultry industry caused by HPAI outbreaks. As a company, they are aligned with the poultry industry's goals to control and prevent disease, enhance disease surveillance, facilitate trade, improve biosecurity measures globally, support food security worldwide, enhance consumer confidence and align with regulatory compliance requirements. Achieving these goals through compartmentalization benefits consumers but challenges prevail. The cost of implementation and maintenance is high and trading partner acceptance is not guaranteed. Reciprocity is the mutual recognition of disease-free compartments between trading countries. Lack of global standards for compartmentalization contributes to the challenge of achieving reciprocity. Loss of compartment status can also damage trust.

Given that no system can fully guarantee disease freedom, and not all risks can be known, it is to be expected that disease will sometimes occur. Detecting infection early and suffering loss of certification ultimately serves the goal of being able to distribute disease-free products around the world. Failures also provide an opportunity to improve biosecurity and surveillance programs and reduce risks of future failure. Regaining the status and rebuilding reputation is built through thorough investigation and taking effective corrective and preventive actions making compartmentalization an adaptive and self-correcting process.

The program developed to achieve an American AI clean compartment by the company was approved in 2016 through the USDA Animal and Plant Health Inspection Service (APHIS) and the NPIP. They were the first to be approved and in 2017 the program gained trading acceptance from eight countries in Asia, Africa and South America. The requirements of the program are publicly available on the NPIP website [47]. It was noted that the timelines for certification and re-certification need to be accelerated. In follow-up discussions there were differing opinions about whether and how vaccination and compartmentalization strategies could be combined in the near future.

9.4. Surveillance in vaccinated populations and safe trade: multiple perspectives

9.4.1. Nonprofit trade organization representing european poultry meat sector

Association of Poultry Processors and Poultry Trade in the EU Countries' (AVEC) members represent 95 % of poultry producers in Europe and European poultry breeders (EPB) and the European Live Poultry and Hatch Eggs Association (ELPHA). This encompasses the entire poultry chain except farmers directly. Vaccination can ensure business continuity and could reduce trade barriers (market access) and trade distortions (market vulnerability and price fluctuations) if more widely adopted.

Surveillance in Europe is performed according to a comprehensive and rigorous EU commission plan.

The plan is science-based, supported by EFSA and has received an official recognition by Singapore. However, industry faces several challenges including the availability of labs and resource limitations (veterinarians, vaccine crews). Once surveillance is operational, the resource requirements are not linear; there are seasonal peaks that must be anticipated and planned for to avoid disruptions. The surveillance plans must also get bi-lateral approval from trading partners. There is no worldwide recognition program in place and some countries still declare country specific bans (e.g., UK, USA, Canada and Japan). From a risk management perspective, decision makers were invited to consider the following questions:

- Should industry prioritize vaccination even if it means potential trade restrictions?
- Should trade considerations drive the strategy towards selective vaccination and enhanced monitoring?
- What are the tradeoffs between public health, industry needs, and international trade?

- How to implement effective surveillance in vaccinated populations to satisfy both health and trade requirements?
- How can science help make surveillance more cost efficient without jeopardizing the vaccinations?
- How does the acceptance of regionalization or compartmentalization work with AI vaccination?

9.4.2. Preventative vaccination in France

As discussed in previous sections, between 2015 and 2023 France experienced a dramatic rise in HPAI outbreaks that peaked in 2021. There was a total of 2840 outbreaks resulting in the slaughter of 40 million poultry and compensation to farmers cost 1642 million euros. The decision to vaccinate was based on multiple factors including zoonotic risk, excessive burden on public finances, moral distress of breeders, exhaustion of veterinary services and poor societal acceptance of mass killings. The decision created vaccination and surveillance program design challenges for France as an exporting country.

From the industry sector perspective, it was critical to preserve market access. For importing countries, it was important to ensure that imported products are not infected by HPAI virus. France's vaccination strategy was built using clear objectives and rules. The objective of vaccination was to slow the spread of virus and complement control measures to achieve eradication. To that end, biosecurity would remain the cornerstone of HPAI control, vaccination would not dispense with the elimination of outbreaks (i.e., vaccinated flocks can be culled if infection is confirmed) and vaccination would be compulsory except in special cases. The populations to be vaccinated were clearly delineated. Vaccination was restricted to ducks and prohibited in all other species. It was mandatory for production ducks and voluntary for breeding ducks but prohibited in breeding ducks whose products are exported.

A custom data recording system was developed to enable vaccination traceability. A reinforced post-vaccination surveillance program comprised of reinforced passive surveillance and active surveillance strategies was implemented in accordance with EU commission rules and WOAH standards. At a national level, the cost of surveillance was about 40 % of the total cost of vaccination. Prior to implementation, enhanced communication and advocacy were conducted to explain the vaccination and surveillance plans with commitment transparency or processes and supporting evidence at all stages (e.g., field trials, scientific opinions, strategy, etc.). The vaccination was launched in October 2023.

The French government have published a report containing a summary and results of the vaccination campaign [448]. From the post-vaccination surveillance program, a total of 32,371 samples of dead birds were analyzed and 822,654 samples from live birds were analyzed by RT-q PCR analysis. There were no positive HPAI virological results. There was a corresponding reduction in outbreaks (<10). Trade has been maintained with most partners, but a few countries have placed embargos on imports, so negotiations are continuing with them.

9.4.3. EU experience with HPAI vaccination

In response to increased global demand, the EU poultry meat output has increased by 44.5 % in 2007–2022 which means more animals and a larger host population susceptible to HPAI. There has been a change in the seasonal infection patterns across Europe creating a new epidemiological situation. Since 2021, there has been considerable research and limited vaccine trials conducted in France, Italy, the Netherlands and Hungary in response to country specific concerns. France conducted vaccine field trials in ducks, Italy performed laboratory vaccine efficacy studies in turkeys, the Netherlands studied vaccine efficacy in chicks for laying hens under high containment conditions and Hungary has looked at vaccine safety and efficacy in breeding geese.

Collectively these studies have shown promising safety and efficacy for some vaccines. However, in geese progeny there is a rapid decrease in maternal immunity that disappears by the third week. The latter and other issues needed to be addressed so EFSA, set up in 2002 to serve as an impartial source of scientific advice, was consulted. Based on their published opinions [8], the EU created harmonized rules on how to conduct vaccination in an organized manner and were primarily designed to protect the intra-EU trading bloc. The rules cover four main aspects:

- Types of vaccines that are allowable— Live AI vaccines (attenuated or not) are prohibited
- Reinforced surveillance— Define minimal requirements but do not dictate how to achieve them
- Risk mitigation measures— Restrictions put in place include not allowing movement of vaccinated animals out of the country because they may interfere with surveillance efforts in other countries
- Traceability/Certificate—Verify compliance

Vaccination results in France have been described in prior sections. The campaign has been considered a success based on the reduction in outbreaks. The main challenges are surveillance in all flocks which place high demand for veterinary services and laboratory testing capacity and implementation of vaccination has led to what EU considers unjustified trade bans. In terms of preparedness, the EU has the knowledge and the means to mobilize responses, and they acknowledge that change is necessary and that rules must adapt to the evolving circumstances. Although the revision process is slow, the goal is to aim for continuous improvement. The responsibilities of stakeholders were emphasized:

- Scientists need to target research in advance
- Risk assessors need to anticipate the risk assessment questions
- producers need to consider vaccination as a real option and prepare for it properly including costs and logistics.
- Traders and operators need to explore and consolidate markets.
- The pharmaceutical industry needs to prepare to supply the world market with suitable vaccines.
- Policy makers must develop modern and sustainable policies
- Regulators need to adapt legislative frameworks in a timely manner

9.4.4. HPAI control in Japan

Japan is both an importing and an exporting country. Their animal health system includes the poultry population (130 million layer chickens/1500 farms and 140 million broilers/2100 farms as of 2023), local governments (166 livestock hygiene service centers (LHSC) with 2064 veterinarians as of 2024), the Ministry of Agriculture, Forestry and Fisheries (MAFF) and the National Institute of Animal Health (NIAH). The four NIAH laboratories and 166 LHSC are distributed throughout the country. Farmers are required to report any suspicion of HPAI cases including increased mortality or atypical clinical signs. As soon as an LHSC receives the report, veterinarians dispatch to the farm, and if AI is confirmed, the farm is rapidly depopulated.

For the first time, Japan have experienced HPAI outbreaks for five consecutive years with a peak number of 84 poultry cases in 2022–23. That epidemic led to culling of 17.7 million chickens and detection of 242 cases in wild birds and two cases of infection in red fox. Farmers are fully compensated (100 % of the estimated market price) for culling when appropriate measures have been taken, but the killing of 16 million layers led to an egg shortage which created a social problem.

HPAI control measures include stamping-out, strict biosecurity, incineration and burial of culled animals and contaminated materials, cleaning and disinfection of farms, and establishment of movement and shipment restrictive zones. Preventive vaccination is not allowed. Emergency vaccination is allowed but has not been implemented to date because the rigorous Veterinary Services enables immediate detection and quick response to contain the disease by stamping-out policy without secondary spread. With regards to the situation of marketing authorization of HPAI vaccines, four HPAI vaccine products (non-DIVA) have been approved for emergency use. All are inactivated whole virus vaccines (H5 or H7 subtype) that require intramuscular or subcutaneous

delivery.

They highlighted the importance and necessity to discuss how to implement vaccination as a complementary control tool and how to carry out surveillance that can demonstrate the effectiveness of vaccination and absence of infection as described in resolution No.28 of 2023 WOAH General Session [5]. They identified several challenges that should be fully discussed in the government and with relevant stakeholders. Those include:

- How to implement vaccination: Which population? Who will perform vaccination? Who will bear the cost?
- How to implement rigorous surveillance: How to design a plan? Who will take samples? Which tests to use (no DIVA vaccine)? Who will bear the cost?
- How to adopt vaccine best practices and reassess on an ongoing basis to ensure matched strains?

Japan's exports have been negatively impacted by the HPAI outbreaks. Japan is also a major importer of live poultry, poultry meat and egg products from Europe, UK, United States, Brazil and other countries. They have suspended imports from France because of their vaccination campaign in ducks which violates an existing agreement.

Japan proposed that WOAH issue guidance to support the implementation of standards for control measures including biosecurity, vaccination and surveillance to serve both importing and exporting countries. They also suggested that WOAH develop a platform to share information on new tools and effective vaccines already approved by members, which would be helpful for other WOAH members. Given that IABS meetings have proved to be a useful mechanism for promoting global dialogue, they recommended that WOAH continue hosting them.

9.4.5. Biosecurity and bio-surveillance in Singapore

"Singapore is a 730 square kilometer city state in Southeast Asia with a population of six million with a high per capita consumption of poultry and eggs. HPAI poses a threat to public health as well as food security. Because Singapore is a transit point for migratory wild birds, they use a multilayer biosecurity system to safeguard population health. At the pre-border level, tight control of animal and animal product imports is maintained through health certificates, source accreditation, zoning and compartmentalization arrangements as well as risk assessment and horizon scanning by watching for early detection of overseas disease events. At the border, inspections are conducted on all imports of poultry, birds, eggs and avian products at the point of entry. They have quarantine centers staffed by veterinarians to house incoming animals as necessary. They work with border authorities to curb any potential smuggling and they conduct sampling and bio-surveillance at the border and post arrival.

Singapore maintains post-border biosecurity and surveillance through a multitude of activities. For example, they use satellite tracking to better understand wild bird migratory pathways. A risk-based approach to bio-surveillance is used across many sites and testing is increased during the migration season. This is underpinned by an active notifiable disease reporting system.

More broadly, bio surveillance is conducted on water and environmental samples, and vectors such as ticks and sandflies that transmit vector-borne diseases. This is part of Singapore's "Whole-of-Government" and One Health approach to bio surveillance that includes scanning and early detection, management of hosts and vectors, interagency information integration and support of science and technology. The need for One Health collaboration, including with research partners, was emphasized.

With respect to vaccination, Singapore does not conduct H5 vaccination in farms or other avian establishments during peacetime but H9 vaccination is allowed in poultry as part of production disease control. Limited, risk-based H5 vaccination is done for high-risk species kept in open exhibits such as swans in botanical gardens. H5 vaccines are stockpiled for emergency use if the threat of HPAI is imminent. All vaccines are periodically reviewed for effectiveness against circulating strains. Also, the H5 vaccination policy is periodically reviewed during peacetime and emergency situations with a focus on shifts in global attitudes towards vaccination and changes to HPAI risks with newly emerging strains.

10. Conclusions

The conclusions that emerged from all presentations and discussions were made publicly available on the IABS website in November 2024. They are as follows:

1.1. Since the last International Alliance for Biological Standardization (IABS) workshop on high pathogenicity avian influenza (HPAI) vaccination (22–23 October 2022), the H5Nx goose/Guangdong (Gs/GD) lineage of HPAI has seen substantive changes in ecology and epidemiology including extending across South America and into the Antarctic to now affect six continents; negatively impacting a diverse and large number of wild birds and mammal species including mainly carnivores but also dairy cattle; mammal-to-mammal transmission in dairy cattle, sea lions and elephant seals; and some genetic changes indicating increased risk to mammals, including humans.

1.2. Biosecurity and stamping-out have not been fully effective at preventing and controlling the H5N1 Gs/GD HPAI panzootic and the virus is persisting in and being spread by wild bird populations.

1.3. The World Organization for Animal Health (WOAH) Terrestrial Animal Health Code (Terrestrial Code) supports use of vaccination as a complementary tool in prevention and control of HPAI in poultry, and such use should not impact HPAI freedom of a country/zone/compartment when supported by appropriate surveillance.

1.4. Vaccination should facilitate safe trade by decreasing the risk of HPAI in poultry and poultry products.

1.5. Vaccination shall not be the sole measure for prevention and control of HPAI, which should also be accompanied by biosecurity measures, movement controls and stamping-out of infected flocks.

1.6. The primary barriers to increasing uptake of vaccines for HPAI in poultry include:

1.6.1. Non-tariff trade barriers on poultry and poultry products from vaccinating countries,

1.6.2. Undefined recommendations in the WOAH Terrestrial Code for appropriate surveillance to demonstrate HPAI freedom in vaccinated populations,

1.6.3. Limited global availability of vaccines against H5Nx Gs/GD HPAI,

1.6.4. Perceived high cost of vaccination and accompanying surveillance, and

1.6.5. Concerns that vaccinated farms will still have all birds destroyed even if only a single bird is found to be infected with HPAI virus.

1.7. HPAI vaccination with fit-for-purpose vaccines and appropriate surveillance can be an effective and successful tool in controlling HPAI outbreaks and stopping sustained HPAI transmission and spread based on the experience of several countries or regions.

1.8. Laboratory contact transmission studies and field experience with adequate surveillance in well-vaccinated flocks exposed to virus indicate "silent infection" (i.e., sustained transmission without birds getting sick or dying) occurs rarely in chickens.

1.9. If transmission does occur, birds with low or no protection will develop disease thus allowing detection of virus in a naturally susceptible subpopulation, i.e., daily mortality or morbidity

surveillance samples. In domestic ducks, silent infection may occur in non-vaccinated flocks.

1.10. Sustained transmission with elevated mortality can occur in vaccinated chicken flocks if the vaccine antigen does not adequately match the field challenge virus, or if the flock is poorly or sub-optimally immunized.

1.11. Vaccinated flocks are a much lower threat of having sustained infection than non-vaccinated flocks, and of transmitting the infection to other hosts because of reduced shedding and environmental contamination.

1.12. If infection does occur in a vaccinated flock, it will lead to much lower mortality than a non-vaccinated flock assuming good match between the field and vaccine strain, and good practices by the vaccinators.

1.13. Additional doses of vaccines (i.e., booster vaccinations) may be required for protection over the long production cycle of some poultry species and production systems.

1.14. Serological monitoring, by HI test using homologous antigen to circulating strains, can inform the success of the vaccination process by determining if the vaccinated birds have a uniform, protective immune response.

1.15. The use of expensive and unnecessary surveillance testing methods inhibits uptake of vaccination.

1.16. An appropriate surveillance system will draw on multiple sources of information.

1.17. Risk-based surveillance (e.g., targeting dead or clinically affected birds) is a more sensitive, cost-effective and efficient approach for early detection, especially for chickens and other species that are likely to die from HPAI, compared to random sampling of healthy birds.

1.18. Routine testing of vaccinated chicken flocks by random sampling of healthy birds is inefficient and unnecessary as other signals will be present if HPAI virus is circulating.

1.19. There are potential drawbacks and practical concerns using non-vaccinated sentinels for surveillance tool in vaccinated poultry populations based on countries' experiences.

1.20. Surveillance must consider the requirements of health certificates necessary for trading of day-old chicks, hatching eggs and meat.

1.21. Based on countries' experience, constant review on the vaccine effectiveness against the circulating field strains is necessary with a view to updating the HPAI vaccine strains as indicated.

1.22. Virological surveillance in wild birds and poultry (vaccinated and non-vaccinated) will provide isolates that can be used in determining HPAI status and assessing the national HPAI prevention and control programs and antigenic match of vaccines to circulating strains. Field viruses and their genomic information should be shared with national and WOAH avian influenza reference laboratories, and the WOAH and Food and Agriculture Organization Animal Influenza Expert Network (OFFLU) Avian Influenza Matching (AIM) program for genomic and antigenic analysis to assess protection of available inactivated vaccines, and for recommendations for timely updates to vaccines as needed.

1.23. Serological surveillance to detect infected among vaccinated animals ('DIVA') has limited use in vaccinated populations and is not an essential component of surveillance systems. It can provide evidence of freedom from infection in a retrospective manner; however, its technical limitations and interpretation need to be recognized.

1.24. Serological surveillance for demonstration of HPAI virus freedom may not be useful under certain situations, e.g., in the presence of circulating LPAI viruses in the field (especially H5, H7, and H9), with the concurrent use of LPAI vaccines, or with the use of inactivated whole virus vaccines. In addition, even with a highly specific DIVA serological test, positive results due to infection with influenza A viruses other than the target strain will regularly appear leading to unnecessary suspicion of infection which would need further testing by other serological tests or collection of additional samples for RT-qPCR testing for active virus infection.

1.25. There is a worldwide shortage of government personnel to collect surveillance samples and in government laboratory capacity to test such samples in a national or regional surveillance program.

1.26. The Terrestrial Code is well-developed and utilized in developing HPAI-free compartments, especially for poultry genetics, but importing country acceptance needs additional transparency and communication on the validity of the process.

1.27. It is evident that HPAIV vaccination can be beneficial not only for large commercial poultry producers, but also for smaller production systems and hobby flocks. In low- and middle-income countries, these flocks play a significant role in family income generation and contribute substantially to the basic supply of animal protein. In high-income countries such as in Europe and North America, the small hobby poultry flocks are a growing phenomenon.

1.28. The primary objective of vaccinating small and hobby poultry flocks is to safeguard against clinical manifestations of HPAI and to minimize the risk of infections during frequent and intensive contacts between poultry and human caretakers, especially children. This sector does not contribute to transboundary trade and has negligible risk of HPAI virus transmission to commercial poultry in high-income countries.

11. Recommendations

The recommendations that emerged from all presentations and discussions were made publicly available on the IABS website in November 2024. They are as follows:

1. Trade policy should be based on science, not politics or protectionism.
2. Molecular diagnostics, primarily quantitative real-time polymerase chain reaction (RT-qPCR), should continue to be the primary test for sensitive, specific detection of HPAIV infections during the period of active infection in both vaccinated and non-vaccinated poultry.
3. WOAH international standards with the inputs of scientists will establish mechanisms and relationships that provide assurance for best vaccination practices. Because of shortage of government personnel, surveillance samples collected by private field veterinarians or trained people should be accepted under a government accreditation program. In addition, private laboratories should be approved by the competent authorities in the member state through an auditable certification program to perform tests, allowing the results to be utilized in the official prevention and control program.
4. Virological surveillance should be conducted in wild birds and poultry (vaccinated and non-vaccinated), to support identification of HPAI virus infections for assessing successfulness of control programs and any detected viruses should undergo further evaluation.
5. Strengthening international collaboration, as well as data, virus isolate and genomic sequence sharing, should be promoted to facilitate development and deployment of efficacious vaccines and timely updates to vaccine formulations, and support implementation and updates to vaccination programs.
6. Appropriate surveillance of vaccinated poultry, to find active infections, should be risk-based, multi-layered and primarily using highly specific and sensitive RT-qPCR assays.:
   - 6.1 The approaches that are suitable for a particular context vary but should provide equivalent outcomes in terms of surveillance sensitivity and time to detection.
   - 6.2. According to a recent EFSA (European Food Safety Authority) opinion, primary samples should focus on dead birds up

to 15 per flock (i.e. "bucket surveillance" with birds collected over 24-h period and maximum of 48 h), but if insufficient numbers of dead birds are available, clinically ill birds with specific HPAI signs (e.g. neurologic signs, blue [necrotic] combs, etc.) are viable supplemental samples, and if insufficient, clinically ill birds with non-specific signs (e.g. listlessness, hunched posture, etc.) are suitable.

6.3. Bucket surveillance is an established, sensitive surveillance system for detecting early infection and confirming HPAI freedom in vaccinated and non-vaccinated poultry, especially in chickens, and utilize pooling of samples to reduce cost without loss of sensitivity.

6.4. Because of low mortality in ducks from HPAI virus infection, bucket surveillance alone may provide insufficient number of specimens and should be supplemented with birds having clinical signs and environmental samples.

6.5. Bucket surveillance should be used to detect sustained transmission. If a single positive dead bird is found, retesting should be done to confirm sustained transmission which would lead to culling.

6.6. Sampling of environmental matrices (e.g., dust, aerosols, water, etc.), for virological surveillance, if found suitable in validation studies, is a potentially sensitive and cost-effective opportunity for early HPAI virus detection in vaccinated flocks before the onset of clinical signs or mortality.

6.7. Potential environmental samples for virological surveillance could include biofilm of poultry drinkers, swabs from boots used in the poultry house, swabs of ventilation louvers, swabs of egg belts, wastewater from processing plants, eggshell wash fluid, environmental samples used for regulatory testing for salmonella, etc.

6.8. There is a lack of general utility for surveillance sampling healthy chickens in a vaccinated population, but some potential for such use in ducks.

6.9. Surveillance must be cost-effective, efficient and sustainable.

7. Serological surveillance, including use of DIVA-compatible vaccines, should only be used as part of a carefully considered surveillance strategy in vaccinated poultry, such as informing changes in a vaccination program.

8. Virological surveillance, using RT-qPCR, is DIVA-compatible with any type of vaccine

9. Undertake targeted research addressing uncertainty in surveillance and biosecurity to reassure trade partners

10. The vaccination process should be assessed by monitoring for protective humoral immune response, especially if using inactivated vaccines. This informs the program on:

10.1. Provides an independent assessment of vaccine quality

10.2. Effectiveness of the vaccination process under field conditions to induce flock level immunity,

10.3. Timing of additional or booster vaccinations,

10.4. Targeting surveillance to flocks with suboptimal immune responses because of much higher probability of infection and transmission, and

10.5. The need for investigating HPAI vaccines for antigenic updates in a timely manner

11. The potential to create vaccination subpopulations whose existence would reduce inhibition of trade in non-vaccinated poultry and poultry products, should be explored.

11.1. Inactivated vaccines that are a poor antigenic match to circulating strains should not be used and such vaccines should be updated. Vector vaccines can provide broader cross protection against antigenically diverse strains but protection against antigenically variant strains should be assessed especially if there is evidence of infection in vaccinated flocks.

11.2. The registration of vaccines should be based on dossiers including quality, safety and efficacy data. Extrapolation of data for minor species should be considered, to avoid repetition in clinical trials already completed elsewhere.

11.3. Development and adoption of multi-strain approaches in vaccine registration concepts should facilitate the exchange of vaccine antigens (e.g., replacement by new invading strains or the emergence of escape mutants) avoiding time-consuming and costly needs of a full licensing process, if the same vaccine platform is utilized. In countries where such a process exists, sharing of the expedited process between countries will accelerate adoption.

11.4. Discussions with the biologics industry should be extended to the input into the design (not only Specificity and Sensitivity) of the tools used for surveillance. For example, an external positive control should be included in RT-qPCR kits to assist the end user in validating kits to new substrates, such as environmental samples or milk, thus facilitating more rapid adapting of surveillance tools while avoid repeating development and validation by the manufacturer.

11.5. Vaccination of small and hobby poultry flocks should be considered to reduce risk of human infection, and because of low transmission risk to commercial poultry, surveillance of individual hobby poultry premises is not necessary.

11.6. Publish Conclusions and Recommendations from the meeting on the IABS website and disseminated to partners.

11.7. IABS commissions the writing of a concept paper for surveillance of vaccinated poultry populations against HPAI to be published in the peer-reviewed journal Biologicals. This will help meet the need for stronger international guidance to facilitate the updating of trade agreements when vaccination is implemented.

11.8. Based on the outcome of the concepts paper, IABS, in consultation with OFFLU experts, will propose an update to the current WOAH Standards, particularly for vaccinated poultry, and if appropriate, develop guidelines to assist countries in biosecurity, surveillance and vaccination standards in support of safe trade.

11.9. After 2 years, IABS convene a workshop on vaccination for prevention and control of HPAI, to discuss, analyze and disseminate information on the progress in surveillance development and implementation, and on utilization of vaccination for prevention and control of HPAI in poultry.

CRediT authorship contribution statement

Nancy C. Sajjadi: Conceptualization, Visualization, Writing – original draft, Writing – review & editing. **Celia Abolnik:** Writing – review & editing. **Francesca Baldinelli:** Writing – review & editing. **Ian Brown:** Writing – review & editing. **Angus Cameron:** Writing – review & editing. **Sjaak de Wit:** Writing – review & editing. **Madhur Dhingra:** Writing – review & editing. **Olivier Espeisse:** Writing – review & editing. **Jean Luc Guerrin:** Writing – review & editing. **Timm Harder:** Writing – review & editing. **Jeremy Ho:** Writing – review & editing. **Tze-Hoong Chua:** Writing – review & editing. **Khaled Hussein:** Writing – review & editing. **Nicholas Lyons:** Writing – review & editing. **Isabella Monne:** Writing – review & editing. **Yukitake Okamuro:** Writing – review & editing. **Damian Tago Pacheco:** Writing – review & editing. **Gounalan Pavade:** Writing – review & editing. **Nicolas Poncon:** Writing – review & editing. **Teguh Yodiantara Prajitno:** Writing – review & editing. **Jose Gonzales Rojas:** Writing – review & editing.

David Swayne: Writing – original draft, Writing – review & editing. **Arjan Stegeman:** Conceptualization, Writing – original draft, Writing – review & editing.

Acknowledgements

The Chair and Co-Chair of the meeting would like to thank the scientific organizing committee, moderators, speakers and participants for their constructive contributions to the conference. All of the moderators and presenters were given the opportunity to review the manuscript for accuracy. Only those who responded and agreed to acceptance of the final report have been included as authors. Please note that Francesca Baldinelli is employed with the European Food Safety Authority (EFSA) in the Unit BIOHAW that provides scientific and administrative support to the Panel on Animal Health and Welfare in the area of animal health. However, the present report is published under the sole responsibility of the author and may not be considered as an EFSA scientific output. The positions and opinions presented in this report are those of the author alone and do not necessarily represent the views or any official position or scientific works of EFSA. To learn about the views or scientific outputs of EFSA, please consult its website at http://www.efsa.europa.eu. Note also that there have been several developments in HPAI detection and disease outcomes since the close of the conference in October 2024. This report has not been updated to include those findings.

References

[1] United States Center for Disease Control [internet]. Highly pathogenic and low pathogenic avian influenza A viruses. https://www.cdc.gov/bird-flu/virus-transmission/avian-in-birds.html. [Accessed 3 March 2025].
[2] Lee DH, Criado MF, Swayne DE. Pathobiological origins and evolutionary history of highly pathogenic avian influenza viruses. Cold Spring Harb Perspect Med 2021; 11(2). https://doi.org/10.1101/cshperspect.a038679.
[3] Gross J, Volmer R, Bessiere P. High pathogenicity avian influenza emergence: blame it on chickens or on humans raising chickens? PLoS Pathog 2024;20(10): e1012608. https://doi.org/10.1371/journal.ppat.1012608.
[4] International Alliance for Biological Standardization [internet]. IABS meeting on high pathogenicity avian influenza vaccination strategies to prevent and control HPAI: removing unnecessary barriers for usages (october 2022): conclusions and recommendations. https://www.iabs.org/documents/2022-meetings-and-webinars/hpai-high-pathogenicity-avian-influenza/final-conclusions-and-recommendations-hpai/?layout=file. [Accessed 3 March 2025].
[5] World Organization of Animal Health [internet]. Strategic challenges in the global control of high pathogenicity avian influenza-WOAH Resolution No. 28. Last accessed March 3, 2025. https://www.woah.org/app/uploads/2023/06/a-resos-2023-all.pdf.
[6] Koopmans MPG, Behravesh CB, Cunningham AA, Adiasamito WB, Almuhairi S, Bilivogui P, et al. The panzootic spread of highly pathogenic avian influenza H5N1 sublineage 2.3.4.4b: a critical appraisal of One Health preparedness and prevention. Lancet Infect Dis 2024 Dec;24(12):e774–81. https://doi.org/10.1016/s1473-3099(24)00438-9.
[7] Nielsen SS, Alvarez J, Bicout DJ, Calistri P, Canali E, Drewe JA, et al., European Food Safety Authority EFSA Panel on AHAW, European Union Reference Laboratory for Avian Influenza. Vaccination of poultry against highly pathogenic avian influenza – Part 1. Available vaccines and vaccination strategies. DOAJ 2023; 21(10). https://doi.org/10.2903/j.efsa.2023.8271.
[8] Nielsen SS, Alvarez J, Bicout DJ, Calistri P, Canali E, Drewe JA, et al., European Food Safety Authority (EFSA) Panel on Animal Health and Animal Welfare (AHAW), European Union Reference Laboratory for Avian Influenza. Vaccination of poultry against highly pathogenic avian influenza – Part 2. Surveillance and mitigation measures. J. EFSA. 2024;22(4). https://doi.org/10.2903/j.efsa.2024.8755.
[9] Banyard AC, Bennison A, Byrne AMP, Reid SM, Lynton-Jenkins JG, Mollett B, et al. Detection and spread of high pathogenicity avian influenza virus H5N1 in the Antarctic Region. Nat Commun 2024;15:7433. https://doi.org/10.1038/s41467-024-51490-8.
[10] Verhagen JH, Fouchier RAM, Lewis N. Highly pathogenic avian influenza viruses at the wild–domestic bird interface in Europe: future directions for research and surveillance. Viruses 2021;13(2):212. https://doi.org/10.3390/v13020212.
[11] Paz M, Franco-Trecu V, Szteren D, Costábile A, Portela C, Bruno A, et al. Understanding the emergence of highly pathogenic avian influenza A virus H5N1 in pinnipeds: an evolutionary approach. Virus Res 2024;350:199472. https://doi.org/10.1016/j.virusres.2024.199472. ISSN 0168-1702.
[12] Caserta LC, Frye EA, Butt SL, Laverack M, Nooruzzaman M, Covaleda LM, et al. Spillover of highly pathogenic avian influenza H5N1 virus to dairy cattle. Nature 2024;634:669–76. https://doi.org/10.1038/s41586-024-07849-4.
[13] Halwe NJ, Cool K, Breithaupt A, Schön J, Trujillo JD, Nooruzzaman M, et al. H5N1 clade 2.3.4.4b dynamics in experimentally infected calves and cows. Nature 2025; 637:903–12. https://doi.org/10.1038/s41586-024-08063-y.
[14] Sacristán C, Ewbank AC, Porras PI, Pérez-Ramírez E, de la Torre A, Briones B, et al. Novel epidemiologic features of high pathogenicity avian influenza virus A H5N1 2.3.3.4b panzootic: a review. Transbound Emerg Dis 2024;(1). https://doi.org/10.1155/2024/5322378. Article ID 5322378.
[15] Li YT, Su YCF, Smith GJD. H5Nx Viruses emerged during the suppression of H5N1 virus populations in poultry. Microbiol Spectr 2021; Oct 31;9(2):e0130921. https://doi.org/10.1128/spectrum.01309-21.
[16] Harder TC, de Wit S, Gonzáles JL, Ho JH, Mulatti P, Prajitno TY, et al. Epidemiology-driven approaches to surveillance in HPAI-vaccinated poultry flocks aiming to demonstrate freedom from circulating HPAIV. Biologicals 2023;83: 10169. https://doi.org/10.1016/j.biologicals.2023.101694.
[17] World Organization of Animal Health and Global Framework for the Progressive Control of Transboundary Diseases [internet]. Technical meeting on HPAI vaccination https://rr-americas.woah.org/en/events/technical-meeting-on-hpai-vaccinationandUpdateonavianinfluenzasituationinhumansandcattlehttps://rr-americas.woah.org/en/news/update-on-avian-influenza-situation-in-humans-and-cattle/.
[18] Food and Agricultural Organization of the United Nations (FAO) [internet]. Global avian influenza viruses with zoonotic potential situation update. https://www.fao.org/animal-health/situation-updates/global-aiv-with-zoonotic-potential. [Accessed 3 March 2025].
[19] Fusaro A, Zecchin B, Giussani E, Palumbo E, Agüero-García M, Bachofen C, et al. High pathogenic avian influenza A(H5) viruses of clade 2.3.4.4b in Europe—why trends of virus evolution are more difficult to predict. Virus Evol 2024;10(1). https://doi.org/10.1093/ve/veae02.
[20] Caliendo V, Lewis NS, Pohlmann A, Baillie SR, Banyard AC, Beer M, et al. Transatlantic spread of highly pathogenic avian influenza H5N1 by wild birds from Europe to North America in 2021. Sci Rep 2022;12:11729. https://doi.org/10.1038/s41598-022-13447-z.
[21] Alkie TN, Byrne AMP, Jones MEB, Mollett BC, Bourque L, Lung O, et al. Recurring trans-Atlantic incursion of clade 2.3.4.4b H5N1 viruses by long distance migratory birds from Northern Europe to Canada in 2022/2023. Viruses 2023; Aug 30;15(9): 1836. https://doi.org/10.3390/v15091836eé. PMID: 37766243; PMCID: PMC10536465.
[22] Convention on the conservation of migratory species of wild animals (CMS) and food and agricultural organisation of the united Nations (FAO) scientific task Force on avian influenza and wild birds [internet]. Statement on H5N1 high pathogenicity avian influenza in wild birds - unprecedented conservation impacts and urgent needs. https://openknowledge.fao.org/server/api/core/bitstreams/9b880198-02d2-4eda-a860-d1a56299bc31/content. [Accessed 3 March 2025].
[23] Warren CJ, Brookes SM, Arnold ME, Irvine RM, Hansen RDE, Brown IH, et al. Assessment of survival kinetics for emergent highly pathogenic clade 2.3.4.4 H5Nx avian influenza viruses. Viruses 2024;16(6):889. https://doi.org/10.3390/v16060889.
[24] Koel BF, van der Vliet S, Burke DF, Bestebroer TM, Bharoto EE, Yasa IWW, et al. Antigenic variation of clade 2.1 H5N1 virus is determined by a few amino acid substitutions immediately adjacent to the receptor binding site. mBio 2014;5(3): e01070. https://doi.org/10.1128/mbio.01645-14.
[25] Tate MD, Job ER, Deng YM, Gunalan V, Maurer-Stroh S, Reading PC. Playing hide and seek: how glycosylation of the influenza virus hemagglutinin can modulate the immune response to infection. Viruses 2014;6(3):1294–316. https://doi.org/10.3390/v6031294.
[26] Lee J, Lee C-W, Suarez DL, Lee SA, Kim T, Spackman E. Efficacy of commercial recombinant HVT vaccines against a North American clade 2.3.4.4b H5N1 highly pathogenic avian influenza virus in chickens. PLoS One 2024;19(7):e0307100. https://doi.org/10.1371/journal.pone.0307100.
[27] Filaire F, Lebre L, Foret-Lucas C, Vergne T, Daniel P, Lelièvre A, et al. Highly pathogenic avian influenza A(H5N8) clade 2.3.4.4b virus in dust samples from poultry farms, France, 2021. Emerg Infect Dis 2022;28(7):1446–50. https://doi.org/10.3201/eid2807.212247.
[28] Bessière P, Hayes B, Filaire F, Lèbre L, Vergne T, Pinson M, Croville G, et al. Optimizing environmental viral surveillance: bovine serum albumin increases RT-qPCR sensitivity for high pathogenicity avian influenza H5Nx virus detection from dust samples. Microbiol Spectr 2023;11(6):e0305523. https://doi.org/10.1128/spectrum.03055-23.
[29] Peng Y, Li X, Zhou H, Wu A, Dong L, Zhang Y, et al. Continual antigenic diversification in China leads to global antigenic complexity of avian influenza H5N1 viruses. Sci Rep 2017;(7). https://doi.org/10.1038/srep43566.
[30] Milani A, Fusaro A, Bonfante F, Zamperin G, Salviatob A, Mancin M, et al. Vaccine immune pressure influences viral population complexity of avian influenza virus during infection. Vet Microbiol 2017;203:88–94. https://doi.org/10.1016/j.vetmic.2017.02.016. ISSN 0378-1135.
[31] Luczo JM, Spackman E. Epitopes in the HA and NA of H5 and H7 avian influenza viruses that are important for antigenic drift. FEMS Microbiol Rev 2024;48(3). https://doi.org/10.1093/femsre/fuae014.
[32] French Ministry of Agriculture and Food Sovereignty [internet]. Report- [HPAI Vaccination Campaign October 2023 -March 2024]. Last accessed April 1, 2025 from: English contents | Ministère de l'Agriculture et de la Souveraineté alimentaire.
[33] Eur-Lex Access to European Law [internet]. Rules for the use of certain veterinary medicinal products for the purpose of prevention and control of certain listed

diseases. Last accessed March 3, 2025. https://eur-lex.europa.eu/eli/reg_del/2023/361/oj.
[34] Chen H, Deng G, Li Z, Tian G, Li Y, Jiao P, et al. The evolution of H5N1 influenza viruses in ducks in southern China. Proc Natl Acad Sci 2004;101(28):10452–7. https://doi.org/10.1073/pnas.0403212101.
[35] Mingxian C, Yanming H, Xingbo W, Xiyi B, Liuyang D, Yan Y, et al. Genetic characterization and evolution of H6N6 subtype avian influenza viruses. Front Microbiol 2022;13. https://doi.org/10.3389/fmicb.2022.963218.
[36] Tian G, Zhang S, Li Y, Bu Z, Liu P, Zhou J, et al. Protective efficacy in chickens, geese and ducks of an H5N1-inactivated vaccine developed by reverse genetics. Virology 2005;341(1):153–62. https://doi.org/10.1016/j.virol.2005.07.011.
[37] Liu J, Chen P, Jiang Y, Wu L, Zeng X, Tian G, et al. A duck enteritis virus-vectored bivalent live vaccine provides fast and complete protection against H5N1 avian influenza virus infection in ducks. J Virol 2011;85. https://doi.org/10.1128/jvi.05420-11.
[38] Cameron AR, Meyer A, Faverjon C, Mackenzie C. Quantification of the sensitivity of early detection surveillance. Transbound Emerg Dis 2020;67(6):2532–43. https://doi.org/10.1111/tbed.13598. Epub 2020 May 14. PMID: 32337798; PMCID: PMC7267659.
[39] Faverjon C, Meyer A, Howden K, Long K, Peters L, Cameron A. Risk-based early detection system of African Swine Fever using mortality thresholds. Transbound Emerg Dis 2021;68(3):1151–61. https://doi.org/10.1111/tbed.13765. Epub 2020 Aug 16. PMID: 32748561.
[40] Gargallo G, Davies JG, Faverjon C, Kampichler C, Baillie SR, Cameron A, et al. Development of a prototype early warning system for avian influenza in the EU based on risk-mapping. EFSA Support. Publications 2022. https://doi.org/10.2903/sp.efsa.2022.EN-7762.
[41] Martin PA, Cameron AR, Greiner M. Demonstrating freedom from disease using multiple complex data sources 1: a new methodology based on scenario trees. Prev Vet Med 2007;79(2–4):71–9. https://doi.org/10.1016/j.prevetmed.2006.09.008. Epub 2007 Jan 16. PMID: 17224193.
[42] Rauw F, Palya V, Van Borm S, Welby S, Tatar-Kis T, Gardin T, et al. Further evidence of antigenic drift and protective efficacy afforded by a recombinant HVT-H5 vaccine against challenge with two antigenically divergent Egyptian clade 2.2.1 HPAI H5N1 strains. Vaccine 2011;29(14):2590–600. https://doi.org/10.1016/j.vaccine.2011.01.048.
[43] Guinat C, Tago D, Corre T, Selinger C, Djidjou-Demasse R, Paul M, et al. Optimizing the early detection of low pathogenic avian influenza H7N9 virus in live bird markets. J R Soc Interface 2021;18. https://doi.org/10.1098/rsif.2021.0074.
[44] Swayne DE, Suarez DL, Spackman E, Jadhao S, Dauphin G, Kim-Torchetti K, et al. Antibody titer has positive predictive value for vaccine protection against challenge with natural antigenic-drift variants of H5N1 high-pathogenicity avian influenza viruses from Indonesia. J Virol 2015;89(7):3746–62. https://doi.org/10.1128/jvi.00025-15.
[45] Collins H, Evans R, Durant D, Wiesel M. Experts and the will of the people-society, populism and science. Palgrave Pivot Charm; 2020. https://doi.org/10.1007/978-3-030-26983-8. eBook ISBN978-3-030-26983-8. Published October 11, 2019.
[46] United Kingdom Department of Environment, Food & Rural Affairs [internet]. Report-compartments for the protection against avian influenza and Newcastle disease in poultry breeding companies in great britain. https://britishpoultry.org.uk/wp-content/uploads/2024/10/Review-Schedules-Poultry-Compartments-FINAL-APRIL-2024-CLEAN-COPY.pdf. [Accessed 3 March 2025].
[47] United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) [internet]. National Poultry Improvement Plan (NPIP): Compartmentalization for protection against Avian Influenza and/or Newcastle disease in primary poultry breeding in the United States of America- Specifications For Management Guidelines and Protocols. Last accessed March 3, 2025. https://www.poultryimprovement.org/documents/SubpartF-Compartmentalization-2021.pdf.