PROPOSED U.S. POULTRY AND EGG INDUSTRY VACCINATION PLAN FOR HIGHLY PATHOGENIC AVIAN INFLUENZA (HPAI)

Submitted by the HPAI Vaccination Working Group Convened by U.S. Egg Producers as represented by United Egg Producers and American Egg Board 1 April, 2025

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Executive Summary

U.S. egg producers continue to be severely affected by the current H5N1 high pathogenicity avian influenza (HPAI) outbreak that began in 2022. As of April 1st, 2025, over 131 million egg laying hens from over 130 farms have been depopulated due to infection with HPAI (1).

High environmental contamination of H5N1HPAI virus from diverse wild birds, as well as wild and domestic mammals (2), have produced an ongoing risk for exposure, infection, severe illness and death in egg laying chickens due to their high susceptibility to infection. Enhancements to on-farm biosecurity have reduced poultry-farm-to-poultry-farm transmission of the virus, but the infection, amplification and dissemination of HPAI by many species of migratory and resident wild birds serves as an ongoing source of virus. In addition, outbreaks of HPAI virus in dairy cattle have served as a source of infection to layer flocks.

Egg producers' investments in enhanced biosecurity measures implemented since the 2014-2015 HPAI outbreak, along with mass culling of poultry, are no longer adequate to prevent and control this devastating disease (3). Additionally, depopulation of infected flocks has resulted in shortages of eggs for consumers.

It is time for a new approach to reduce layer and pullet flock susceptibility to HPAI virus by considering a vaccination strategy that can increase resistance to HPAI virus infection, reduce viral shedding, and importantly, reduce the risk of a potential mutation event that may lead to further HPAI infection in humans.

Vaccination Implementation

HPAI vaccination would serve as a complementary tool to existing biosecurity practices to better prevent outbreaks and control the disease. Use of safe, pure, potent, efficacious, U.S. Department of Agriculture (USDA)-licensed vaccines, when applied appropriately to egg type chickens, will increase resistance to becoming infected by the H5N1 HPAI virus. Additionally, vaccination will reduce virus shedding and thus decrease environmental contamination, reducing potential transmission, and overall outbreaks of HPAI in flocks (2).

An HPAI vaccination program should be implemented using Avian Influenza Vaccine and Vaccination Stewardship practices (4) and utilizing only USDA-licensed H5 avian influenza vaccines. Currently, several USDA licensed vaccines are available that have demonstrated experimental efficacy and field effectiveness against the 2.3.4.4b H5N1 HPAI virus circulating in the U.S. These licensed products include an inactivated avian influenza vaccine, several recombinant herpesvirus of turkeys vaccines with H5 avian influenza gene inserts (rHVT-H5), and an RNA particle vaccine (5). Manufacturing capacity, storage and

distribution logistics and vaccination methods and practice to accurately apply these vaccines to egg layer type chickens are currently available and utilized in the U.S.

Utilizing an emergency vaccination program according to international standards (6), the plan would focus on vaccination of day-old chicks in the hatchery with a rHVT-H5 vaccine, plus a boost during the pullet grow-out phase with a non-replicating H5 vaccine. This is an intermediate-to-long-term strategy to achieve individual flock immunity over a 12-18-month period with vaccinated pullets replacing non-vaccinated hens through a production phased response. Alternatively, pullets in grow-out could be vaccinated with two or three doses of non-replicating H5 vaccine.

Vaccination of chicks in-hatchery and pullets in grow-out phase is more logistically feasible in comparison to an emergency vaccination of hens *in-lay*, which is challenging in caged layers and potentially unachievable in cage-free operations due to difficulty in catching individual birds for vaccination. All currently USDA-licensed vaccines for H5 avian influenza require injection (*in ovo*, subcutaneous or intramuscular) to individual birds.

Regional Approach

Vaccination for HPAI has not historically been well accepted internationally; however, use of a targeted regional approach through defined "vaccination zones" may be a highly effective tool for maintaining trade for poultry and poultry products. At the beginning of the 2014 outbreak of HPAI in the United States, USDA-Animal Health Inspection Service (APHIS), Veterinary Services (VS) had a few agreements to zone out infected flocks to maintain trade with our trading partners (7). USDA currently has approximately 80 such zoning agreements with trading partners. Therefore, use of a targeted approach with vaccinated flocks will allow USDA-APHIS to present the vaccination plan to trading partners prior to implementation to ensure that no significant reduction to the export market will occur.

The initial approach for vaccination for the current outbreak should be to define vaccination zones by state or county through a risk-based prioritization program. Within a region, only egg laying type chickens and turkeys would be vaccinated. Meat-type chickens (broilers) or their breeding stocks, and genetic stocks of all poultry species would not be vaccinated without further planning and stakeholder engagement. It should be noted that the location of vaccination for a given flock may encompass multiple states/counties as layer pullets are only hatched in specific locations and distributed across the country and pullets may be grown in facilities distant from their scheduled placement on layer farms

Vaccination Monitoring and Surveillance

Effectiveness of the vaccination plan should be monitored to ensure flock immunity is achieved. Monitoring vaccination effectiveness can be achieved by testing serum from a sample of vaccinated chickens for specific H5 antibodies or expression of rHVT-H5 vaccine in feather follicles or the spleen to assess the success of the vaccination program to provide protection.

Surveillance should be performed to identify HPAI infections, and must be cost-effective, efficient, and sustainable (8). Appropriate surveillance of vaccinated poultry is risk-based, multi-layered and primarily virological in nature to find any potential active virus infections. Surveillance should focus on samples from dead chickens, supplemented by clinically ill birds and specific environmental samples with validation. Currently, such testing is in use in control zones to monitor premises near an infected farm, in conjunction with USDA's National Animal Health Laboratory Network (NAHLN). Vaccinated flocks with active virus infections may occur rarely and will be depopulated.

Serological surveillance to find antibody evidence of HPAI virus infection may also have some limited utility for annual reassessment to inform any changes to the vaccination program (8). Importantly, the currently available USDA-licensed rHVT-H5 and RNA vaccines provide a significant advantage for serological surveillance, as this can be achieved through existing commercial tests, as opposed to inactivated virus vaccines that would need a validated anti-neuraminidase serology test to differentiate antibodies induced by the vaccine from those induced by HPAI virus infection.

Impact on Human Exposure and Zoonotic Potential

In the U.S., a few cases of human infection have been associated with occupational exposure in poultry depopulation crews. Use of poultry vaccination against HPAI in other countries has demonstrated drastic reduction in human exposure to the HPAI virus and subsequent development of fewer human infections. The use of vaccination in the U.S. poultry and egg industry will reduce human infection risks to culling crews by decreasing the number of infected flocks needing depopulation and the quantity of HPAI virus contamination into the poultry environment.

Executive Summary Conclusion

This proposed HPAI vaccination plan for the U.S. poultry and egg industry is submitted on behalf of U.S. egg producers, as represented by United Egg Producers and American Egg Board. More specific implementation details as part of a final plan will be developed in collaboration with leadership from USDA, state agriculture agencies, and other livestock producers.

Introduction

Wild waterfowl have historically been natural asymptomatic carriers of low pathogenicity avian influenza (LPAI) viruses. Some H5 and H7 LPAI viruses have mutated to become the H5 and H7 high pathogenicity avian influenza (HPAI) viruses that cause extreme mortality in domestic poultry species. Between 1959 and 2023, most HPAI viruses were controlled and eradicated from commercial poultry by enhanced biosecurity, rapid surveillance and implementation of stamping-out programs that included indemnification for affected flocks (2). A specific H5 HPAI virus arose through mutation in Asia over 29 years ago. The Goose/Guandong (Gs/GD) lineage virus has expanded its host range and spread globally to six continents via wild aquatic birds including waterfowl and shorebirds. Wild bird populations are shedding large quantities of virus into the environment and have varying clinical presentations, ranging from asymptomatic infections to severe disease and death.

The emergence of H5 HPAI in the U.S. from wild waterfowl in 2014 resulted in the worst animal health disaster in the history of the country. The 2014-15 U.S. HPAI outbreak precipitated significant investments in egg and pullet farm biosecurity due to common lateral spread of virus between premises. Additionally, the industry implemented the U.S. Department of Agriculture (USDA) National Poultry Improvement Plan (NPIP) Program Standards for Biosecurity Principles including audits to reduce the potential for virus introduction and lateral spread between farms (9).

Unfortunately, since 2021, continuous infections of migratory birds have disseminated the highly contagious H5N1 2.3.4.4b clade HPAI virus through all the major flyways in the world. As a result, major repeat losses are occurring in poultry flocks in many countries. In the U.S., the virus has resulted in extreme losses in commercial meat turkey and egg production flocks.

Despite efforts to enhance structural, operational, and cultural biosecurity on farms, the direct introduction of HPAI virus (via wild birds or environmental contamination near barns) to pullet and layer flocks are still occurring at a rapid pace. Emergence of H5N1 B3.13 genotype HPAI virus in dairy cattle has also complicated the situation (increased biosecurity risks) by amplifying viral load that can spread from dairy farms to poultry flocks. Furthermore, the detection of HPAI virus in additional wild and peri-domestic bird species and spillover to wild and captive mammals demonstrate the situation is ever expanding and becoming untenable (10). We are facing a new paradigm where both wild waterfowl and other animal species perpetuate and shed HPAI virus in areas where poultry production occurs.

Proposed HPAI Vaccination Plan

U.S. egg producers continue to be severely affected by the H5N1 HPAI outbreak that began in 2022. As of April 1st, 2025, over 131 million egg laying hens from over 130 farms have been depopulated due to infection with HPAI (1). Depopulation of infected flocks has resulted in shortages of eggs for consumers. To reduce the welfare and economic impact of HPAI on egg layer flocks, an emergency response vaccination must be considered to complement existing biosecurity practices and prevent infections and outbreaks.

The World Organization for Animal Health (WOAH) states that to control HPAI, policies should consider biosecurity, surveillance, indemnification schemes, and under certain specific conditions, vaccination of poultry may be recommended (3). Vaccination would serve as a complementary tool to existing biosecurity practices to better prevent outbreaks and control HPAI. Use of safe, pure, potent, efficacious, USDA-licensed vaccines, applied appropriately to egg type chickens, will have the following benefits (2):

- Increase resistance of chickens to infection by requiring a high quantity of virus exposure to cause infection.
- Reduce the number of flocks that become infected and must be depopulated, safeguarding bird welfare and stabilizing the overall egg supply.
- The few birds that may get infected will have decreased quantity of virus shedding into the environment, reducing risk of lateral transmission.
- Reduced replication of the HPAI virus in egg-laying flocks will lower the risk of infections in workers and decrease risk for potential mutational events that may lead to further HPAI infection in humans.

Vaccination Goals and Implementation

Vaccination Goals

This vaccination plan is designed to address the unique situation of the H5N1 clade 2.3.4.4b of the Eurasian Gs/GD lineage of HPAI that has created the global panzootic including the U.S. since 2022. This plan would not be applicable to emergent H5 and H7 HPAI outbreaks that are caught early after a LPAI to HPAI mutation, such as the H7N3 HPAI event that occurred in South Carolina turkey flocks in 2020 or the H7N9 HPAI event in Mississippi broiler breeders in 2025. Rapid diagnosis and surveillance of the South Carolina H7N3 outbreak in turkeys allowed for stamping-out programs to fully eradicate H7N3 from the index farm. However, this vaccination plan could be applicable to future HPAI outbreaks that exceed the capacity of stamping-out programs to achieve eradication in a short time frame, as has proven to be the case with the H5N1 clade 2.3.4.4b.

Vaccine and Vaccination Stewardship

The decision to vaccinate poultry to protect against HPAI necessitates a review of the national, regional and local conditions, including:

- 1) Assess whether biosecurity, surveillance and stamping-out methods in place are sufficient to prevent and control HPAI virus infection and if their use is sustainable.
- 2) Assess likelihood of virus elimination with the addition of vaccination as a complementary tool.
- 3) A plan to regularly assess progress on disease control and adjust any vaccination program accordingly.

After vaccination approval, the success of vaccines and their application through vaccination against HPAI requires the utilization of the 12 principles of Avian Influenza Vaccine Stewardship (4). These principles conceptually rely upon the use of best practices, transparency, rigor, and individual and organizational responsibility. The 12 principles include:

- 1) Vaccines should not be used as a replacement or substitute for other methods of disease prevention such as biosecurity, surveillance and stamping-out, but added as a complementary tool for an additional layer of protection.
- 2) The decision to use vaccine is just the beginning of the process, not the end, with ongoing requirements for assessment of the vaccination process at the individual farm level, veterinary medical oversight, collection and reporting of data and interaction with regulatory authorities.
- 3) Selection of appropriate vaccines that will provide protection against the circulating field viruses.
- 4) Vaccines should be used in accordance with the vaccine manufacturer's label instructions regarding age and dose of administration.
- 5) Selected vaccinated flocks should be monitored to ensure the vaccine is producing the desired immune response and for planning the timing of potential boosters, if required.
- 6) Surveillance is necessary to not only identify potential infections, but also to further analyze and sequence the virus for antigenic analysis to determine if an update to the vaccine is required.

- 7) Be aware of the possible introduction of novel 2.3.4.4b antigenic variants, new genotypes, and new subtypes from wild birds and adjust diagnostics and surveillance systems for early recognition and assessment.
- 8) Remove licensure of vaccines that no longer afford protection from disease and virus shedding.
- 9) Ensure vaccination is performed in a manner that does not transmit the virus.
- 10) Regularly (annually) re-assess the need for and nature of vaccine programs and modify the programs accordingly.
- 11) Special attention should be given to farms or locations where infection occurs or persists, despite appropriate usage of vaccines.
- 12) Examine ways to modify production practices that may facilitate transmission and replication of the virus.

Licensed Vaccines

An HPAI vaccination program should only utilize USDA-licensed (or conditionally licensed) H5 avian influenza vaccines. Several H5 vaccines have USDA-license (5) in two broad categories: Live Vectored and Non-Live (inactivated and non-replicating) vaccines. The USDA-licensed vaccines include:

- 1) H5 subtype, DNA (1057.D0),
- 2) H5N1 subtype, killed virus (1057.R1),
- 3) H5N3 subtype, killed virus (1057.R3),
- 4) RNA (1905.D0),
- 5) H5 subtype, serotype 3, live Marek's Disease Vector (1L81.R0, avian influenzabursal disease-Marek's Disease Vaccine),
- 6) H5 subtype, live fowl pox vector (1061.R0, avian influenza fowl pox vaccine), and
- 7) H5 subtype, serotype 3, live Marek's disease vector (1062.R0, avian influenza Marek's disease vaccine).

In addition to the above vaccines, in late February 2025, an H5N2 subtype, killed virus vaccine with a 2.3.4.4b clade seed strain received a conditional license from USDA (11).

Currently, some of the USDA licensed vaccines have demonstrated experimental efficacy in chickens against the 2.3.4.4b H5N1 HPAI virus circulating in the U.S., including one

inactivated avian influenza vaccine, two rHVT-H5, and an RNA vaccine (12,13). Additional experiments in Europe support efficacy of the two rHVT-H5 vaccines (Belgium, Dutch and Italian studies) for chickens and turkeys (14,15,16). The USDA-licensed vaccines have shown to be efficacious against the H5N1 2.3.4.4b clade HPAI viruses and fit-for-purpose in egg-type chickens and their commercial productions systems. Two additional vaccines – self-amplifying RNA (2.3.4.4b clade) and hemagglutinin subunit (2.3.2 clade) - have been licensed and used successfully in ducks in France against the H5N1 2.3.4.4b clade of HPAI (17).

Manufacturing capacity, storage, distribution logistics, vaccination methods and experience to accurately apply these vaccines to egg layer type chickens are currently available in the U.S. Today, various live and killed vaccines are routinely applied to protect and keep egg type chickens healthy from domestic diseases such as infectious bursal disease, infectious bronchitis, Newcastle disease, and others (18).

Furthermore, many experimental vaccines are in various early stages of development and exploration. These new vaccines could be included if they receive USDA-licensure based on safety, purity, potency and efficacy against an H5 2.3.4.4b challenge HPAI virus and have demonstrated commercial production and distribution capacity.

Vaccination Implementation

Vaccination can be applied in a systematic or emergency program (6). This proposed plan would use emergency vaccination as an adjunct to the application of other essential biosecurity and disease control measures to reduce and control ongoing HPAI outbreaks. The emergency vaccination will follow a risk-based strategy with application to layer and layer pullet populations with highest risk of H5N1 HPAI infection in specific high-risk geographic areas. The program will not vaccinate all poultry types, nor be a nationwide vaccination program. Chicken broilers and their breeders are intentionally excluded from the vaccination plan. Poultry eggs and egg products for export will come from nonvaccinated flocks. The emergency vaccination program should utilize international standards as a targeted vaccination strategy (6).

Vaccination of egg-type chicks *in-hatchery* and pullets in grow-out phase is more logistically feasible in comparison to an emergency vaccination of hens *in-lay*. Vaccination of caged *in-lay* hens is challenging and potentially unachievable in cage-free operations due to difficulty in catching individual birds for vaccination. All currently USDA-licensed vaccines for H5 avian influenza require injection (*in-ovo*, subcutaneous [SQ], or intramuscular [IM]) into individual birds. All vaccines should be utilized at the recommended manufacturer's dose and age of application.

Proposed vaccination schedule for egg layer pullets		
Prime	Day 0 (or <i>in-ovo</i> , 18-days of incubation)	rHVT-H5 vaccine via SQ injection
Boost	Between weeks 7-15*	Inactivated or non-replicating H5 vaccine via IM or SQ injection

*Delay boost as late as feasible to prolong immunity conferred, but before onset of egg production. Follow vaccine manufacturer product labels for age and route of administration.

This proposed plan focuses on vaccination of day-old layer pullet chicks in the hatchery using existing infrastructure and routine vaccination practices along with a boost vaccination during the pullet grow-out phase following normal industry protocols with an inactivated or non-replicating H5 vaccine. This is an intermediate-to-long-term strategy to achieve individual flock immunity over a 12-18-month period with vaccinated pullets replacing non-vaccinated hens through a production phased response. Alternatively, pullets in grow-out could be vaccinated SQ or IM with at least two doses of inactivated or non-replicating H5 vaccine.

Regional Approach

Establishing HPAI-free zones and compartments during outbreaks has been a highly effective tool for maintaining trade for poultry and poultry products in the U.S. and worldwide (19). At the beginning of the 2014 outbreak of HPAI in the U.S., USDA-Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) developed several such zoning agreements with our trading partners, and by the end of 2015, there were 14 agreements in place (7). USDA currently has approximately 80 such zoning agreements with trading partners.

The size of HPAI containment zone restrictions placed by a country can be 10 kilometers, a county or at a state level. Many existing zoning agreements are at a county level. Veterinary Services can use a similar approach to discuss the use of avian influenza vaccine with a zoning approach to ensure ongoing trade outside the vaccination zone (8). These *vaccination zones* would not be allowed to ship product internationally from vaccinated flocks unless trading partners are willing to accept products and animals from vaccinated flocks. Unvaccinated flocks and their products within the zone should be free to move internationally in accordance with existing agreements.

We strongly recommend that USDA-APHIS-VS develop a zoning-based vaccination plan that will be shared with our trading partners to reduce losses in the egg and turkey industries in the U.S. We recommend USDA initially limit vaccination to states with appropriate hatcheries, pullet rearing farms, and lay farms to complete vaccination protocols and monitor vaccinated flocks. States in the upper Midwest and Mid-Atlantic regions have appropriate layer and turkey production infrastructure and historically significant losses due to HPAI. Upper Midwest and Mid-Atlantic states would therefore be a logical starting point for the initial vaccination zones. Additional counties or states may seek approval based on bird movements and local risk assessments. This regional approach supports a manageable rollout to consult trading partners, secure the initial vaccine doses, develop vaccine and vaccination program logistics and tracing, establish surveillance protocols, and prepare diagnostic assay capacity in the NAHLN.

Acceptance by trading partners is critically important for the success of this approach to vaccination. The vaccination plan should be presented to trading partners prior to vaccine implementation to ensure that no significant reduction to the export market occurs. In discussions with trading partners, USDA should place emphasis on the vaccination plan being restricted to specific commodities (eggs and turkeys), and the smallest zones possible, such as county level. Any flock determined to be HPAI virus positive will be depopulated.

Vaccination Monitoring and Surveillance

Vaccination for avian influenza has not been well accepted internationally because of the possibility of subclinical infection; therefore, it is necessary to have appropriate surveillance to assure importing countries and consumers that vaccinated flocks are free from HPAI virus.

Monitoring for Effective Vaccination (Vaccination Excellence)

The success of the vaccination program will be assessed to determine the development of protective immunity which translates into effective herd immunity against HPAI infections and their sustained transmission. Circulating (humoral) antibodies against the hemagglutinin protein are the foundation of protective immunity produced by all types of avian influenza vaccines and are assessed primarily by hemagglutinin inhibition assay (20). In addition, other tests such as H5-ELISA have been developed, validated and licensed in some countries and are amenable to high-throughput serum sample testing and analysis (21). With live-vectored vaccines, additional protection is produced through cellular and

mucosal immunity. Routine laboratory assessment of such immunity is not practical but can be accomplished on an *ad hoc* basis in research studies.

This assessment of humoral immunity is an established, laboratory correlate of protection, both for assessing the vaccination process (number of chickens with immune response) and the level of protection (specificity and magnitude of the antibody response). Analysis of the collective data is critical in establishing flock ("herd") immunity of the farm and informs the timing for the need of any booster vaccinations. For rHVT-H5 vaccines, success of vaccination can be assessed by measuring the replication of the vaccine virus in feather follicles or spleen.

Serological monitoring of vaccinated flocks will require additional discussion with USDA, diagnostic laboratories and manufacturers of diagnostic assays.

Surveillance for HPAI Virus Infection

The U.S. has a National Avian Influenza Surveillance System (NAISS) administered by USDA-APHIS-VS in partnership with other Federal and State agencies and the commercial poultry industry (22). This system utilizes the NPIP, Live-Bird Marketing System (LBMS) and other State-sponsored programs to generate data in support of determining HPAI-free status and to detect infections that result in actions to eradicate (9). The core laboratory support comes from NAHLN and the National Veterinary Services Laboratories. This system conducts virological and serological surveillance in non-vaccinated commercial poultry, backyard poultry and LBMS for both HPAI and H5/H7 LPAI. The existing assays (both commercial and accredited laboratory assays), NAHLN laboratory workforce capacity, and USDA-APHIS-VS logistics should be utilized for HPAI surveillance in vaccinated flocks in control zones near an HPAI infected poultry premise have been established by the Secure Poultry Supply Plan (23). States have implemented the flock sampling protocols which may be adapted to monitoring vaccinated flocks for infection.

Global Guidance on Vaccine Surveillance

At the global level, WOAH provides guidelines for declaration of country, zone or compartment to be HPAI-free based on surveillance to demonstrate absence of infection (24). The use of vaccination is recommended under specific conditions, as a complementary tool when stamping-out policy alone is not sufficient, and the use of vaccination should not affect a countries' HPAI status as free country or zone if surveillance supports the absence of infection. These principles were reinforced in a recent WOAH policy briefing that stated, "...stricter biosecurity measures and mass culling of poultry may no longer be sufficient to control the disease," and "...poultry vaccination can no longer be excluded from the available alternatives and should be considered a complementary tool" (3).

Surveillance Procedures

The goal of surveillance is to detect infections from HPAI viruses. Such surveillance must be cost-effective, efficient and sustainable for both non-vaccinated and vaccinated poultry (8). For vaccinated populations, appropriate surveillance should focus on finding active infections and be risk-based, multi-layered and primarily virological utilizing highly specific and sensitive qRT-PCR assays. This virological surveillance is DIVA-compatible (detecting infection in vaccinated animals) with any type of USDA-licensed vaccine and has been extensively used in non-vaccinated poultry populations.

The virological surveillance should focus on daily mortality samples, also called *bucket surveillance*. Bucket surveillance is an established, sensitive surveillance system for detecting early infection and confirming HPAI freedom in both vaccinated and non-vaccinated poultry, especially in chickens, and can utilize pooling of samples to reduce cost without loss of sensitivity. Bucket surveillance in vaccinated poultry relies on a small and highly-susceptible subpopulation of chickens that did not develop a protective immune response either because of being missed during the vaccination process or their immune system did not mount a protective humoral or cellular immune response – making them susceptible to infection with a clinical outcome of severe illness and death. If inadequate numbers of dead chickens are available, sick chickens can be used as a supplement. However, in well-vaccinated flocks, there is a lack of general utility in random sampling healthy chickens, as most will be immune from infection.

Bucket surveillance should be used to detect sustained transmission in a flock. If a single positive dead bird (or pooled sample) is found, retesting should be done to confirm sustained transmission. If confirmed, a positive virus detection would lead to culling of the flock.

We recommend vaccinated pullet and layer flocks should have at least two pools of 11 oropharyngeal swabs collected and tested by qRT-PCR every 14-day period, which corresponds to one avian influenza incubation period. Use of premovement isolation periods in vaccinated flocks will enhance surveillance accuracy before pullets are moved to layer farms or end of lay flocks are moved off farm (23).

Environmental samples should be explored as alternative surveillance samples as they potentially could be sensitive and cost-effective opportunities for early HPAI virus detection. Such samples could include various matrices (dust, aerosols, water, etc.) obtained from biofilm of poultry drinkers, swabs from boots used in the poultry house,

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swabs of ventilation louvers, swabs of egg belts, wastewater from processing plants, eggshell wash fluid, environmental samples used for regulatory testing for salmonella, and other types of environmental samples. The virological surveillance system for vaccinated poultry should have an equivalent sensitivity to the virological surveillance system for nonvaccinated poultry.

Virological surveillance will potentially provide HPAI virus isolates from both vaccinated and non-vaccinated flocks. These virus isolates would be further studied by sequencing, genetic analysis, antigenic assessments and vaccination challenge and transmission studies to determine if the vaccines applied are still effective at providing protection. If sufficient antigenic drift has occurred in the field HPAI viruses to render them resistant to one or more vaccines, then such data should be used to update inactivated seed strains and remove ineffective vaccines from use.

Serological surveillance, including use of DIVA-compatible vaccines, can at best only be part of a carefully balanced surveillance strategy in vaccinated poultry such as for assessing the presence of low infection rates before making major changes in or stopping a vaccination program. Serological surveillance has inherent issues with false positive results, necessitating retesting with more specific and sensitive tests or follow-up with virological testing to confirm active infection. In addition, potential infections by a LPAI viruses within a H5 vaccinated flock will necessitate additional differentiating serological tests, taxing diagnostic laboratory resources. Experience in France with vaccinated ducks during 2023-2025 has emphasized the limited value of serological DIVA surveillance as compared to the specific, sensitive virological surveillance using qRT-PCR.

Impact on Human Exposure and Zoonotic Potential

Vaccination of poultry is not a threat to public health, in fact, it helps to protect humans from infection by preventing or greatly reducing poultry infection and environmental contamination, i.e., the source of virus for human exposure and infection (25). The U.S. has experienced 70 human infections from H5N1 2.3.4.4b HPAI viruses since 2022. Most of the human cases have had occupational exposure to high doses of virus, principally dairy farm workers (41 cases) or poultry depopulation crews (24 cases) (26). Vaccination of poultry for H5 HPAI in China and Vietnam has resulted in a great reduction or elimination of human HPAI virus infections (27).

Summary Recommendations

This proposed HPAI vaccination plan for the U.S. poultry and egg industry is submitted on behalf of U.S. egg producers, as represented by United Egg Producers and American Egg Board.

HPAI is an ongoing threat to the egg industry due to the extensive dissemination of virus from wild birds and mammalian species. Biosecurity practices alone have not provided adequate protection. The industry believes it is time to enhance our overall strategy to control the virus through implementing vaccination in egg laying flocks. Vaccination should be combined with a robust surveillance plan as part of a strategy to stamp-out virus in commercial flocks.

The protocols must be acceptable to the federal government, state animal health officials, the poultry industry, livestock partners, and trading partners. We look forward to further discussions with USDA about this proposal.

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