

NAHEMS GUIDELINES: VACCINATION FOR CONTAGIOUS DISEASES

APPENDIX C: VACCINATION FOR HIGH PATHOGENICITY AVIAN INFLUENZA

FAD PReP

**Foreign Animal Disease
Preparedness & Response Plan**



NAHEMS

**National Animal Health
Emergency Management System**



United States Department of Agriculture • Animal and Plant Health Inspection Service • Veterinary Services

AUGUST 2015

The Foreign Animal Disease Preparedness and Response Plan (FAD PReP)/National Animal Health Emergency Management System (NAHEMS) Guidelines provide a framework for use in dealing with an animal health emergency in the United States.

This FAD PReP/NAHEMS Guidelines was produced by the Center for Food Security and Public Health, Iowa State University of Science and Technology, College of Veterinary Medicine, in collaboration with the U.S. Department of Agriculture Animal and Plant Health Inspection Service through a cooperative agreement.

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THE IMPERATIVE FOR FOREIGN ANIMAL DISEASE PREPAREDNESS AND RESPONSE

Why Foreign Animal Diseases Matter

Preparing for and responding to foreign animal diseases (FADs)—such as highly pathogenic avian influenza (HPAI) and foot-and-mouth disease (FMD)—are critical actions to safeguard the nation’s animal health, food system, public health, environment, and economy. FAD PReP, or the *Foreign Animal Disease Preparedness and Response Plan*, prepares for such events.

Studies have estimated a likely national welfare loss between \$2.3–69 billion¹ for an FMD outbreak in California, depending on delay in diagnosing the disease.² The economic impact would result from lost international trade and disrupted interstate trade, as well as from costs directly associated with the eradication effort, such as depopulation, indemnity, carcass disposal, and cleaning and disinfection. In addition, there would be direct and indirect costs related to foregone production, unemployment, and losses in related businesses. The social and psychological impact on owners and growers would be severe. Zoonotic diseases, such as HPAI and Nipah/Hendra may also pose a threat to public health.



Challenges of Responding to an FAD Event

Responding to an FAD event—large or small—may be complex and difficult, challenging all stakeholders involved. Response activities require significant prior preparation. There will be imminent and problematic disruptions to interstate commerce and international trade.

A response effort must have the capability to be rapidly scaled according to the incident. This may involve many resources, personnel, and countermeasures. Not all emergency responders may have the specific food and agriculture skills required in areas such as biosecurity, quarantine and movement control, epidemiological investigation, diagnostic testing, depopulation, disposal, and possibly emergency vaccination.

Establishing commonly accepted and understood response goals and guidelines, as accomplished by the FAD PReP materials, will help to broaden awareness of accepted objectives as well as potential problems.

¹ Carpenter TE, O'Brien JM, Hagerman AD, & McCarl BA. 2011. "Epidemic and economic impacts of delayed detection of foot-and-mouth disease: a case study of a simulated outbreak in California." *J Vet Diagn Invest.* 23:26-33.

² Estimates based on models may vary: Ekboir (1999) estimated a loss of between \$8.5 and \$13.5 billion for an FMD outbreak in California. Ekboir JM. 1999. "Potential Impact of Foot-and-Mouth Disease in California: the Role and Contribution of Animal Health Surveillance and Monitoring Services." *Agricultural Issues Center.* University of California, Davis.

Lessons Learned from Past FAD Outbreaks

The foundation of FAD PReP is lessons learned in managing past FAD incidents. FAD PReP is based on the following:

- Providing processes for emergency planning that respect local knowledge.
- Integrating State-Federal-Tribal-industry planning processes.
- Ensuring that there are clearly defined, obtainable, and unified goals for response.
- Having a Unified Command with a proper delegation of authority that is able to act with speed and certainty.
- Employing science- and risk-based management approaches to FAD response.
- Ensuring that all guidelines, strategies, and procedures are communicated effectively to responders and stakeholders.
- Identifying resources and trained personnel required for an effective incident response.
- Trying to resolve competing interests prior to an outbreak and addressing them quickly during an outbreak.
- Achieving rapid FAD detection and tracing.

FAD PReP Mission and Goals

The mission of FAD PReP is to raise awareness, expectations, and develop capabilities surrounding FAD preparedness and response. The goal of FAD PReP is to integrate, synchronize, and deconflict preparedness and response capabilities as much as possible before an outbreak by providing goals, guidelines, strategies, and procedures that are clear, comprehensive, easily readable, easily updated, and that comply with the National Incident Management System.

In the event of an FAD outbreak, the three key response goals are to: (1) *detect, control, and contain the FAD in animals as quickly as possible*; (2) *eradicate the FAD using strategies that seek to stabilize animal agriculture, the food supply, the economy, and to protect public health and the environment*; and (3) *provide science- and risk-based approaches and systems to facilitate continuity of business for non-infected animals and non-contaminated animal products*. Achieving these three goals will allow individual livestock facilities, States, Tribes, regions, and industries to resume normal production as quickly as possible. They will also allow the United States to regain FAD-free status without the response effort causing more disruption and damage than the disease outbreak itself.

FAD PReP Documents and Materials

FAD PReP is not just one, standalone FAD plan. Instead, it is a comprehensive U.S. preparedness and response strategy for FAD threats, both zoonotic and non-zoonotic. The following section provides examples of the different types of FAD PReP documents available.

- Strategic Plans—Concept of Operations
 - *APHIS Foreign Animal Disease Framework: Roles and Coordination* (FAD PReP Manual 1-0): This document provides an overall concept of operations for FAD preparedness and response for APHIS, explaining the framework of existing approaches, systems, and relationships.
 - *APHIS Foreign Animal Disease Framework: Response Strategies* (FAD PReP Manual 2-0): This document provides significant detail on response strategies that will be conducted in an FAD outbreak.
 - *Incident Coordination Group Plan* (FAD PReP Manual 3-0): This document explains how APHIS headquarters will organize in the event of an animal health emergency.
 - *FAD Investigation Manual* (FAD PReP Manual 4-0): This field-ready manual provides detailed information on completing an FAD investigation from start to finish.

- *A Partial List of FAD Stakeholders* (FAD PReP Manual 5-0): This guide identifies key stakeholders with whom the National Preparedness and Incident Coordination (NPIC) Center collaborates.
- NAHEMS Guidelines
 - These documents describe many of the critical preparedness and response activities, and can be considered as a competent veterinary authority for responders, planners, and policy-makers.
- Industry Manuals
 - These manuals describe the complexity of industry to emergency planners and responders and provide industry a window into emergency response.
- Disease Response Plans
 - Response plans are intended to provide disease-specific information about response strategies. They offer guidance to all stakeholders on capabilities and critical activities that would be required to respond to an FAD outbreak.
- Standard Operating Procedures (SOPs) for Critical Activities
 - For planners and responders, these SOPs provide details for conducting critical activities such as disposal, depopulation, cleaning and disinfection, and biosecurity that are essential to effective preparedness and response to an FAD outbreak. These SOPs provide operational details that are not discussed in depth in strategy documents or disease-specific response plans.
- Continuity of Business Plans (commodity specific plans developed by public-private-academic partnerships)
 - Known as the Secure Food Supply Plans, these materials use science- and risk-based information to facilitate market continuity for specific products in an outbreak.
 - More information on these plans can be found at the following: www.secureeggssupply.com, www.securepork.org, www.securemilksupply.org, www.securebroilersupply.com.
- APHIS Emergency Management
 - APHIS Directives and Veterinary Services (VS) Guidance Documents provide important emergency management policy. These documents provide guidance on topics ranging from emergency mobilization, to FAD investigations, to protecting personnel from HPAI.

For those with access to the APHIS intranet, these documents are available on the internal APHIS FAD PReP website: <http://inside.aphis.usda.gov/vs/em/fadprep.shtml>. Most documents are available publicly, at <http://www.aphis.usda.gov/fadprep>.

PREFACE

The Foreign Animal Disease Preparedness and Response Plan (FAD PReP)/National Animal Health Emergency Response System (NAHEMS) Guidelines provide the foundation for a coordinated national, regional, state and local response in an emergency. As such, they are meant to complement non-Federal preparedness activities. These guidelines may be integrated into the preparedness plans of other Federal agencies, State and local agencies, Tribal Nations, and additional groups involved in animal health emergency management activities.

This Appendix C: Vaccination for High Pathogenicity Avian Influenza is a supplement to FAD PReP/NAHEMS Guidelines: Vaccination for Contagious Diseases, and covers the disease-specific strategies and general considerations of vaccination. Both documents are components of APHIS' FAD PReP/NAHEMS Guidelines series, and are designed for use by APHIS Veterinary Services (VS), and other official response personnel in the event of an animal health emergency, such as the natural occurrence or intentional introduction of a highly contagious foreign animal disease in the United States.

Appendix C: Vaccination for High Pathogenicity Avian Influenza, together with the FAD PReP/NAHEMS Guidelines: Vaccination for Contagious Diseases, provide guidance for USDA employees, including National Animal Health Emergency Response Corps (NAHERC) members, on principles of vaccination for high pathogenicity avian influenza for animal health emergency deployments. This Appendix C: Vaccination for High Pathogenicity Avian Influenza provides information for personnel associated with vaccination activities. The general principles discussed in this document are intended to serve as a basis for understanding and making sound decisions regarding vaccination in an avian influenza emergency. As always, it is important to evaluate each situation and adjust procedures to the risks present in the situation.

The FAD PReP/NAHEMS Guidelines are designed for use as a preparedness resource rather than as a comprehensive response document. For more detailed vaccination information, see plans developed specifically for the incident and the references at the end of this document.

NOTE: This Appendix C: Vaccination for High Pathogenicity Avian Influenza 2015 is an update of the 2011 content.

APHIS DOCUMENTS

Key APHIS documents complement this “Appendix C: Vaccination for High Pathogenicity Avian Influenza, Strategies and Considerations” and provide further details when necessary. This document references the following APHIS document:

- Highly Pathogenic Avian Influenza (HPAI) Response Plan: The Red Book, USDA APHIS

This document and other Foreign Animal Disease Preparedness and Response Plan (FAD PReP) resources are available on the Inside APHIS website for APHIS employees, at <http://inside.aphis.usda.gov/vs/em/fadprep.shtml>, and also at <http://www.aphis.usda.gov/fadprep>.

For the full listing of all references, including other APHIS documents, see section 21. References.

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Appendix C: Vaccination for High Pathogenicity Avian Influenza *Strategies and Considerations*

1. PURPOSE

This Appendix is intended to provide relevant information for federal and state officials and other interested parties who will participate in making decisions related to vaccination as an aid in controlling an HPAI outbreak in the U.S. The following topics are presented and discussed:

- Important characteristics of high pathogenicity avian influenza (HPAI)
- Characteristics of vaccines
- Strategies for vaccine use
- Various factors that must be considered when designing an effective vaccination program

The United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Veterinary Services (VS) has a separate document, *HPAI Response Plan: The Red Book* that identifies the capabilities needed to respond to an HPAI outbreak in the United States as well as identifying all the critical activities involved in responding with the corresponding time-frames. Please refer to that document for those specific details.

2. BACKGROUND

High pathogenicity avian influenza (HPAI) has become an increasing concern worldwide. The number of major avian influenza outbreaks has increased dramatically since the Asian lineage H5N1 HPAI viruses were first recognized in Hong Kong in the late 1990s. More than 200 million birds were affected by HPAI viruses (HPAIV) in 1999-2005, while only 18 outbreaks affecting 23 million poultry were reported during the four previous decades ([1]; [2] cited in [3]). In addition to the widespread and continuing epizootics caused by Asian lineage H5N1 viruses and their reassortants, HPAI outbreaks have been caused by other viruses. Some countries affected included Italy (H7N1) in 1999-2000, Chile (H7N3) in 2002, the Netherlands (H7N7) in 2003, Canada (H7N3) in 2004 and 2007, South Africa (H5N2) in 2004, 2006 and 2011, the U.S. (Texas; H5N2) and Pakistan (H7N3) in 2004, North Korea (H7N7) in 2005, the U.K. (H7N7) in 2008, Spain (H7N7) in 2009 and Mexico (H7N3) in 2012 [1;4-8]. Reassortment has generated new subtypes of Asian lineage H5N1 HPAI lineage viruses among poultry in Asia [9-14], among which HPAI H5N8 viruses have emerged to become a significant global threat. Asian lineage H5N8 HPAI viruses became widespread among birds in Asia and Europe in 2014 [15;16], and reached North America in 2014, apparently in migratory wild birds [17-19]. They have reassorted with North American LPAI viruses to produce new HPAI variants, such as H5N1 and H5N2, which contain gene segments of both Eurasian and North American origin [15;17-24]. Some of these viruses have caused outbreaks in North American poultry. The introduction of Asian lineage H5N1 and H5N8 HPAI viruses to new regions by wild birds has made it more difficult to exclude these viruses by traditional means, such as controls on poultry movement [15-18;25-33]. The serious nature of some zoonotic avian influenza infections, an issue first recognized in the 1990s, adds urgency to the control of this disease.

Avian influenza viruses (AIV) have spread rapidly in some outbreaks, especially in areas with a high density of poultry operations. Vaccination can be used as a supplemental emergency measure to slow virus transmission during an outbreak, or as a prophylactic technique when the risk of introduction is

high. In 2004, participants in the World Organization for Animal Health (OIE) International Conference on the Control of Infectious Animal Diseases by Vaccination in Buenos Aires, Argentina concluded that mass slaughter is no longer acceptable as the main technique for disease control and eradication, due to ethical, ecological and economic concerns [34]. They recommended that methods for disease prevention, control and eradication be reviewed, and advised an increased emphasis on vaccination. The OIE Terrestrial Animal Health Code allows vaccination to be used for avian influenza without affecting the country's disease status or impacting animal trade, if surveillance convincingly demonstrates the absence of viruses in poultry [35]. Vaccination must be part of an overall control plan that prevents the inapparent spread of these viruses among domesticated birds.

3. OVERVIEW OF AVIAN INFLUENZA

Summary

Avian influenza viruses are a heterogeneous group of viruses with varying pathogenicity. They are divided into low (LPAI) and high (HPAI) pathogenicity viruses. HPAI viruses are defined by their high virulence in chickens and/or their amino acid motif at the cleavage site of the hemagglutinin (HA) precursor. All other viruses are considered to be LPAI.

LPAI viruses are maintained in aquatic wild birds, and are occasionally transferred from these hosts into poultry. Once a virus becomes adapted to circulation among poultry, it is unusual for it to re-establish itself in wild bird populations.

HPAI viruses evolve from H5 and H7 LPAI viruses, usually while these viruses are circulating in poultry. The timing of this change is unpredictable. Once an LPAI virus has an HA cleavage site consistent with HPAI viruses, it is considered to be an HPAI virus, regardless of its virulence in poultry. Some of these viruses may initially cause only mild clinical signs in a flock; however, typical HPAI signs may develop with time as the virus evolves to become more pathogenic. All H5 and H7 LPAI viruses in poultry, as well as HPAI viruses, are notifiable to the OIE. HPAI viruses are generally not found in wild birds, except in the vicinity of outbreaks among poultry. However, there have been some exceptions, including the Asian lineage H5N1 and H5N8 viruses. Whether Asian lineage H5 HPAI viruses are maintained long-term in wild bird populations is still uncertain.

Avian influenza viruses have evolved into two distinct genetic lineages, which are called the Eurasian and North American lineages. The amount of reassortment between these lineages in wild birds seems to differ between regions, with very few reassortant viruses in some areas or populations, but significant reassortment where there is overlap between migratory flyways.

Antibodies are important in protecting birds from avian influenza. Cell-mediated and mucosal responses may also be protective, although their roles are still poorly understood. Immune responses to AIV may differ between avian species. Protective antibody responses are mainly directed against the viral hemagglutinin, a highly variable surface glycoprotein. There are 16 HA types in birds, and the current vaccines provide no significant cross-protection between types. Antibodies to the second major surface glycoprotein, the neuraminidase (NA), also seem to provide some protection, but they are less important than responses to the HA. As with the HA, vaccines do not provide immunity to NA types not contained in the vaccine. Vaccines with heterologous NAs are effective if the HA type matches that of the outbreak strain.

Immune responses to some internal AIV proteins, including the nucleoprotein, matrix (M) proteins and non-structural protein 1 (NS1), are useful in recognizing infections in vaccinated or nonvaccinated birds, but they do not appear to contribute significantly to immunity from the current vaccines.

Strains of influenza viruses change continuously, a process known as ‘antigenic drift.’ As a result of antigenic drift, an influenza virus can give rise to numerous variants after it has circulated among poultry for a time. This has occurred with the A/goose/Guangdong/1996 lineage (‘Asian lineage’) of H5N1 viruses. Multiple genotypes and at least ten distinct phylogenetic clades (0–9) have been identified in this lineage. Second, third, fourth and fifth order clades have also emerged from within these clades, and will continue to emerge while these viruses circulate. Not all H5N1 viruses are continuing to circulate; over time, some clades and genotypes disappear or become uncommon. This lineage has also reassorted with other AIV, generating reassortant H5 HPAI viruses with different NA and other viral proteins.

The rate of antigenic drift in AIV historically seems to have been low: there are examples of poultry being protected by vaccine strains isolated 20 years or more before the challenge virus. Vaccination, especially long term, can increase selection pressures if the virus continues to circulate. Long-term vaccination programs have been conducted in Mexico against H5N2 LPAI viruses, and in some Asian and Middle Eastern countries against H5N1 HPAI and H9N2 LPAI viruses, without eliminating virus circulation. Vaccine-resistant strains have subsequently emerged. Such variants have not been reported from some countries that applied vaccination in a more limited manner.

The incubation period in poultry can be a few hours to a few days in individual birds, and up to 2 weeks in the flock. A 21-day incubation period, which takes into account the transmission dynamics of the virus, is used in avian populations for regulatory purposes. The effect of the virus varies with the host species and the specific AIV isolate. In chickens and turkeys, HPAI viruses usually cause a severe, systemic illness with high morbidity and mortality rates. The clinical signs range from sudden death to a more prolonged illness with nonspecific respiratory and systemic signs. Not all gallinaceous birds are equally affected. In pheasants and quail, the effects vary from a mild and transient illness to a serious and uniformly fatal disease. Waterfowl are often infected asymptotically or develop only mild illness when infected with HPAI viruses, but they can be severely affected by some strains, including some (but not all) H5N1 Asian lineage viruses. Virus shedding from subclinically infected birds, such as waterfowl, can be important in maintaining HPAI viruses in poultry populations.

In addition to birds, HPAI (and LPAI) viruses occasionally affect mammals including humans. The Asian lineage H5N1 HPAI viruses have been isolated from many different avian species including poultry, wild birds and zoo birds, and sporadically from various mammals. In mammals, the consequences of infection have ranged from no clinical signs to death, but many human cases have been fatal. Some reassortants belonging to this H5 lineage can also infect mammals experimentally or in nature, with or without clinical signs. At present, experimental studies with H5N8 HPAI viruses suggest they might be less virulent in mammals than some recent H5N1 isolates.

The species of poultry, viral strain, structure of the poultry population and other parameters can influence transmission, and should be taken into consideration when designing a control plan. There may be differences in the ease with which different AIV strains are spread. Backyard

poultry may either act as reservoirs of HPAI or become infected mainly through virus shed from commercial flocks. Where these birds are numerous, they can contribute significantly to transmission even if they are less likely to become infected than commercial flocks.

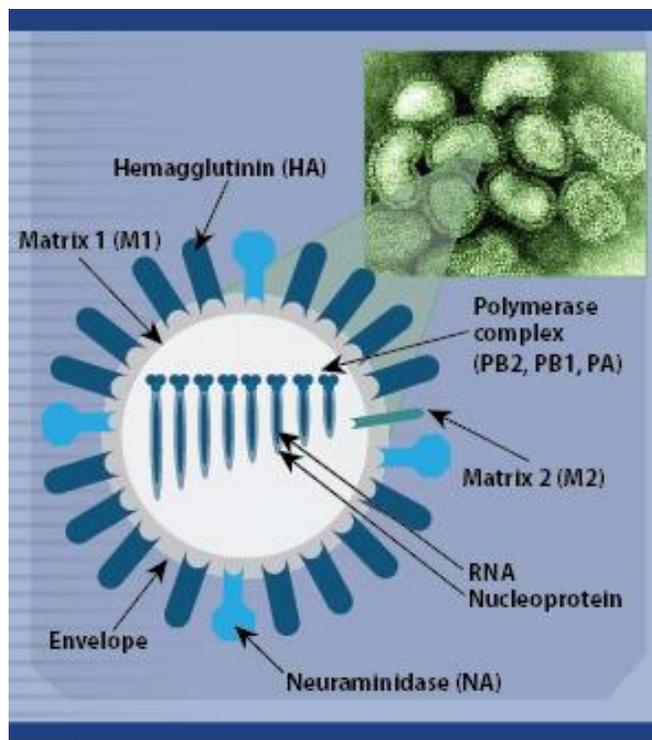
Avian influenza viruses can be shed in the feces as well as in respiratory secretions, although the levels may be higher in one location than another. Fecal-oral transmission usually maintains the virus in wild waterfowl populations. HPAIV isolates can be transmitted efficiently between ducks, whether or not they cause clinical signs in these birds. Once a virus has entered a flock of gallinaceous poultry, it can spread by both the fecal–oral route and in aerosols, due to the close proximity of the birds. Chickens and turkeys can begin shedding HPAI viruses as early as 1-2 days after infection. In these two species, the infectious period tends to be limited by the death of the bird. More resistant species might transmit viruses longer. The persistence of AIV in the environment can be influenced by many factors, and is generally reported to be longer at low temperatures. Aerosol transmission between farms is ordinarily thought to be unlikely, and AIV transmission usually follows the movement of infected birds and vehicles, people or other fomites. Seasonality has been reported with the Asian lineage H5N1 viruses, which tend to reemerge during colder temperatures. AIV seasonality also occurs in wild birds.

Effective vaccination can decrease transmission between animals by 1) decreasing the susceptibility of animals to infection, and 2) reducing virus shedding, if a vaccinated animal becomes infected. In addition to reducing transmission between flocks, decreased virus shedding reduces contamination of the environment and the risk to humans. However, vaccination may allow birds to survive longer without clinical signs, and if virus shedding is not substantially reduced, transmission could be enhanced.

Avian influenza viruses are a highly heterogeneous group of viruses with varying pathogenicity. AIV belongs to the family Orthomyxoviridae, the genus influenza virus A and the viral species influenza A virus [36].

3.1 AIV Proteins and their Roles in Vaccination

Influenza A viruses are enveloped viruses with an eight-segmented, negative sense, single-stranded RNA genome that encodes more than 12 proteins, including some gene products of unknown function [37-41].



Graphic illustration by: Dani Ausen and Katlyn Harvey, Iowa State University; Photo source: ISU EEDA Course, Section by Corrie Brown, University of Georgia, Athens

Three AIV proteins - the viral hemagglutinin (HA), neuraminidase (NA), and matrix 2 (M2) proteins—are incorporated into the envelope of the virion. The HA glycoprotein is the most abundant of these three proteins, and is estimated to comprise 80% of the total [37]. NA proteins account for most of the remainder, while the transmembrane protein M2 is present in very small amounts, approximately 16-20 molecules per virion. The HA protein is responsible for the initial binding of the virion to host cells, via sialic acid [37;42]. The viral NA cleaves sialic acids from glycoproteins and glycolipids, preventing the aggregation of virions and allowing them to be released efficiently from the cell [37;42]. It is required for influenza viruses to spread efficiently [42]. The enzymatic activity of the NA might also facilitate the infection of cells, by helping the virion penetrate the blanket of mucus in the respiratory tract or the glycocalyx surrounding the cell [43]. The M2 protein is a proton-selective ion channel, which acidifies the viral core and allows the viral ribonucleoproteins to be released into the cytoplasm of the host cell [37;44;45]. In infected or vaccinated animals, the immune response is primarily targeted at the HA, which is critical in vaccine matching, and to a lesser extent to the NA [46-49]. The extracellular domain (M2e) of M2 is abundant on AIV-infected cells, and has been investigated in tests to differentiate vaccinated from infected animals (DIVA tests) [44;45;50]. There has also been some research attempting to produce a ‘universal’ influenza vaccine based on M2 for birds, as well as for mammals [51;52]. Universal vaccines would theoretically protect the recipient from all viruses, regardless of subtype.

The viral internal proteins are involved in nucleic acid replication, alter antiviral responses in the host cell or influence virulence [37;38;53]. They include the abundant matrix protein M1 and several proteins that make up the viral ribonucleoproteins (vRNPs). The vRNPs consist of viral RNA wrapped around the nucleoprotein (NP) and very small amounts of a protein known as either nonstructural protein 2 (NS2) or nuclear export protein (NEP) [37;49;53]. In addition to this minor structural role, NS2/NEP has an important nonstructural role in exporting newly made viral RNA from the nucleus [53]. The nucleoprotein complexes are tightly associated with the three polymerase proteins, polymerase acidic protein (PA), polymerase basic protein 1 (PB1) and polymerase basic protein 2 (PB2) [53]. An additional AIV protein, PB1-F2, can be found in either a truncated or full length form (both of which appear to be functional) in different influenza A virus strains [38;53]. Most avian influenza viruses contain a full-length version, although truncated forms have also been detected [38;54]. PB1-F2 appears to modulate immune responses in mice, and may increase pathogenicity in some virus/host combinations in birds and mammals [38]. Additional gene products encoded by polymerase genes (e.g., PB1-N40, PA-X, PA-N155 and PA-N182) have also been identified in influenza A viruses [39-41]. None of the internal proteins are important in vaccine responses in birds [55], but antibody responses to the conserved matrix protein and nucleoprotein are used in avian influenza diagnosis.

NS1 is a multifunctional protein which has been implicated in the inhibition of interferon-mediated host defenses [53;56;57], and also regulates the translation and splicing of mRNA [37;53;56]. There are two major lineages of NS1, A and B, which diverge approximately 35% in their nucleotide sequence ([57]; [58-60] cited in [61]). AIV can contain either of these two lineages [57]. NS1 was thought to be the only AIV protein completely absent from the virion [53]; however, it has been recently identified in purified virions, although the amount is low [62]. It has been investigated for use in DIVA tests [45;48;56;57;61;63-65]. The NS2/ NEP has been detected in vRNPs [53]. The amounts in the virion are, however, very small and do not prevent it from being considered for DIVA tests. Currently, there are no published reports of its use. Finally, it should be noted that various host proteins have also been identified in purified influenza virions, including some proteins that appear to be enriched from host cells and may be as abundant as the viral NA [62].

Table 1: Avian Influenza Virus Proteins			
Abbreviation	Protein	Location	Use in vaccination
HA	Hemagglutinin	Envelope	Primary target of immune response; Vaccine HA must match field virus
NA	Neuraminidase	Envelope	Target of immune response; Can match field virus or differ; Immune response to this protein is used in a DIVA strategy
M2	Matrix 2	Envelope/ transmembrane	Immune response to extracellular portion of M2 (M2e) has been tested for use in DIVA strategy; M2 also a target for 'universal' AIV vaccines
M1	Matrix 1	Internal (structural protein)	Immune responses to this protein used in some diagnostic tests; Not a DIVA strategy except with recombinant vaccines that lack internal proteins

Abbreviation	Protein	Location	Use in vaccination
NP	Nucleoprotein	Internal (structural protein)	Immune responses to this protein used in some diagnostic tests; Not a DIVA strategy except with recombinant vaccines that lack internal proteins
NS1	Nonstructural protein 1	Nonstructural protein	Immune response to NS1 has been tested for use in a DIVA strategy
NS2/ NEP	Nonstructural protein 2/ Nuclear export protein	Internal (Nonstructural role and minor structural role)	None
PA	Polymerase acidic protein	Internal	None
PB1	Polymerase basic protein 1	Internal	None
PB1-F2			None
PB2	Polymerase basic protein 2	Internal	None

Proteins important in avian influenza vaccines or diagnostic tests are discussed further in the relevant sections.

3.2 AIV Subtypes and Strains

The HA and NA proteins, which are highly variable, are the basis for classifying avian influenza viruses into subtypes. Sixteen hemagglutinins (H1 to H16), and 9 neuraminidases (N1 to N9) have been found in viruses from birds, while two additional HA and NA types have been identified, to date, only in bats [53;66-71]. All AIV subtypes, including most possible combinations of the hemagglutinin and neuraminidase, have been detected in wild bird reservoir hosts [3;72]. Some hemagglutinins, such as H14 and H15, are uncommon and seem to occur only in limited geographic areas or populations, or perhaps are maintained in wild bird species or locations that are not usually sampled [72].

Avian influenza viruses have evolved into two distinct genetic lineages (based on the HA protein), which are called the Eurasian and North American lineages [72;73]. This separation is thought to have resulted from the migration patterns of their waterfowl hosts, which predominantly migrate from north to south along these land masses ([74;75] cited in [73]). The amount of reassortment between these lineages seems to differ between regions, with very few reassortant viruses in some areas or wild bird populations, but significant reassortment where there is overlap between migratory flyways, such as in Alaska and Iceland [72;76-86]. Avian influenza virus surveillance in Central and South America has been limited, but the viruses detected include a unique South American sublineage (or lineage) as well as viruses closely related to the North American lineage [87]. Some analyses suggest that the viruses in Australia and New Zealand might be geographically isolated to some extent, although there is also some evidence for reassortment with Eurasian viruses [88-91].

Strains of influenza viruses are described by their type (“A” for all influenza A viruses), host, place of first isolation, strain number (if any), year of isolation, and subtype [92;93]. For example, A/goose/Guangdong/1/96 (H5N1) is a type A influenza virus first isolated from a goose in Guangdong province, China in 1996, and has the subtype H5N1.

3.3 Low and High Pathogenicity Avian Influenza

Avian influenza viruses are divided into two pathotypes: high pathogenicity (HPAI) or low pathogenicity (LPAI). This distinction is based on the genetic features of the virus, as well as the severity of disease in experimentally inoculated chickens [94]. Although there are exceptions, HPAI viruses usually cause serious illness in chickens and turkeys, while infections with LPAI viruses are generally much milder or subclinical in all species of birds. With rare exceptions (see section 3.3.1), HPAI viruses found in nature have always contained H5 or H7 hemagglutinins [95-98]. LPAI viruses can contain any of the 16 avian HAs including H5 and H7. Poultry viruses that must be reported to the OIE (formerly called “notifiable avian influenza viruses”) include HPAI viruses and all LPAI viruses containing H5 or H7, which have the potential to evolve into HPAIV [35].

Although other genes can contribute to AIV virulence, the structure of the HA proteolytic cleavage site is a major determinant of pathogenicity in chickens and turkeys [53;95]. For the virion to become infectious, host proteases must split the hemagglutinin precursor protein, HA0, into two fragments, HA1 and HA2 [53;73;95]. HA1 contains the receptor binding domain and is the most variable region of the protein. HA2 is responsible for fusion of the viral and endosomal membranes, which releases the virus into the cytosol [37]. The HA0 cleavage sites of HPAI and LPAI viruses differ in their susceptibility to proteases, affecting their distribution in the body. LPAI viruses are cleaved by trypsin-like enzymes found in the respiratory and intestinal tracts (e.g., plasmin, trypsin, trypsin-like enzymes, and miniplasmin) or by certain bacterial proteases [45;53;95]. This characteristic is generally thought to limit their distribution to the respiratory and gastrointestinal tracts [45;53;95;99-101]. In contrast, the HA0 of an HPAI virus is cleaved intracellularly, within the Golgi apparatus, by proteases of the subtilisin family (e.g., furin) [53]. Such proteases are found in cells throughout the body, and HPAI infections in susceptible gallinaceous birds are systemic ([45;95]; [100;102-104] cited in [105]).

HPAI viruses evolve from H5 and H7 LPAI viruses, typically while these viruses are circulating among poultry [3;67;106]. The timing of this change is unpredictable [3]. Once an LPAI virus has an HA0 cleavage site consistent with HPAI viruses, it is considered to be an HPAI virus, regardless of its pathogenicity in poultry [94]. A table containing all known hemagglutinin cleavage site sequences can be found on the OIE/FAO Net on Avian Influenza (OFFLU) Web site at http://www.offlu.net/fileadmin/home/en/resource-centre/pdf/Influenza_A_Cleavage_Sites.pdf.

3.3.1 OIE Definition of HPAI

The formal definition of an HPAI virus, according to the OIE, is an AIV that either 1) has an intravenous pathogenicity index (IVPI) greater than 1.2 in 6-week-old chickens, or 2) kills at least six of eight (75%) 4-to 8-week-old chickens within 10 days after intravenous inoculation [35;94]. When H5 and H7 viruses do not meet the definition of an HPAI virus under these criteria, they are sequenced to determine the pattern of amino acids at the proteolytic cleavage site. If the amino acid motif is similar to those that have been observed in HPAI viruses, the virus is considered to be HPAI [35;94]. All other H5 and H7 viruses are classified as LPAI viruses. Alternatively, sequencing may be done as the initial step, followed by pathogenicity testing if the virus does not have a cleavage site consistent with HPAIV. Molecular methods cannot be used alone to define a virus as LPAI, because cultures might contain mixed populations of viruses [94]. HPAI viruses may sometimes be found initially in asymptomatic flocks, or in

flocks that have clinical signs consistent with LPAI [5;107]. Typical HPAI signs may develop with time as these viruses evolve to become more pathogenic.

There are reports of viruses found in nature or generated in the laboratory that technically fit the definition of HPAIV, although they did not contain H5 or H7. An H10N4 virus and an H10N5 virus would have been defined as HPAIV by intravenous chicken inoculation tests, although they did not cause death or clinical signs after intranasal inoculation, and did not contain a typical HPAIV cleavage site [94;97]. Another H10 virus also fit the HPAI definition; however, this virus affected the kidneys and had a high mortality rate in intranasally inoculated young chickens [108]. In addition, laboratory insertion of genetic sequences from HPAI viruses has created non-H7, non-H5 viruses that were either 1) pathogenic only after intravenous inoculation, or 2) highly virulent after both intravenous and intranasal inoculation [109]. The latter viruses contained H2, H4, H8 or H14 hemagglutinins in this experiment. Whether such viruses could evolve naturally from LPAI viruses is still uncertain.

3.4 Immune Responses to AIV Proteins

Although immunity to AIV is incompletely understood, humoral immune responses are known to be important in protecting infected or vaccinated gallinaceous birds [49]. Cell-mediated immunity (CMI) also seems to play a role during infections [49;110-114]. There is still relatively little information about mucosal responses; however, they are expected to occur in naturally infected birds, but not in birds immunized parenterally with inactivated vaccines.

Humoral immune responses are primarily directed to the viral HA, and to a lesser extent, to the NA [46-49;53]. Antibodies to the HA protein are neutralizing, probably because they interfere with the attachment of the virion to the host cell, or block fusion between the viral and endosomal membranes ([115] cited in [116]). Antibodies to the NA may also provide some protection in birds ([117] cited in [42]), but their role is incompletely understood [42]. They do not prevent infections, but are thought to result in virus aggregation, in effect decreasing the amount of virus that is released to infect new cells ([42;45]; [118] cited in [116]). Immunity to the NA was reported to provide partial protection from clinical signs without significantly decreasing virus shedding in one experiment [55]; to delay but not prevent death in another study [119]; and to protect chickens from clinical signs and decrease virus shedding in two studies [116;120]. The currently available vaccines do not provide significant cross-protection between the 16 avian HA types [46-49]. Because the HA is the major target of humoral immunity, an effective vaccine must contain the same HA type as the field virus. Likewise, vaccine-induced immunity to one NA does not protect birds from the other 8 types [42]. However, the NA is less important in immunity, and a vaccine with a heterologous NA (or no NA) can be protective if the HA is a good match [44-46].

Immune responses to some internal AIV proteins, including the nucleoprotein, matrix proteins and NS1, can be useful in recognizing infections in vaccinated and/or nonvaccinated birds, but they do not appear to contribute significantly to immunity from the current vaccines [55]. CMI responses to conserved internal proteins may, however, be involved in heterosubtypic immunity, a phenomenon where infected birds are sometimes protected from subsequent infection by a virus containing a different HA [110-114;121;122]. For example, previous exposure to H1N1 or H1N2 LPAI viruses has been reported to partially protect chickens from challenge by H5N1 HPAI viruses [122]. At present, the main significance of heterosubtypic immunity in vaccination is its potential for affecting surveillance by clinical signs in nonvaccinated (e.g., sentinel) birds, if they are regularly exposed to AIV in the field.

Immune responses to AIV are not necessarily identical in different species of birds. In particular, they might differ between waterfowl and gallinaceous poultry [123]. In one recent study, naive chickens infected with an H5N3 LPAI virus did not shed this virus from the respiratory tract on second exposure,

but there was no effect on the shedding of an unrelated H7N2 LPAI virus [124]. In ducks, the same protocol resulted in decreased the shedding of both H5N3 and H7N2 LPAI viruses from the intestinal tract, although neither was completely prevented.

3.5 Antigenic Drift and the Effect of Vaccination

3.5.1 Antigenic Drift, Reassortment and Antigenic Shifts

Strains of influenza viruses evolve as they accumulate point mutations during virus replication, a process known as ‘antigenic drift’ [93]. The viral RNA polymerase lacks proofreading ability, facilitating such mutations. While the structure of the internal viral proteins must be largely conserved to retain their function, mutations are more readily perpetuated in the HA and NA. Antigenic drift allows influenza viruses to evade immunity from previous infections or vaccination. An abrupt change in the subtypes found in a host species is called an antigenic shift. Antigenic shifts can result from the direct transfer of a whole virus from one host species into another, the re-emergence of a virus that was found previously in a species but is no longer in circulation, or reassortment between influenza viruses [92]. Reassortment can occur between all influenza A viruses, including those that usually infect different host species (e.g., avian influenza viruses and human influenza viruses) [125]. Antigenic shifts periodically give rise to novel influenza viruses, to which the host species usually has no pre-existing immunity.

As a result of antigenic drift, an influenza virus can give rise to numerous variants after it has circulated among poultry for a time. This has occurred with the A/goose/Guangdong/1996 lineage (‘Asian lineage’) of H5N1 viruses. Multiple genotypes and at least ten distinct phylogenetic clades (0–9) have been identified in this lineage [111;126-131]. Second, third, fourth and fifth order clades have emerged, mainly from clades 1 and 2, and the most prominent clades can differ between countries or regions [131;132]. Only a proportion of the known H5N1 clades are actively circulating; others are considered to be extinct [131]. HPAI H5N2, H5N5 and H5N8 viruses belonging to this lineage have also been reported in Asia, as the result of reassortment between H5N1 viruses and other AIV [9-14;16]. Reassortant H5N8 HPAI viruses belonging to clade 2.3.4.4 [22;131;133] spread widely in Asia and Europe in 2014 [15;16] and reached North America in 2014 [17-19]. These viruses have generated additional HPAI reassortants of various subtypes (e.g., H5N1, H5N2), including some that contain some gene segments from the North American lineage [15;17-24;131]. One H5N2 HPAI virus has caused numerous outbreaks among poultry in North America [21;22]. This virus contains the Eurasian clade 2.3.4.4 H5 hemagglutinin, a North American NA, and internal genes from both lineages (polymerase acidic protein subunit, matrix, PB2, and nonstructural protein genes from the Eurasian lineage, and nucleoprotein and PB1 genes from the North American lineage) ([21]; [24] cited in [22]).

3.5.2 Vaccination and Antigenic Drift

Vaccination can increase selection pressures and contribute to antigenic drift if the virus continues to circulate. Vaccines for human influenza viruses are changed frequently in response to antigenic drift in long-lived human populations [47;73;134;135]. Historically, this was thought to be unnecessary for avian influenza. Long-term avian influenza vaccination programs were rare before 1995, and there was little selection pressure from vaccines before that time [136]. In addition, commercial chickens and turkeys have short lifespans and usually have not been exposed to avian influenza viruses, whereas people are exposed repeatedly through infection or vaccination, and the level of population immunity is greater [134]. Factors such as these appear to have resulted in a historically lower rate of genetic drift in AIV than human influenza viruses [137]. There are numerous examples of poultry being protected by a variety of vaccine strains, including some isolated 20 years or more before the challenge virus [137-142]. However, vaccine-resistant field strains have recently emerged in poultry during long-term vaccination campaigns where control programs have not stopped virus circulation. Antigenic drift was demonstrated during an ongoing H5N2 LPAI vaccination program in Mexico, and probably contributes to the persistence of these

viruses for more than a decade despite a control program [134;136]. (Other factors including selection pressure from maternal antibodies [134;143], and immunity to LPAI viruses in infected, non-vaccinated flocks [136] probably also play a role.) Selection pressures from long-term vaccination campaigns may also have contributed to the evolution of new H9N2 LPAI variants in the Republic of Korea [144] and H5N1 HPAI variants in China, Indonesia, Vietnam and Egypt [8;145-148]. Some countries that did not vaccinate against H5N1 (e.g., Thailand, Turkey and Nigeria) [148], or that applied more limited or targeted vaccination campaigns, such as Pakistan and Russia, did not report the evolution of vaccine resistant strains [8;149].

3.6 Species Affected

3.6.1 Wild Birds

Avian influenza viruses can infect both domesticated and wild birds. Wild birds usually carry only LPAI viruses [72]. These viruses are especially common among birds that live in aquatic environments, particularly members of the order Anseriformes (waterfowl, such as ducks, geese and swans) and two families within the order Charadriiformes, the Laridae (gulls and terns) and Scolopacidae (shorebirds) [66;69;72;80;85;92;150-159]. Infections are not necessarily common in all members of these orders, and some species may maintain viruses long-term, while others act as spillover hosts. Aquatic species belonging to other orders occasionally have high infection rates, and might also be involved in the epidemiology of this disease [154;160;161]; [162] cited in [161]. For instance, infections among seabirds seem to be particularly common in murres (*Uria* spp.) [163].

The most common influenza subtypes in wild birds may differ between species and regions, and can change over time [72;157;158;161;163-165]. Virus diversity seems to be particularly high among charadriiform birds [72;155]. A few avian influenza subtypes, such as H13 and H14 viruses, seem to have a limited host range [72;76;80;158;166-171]. LPAI viruses can also infect wild terrestrial birds, such as raptors and passerines, but infections are ordinarily uncommon, and these species are not thought to be important reservoirs [153;154;172-181]. Higher infection rates have occasionally been reported in some terrestrial species, and in a study from Vietnam, AIV were particularly common in certain terrestrial birds that forage in flocks, with an especially high prevalence in Japanese White-eyes (*Zosterops japonicus*) [174;180]. Most, though not all, infections in wild birds are asymptomatic [92;150;182;183].

HPAI viruses are not usually found in wild birds, although they may be isolated transiently near outbreaks in poultry [176]. Exceptions include the Asian lineage H5N1 and H5N8 viruses (and some reassortants of H5N8, such as an H5N2 HPAI found in North America), which have been found repeatedly in wild birds, an H5N3 virus isolated from an outbreak among terns in the 1960s, an H7N1 virus that was isolated from a sick wild siskin, *Carduelis spinus*, and an H5N2 virus found in a few asymptomatic wild ducks and geese in Africa [16-19;22;25-29;31-33;182-197]. The latter H5N2 viruses are unrelated to Asian lineage H5N1 viruses, but closely related to H5 LPAI viruses circulating among wild and domesticated ducks, and might have emerged in wild birds [186].

3.6.2 Domesticated Birds and Mammals

HPAI viruses are usually found in domesticated poultry. These viruses typically enter poultry populations, from wild birds, as LPAI viruses [95]. Some viruses circulate inefficiently and die out, while others become adapted to the new host. The latter may continue to circulate as LPAI viruses; or if they contain H5 or H7, they may evolve into HPAI viruses. HPAI and LPAI viruses have been found in many domesticated birds, including gallinaceous poultry and game birds, ducks, geese, ratites, pigeons and cage birds; however, some species seem to be more resistant to infection and/or illness than others [25-27;48;66;103;182;190;191;198-222]. For example, there are few reports of AIV in psittacine birds, and pigeons appear to be relatively resistant to infection compared to poultry. Once a virus has adapted to

poultry, it is thought to be unusual for it to re-establish itself in wild bird populations [95]. Whether the Asian lineage H5 viruses are an exception to this rule is currently a matter of debate [28-30;196;223;224].

Mammals that have been infected occasionally in nature by LPAI or HPAI viruses include cats, dogs, pigs, equids, mink, and various wild and captive terrestrial or aquatic species [67;190;192;225-253]. Ferrets can be infected experimentally with many viruses. Persistence of AIV in a mammalian species is rare, with recent examples limited to an H3N2 LPAI virus, apparently of avian origin, that appears to be circulating among dogs (and possibly cats) in parts of Asia since 2007 [254-262].

3.6.3 Host Range of the Asian Lineage H5N1 Avian Influenza Viruses and Reassortants including H5N8

Asian lineage H5N1 HPAI viruses seem to have an unusually wide host range. These viruses can infect a variety of wild birds belonging to many different orders, including the Anseriformes and Charadriiformes, and they have been detected occasionally in species not usually affected by AIV, such as raptors [25-29;182;183;188-197]. Both clinical cases and asymptomatic infections have been described in wild birds [190;193;196;263]. Whether wild birds can maintain these viruses for long periods (or indefinitely), or are repeatedly infected from poultry, is still controversial [28-30;196;223;224]. However, the evidence that wild birds can transfer Asian lineage H5 HPAI viruses to poultry in new geographic regions now appears strong [15-19;30]. Like their H5N1 progenitor, Asian lineage H5N8 HPAI viruses have been detected repeatedly in wild birds, and presumably reached North America by this route [15-19;22;31-33;184]. In the U.S., a reassortant Asian lineage H5N2 virus isolated from outbreaks among poultry is closely related to a virus detected in wild birds [21].

Asian lineage H5N1 viruses can also infect many species of mammals, and their full host range is probably not yet known. These viruses have sporadically affected humans, with many cases being fatal (see also section 20.1) [264-266]. They have also been found in pigs, cats, dogs, donkeys, tigers (*Panthera tigris*), leopards (*Panthera pardus*), clouded leopards (*Neofelis nebulos*), lions (*Panthera leo*), Asiatic golden cats (*Catopuma temminckii*), stone martens (*Mustela foina*), raccoon dogs (*Nyctereutes procyonoides*), palm civets (*Chrotogale owstoni*), plateau pikas (*Ochotona curzoniae*) and a wild mink (*Mustela vison*) [67;190;192;226-238;245;267-270], and serological evidence of infection or exposure has been reported in horses and raccoons [237;271-273]. Experimental infections have been established in cats, dogs, foxes, pigs, ferrets, laboratory rodents, cynomolgus macaques (*Macaca fascicularis*) and rabbits [188;190;191;235;238;240;267;274-283]. Cattle could be experimentally infected with viruses isolated from cats [283], but studies in Egypt detected no antibodies to H5N1 viruses in cattle, buffalo, sheep or goats, suggesting that these species are not normally infected [271]. The effects of H5N1 HPAI viruses in animals have varied. Some reported cases were fatal; however, both mild (or subclinical) and serious cases have been reported in felids and dogs [192;228-231;233-235;238;239;245;276;278;280;281;285], and infected pigs seem to develop mild or no clinical signs [235;267;269-271;279;286].

HPAI H5N2, H5N5 and H5N8 viruses, resulting from reassortment between the Asian lineage H5N1 viruses and other avian influenza viruses, have been reported among poultry in Asia [9-14]. Some of these viruses, such as an H5N2 virus isolated recently from a dog with respiratory signs, may be able to cause illness in mammals [225;287;288]. This H5N2 virus could be transmitted from experimentally infected dogs to dogs, chickens and cats [225;287;288]. No illnesses caused by HPAI H5N8 viruses have been reported in naturally infected mammals, as of July 2015, although seropositive dogs were detected on some infected farms [289]. Initial experiments in ferrets and mice suggest that these H5N8 viruses are less pathogenic for mammals than some H5N1 isolates, with low to moderate virulence in these species [289-291]. One study reported that virus replication was inefficient in experimentally infected dogs,

which developed no clinical signs [289]. Cats were more likely to become infected in this study, and developed mild and transient signs (fever, marginal weight loss).

3.7 Pathobiology and Clinical Signs in Birds

3.7.1 Pathobiology and the Occurrence of Viruses in Tissues

Unlike LPAI viruses, which tend to cause subclinical infections or mild illness [66;212], HPAI viruses cause a multisystemic disease with high morbidity and mortality in chickens and turkeys [95;99;292]. HPAI viruses replicate initially in the respiratory tract, but soon disseminate to the blood and organs in susceptible species [95]. Death is generally correlated with the level of virus replication [95]. It may occur peracutely from altered vascular permeability, which usually ends in hemorrhages, edema and/or multiple organ failure, or as the failure of one or more critical organs if a bird survives longer. The incubation period in poultry can be a few hours to a few days in individual birds, and up to 2 weeks in the flock [66;93;152]. A 21-day incubation period, which takes into account the transmission dynamics of the virus, is used in avian populations for regulatory purposes [35].

In chickens and turkeys, HPAI viruses or their antigens have been detected in a wide variety of visceral organs, as well as in skeletal muscle (meat), blood, bone marrow, feather follicles, brain and other tissues [95;103;105;293-304]. Asian lineage H5N1 HPAI viruses have also been found in meat from ducks [300;305;306] and quail [306]. In some studies, tissues from ducks contained less antigen than tissues from chickens or quail [306;307]. HPAI viruses have been found within the albumen or yolk of chicken, turkey and quail eggs [293;294;304;308-313]. Viruses in feces can contaminate the eggshell.

3.7.2 Clinical Signs in Gallinaceous Poultry

The clinical signs vary with the species of bird. Chickens and turkeys infected with HPAI viruses usually become severely ill [66;92;198], although the specific signs vary in frequency and type with the outbreak [95;314;315]. Nonspecific systemic and/ or respiratory signs are common, but there may be few or no signs when the viral strain kills the birds quickly [66;95;103;198;202;294;295;316-318]. In most cases, the flock mortality rate is high in fully susceptible birds, and may reach 90-100% [66;67;319]. Because a virus can be defined as HPAI based solely on its genetic composition, it is also possible for an HPAI virus to be isolated from chickens or turkeys showing mild signs consistent with LPAI [5]. Chickens or turkeys may differ in their susceptibility to some HPAIV isolates [304;320-322]. Other gallinaceous birds may be less susceptible than chickens and turkeys. In pheasants and quail inoculated with HPAI viruses, the effect varies from a mild and transient illness to a severe and uniformly fatal disease ([198;323]; [103;199] cited in [200]).

3.7.3 Clinical Signs in Ducks and Geese

Domesticated ducks and geese tend to be unaffected or mildly affected by most HPAI viruses ([201;207] cited in [186]; [198;199;201-203] cited in [324]). This may result in inapparently infected flocks and the maintenance of HPAI viruses among waterfowl ([48]; [325;326] cited in [327]). Even modern commercial operations may be affected. In Germany, subclinical H5N1 infections were reported in two commercial fattening duck facilities with high biosecurity in 2007([328] cited in [327]).

Symptomatic infections are also possible in waterfowl. With most viruses, clinical signs are generally limited to respiratory signs (e.g., sinusitis), diarrhea, occasional cases with neurological signs, and possibly increased mortality in the flock; however, some recent Asian lineage H5N1 HPAI strains cause acute, severe neurological disease or sudden death, with a high case fatality rate [25-27;66;182;191;204-211;329;330]. Sensitivity to a given virus can vary between species of waterfowl including different species of ducks. Muscovy ducks (*Cairina moschata*) seem to be particularly susceptible to Asian lineage H5N1 HPAI viruses [331-335]. Pekin ducks (*Anas platyrhynchos* var. *domestica*) and mallards (*Anas*

platyrhynchos) have had lower mortality rates and fewer clinical signs in most experiments, although there are reports of high mortality even in these species, when infected in the laboratory [182;332;334;336-340]. Mule ducks, a cross between Pekin and Muscovy ducks, were also relatively resistant to clinical signs in one study [341]. All birds that are called ducks are not necessarily closely related, and Muscovy ducks and Pekin ducks belong to different tribes (Cairinini and Anatini, respectively) of the subfamily Anatinae [342]. Some HPAIV strains may disproportionately affect young ducks [208;210;292;307;327;343;344]. In one study, older Pekin ducks were reported to shed less virus than young birds ([345] cited in [346]). In another report, virus shedding was similar in both age groups, but severe clinical signs only occurred in younger birds [343].

3.7.4 Clinical Signs in Other Domesticated Birds

Pigeons are thought to be relatively resistant to illnesses caused by AIV, although there have been reports of sporadic deaths and rare outbreaks [95;177;347]. Reported clinical signs in one H5N1 HPAI outbreak included neurological signs, greenish diarrhea and sudden death [347]. Some pigeons that were experimentally infected with H5N1 viruses remained asymptomatic, while others became moderately to severely ill [95;177;347].

There is limited information about avian influenza viruses in ostriches, but HPAI viruses may not necessarily be more pathogenic than LPAI viruses in this species [215;217-221]. Clinical signs tend to be mild in adult ostriches, and more severe in young birds less than 6 months of age, which can develop nonspecific signs (e.g., depression), dyspnea; green urine, diarrhea or hemorrhagic diarrhea, with increased mortality [215;220-222].

Canaries (*Serinus canarius*) became ill after being exposed to a wild siskin infected with an H7N1 HPAI virus [187]. The clinical signs included conjunctivitis, apathy and anorexia, and the mortality rate was high.

Elevated mortality reported in some outbreaks in ostriches, pigeons and other relatively resistant birds might be caused by concurrent infections and other complications [220;347].

3.7.5 Clinical Signs in Wild Birds and Captive Wild Species

Studies in experimentally infected wild birds and observations in captive and wild birds suggest that some species can be severely affected by Asian lineage H5N1 HPAI viruses, while others may have much milder signs or shed viruses asymptotically [26;27;172;182;183;189;191-194;205;263;339;348;349]. During one H5N1 outbreak at a wildlife rescue center, some birds died without preceding clinical signs, while others developed anorexia, extreme lethargy, dark green diarrhea, respiratory distress and/or neurological signs, with death often occurring within 1-2 days [192]. Some birds at the facility were unaffected. Neurological signs, varying from mild to severe, were reported in a number of experimentally infected birds including some species of ducks, geese, gulls, house finches and budgerigars [182;193;205;263]. Other experimentally infected birds, such as zebra finches, had high mortality rates, but only nonspecific signs of depression and anorexia [193].

Asian lineage H5N8 viruses have also been associated with wild bird die-offs in some countries, and these viruses and/or their reassortants have been detected in various wild birds including sick, dead and apparently healthy waterfowl, and sick or dead birds in other orders (e.g., Ciconiiformes Gruiformes, Podicipediformes) [16;20;22;23;31-33;184;350]. The presence of the virus may have been an incidental finding in some birds; however, others had no other diseases or injuries. Some birds in South Korea were systemically infected, and neurotropism appeared to have contributed to death [184]. Renal lesions were also notable. In North America, an H5N8 virus killed four captive gyrfalcon (*Falco rusticolus*) or gyrfalcon-peregrine hybrids that had eaten an infected wild bird [22]. Experimental infections with one

H5N8 isolate were asymptomatic in mallards, and either fatal or asymptomatic in Baikal teal (*Anas formosa*) [351].

Information about the effects of other HPAI viruses on wild birds is limited. Wild waterfowl infected with some HPAI viruses seem to be resistant to clinical signs [27;95;186], but an H5N3 HPAI virus caused high mortality among South African terns in the 1960s [185;187]. A wild siskin naturally infected with an H7N1 HPAI virus was also ill [187].

3.8 Transmission

Avian influenza viruses can be shed in the feces as well as in respiratory secretions, with differing recovery rates from each site depending on the virus, species of bird and other factors [66-68;92;93;352;353].

3.8.1 Virus Transmission among Waterfowl

LPAI viruses usually replicate in the intestinal tracts of their aquatic reservoir hosts, and fecal-oral transmission is the predominant means of spread in these birds [27;72;123]. Some recent isolates of Asian lineage H5N1 HPAI viruses have been found in higher quantities in the respiratory secretions than the feces [27;204;306]. This is generally thought to be a characteristic of AIV that have become adapted to gallinaceous poultry, which retain their respiratory tropism even when transferred back to waterfowl [354].

In the laboratory, wild waterfowl usually excrete AIV in the feces during the first two weeks after they become infected [45], but shedding can vary between individuals [355]. Some experimentally infected mallards began to excrete LPAI viruses 1-2 days after inoculation, and some of these birds shed viruses in the laboratory for at least 21 days [355]. An H7N7 HPAI virus was shed from nonvaccinated ringed teal (*Callonetta leucophrys*) for 5 to 17 days [200], and viral RNA from an H5N1 HPAI virus was detected for up to 19 days in oropharyngeal secretions in a vaccinated domesticated Muscovy duck, although virus isolation was not possible after 3 days [331]. The duration of shedding in a waterfowl flock under field conditions is still unclear [354], but the mean period of virus shedding for most waterfowl in the wild seems to be less than a week [123]. Both virulent and avirulent HPAIV isolates can be transmitted efficiently between ducks [204].

3.8.2 Virus Transmission in Gallinaceous Poultry

Once an avian influenza virus has entered a flock of gallinaceous birds on a farm, it can spread by both the fecal-oral route and in aerosols, due to the close proximity of the birds. Fomites can be important in transmission, and flies may act as mechanical vectors [66;356-358]. The possibility of wind-borne transmission of HPAI viruses between farms was suggested by one study [359], but has not been conclusively demonstrated. In most cases, however, the spread of the virus follows the movements of infected poultry and vehicles, people and other fomites, and long-distance aerosol transmission is considered to be unlikely [360].

HPAI viruses can be found in both the feces and respiratory secretions of chickens and turkeys, with most replication thought to occur in the respiratory tract [100;124;198;202;203;208;293;300;301;309;320;361-367]. These birds can begin shedding HPAIV as early as 1-2 days after infection [100;203;295;301;320;363;365-368]. In chickens and turkeys, the infectious period tends to be limited by the death of the bird. Less is known about transmission in other gallinaceous species. Nonvaccinated golden pheasants (*Chrysolophus pictus*) excreted an H7N7 HPAI virus for up to 23 days in one study [200]. In another experiment, Chinese ring-necked pheasants (*Phasianus colchicus*) shed various LPAI viruses for varying lengths of time, from less than 10 days to as long as 23 days, and in one exceptional

case, up to 45 days [369]. The same LPAI viruses could only be isolated for a week or less in chukar partridges (*Alectoris chukar*).

Experiments in small groups of birds [139;198;200;367;370] and limited field studies [139;198;200;367;370] suggest that the species of poultry, viral strain, structure of the poultry population and other parameters can influence transmission, and should be taken into consideration when designing a control plan [371]. In laboratory experiments, some AIV strains appear to spread more readily between birds than others [295;361-363;372]. Viruses that killed birds rapidly were not transmitted as well in some studies, possibly because this limited the period of virus shedding [198;202;373]. In contrast, one HPAI virus (A/chicken/Pennsylvania/1370/83; H5N2) was infectious longer than a closely related LPAI virus from the same outbreak, and spread more readily to susceptible contacts [364]. Transmission may also be influenced by environmental factors such as the stocking density, size of the room, temperature and airflow [198;364]. Avian influenza viruses are thought to propagate more slowly in caged flocks than in group-housed flocks [219;317;374-378], although the type of housing did not affect the transmission rate in some studies ([346]; [378] cited in [346]). Seasonality has been reported with Asian lineage H5N1 viruses, which have tended to reemerge during colder temperatures [379-381]. This might be the result of factors such as increased virus survival in the cold, increased poultry trade during winter festivals and/or wild bird movements [379;380]. Seasonality has also been reported in wild bird populations. For example, LPAI virus prevalence in North American blue-winged teal (*Anas discors*) is increased at late summer staging areas before migration, when bird densities are high and young “hatch year” birds have not yet developed immunity [382].

3.8.3 Backyard Poultry and Transmission

Backyard poultry may act as reservoirs of HPAI, or they may be infected incidentally through virus shed from commercial flocks [383]. Backyard birds were reported to have had only a marginal role in the 2003 H7N7 epizootic in the Netherlands ([384] cited in [383]). A study from an epizootic in Thailand also estimated that backyard flocks were less likely to become infected than commercial poultry; however, these birds were so numerous that they still made a significant contribution to transmission ([378] cited in [383]).

3.8.4 Virus Survival in the Environment

Fecal-oral transmission of avian influenza viruses in birds may be facilitated by prolonged survival in some environments. The persistence of these viruses can be influenced by many factors such as the initial amount of virus; temperature and exposure to sunlight; the presence of organic material; pH and salinity (viruses in water); the relative humidity (on solid surfaces or in feces); and in some studies, by the viral strain [385-399]. Avian influenza viruses survive best in the environment at low temperatures, and some studies suggest that they are more persistent in fresh or brackish water than salt water [363;385-387;389;391;393;395;396;400-402]. Some viruses may survive for several weeks to several months or more in distilled water or sterilized environmental water, especially under cold conditions [385;386;389-391]. However, the presence of natural microbial flora may considerably reduce their survival in water, and at some temperatures, viruses may remain viable for only a few days (or less, in some environments) to a few weeks [390-392;395;403]. Other physical, chemical or biological factors in natural aquatic environments may also influence persistence [390;391;394;402;403].

In feces, some anecdotal field observations stated that LPAI viruses can survive for at least 44 or 105 days, but the conditions were not specified [385]. Under controlled laboratory conditions, LPAI or HPAI virus persistence in feces ranged from < 1 day to 7 days at temperatures of 15-35°C (59-95°F), depending on the moisture content of the feces, protection from sunlight and other factors [363;393;395;397;400;401;404]. At 4°C (39°F), some viruses survived for at least 30-40 days in two studies [363;395], but they remained viable for times ranging from less than 4 days to 13 days in two

recent reports [393;401]. On various solid surfaces and protected from sunlight, viruses were reported to persist for at least 20 days and up to 32 days at 15-30°C (59-86°F) [400]; and for at least 2 weeks at 4°C if the relative humidity was low [393]; but also for less than 2 days on porous surfaces (fabric or egg trays) or less than 6 days on nonporous surfaces at room temperature [405]. Survival was longer on feathers than other objects in two reports: at least 6 days at room temperature in one study [405] and 15 days at 20°C (68°F) and 160 days at 4°C in another report [401]. Some viruses persisted for up to 13 days in soil (4°C) [393], for more than 50 days (20°C) or 6 months (4°C) in poultry meat (pH 7) [388], and for 15 days in allantoic fluid held at 37°C (99°F) [399]. Exposure to direct sunlight greatly reduced virus survival [393]. Environmental sampling in Cambodia suggested that their persistence in tropical environments might be brief: although RNA from Asian lineage H5N1 HPAI viruses was found in many samples including dust, mud, soil, straw and water, virus isolation was only successful from one water puddle [406].

3.8.4 Vaccination and Virus Transmission

Effective vaccination can decrease transmission between animals by 1) decreasing the susceptibility of animals to infection, and 2) reducing virus shedding, if a vaccinated animal becomes infected. In addition to reducing transmission between flocks, decreased virus shedding reduces the contamination of the environment and potentially lowers the risk to humans [73;407]. However, vaccination may also allow birds to survive longer without clinical signs, and if virus shedding is not substantially reduced, transmission could be enhanced.

3.8.5 Virus Transmission to and between Mammals

People and other mammals are usually infected with avian influenza viruses during close contact with infected birds or their tissues, although indirect contact via fomites or other means is also thought to be possible [67;228;230;231;233;234;236;239;276;408-419]. Respiratory transmission is likely to be an important route of exposure, and the eye may also act as an entry point [420-424]. A few H5N1 HPAI virus infections in animals, and rare cases in humans, have been linked to the ingestion of raw tissues from infected birds [227;228;230;231;233;234;236;276;412;417;418]. Oral transmission experiments provide additional evidence for this route in various mammals [227;234;276;278;279;285;417;425]. In humans, the strongest evidence for oral transmission is that two people became infected with an Asian lineage H5N1 virus after eating uncooked duck blood [417]. Additional routes of exposure also existed in other cases [412;418], such as an H5N1 infection in a woman who had no exposure to poultry except through the raw duck blood and chicken hearts processed in the home and sold at her husband's food stand [412].

Most infected people do not seem to transmit AIV to others, including family members [426-429]. Nevertheless, Asian lineage H5N1 HPAI viruses appear to be capable of person-to-person transmission in rare instances [413-416], and one H7N7 HPAI virus was found in a few family members of poultry workers in the Netherlands (although transmission via fomites can be difficult to rule out in such studies) [429;430]. Likewise, a zoonotic H7N9 LPAI virus in China does not seem to spread readily between people, but human-to-human transmission was suspected in a few family clusters [408;410;411;431-436]. Close, unprotected contact, seems to be necessary to transmit any of these viruses [413-416;435;436]. Some laboratory experiments, outbreak reports and field studies have also demonstrated or suggested that H5N1 viruses may be transmitted to a limited extent from sick cats or zoo felids to other felids [231;276], and perhaps for a short time within pig herds [286]. Dogs inoculated with an Asian lineage H5N2 HPAI reassortant isolated from a sick dog could transmit this virus to dogs, chickens and cats [225;287;288]. In other studies, H5N1 HPAI viruses were not transmitted between asymptomatic, naturally infected cats [239], between small numbers of experimentally infected dogs and cats [280] or between pigs [267]. Likewise, one Asian lineage H5N8 HPAI virus did not spread readily between either dogs or cats in a

laboratory experiment [289]. Sustained transmission of avian influenza viruses is a rare event in mammals [92;235] and has never been reported with any of the Asian lineage H5 HPAI viruses.

4. DETECTION OF INFECTED ANIMALS

Summary

Clinical signs and mortality may be useful in recognizing infected flocks of highly susceptible species such as chickens and turkeys; however, the signs and lesions of HPAI are not pathognomonic, and laboratory tests are necessary for confirmation. In general, clinical signs are not an effective way to recognize flocks of infected waterfowl.

Laboratory tests must be validated for the species of bird and purpose of the test. Some tests used in chickens and turkeys are not reliable or not validated in other avian species.

Virological methods used to detect infected birds include virus isolation, genetic methods (e.g., RRT-PCR) and antigen detection. Virus isolation and characterization should be used during the initial confirmation of the outbreak, but it is slow and requires a laboratory with a high biosecurity level. Faster genetic methods, such as RRT-PCR, are often used to identify infected flocks after the initial characterization of the virus. Genetic techniques can also help evaluate changes in circulating viruses, which may affect vaccine efficacy. Antigen-capture kits have low sensitivity, and are not useful for screening asymptomatic flocks. However, they might be used as a flock test with samples from sick or dead birds.

Serology can be used in surveillance to help substantiate freedom from infection, as well as to monitor responses to vaccination. Agar gel immunodiffusion (AGID) and nucleoprotein-specific ELISA tests are traditionally used for avian influenza surveillance in nonvaccinated birds. These tests can detect antibodies to any influenza A virus, regardless of the subtype. HA- and NA-based serological tests are specific for one of the 16 avian HA or 9 avian NA types, respectively. Antibodies to the HA can be detected with the hemagglutination inhibition (HI) test, ELISAs and serum neutralization (SN). In addition to its use in surveillance, the HI test can assess serological responses to vaccines after immunization, and evaluate serological relatedness between vaccine and field viruses. Neuraminidase inhibition (NI) and indirect immunofluorescent antibody (iFA) tests can detect antibodies to the NA. ELISAs have also been developed for some NA types. Antigenic drift or other changes in field viruses might affect the sensitivity of some HA- and NA-based serological tests.

4.1 Clinical Signs

Clinical signs (see section 3.7) may be useful in the recognition of HPAI infected flocks that contain highly susceptible species such as chickens and turkeys; however, the signs and lesions are not pathognomonic, and laboratory tests are necessary for confirmation. Clinical signs can be highly variable or absent in some species such as ducks [25-27;66;182;191;198;199;201-211;292;307;327;343], pheasants ([103;437] cited in [200]) and wild birds [27;66;67;72;92;106;172;182;183;189;191-194;205;348;438], and are particularly unreliable in detecting infected flocks of waterfowl [48;327;328;342;439].

It is still uncertain how quickly an outbreak can be recognized, based on clinical signs and changes in production parameters (e.g., decreased feed consumption). Some models and studies suggest that recognizing an HPAI outbreak based on an increase in mortality might take an average of 5 days to a week or longer [346;375;440].

4.2 Virological Methods to Detect Infected Birds

Virological assays used to detect infected birds include virus isolation, genetic methods (e.g., RRT-PCR), and antigen capture assays [94;439;441]. Both cloacal and oropharyngeal samples, as well as tissue samples from dead birds, should be collected [94;441].

4.2.1 Virus Isolation

Virus isolation and characterization should be used during the initial diagnosis of an HPAIV outbreak [94]. Virus isolation can detect active infections as soon as 24 hours after infection in an individual host, and flocks may be positive for several weeks [441]. The designation of a virus as LPAI or HPAI for official control purposes is based on *in vivo* pathogenicity tests, as well as the detection of molecular patterns in the HA0 cleavage site (see section 3.3.1 for details) [94]. Drawbacks to virus isolation include the high levels of biosecurity needed for culture (usually biosafety/biocontainment level 3), and the time necessary to conduct the test, which may be several days to weeks [94;439;441].

4.2.2 Molecular Techniques

Molecular methods in use or described for AIV include traditional reverse transcription-polymerase chain reaction (RT-PCR) assays, real-time RT-PCR (RRT-PCR), and the nucleic acid sequence-based amplification (NASBA) test [439;441]. The National Animal Health Laboratory Network (NAHLN) uses an official avian influenza RRT-PCR diagnostic procedure, with specific RNA extraction and RT-PCR amplification kits [439]. If appropriate primers are used, some RT-PCR techniques can rapidly detect and identify H5 and H7 viruses in clinical specimens. RRT-PCR tests are valuable in the identification of infected premises after the virus has been characterized initially, and are currently the preferred method for surveillance [94]. Genetic techniques (e.g., sequencing and phylogenetic analysis) are also valuable for evaluating changes in circulating viruses, which may affect vaccine efficacy [94]. Molecular diagnostic tests should be validated for the avian species and specimens with which they will be used [439].

4.2.3 Antigen Capture

Several antigen-capture kits for influenza A viruses are commercially available [94;439]. Most are enzyme immunoassays or lateral flow devices, and recognize the viral nucleoprotein, which is highly conserved between viruses. These tests can detect any AIV subtype [94;439]. Some antigen-capture tests were developed for diagnosis in humans, and have been adopted as veterinary assays, but others are specific for animals [439;441]. The latter include some type A influenza and H5 subtype specific tests [439]. Antigen capture tests can be useful during eradication efforts, as they can usually provide results in 15–30 minutes, and minimal laboratory equipment is required [439;441]. Although their specificity for AIV is reported to be high [442;443], their sensitivity is low [442-444]. These tests are also reported to be less sensitive in waterfowl and wild birds than chickens, probably because the concentrations of viral antigens are lower [443]. However, they had good sensitivity in testing brain swabs from some symptomatic waterfowl. Due to their low sensitivity, antigen capture tests are not appropriate for surveillance in apparently healthy birds [441;443;444]. They may be useful as a flock test, using samples from sick or dead birds [94;441-443]. During H5N1 HPAI outbreaks in Hong Kong, the Directigen™ test could detect HPAIV antigens in at least one sample from all H5N1-infected farms or live bird markets, if at least three sick or dead chickens were tested (Dr. T. Ellis, personal communication in [443]). Antigen detection tests should be used with confirmatory tests such as RT-PCR or virus isolation [94;439;441;443]. They have not been validated for all species of birds [94;444].

Assay	Relative sensitivity	Relative specificity	Time Needed to Run Assay
Virus isolation	Very high	Moderate	1-2 weeks
RRT-PCR	Very high	Very high	3 hours
Commercial antigen detection tests	Low	High	15 minutes

1. Based on an AIV review by E. Spackman, D.L. Suarez and D.A. Senne [441]

4.3 Serological Tests to Detect Infected Birds and Evaluate Vaccine Responses

Serology is used in surveillance, to help demonstrate the absence of virus circulation in vaccinated or nonvaccinated flocks (see also DIVA tests, section 5) and substantiate freedom from infection, as well as to monitor and evaluate responses to vaccination. Serology is a poor method of diagnosing acutely infected, nonvaccinated flocks during an outbreak, as most birds die rapidly without mounting a measurable immune response [439].

AIV-infected poultry produce antibodies to the viral HA, NA, nonstructural proteins (NSPs), nucleoprotein, matrix proteins and polymerase complex proteins [35], although titers to each protein vary and may appear or diminish at different rates. Agar gel immunodiffusion (AGID) and nucleoprotein-specific ELISA tests are traditionally used for AIV surveillance in nonvaccinated flocks [57;63]. These tests, which recognize antibodies to the conserved nucleoprotein and matrix proteins, can detect antibodies to any influenza A virus, regardless of the subtype [57;94]. The AGID test may be able to identify infected poultry as soon as 5 days after infection, and these antibodies remain detectable for weeks or months [441]. This test is reliable in chickens and turkeys, but not in some other species of birds including ducks [35;94;441]. ELISAs are more sensitive [94], but less specific than AGID, i.e., they are more prone to false positives [441]. They are usually confirmed with other tests [441]. Some commercial ELISAs are specific for chickens and turkeys [441], but competitive or blocking ELISAs can be used in all avian species [35;94;441]. ELISA tests that have been validated for veterinary use are preferred to those marketed for the detection of human influenza viruses [94].

HA- and NA- based serological tests are specific for one of the 16 avian HA or 9 NA types, respectively, and do not detect all AIV. Antibodies to these two proteins have been found in some nonvaccinated, experimentally infected chickens and turkeys by day 6 or 7 [445-447]. When these antibodies first appear might be influenced by the species of bird, viral strain and infectious dose [447]. Tests that detect antibodies to the HA include the hemagglutination inhibition (HI) test, serum neutralization (SN) and some subtype-specific ELISAs [35;94]. The HI assay is a quantitative test based on the ability of antisera to block the agglutination of red blood cells by the viral HA [448]. Differences in techniques between laboratories can affect the reported titers [94]. Although HI can be used in a variety of avian species [441], the SN test is the preferred serological test in mammals and some species of birds [35]. Neuraminidase inhibition (NI) and indirect immunofluorescent antibody (iIFA) tests can detect antibodies to the neuraminidase, and a limited number of ELISAs that detect specific neuraminidase types have also been developed [35;45;64;94;447;449-452]. HA- and NA- based tests can be used for various purposes, including in surveillance (provided the subtype is known), and to identify the subtype of a field virus after virus isolation (although genetic tests such as RT-PCR are also used) [35;441]. The HI test can also assess

the magnitude of serological responses to a vaccine, and evaluate serological relatedness between vaccine and field viruses [94]. Significant antigenic drift can affect the sensitivity of HA- and NA-based assays [441], and this might be a concern during some long-term vaccination campaigns [453].

Assay	Relative sensitivity	Relative specificity	Time Needed for Assay
Hemagglutination inhibition	High	Moderate to high	2 hours
Neuraminidase inhibition	Moderate	Moderate to high	6-7 hours ²
AGID	Moderate	High	2 days
Commercial ELISAs	Moderate	Moderate	2-3 hours

1. Based on an AIV review by E. Spackman, D.L. Suarez and D.A. Senne [441]
2. Personal communication, Dr. M.L. Killian, USDA APHIS National Veterinary Services Laboratories

4.4 Validation of Assays (OIE Website)

Both virological and serological assays should be validated for the species of birds and the specific purpose [94]. The OIE Register (<http://www.oie.int/en/our-scientific-expertise/registration-of-diagnostic-kits/background-information/>) lists kits that have been certified by the OIE.

5. AVIAN INFLUENZA VACCINES AND DIVA TESTS

Summary

Birds can be effectively protected against the HA type(s) in a vaccine, whether or not the NA matches the field virus. Commercially available avian influenza vaccines include 1) inactivated, whole virus vaccines and 2) recombinant vectored vaccines based on the HA, with or without the NA protein. Currently, the latter include fowlpox-vectored, Newcastle disease-vectored and turkey herpesvirus-vectored vaccines.

Inactivated avian influenza vaccines for poultry are usually made as oil emulsions, using nonpurified, unconcentrated allantoic fluid from infected eggs. Some inactivated vaccines contain field strains of LPAI viruses. Other vaccine strains are engineered, via reverse genetics, to contain the HA and NA of choice. Inactivated avian influenza vaccines must be given individually to birds by injection, and can be administered repeatedly. Their efficacy in chickens is not optimal until the bird reaches 2–3 weeks of age. One week old turkeys were also reported to have suboptimal immune responses. In addition to gallinaceous poultry, inactivated vaccines have been used in other avian species including waterfowl and zoo birds. Their efficacy may differ between species.

Live, fowlpox-vectored H5 vaccines are also produced, but commercial fowlpox-vectored H7 vaccines are not yet available. One H5 vaccine (TROVAC™ H5) expresses the HA of

A/turkey/Ireland/1378/83 (H5N8), and has been licensed for emergency use in the U.S. since 1998. Another vaccine, produced in China, contains the HA and NA genes from an early Asian lineage H5N1 virus, A/goose/Guangdong/1/96. Fowlpox-vectored vaccines only replicate well in chickens, and have been licensed for use in this species, although they have been tested as nonreplicating vaccines in other birds. Fowlpox-vectored avian influenza vaccines must be given individually to birds by injection, and are ineffective in birds with active immunity to the vector. The TROVAC™ H5 vaccine is usually administered to day-old chicks at the hatchery, but it can be used in older, seronegative birds.

A Newcastle disease virus-vectored live vaccine, which contains the HA from Asian lineage H5 viruses, has been licensed in China since 2006, and was updated with a new clade 2.3 H5 insert in 2008. It is reported to protect birds against both highly pathogenic Newcastle disease and Asian lineage H5 HPAI viruses. Another live NDV-vectored avian influenza vaccine, which contains an H5 from a North American (Mexican) lineage LPAI virus, is licensed in Mexico. It is also labeled for use against both avian influenza and Newcastle disease. A killed version of the latter vaccine has been described in the literature. NDV-vectored vaccines can, at least theoretically, be given by mass administration methods such as sprays or drinking water. Eye drops have been used to administer these vaccines in most published experiments. Administration via drinking water was also effective in one study; however, the vaccine was administered individually to the birds.

A commercial turkey herpesvirus-vectored H5 vaccine contains the HA from a clade 2.2 Asian lineage H5N1 virus isolated in Europe, and has been licensed in a limited number of countries including the U.S. It is labeled for administration by injection to 1-day-old birds at the hatchery, and can also be used to help protect birds from Marek's disease. The formulation (cell-associated) of the current preparation may be an issue, as the vaccine must be shipped and stored in liquid nitrogen, and thawed in limited amounts shortly before use.

An effective surveillance strategy must be established during a vaccination campaign. This is necessary to detect infected flocks, which might otherwise be found by clinical signs, and to prevent the virus from being maintained in the vaccinated population. Surveillance is also used to assess antigenic and genetic changes in the field virus (including the emergence of vaccine-resistant strains), and to demonstrate the effectiveness of the vaccine and vaccination campaign. After an eradication campaign, surveillance demonstrates freedom from infection to trading partners. Avian influenza vaccination programs can be compatible with the continuation of international trade, if the surveillance program convincingly demonstrates that infection is absent from the exporting compartment. This program must meet the standards in the OIE Terrestrial Animal Health Code, to avoid unjustified trade restrictions.

Infections with field viruses can be recognized in vaccinated flocks by various means including serology (DIVA tests), virology and the use of sentinel birds. The method(s) used are influenced by the type of vaccine (e.g., homologous or heterologous, inactivated or recombinant vectored). DIVA strategies are based on recognizing serological responses to AIV antigens that occur in field viruses or are expressed in infected cells, but are not found in vaccines. These strategies might be effective only in populations with good biosecurity and little or no exposure to LPAI viruses. DIVA tests should be validated for the species and the purpose for which they will be used.

Three serological DIVA strategies - the heterologous neuraminidase strategy, the detection of antibody titers to nonstructural proteins (NSPs), and the detection of titers to the M2e protein - have been investigated for use with whole inactivated vaccines.

The heterologous neuraminidase DIVA strategy consists of a vaccine that contains a different NA type than the field virus, combined with a serological test that detects antibodies to the NA of the field virus. The main limitations of this strategy are that it may not identify a field virus with an unexpected NA, and it cannot identify infections with a virus that has the same NA type as the vaccine strain. Tests that might be used include neuraminidase inhibition, iIFA and ELISAs specific for the NA type. ELISAs are currently available only for a limited number of NA types. Some serological tests have been validated for use with this strategy (although field validation of some assays may still be lacking, or absent in some types of birds). The heterologous neuraminidase DIVA was field tested in Italian vaccination campaigns against LPAI viruses, and has also been used by other countries.

Another proposed DIVA strategy is the detection of antibodies to nonstructural proteins, viral proteins that are present during replication in cells, but are not packaged into the virion except in very small quantities. Birds that have been vaccinated with an inactivated (i.e., nonreplicating) vaccine should not develop antibodies to NSPs unless they become infected. This DIVA strategy has the advantage of detecting infections with any AIV subtype. A DIVA test based on the nonstructural protein NS1 has shown some promise as a flock test in some studies, but it is not yet validated. One concern with this strategy is that inactivated vaccines produced for birds can contain low levels of NSPs, and may induce low titers to NS1. Serological responses to NS1 may also be weak in vaccinated birds that become infected, because vaccination may limit virus replication. At present, the NS1 DIVA appears to require further development to be practical.

The M2e DIVA strategy is based on the detection of the extracellular domain of an integral membrane protein that is present in minimal amounts on virions, but abundant on AIV-infected cells. This DIVA strategy can also detect infections with any AIV subtype, and has been promising for use as a flock test in both chickens and ducks. One issue with the M2e DIVA is that the duration of the response seems to be short, which could be limit its use and/or require frequent flock testing. This DIVA strategy also remains to be fully validated before use.

Companion DIVA tests for recombinant vectored avian influenza vaccines can be based on any protein not contained in the vaccine. Assays that could theoretically be used with the current fowlpox-vectored and NDV-vectored vaccines include AGID, nucleocapsid or matrix-based ELISAs, tests to detect antibodies to NSPs (e.g. NS1) and tests that detect any neuraminidase not contained in the vaccine. These DIVA strategies have not yet been validated with vectored avian influenza vaccines.

Vaccination may limit virus replication in birds that become infected. As a result, infected birds may develop only low titers to AIV proteins. This must be considered when surveillance relies on DIVA testing. It also limits these assays to use as flock tests. Poor responses are a particular concern with the NS1 protein, which is a weak antigen. Weak antibody responses to the neuraminidase of the challenge virus have also been reported in some vaccinated birds tested with the heterologous neuraminidase DIVA strategy.

The use of nonvaccinated sentinel birds is an alternative or complementary method to detect infections in vaccinated flocks. This strategy is suitable for use with all avian influenza vaccines,

as well as in flocks where the birds may have been previously exposed to AIV. Sentinel birds can also provide an additional layer of security with serological DIVA strategies. Sentinels should be monitored daily for clinical signs, and any illness or deaths must be investigated to rule out avian influenza. These birds should also be tested regularly with serological and/ or virological tests.

An additional safeguard in vaccinated flocks is to sample baseline daily mortality or sick vaccinated birds, using virological tests. The ability of clinical surveillance to detect infections in vaccinated birds may be limited.

5.1 Overview of Protective Immunity and DIVA Tests

Birds are protected from AIV by immunity to the HA, and to a lesser extent, the NA [46-49]. In practical terms, they can be effectively protected against the HA type(s) in the vaccine, whether or not the NA matches the field virus [44-46]. Licensed avian influenza vaccines include 1) inactivated, whole virus vaccines, and 2) recombinant vectored vaccines based either on the HA protein alone, or both HA and NA proteins.

Strategies to differentiate infected from vaccinated animals (DIVA strategies) are based on recognizing serological responses to antigens that occur in field viruses or are expressed in infected cells, but are not found in vaccines. DIVA strategies that can be used with avian influenza vaccines include:

- Heterologous neuraminidase DIVA: The use of a vaccine that contains a different neuraminidase type than the field virus, combined with a serological test that detects antibodies to the NA of the field virus.
- The use of an inactivated vaccine, combined with a serological test that recognizes responses to viral proteins that are made exclusively or primarily by viruses during replication in the cell.
- The use of a recombinant vaccine based on the HA (or HA and NA), combined with a serological test that recognizes responses to any viral protein not contained in the vaccine.

Each serological DIVA strategy is appropriate only with certain types of avian influenza vaccines. Another strategy is to place nonvaccinated sentinel birds in vaccinated flocks. Sentinel birds can be used with any vaccine. Avian influenza vaccines and their companion DIVA strategies are described in more detail in the following sections.

5.2 Live Attenuated Avian Influenza Vaccines

Conventional live attenuated avian influenza vaccines are not recommended for poultry, both because they could reassort with field viruses, and because live H5 or H7 vaccines could generate HPAI mutants [45;49;94;454;455].

5.3 Inactivated Avian Influenza Vaccines and Companion DIVA Strategies

5.3.1 Inactivated Vaccines

Inactivated avian influenza vaccines are usually made as oil emulsions for potency. They have traditionally been made from LPAI viruses that grow to high titers in embryonating chicken eggs [46;47;49;456;457]. HPAI vaccine strains are uncommonly used (and are not recommended by the OIE) due to safety concerns, the need for high biocontainment manufacturing facilities for these viruses, and the fact that vaccines containing LPAI viruses are effective against HPAI field strains [46;94;458]. Some inactivated vaccines are still made using this traditional approach; others are custom-made by reverse genetics.

Reverse genetics is a technique that can create inactivated avian influenza vaccines with any desired HA and NA type [45;47;49;342;456;457;459]. It can generate a vaccine strain that has an HA tailored to the outbreak strain, combined with the NA of choice, and internal proteins from an AIV strain that grows well for vaccine production (e.g., the human vaccine strain PR8). The HA may come from an HPAI strain, such as the field virus, that has had its HA0 cleavage site mutated to the low pathogenicity form [47;342;459]. It may also come from a related LPAI virus [47]. One strategy is to generate a vaccine strain with HA and NA proteins that both match the field virus, potentially maximizing immunity [342;459]. Another approach is to use an HA similar to the field virus, but a different neuraminidase, which allows the use of the heterologous neuraminidase DIVA strategy (see section 5.3.5) [45;94;459;460]. Reverse genetics also lends itself to modifications that may improve immunity from the vaccine strain. For example, experimental attempts have been made to broaden the specificity of H5N1 vaccine viruses by introducing specific mutations into the HA gene [461].

5.3.2 Production of Inactivated Vaccines

Although some vaccine viruses (including those produced by reverse genetics) may also replicate well in mammalian cell lines [462;463], the viruses for avian vaccines are currently grown in embryonated chicken eggs [45;49;94]. These viruses are then inactivated by physical or chemical methods, which may include formalin, beta-propiolactone or aziridines (e.g., binary ethyleneimine) [49;94;458;464]. Unlike influenza vaccines for humans, which are generally treated with detergent to partially purify the HA and NA into a subunit vaccine, avian influenza vaccines for poultry are usually crude preparations that use unpurified, unconcentrated allantoic fluid [45;49;94]. The production costs for such unpurified vaccines are lower; however, they may induce antibodies to proteins not contained in the virion, which could interfere with the use of some DIVA tests [49]. Some AIV isolates do not grow well enough to produce potent vaccines unless they are concentrated, which is expensive [407]. Concentration may also be done for vaccine storage [94].

The infective allantoic fluid is usually emulsified as a water-in-oil preparation, with the oil acting as an adjuvant [45;49;94]. The type of adjuvant can affect vaccine efficacy, and this may also be influenced by the species of bird [462]. Non-metabolizable mineral oil is reported to be more potent than biodegradable oils in gallinaceous poultry ([465] cited in [45]) and some manufacturers use proprietary adjuvants. Oil adjuvants mainly induce humoral immunity [49;466], although water-in-oil emulsions may activate cytotoxic T lymphocytes (CTLs) under some conditions [49]. Residues of the agent used to inactivate the virus can affect the stability of the emulsion and antigen quality, which might influence vaccine efficacy [45]. Inactivated vaccines are tested to ensure that the virus has been inactivated [94]. Batch control tests, as well as testing for sterility, freedom from contamination of biological materials, and potency are also done [94]. Vaccines should be bought from reputable companies with good quality control. There have been occasional reports of vaccines that contained strains other than those on the label (e.g., A/goose/Guangdong/1/96, H5N1 rather than A/turkey/England/N28/1973, H5N2) [136;467]. Inactivated avian influenza vaccines are expected to maintain their potency for at least 1 year, when stored as recommended [94].

5.3.3 Administration of Inactivated Vaccines

Inactivated avian influenza vaccines must be given individually to birds by injection [457;468;469]. Their efficacy is not optimal in chickens until the bird reaches 2–3 weeks of age [466;469]. Seven-day-old turkeys were, likewise, reported to have limited responses to AIV vaccine antigens [368]. Inactivated vaccines are safer than vectored vaccines, particularly in immunocompromised hosts [457]. They have been effective in a variety of poultry species including chickens, turkeys, geese and ducks, although vaccine efficacy is not necessarily the same in each species [46;48;337]. These vaccines have also been administered safely to a wide variety of zoo birds, and stimulated HI titers expected to be protective in many, though not all, of these birds (see also section 19) [49;470-477]. Among domesticated

waterfowl, Pekin ducks were reported to respond better than Muscovy ducks to at least one commercial vaccine [337].

Inactivated vaccines can be given repeatedly, which may be necessary in long-lived birds such as breeders, layers and turkeys [48;457] or zoo birds. More than one dose may also be necessary for full efficacy in some birds, depending on the vaccine and host species [49;327;337;342;477;478]. Boosters are likely to be needed for good efficacy in the field, even if a single dose is adequate to stop AIV transmission in the laboratory [8;327;342;468;479;480].

5.3.4 Companion DIVA Strategies for Inactivated Vaccines

Potential companion DIVA strategies for inactivated vaccines include 1) the heterologous neuraminidase method, or 2) serological tests that detect antibodies to AIV proteins that are absent or minimal in the virion, i.e. nonstructural proteins.

5.3.4.1 Heterologous Neuraminidase DIVA Strategy

The heterologous neuraminidase DIVA strategy requires the use of a vaccine with the same HA type as the field virus, but a different NA [94;451;460]. The HA component of the vaccine provides protection from the outbreak strain, while the neuraminidase allows infections to be recognized in vaccinated birds [460]. The main limitations of this strategy are that it may not identify infections with a new field virus that has an unexpected NA, and it cannot identify infections with field viruses of the same NA type as the vaccine strain [1;45;48;94;481]. To help mitigate this problem, the development of H5 and H7 vaccine strains with neuraminidase types that are not common in H5 or H7 viruses (e.g., N5 or N8), has been investigated [1;482]. A different experimental approach is to produce vaccines that contain NA genes not normally found in AIV. For instance, one laboratory has produced an experimental vaccine that contains an avian HA gene from H5 viruses, but an NA from a human influenza B virus [483]. The heterologous neuraminidase method is the only DIVA strategy that has been field tested in a campaign that resulted in virus eradication [48]: during LPAI outbreaks in Italy, iIFA assays were used to detect infections with H7N1 and H7N3 field viruses in vaccinated poultry [447;484]. Six additional countries are reported to have employed a heterologous neuraminidase strategy in vaccination campaigns, although details were not provided [149].

Assays used to detect infected birds in this strategy should be highly sensitive [451]. The replication of challenge/ field viruses is decreased in vaccinated birds, and may result in diminished antibody responses to the NA ([142]; [485;486] cited in [451]). In one challenge study, antibodies to a heterologous neuraminidase were only detected in a few vaccinated birds after challenge, although an HI anamnestic response was reported in 60% of the birds [142]. Higher response rates have been reported in vaccinated birds in some other studies (e.g., [459]). Based on a limited number of studies, it appears that responses to the NA protein of an infecting virus are also slightly delayed in vaccinated compared to nonvaccinated birds [452]. However, both turkeys and chickens appear to respond by 2 weeks after infection, and some individual birds may respond by 1 week [452].

Serological tests that can be used in the heterologous neuraminidase DIVA strategy include iIFA, the neuraminidase inhibition (NI) assay and ELISAs [45;64;447;449-451;459]. The conventional NI test and iIFAs are labor-intensive [481], cannot be adapted for automation and high throughput flock screening, are subjective, and depend on the skill of the operator in test interpretation [45;73;447;451]. In addition, there is the necessity of propagating and handling infectious viruses [451]. An advantage to the iIFA is that the characteristic distribution and pattern of fluorescence results in a high degree of confidence in test interpretation, if the sample is read by skilled technicians [447]. A modified neuraminidase inhibition assay, which uses a fluorescent substrate, is fast and quantitative ([55;487] cited in [73]). This test was promising in chickens vaccinated with various North American or Asian lineage vaccines, and challenged

with H5N1 HPAI viruses [73]. Indirect and competitive ELISAs that detect antibodies to at least 4 neuraminidases (N1, N2, N3 and N7) have also been developed [45;449-452]. The level of validation varies, with some tests showing sufficient sensitivity and specificity to identify vaccinated birds that become infected with a challenge virus in the laboratory [452]. Some ELISAs, including at least one in the U.S., are marketed as commercial test kits [452]. Field validation for use as DIVA tests may still be lacking [452].

5.3.4.2 DIVA Tests Based on Differential Immune Responses to AIV Proteins: NS1

The detection of antibodies to nonstructural proteins (NSPs) has also been proposed as a DIVA strategy with inactivated vaccines [45;48;56;61;63;64;451;452]. NSPs are viral proteins that are present during replication in cells, but are not packaged into the virion. These proteins are absent from sufficiently purified inactivated vaccines. Because these vaccine viruses do not replicate in birds, vaccinated birds should not develop antibodies to NSPs unless they become infected. In practice, commercial inactivated avian influenza vaccines are usually unpurified [45;49;94], and may contain low levels of NSPs.

The NS1 protein has been investigated for use in DIVA tests, and some commercial or experimental NS1 ELISAs have been produced [45;57;61]. (NB: Although NS1 was previously thought to be absent from purified virions, a study published in 2014 found very low but detectable levels [62]. However, these amounts are small enough not to interfere with its use in DIVA strategies.) Large amounts of NS1 are made in AIV-infected cells during virus replication [57;63]. It is highly conserved ([57]; [58;59] cited in [56]), and NS1-based serological tests can detect infections with any subtype [64], provided the test takes into account the occurrence of two different forms (NS1A and NS1B) [61]. One issue with the NS1 DIVA strategy is that birds immunized with commercial inactivated avian influenza vaccines can develop low titers to this protein [57;61;65]). NS1 is also a weak antigen compared to other proteins in influenza viruses [57]. Serological responses in infected birds might be poor, especially when virus replication is limited by vaccination ([63;142]; [485;486] cited in [451]).

In an early study, an ELISA detected reactions to NS1 in LPAI virus-infected poultry, but not in birds immunized with commercial vaccines [57]. These sera were diluted 1:200 to eliminate low-level reactions from the use of unpurified vaccines. Another study also reported that anti-NS1 titers could be found in nonvaccinated, LPAI virus infected birds [65]. However, other researchers found that antibodies to NS1 appeared only transiently before declining or disappearing over the next few weeks, in LPAI virus-infected poultry [63;488]. Two LPAI viruses induced NS1 titers in few or no birds [61]. One group from South Korea reported that attempts to validate a NS1 strategy for use with H9N2 LPAI vaccination were unsuccessful, although a different strategy (M2e, see section 5.3.7) was promising in this setting [489].

The NS1 DIVA has been somewhat more promising in poultry infected with HPAI viruses. In chickens infected with A/turkey/England/63 (H7N3), HI titers and antibodies to NS1 were both detected as early as 1 week in surviving birds [61]. Anti-NS1 titers remained relatively unchanged during the 2 months the birds were followed. In another experiment, antibodies to NS1 could be found in up to 40% of vaccinated birds challenged with an H5N2 HPAI virus, although they occurred in only a small percentage of vaccinated birds challenged with LPAI viruses [63]. One study reported that an NS1-ELISA could identify antibodies to this protein in some, but not all, vaccinated or nonvaccinated chickens that had been challenged with an H7N3 HPAI virus and were positive by virus isolation [61]. Based on these studies, the NS1 DIVA might be useful as a flock test in HPAIV-infected birds, but it is unreliable for detecting infections in individual birds. Additional birds may also need to be tested to compensate for the poor seroconversion [63].

At present, the NS1 DIVA is not fully validated in experimentally infected animals [45;48;452]. While it may be possible to develop this strategy, the inconsistent antibody responses to the NS1 protein may be an issue, and it does not appear to be a practical method for use in the near future [452].

5.3.4.3 DIVA Tests Based on Differential Immune Responses to AIV Proteins: M2e

The M2e AIV protein has been investigated for use in DIVA testing of chickens and ducks [44;50;336;489-491]. M2e, which is the extracellular domain of an integral membrane protein, is present in minimal amounts on virions, but abundant on AIV-infected cells [44;45]. This DIVA strategy should be able to detect infections with any AIV, regardless of the subtype. One ELISA was reported to recognize sera reactive to various H1, H2, H3, H5, H7, H9 and H11 LPAI or HPAI viruses [491].

Several studies have demonstrated that antibodies to M2e are present in most nonvaccinated chickens or ducks infected with H5 or H7 HPAI viruses and chickens infected with H9 LPAI viruses, but absent in the majority of birds immunized 1-3 times with inactivated vaccines [44;50;336;489-491]. One group reported that antibodies to M2e were also absent from chicks with high levels of maternal antibodies due to vaccination [50]. Field samples from nonvaccinated or vaccinated chickens may have false positive rates up to approximately 5% in some M2e ELISAs [490;491]; however, this appears to be related to the composition of the assay, and a recent study reported few false positives [50].

Several studies have demonstrated that vaccinated ducks [44;336] or chickens [50;490;491] seroconverted to M2e when they became infected with H5N1 HPAI viruses after challenge. In one study, titers to M2e were elevated after challenge despite no significant increase in HI titers [491]. However, another group reported that, in chickens vaccinated up to 3 times, the magnitude of the M2e titer after challenge was inversely correlated with the number of vaccinations [50]. In one early study, an M2e ELISA could not detect infected chickens that had been immunized with an H7N1 vaccine and challenged with an H7N7 HPAI virus, although it was effective in vaccinated ducks challenged with H5N1 [44]. The reason for this is unclear. All later challenge studies in chickens used H5N1 HPAI viruses [50;490;491] or H9N2 LPAI viruses [489].

Antibodies to M2e can be detected by 7-14 days after challenge in some vaccinated chickens [50;489] and by 2 weeks in ducks [44]. However, the duration of the response may be short, which could be a limitation to this DIVA strategy in the field and/or require frequent flock testing [50]. In one study, all vaccinated layer chickens challenged 6 weeks later with an H5N1 HPAI virus developed M2e antibodies, and titers persisted for at least 8 weeks, peaking around 2-4 weeks after challenge [50]. However, titers were lower and antibodies persisted for only 4 weeks when the birds were challenged 2 weeks after immunization, at a time when they were presumably more resistant. Another group detected M2e titers in only 22% of vaccinated chickens, 9 weeks after they had been challenged with an H9N2 LPAI virus [489]. These birds had received a single dose of vaccine. Chickens vaccinated twice had even lower titers. Fewer than 20% of the latter group had antibodies to M2e at any time. It is unclear whether vaccination prevented virus replication, or M2e titers were too low to be detected. In this study, M2e titers in nonvaccinated chickens began to decline 3-6 weeks after inoculation with an H9N2 virus, but 43-100% of these birds still had detectable titers after 26 weeks. Others have also reported that antibodies to M2e seem to decline more rapidly in naturally infected birds than responses to other AIV proteins [492].

As with other DIVA tests, the M2e strategy appears to be best suited for development as a flock test, and not as a test for individual birds [50]. One experimental M2e ELISA was tested with serum samples collected from chickens during H9N2 LPAI vaccination campaigns in South Korea [489]. These authors reported that avian influenza could be diagnosed in a chicken house when more than 20% of sera from vaccinated chickens reacted in this assay. The various M2e ELISAs, as well as the DIVA strategy overall, remain to be fully validated before use. One additional consideration is whether changes in field viruses

might affect test sensitivity. Although M2 is less variable than the HA protein, several lineages of this protein (with variability particularly in M2e) have been identified in China [493].

5.4 Recombinant Vected Avian Influenza Vaccines and Companion DIVA Strategies

5.4.1 Overview of Recombinant Vected Avian Influenza Vaccines

Vected vaccines based on the AIV hemagglutinin, with or without the neuraminidase, are also in use. They consist of genes for AIV proteins inserted into the vector construct, which contains a promoter and other sequences that allow the genes to be expressed *in vivo* after injection [49]. These vaccines have some of the advantages of a live virus vaccine in mimicking an infection, without the risk of gene reassortment [49;457;494]. The use of the HA gene alone also improves safety during vaccine manufacturing, compared to the use of whole virus [45;457]. Depending on the specific vector and the route of administration, vected vaccines may stimulate mucosal and cell-mediated immunity, as well as humoral immunity [45;49;94;457;494]. In addition, the AIV gene insert can be changed relatively quickly if the field virus changes [47;49;457].

Vected avian influenza vaccines can induce immunity to the vector, as well as to the AIV proteins it contains. This may be useful in some vaccination programs, e.g., if the vector is Newcastle disease virus (NDV) and a separate vaccination is not needed for this disease [45]. It can also be a disadvantage, because the vaccine may not be effective in animals that have already been exposed to the vector [46;49;94;460;469;495]. Maternal antibodies to AIV seem to interfere less with some vected vaccines than inactivated vaccines, especially when used in a prime-boost protocol [94]. However, their effects may not be negligible in some circumstances (see section 15.9, Maternal Antibodies).

Recombinant live vected vaccines should only be used in species where their efficacy has been proven [94]. Such vaccines are, theoretically, limited to use in species susceptible to the replication of that vector [46;140;496]. However, some vaccines have also been investigated in non-susceptible species, given as non-replicating vaccines at higher doses (for details, see section 5.4.2 below) [331;336].

Vected AIV vaccines are sometimes administered as single doses to short-lived birds such as broilers [497]. The 2015 OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals questions whether a single dose will provide sufficient immunity in the field, without boosting [94]. (See section 15.8.1 for a discussion of prime-boost vaccination protocols.)

5.4.2 Fowlpox Vected Vaccines

Live, fowlpox-vected recombinant vaccines are produced commercially for H5 viruses [469;496;498;499], and are in development for H7 viruses [460;469;500]. Current H5 vaccines contain the HA alone, or both the HA and NA, but no other AIV proteins [46;116;469;501;502]. The TROVAC™ H5 vaccine (Merial Select Inc., Gainesville, Georgia, USA) expresses the HA of A/turkey/Ireland/1378/83 (H5N8), and has been licensed for emergency use in the U.S. [46;466;469]. This vaccine is produced in chicken-embryo-origin fibroblast cells [503]. It is supplied as a lyophilized powder, which is reconstituted with an aqueous diluent at the time of use. A fowlpox-vected H5N1 avian influenza vaccine, produced in China, contains the H5 and N1 genes from an early (clade 0) Asian lineage H5N1 virus, A/goose/Guangdong/1/96 [501].

Fowlpox-vected avian influenza vaccines must be given individually to birds by injection [47]. These vaccines are ineffective in birds with pre-existing active immunity to the vector (i.e., immunity produced by the bird's own immune system after exposure to fowlpox virus or fowlpox-vected vaccines) [46;460;469;495]. Because exposure to fowlpox is common and can sometimes be unpredictable [460],

the vaccine is usually administered to day-old chicks at the hatchery [45-47;466;469;496]. Maternal antibodies to fowlpox do not seem to interfere significantly with immunity in day-old birds ([469;504]; [505] cited in [94]). Fowlpox-vectored vaccines can also be given to older birds, if they have never been exposed to the vector [469].

The fowlpox virus replicates well only in chickens, and these vaccines have been licensed for use in this species [46;140;496]. Their use has also been explored in other avian species [331;336;502;506], because the vector can enter cells that are not permissive for replication and express some early genes ([507] cited in [331]). In this situation, the vector is nonreplicating, and the vaccine is given at a high dose [331]. Fowlpox-vectored H5 vaccines were less effective than inactivated vaccines in two challenge experiments in Muscovy ducks [331], as well as in two Chinese breeds of ducks (although an experimental fowlpox vaccine incorporating cytokine expression was more effective in the latter study) [508]. In Pekin ducks, priming with a fowlpox-vectored vaccine and boosting with an inactivated vaccine was more effective than giving two doses of either vaccine [336]. Another study reported that a similar prime-boost regimen in Pekin ducks or Muscovy ducks was at least as immunogenic as 2 doses of an inactivated vaccine [506].

5.4.3 Newcastle Disease Virus Vectored Vaccines

Newcastle disease virus has also been used as a vector for avian influenza vaccines. Experimental NDV- vectored avian influenza vaccines have been effective in chickens challenged with HPAI viruses [119;501;509-516] and in mule ducks, when given as 2 doses [341]. One dose was effective in Muscovy ducks, but protection was short-lived [344]. Better protection was obtained in the latter study when the vaccine was used in combination with a fowlpox- vectored vaccine. One NDV- vectored vaccine, which contains an Asian lineage H5 from A/goose/Guangdong/1/96, was licensed in China in 2006 [501]. It was used extensively in China, and is reported to protect birds against both highly pathogenic Newcastle disease and Asian lineage H5 HPAI viruses [45;49;94;342;501]. In 2008, the HA gene was replaced by H5 from a more recent H5N1 strain, A/duck/Anhui/1/06 (clade 2.3) [501]. A live NDV- vectored H5 avian influenza vaccine has also been licensed in Mexico [45;49;517]. This vaccine (NewH5™, Laboratorio Avi-Mex, SA de CV, Mexico) contains the H5 from the LPAI virus A/chicken/Mexico/435/2005 (H5N2) [494], and is labeled for use against both avian influenza and Newcastle disease. The vector is propagated in SPF embryonated eggs and lyophilized. A killed version of the NDV- vectored H5 vaccine (K-NewH5™, Laboratorio Avi-Mex, SA de CV, Mexico) has been described in the literature [457]. The vector construct is grown in embryonated eggs, and the allantoic fluid is inactivated with formalin and emulsified in mineral oil.

NDV- vectored vaccines are capable of being given by mass administration methods such as sprays or drinking water [45;47;481;501;512;517]; however, eye drops and/or combined ocular and intranasal administration have been used in most published experiments [45;344]. One vaccine resulted in similar clinical protection, virus excretion and HI titers whether it was administered to chickens by the oculonasal route or via drinking water [515]. However, the vaccine was administered individually to all birds in this study, in measured doses, rather than by free access to water containing the vaccine.

A single dose of the Chinese NDV- vectored vaccine was sufficient to protect chickens challenged with HPAI viruses in the laboratory, but repeated doses have been necessary for good immunity in field studies in China [45]. The possibility that pre-existing immunity to NDV might decrease the effectiveness of these vaccines should also be considered [45;49;94;481]. Birds in commercial production may be vaccinated multiple times with NDV vaccines over a lifetime, and chicks may have maternal antibodies to this virus [49]. The 2015 OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals states that NDV- vectored vaccines are largely ineffective when administered as a single dose to poultry that have maternal antibodies or are well-immunized against Newcastle disease [94]. Few studies examining the effects of maternal antibodies to NDV have been published. One suggested that chicks with high maternal

antibody titers to NDV can be vaccinated successfully with a commercial live NDV-vectored vaccine [494]. However, protection was based only on clinical signs; virus shedding was not measured. In another study, antibodies to NDV had no inhibitory effect on serum and duodenal antibody responses to the HA, in 7-day-old mule ducks that received 2 doses of an NDV-vectored H5 vaccine (a single dose was not tested) [341]. (Paradoxically, these antibodies appeared to be higher in ducks with maternal antibodies to NDV). The maternal antibodies did, however, inhibit immune responses to the NDV component of the vaccine.

NDV-vectored H7 vaccines are not yet commercially available. Although early H7 vaccines provided limited clinical protection [510], newer vaccines protected chickens from clinical signs after challenge by HPAI viruses, and decreased or prevented virus shedding in some studies [513;514;518]. In one of these studies, an NDV-vectored H7 vaccine appeared to be less immunogenic than an NDV-vectored H5 vaccine that expressed similar amounts of protein, and two doses of the H7 vaccine were required for good protection in chickens [514].

5.4.4 Turkey Herpesvirus Vectored Vaccines

A commercially available turkey herpesvirus (HVT)-vectored vaccine (Vectormune HVT AI™, Ceva Santé Animale, Libourne, France) contains the HA from the clade 2.2 Asian lineage H5N1 HPAI virus A/Swan/Hungary/499/2006. The vector is a genetically engineered Marek's disease virus of serotype 3 (turkey herpesvirus) [519]. This vaccine has been licensed in a small number of countries, including the U.S., Egypt and Bangladesh (as of 2014), and it has been used in Egypt since 2012 [458;497]. It is labeled for administration by injection to 1-day-old chicks at the hatchery, and also helps protect birds from Marek's disease [519;520]. In theory, HVT-vectored vaccines could also be injected *in ovo*, 2-3 days before hatching ([521] cited in [522];[523]); however, efficacy and lack of interference with any other *in ovo* vaccines should be demonstrated by this route. The current vaccine is fragile and requires careful handling. It is supplied in a frozen, cell-associated form, and must be shipped and stored frozen in liquid nitrogen [519]. Once it has been removed from the liquid nitrogen (using appropriate personal protective equipment), it must be thawed, mixed with diluent and used quickly. The manufacturer recommends thawing and using no more than 3 ampoules at one time. A lyophilized cell-free form of this vectored vaccine was promising, and easier to handle, in one recent study [524].

Commercial or experimental HVT-vectored H5 vaccines have been effective in chickens, and are labeled for use in this species [497;519;520;522;524;525]. Unpublished work from the manufacturer suggests that these vaccines can also replicate in some waterfowl, although seroconversion was not observed [526]. Virus replication appeared to be best in Muscovy ducks and geese, intermediate in mule ducks, and low in Pekin ducks. Challenge studies are still required to evaluate protection in the various species [526]. HVT-vectored vaccines can establish persistent infections in some species of birds, including some waterfowl, which may allow long-term stimulation of immunity [526].

Experimental HVT-vectored H7 vaccines that can protect chickens from clinical signs and reduce virus shedding have also been described [523;527], but are not commercially available at this time.

5.4.5 Companion DIVA Tests for Recombinant Vectored Avian Influenza Vaccines

Companion DIVA tests for vectored avian influenza vaccines can be based on any protein not contained in the vaccine. There appears to be little published work, at present, investigating their use, and there are no reports that DIVA strategies have been used with these vaccines in the field.

The AGID test and nucleocapsid or matrix protein-based ELISAs could theoretically be used with the vectored vaccines that express only the HA and/or NA [35;45;94;451;460;469;481]. Both of these assays have the advantage of recognizing infections with any subtype of AIV. Some researchers have reported

that AGID could detect titers to the nucleoprotein and matrix proteins, in chickens vaccinated with a fowlpox-vectored H5 vaccine and challenged with H5N2 or H5N1 viruses [366;528]. Antibodies were not found in vaccinated chickens before challenge. In another study, two commercial ELISAs could not detect antibodies to the NP in some chickens vaccinated with fowlpox-vectored H5 vaccines, even among birds that shed H5N1 HPAI viruses after challenge [466]. This study, as well as an experiment reporting the absence of AGID titers in some vaccinated, challenged chickens ([529] cited in [466]), suggest that this strategy should be used with caution and only as a flock test. The absence of titers to the NP and matrix proteins may be due to limited virus replication, and might be compensated by increasing the number of samples from the flock [466].

DIVA strategies could also be based on other AIV proteins not contained in the vaccine construct, such as non-structural proteins or M2e; however, these strategies do not seem to have been explored [35;45]. Tests to detect the neuraminidase would be appropriate with vaccines containing only the HA, as well as with the Chinese fowlpox-vectored H5N1 vaccine if the field virus contained a NA other than N1.

5.5 General Limitations of Serological DIVA Tests

Vaccination may limit virus replication, and decrease the immune response to AIV proteins [45]. For this reason, the sensitivity of the serological tests used in DIVA strategies must be high [45;451], and titers may nevertheless be low or absent in some birds [57;61;63;64;142;451;466;485;486;530]. For this reason, DIVA assays must be used as flock tests [61;142;466]. The number of samples tested might need to be increased to compensate for weak seroconversion [63].

DIVA strategies based on differential responses to AIV proteins are effective only in populations with little or no exposure to these viruses [45;342]. These strategies might be ineffective in ducks, geese, zoo birds or free range flocks, which may be exposed more often to LPAI viruses than commercial poultry reared under high biosecurity [45;342]. DIVA tests have not yet been validated in some species, including ducks [342].

5.6 Evaluation and Validation of DIVA Tests

Commercial assay kits or in-house tests should be validated for the species and purpose for which they will be used [94;452]. Assessment by an outside laboratory is also desirable to ensure that the results are reproducible [452]. Depending on their purpose, validated tests can vary in their sensitivity and specificity. The recognition of a test as validated is usually done by a nation or international body [439]; a test may be validated in some countries but not others. However, diagnostic tests (or vaccines) that have been licensed in a country with a well-defined and rigorous procedure for this process are often either accepted by other countries or can be licensed through an expedited process [452]. Validated tests are often marketed commercially [439]. The OIE Register of Diagnostic Tests (<http://www.oie.int/en/our-scientific-expertise/registration-of-diagnostic-kits/background-information/>) lists assays that are currently validated by the OIE, using 'fitness for purpose' as a criterion. Tests that have been validated must continue to be monitored for their performance, especially on new isolates [439].

Currently, the heterologous neuraminidase strategy is the only DIVA method that has been validated in the field and used during an outbreak. Some tests to detect internal virus proteins (e.g., AGID and ELISAs) are well known and standardized for AIV detection in nonvaccinated birds, but they do not appear to have been validated yet for use in a DIVA strategy. They can only be used as DIVA tests with recombinant vectored vaccines [447]. The NS1 and M2e DIVA strategies are still under investigation [44;50;61;63;336;489-491] and should be validated if they are chosen.

5.6.1 Assays Used in the Heterologous Neuraminidase DIVA Strategy

Indirect immunofluorescence tests have been validated for some species, and some iIFAs have been field tested in Italian vaccination campaigns [447;484]. In preliminary validation of an N1 iIFA using turkey sera, its relative sensitivity and specificity were 98.1% and 95.7%, respectively, compared to the HI test [484]. There was almost perfect agreement between the two testing methods. Full laboratory validation has been published for an N3 iIFA, in turkeys and chickens [447]. The diagnostic sensitivity of this test, using turkey sera, was 99.2% (95% CI: 96.9-99.9) and the specificity was 100%. The positive predictive value was 100% and the negative predictive value was 99.3% (95% CI: 97.6-99.9). In chickens, the diagnostic sensitivity was 94.1% (95% CI: 91.3-96.8) and the specificity was 99.0% (95% CI: 98.0-100%). The positive predictive value was 98.5% (95% CI: 94.5-99.8) and the negative predictive value was 96.1% (95% CI: 92.5-98.3). There was almost perfect agreement between the H7 HI test and the N3 iIFA test for both chickens and turkeys. This test was also examined with field samples in vaccinated turkeys and chickens [447]. Its performance appeared to be better in turkeys; however, this conclusion was based on a limited number of samples from chickens. The authors recommend that, until the test can be investigated further in vaccinated caged layers, the sample size for this category of birds should be increased.

ELISAs can also be used in the heterologous neuraminidase DIVA; however, an ELISA specific for the NA of the field virus must be available. An advantage to using ELISAs is that they can rapidly test large numbers of samples. ELISAs that detect antibodies to some neuraminidases (N1, N2, N3 and N7) have been published [449-452]. Three ELISAs are adequately sensitive and specific to be used as screening tests, and can be used as DIVA tests [452]. While at least one published analysis has compared the performance of competitive ELISAs using field samples, as well as samples from the laboratory [450], a recent (2012) review noted that field validation with large numbers of field samples from vaccinated and vaccinated, exposed birds was still lacking [452].

One study reported that several different DIVA tests had low sensitivity, when they were used to detect infections in vaccinated chickens [64]. Bayesian methods were used to evaluate three assays employed in DIVA strategies - an N7 iIFA test, the neuraminidase inhibition assay and a NS1 ELISA - in vaccinated chickens infected with an H7N7 HPAI virus. The N7 iIFA and NI assays had sensitivities of 95% (95% CI: 89-98%) and 93% (95% CI: 78-99%), respectively, when they were used to detect seroconversion in nonvaccinated chickens. However, their sensitivity in vaccinated chickens was only 64% (95% CI: 52-75%) and 63% (95% CI: 49-75%), respectively. The sensitivity of the NS1 ELISA was 55% (95% CI: 34-74%) for detecting seroconversion, and 42% (95% CI: 28-56%) for detecting infections in vaccinated birds. The estimated specificity for these tests was 92% (95% CI: 87-95%) for the iIFA, 91% (95% CI: 85-95%) for the NI assay, and 82% (95% CI: 74-87%) for the NS1 ELISA. This study also reported that chickens that shed virus longer (at least 3 days) were likely to develop antibodies, while infected birds that did not shed infectious virus often remained seronegative. The former birds are more likely to be biologically relevant to transmission. This analysis suggests that the N7 iIFA and N7 NI assays may be useful in detecting birds that are shedding significant amounts of virus in an infected flock, but might not be as valuable for documenting the absence of AIV after vaccination [64].

5.7 Sentinel Birds

Nonvaccinated sentinel birds can also be used to recognize infections in vaccinated flocks. This strategy can be employed with all avian influenza vaccines, as well as in species that have been previously exposed to AIV [45;48;496]. Sentinel birds have also been recommended for additional surveillance when the heterologous neuraminidase DIVA strategy is used [1]. This combination was field tested during several H7 (LPAI) vaccination campaigns in Italy [1;460;531;532]. Other countries have reported using sentinel birds in vaccination campaigns [149], including France, during a vaccination campaign to protect free-range ducks and geese from Asian lineage H5N1 viruses [1].

If sentinel birds are used, they must be placed in each vaccinated flock [496], and should be randomly spread throughout a facility [452]. They must be seronegative for AIV, and clearly and permanently identified by a tamper-resistant method, to prevent their replacement with other birds [35;48;460;496]. It may be difficult to mark the birds so that they can be recognized readily, especially in large flocks [45;48;94]. Sentinels should be monitored daily for clinical signs, and any illness or deaths must be investigated to rule out avian influenza [496]. They should also be tested regularly by serology and/or virological tests [35;496]. Bleeding and swabbing these birds can be time-consuming [48;460].

Some authors have raised concerns that sentinel birds may become infected and amplify the virus if it enters the flock [452]. While this is theoretically possible, a small percentage of vaccinated birds is also likely not to respond well to a vaccine in the field for various reasons; thus, susceptible (but unmarked) birds will probably already exist in many flocks, and could also act as amplifiers [452]. Because high flock immunity (e.g., > 80%) is expected to interrupt transmission, and the number of sentinels is low (typically 1%), they are unlikely to have any significant effect on the ability of vaccination to inhibit virus transmission [452].

5.8 Virological Tests to Detect Infected Birds

An additional safeguard in vaccinated flocks is to sample baseline daily mortality or sick vaccinated birds, using RRT-PCR or antigen capture ELISAs [94]. Oropharyngeal and cloacal swabs should be collected, either individually or as pooled samples [94].

5.9 Overview of Surveillance During and After Vaccination Campaigns

During a vaccination campaign, good surveillance is necessary to detect infected flocks, which might otherwise be found by clinical signs, and to prevent the virus from being maintained in the vaccinated population [35;48;455]. By verifying that HPAI viruses are absent from vaccinated flocks, surveillance also helps prevent human exposure [455]. Other important aspects of surveillance include the assessment of antigenic and genetic changes in the field virus (including the emergence of vaccine-resistant strains) if vaccination is conducted during an outbreak, and the demonstration of the effectiveness of the vaccine and vaccination campaign [35;48;455;496]. Continued surveillance is necessary after an eradication campaign to demonstrate freedom from infection to trading partners [35;48;455;496]. In addition, countries that declare freedom from HPAI viruses should conduct ongoing active and passive surveillance to substantiate this claim, whether or not vaccination is practiced [35].

Avian influenza vaccination programs can be compatible with the continuation of international trade, if surveillance convincingly demonstrates that infection is absent from the exporting compartment [1;35;48]. The surveillance program must meet the standards in the OIE Terrestrial Animal Health Code, to avoid unjustified trade restrictions [496]. It must also be acceptable to partners in bilateral trade agreements [452]. The surveillance program should be designed to detect infected birds soon after the

virus is introduced [1;46;48;496]. Tests must be repeated every 6 months or less, with the actual interval between tests based on the risk of infection [35]. Vaccinated flocks should also be determined to be AIV-free before movement [496]. In addition, surveillance should be conducted in nonvaccinated flocks [46]. The primary examples of surveillance programs that allowed the continuation of trade were Italy's LPAI vaccination programs between 2000 and 2004, which used a heterologous neuraminidase DIVA strategy [452]. These programs were accepted by Italy's E.U. trading partners for the export of meat and eggs.

5.9.1 Use of Clinical, Serological and Virological Tests in Surveillance

Methods used to detect field virus(es) in vaccinated flocks are influenced by the type of vaccine (e.g., homologous or heterologous, inactivated or recombinant vectored), the vaccination strategy, and the availability of diagnostic laboratories [48;496]. Laboratory testing strategies should be used whenever possible, rather than relying solely on sentinel birds [35]. The sensitivity and specificity of the diagnostic tests should ideally be validated for the vaccination/infection history and the species to be tested.

When testing birds by serology, the specificity of any confirmatory test should be higher than that of the screening test, and its sensitivity should be as high or higher. Some serological tests are not yet validated, or not validated for a species. Nonvaccinated sentinel birds must be used when birds have been vaccinated with homologous vaccines, or if an appropriate DIVA test is unavailable [94;496]. Sentinel birds can also be combined with other strategies, to provide additional confidence that virus is not present [35;48;496]. All flocks with seropositive results not due to vaccination must be investigated [35]. Clustering of seropositive flocks is particularly suspicious of infection, although other causes are possible [35]. HPAI may be ruled out if a thorough epidemiological and laboratory investigation finds no evidence for infection [35].

Virological tests are used to monitor populations that are at risk of infection, investigate seropositive flocks and flocks that have been epidemiologically linked to an outbreak, confirm suspected clinical cases (including illness in sentinel birds), and test 'normal' daily mortality from vaccinated flocks [35;46;48]. Molecular methods such as RT-PCR are most likely to be used, although other tests such as virus isolation may be appropriate in some situations [46;48;94]. Field trials in Germany suggest that caution should be used in relying on virus isolation in geese; in this species, isolation was often unsuccessful in PCR-positive samples [327]. The sensitivity of antigen detection systems is low, and these tests should be used only for screening clinical cases [35]. If an AIV is detected by the investigation, the virus should be isolated and identified, and pathogenicity testing should be done [35].

Clinical surveillance, with the monitoring of clinical signs and production parameters (e.g., increased mortality or reduced feed and water consumption) is also important in surveillance [35]. Passive surveillance is less likely to detect infections in vaccinated than nonvaccinated flocks [531;533]. An evaluation of field data from the Italian monitoring system in 2000-2005 found that active surveillance was the most effective method to detect infected flocks, particularly during a vaccination program [531]. The detection rate was 61% for active surveillance, 32% for passive surveillance and 7% for targeted surveillance after outbreaks were confirmed.

5.9.2 Demonstration of Freedom from Infection After Outbreaks

After an outbreak, an active surveillance program must demonstrate that the infection is no longer present, using virological and serological tests, and regular clinical examination of poultry [35]. Sentinel birds can be used to aid the interpretation of the results from surveillance. Surveillance strategies used to demonstrate freedom from HPAI and H5/H7 LPAI H5 viruses in poultry, at an acceptable level of confidence, must address local considerations such as how often poultry come in contact with wild birds, the level of biosecurity and the production systems, and the commingling of different species of birds. The OIE Member must provide scientific data that explains the epidemiology of these notifiable viruses

in the area and the management of all risk factors. The country should provide the OIE with the performance characteristics and information on the validation of the tests used. The OIE Terrestrial Animal Health Code provides additional details on the requirements for a surveillance system.

6. VACCINE AVAILABILITY AND LICENSING

Summary

Although there is sufficient worldwide production capacity to supply avian influenza vaccines for emergency vaccination, including large-scale campaigns, an unexpected increase in demand could result in temporary shortages. Vaccine banks can mitigate such concerns, and if used, should be established well before a vaccine is needed. The choice of vaccines to bank should be based on continuous surveillance of the antigens found in circulating viruses. Banks with a small number of H5 and H7 types can provide effective vaccines against many field viruses; however, the recent emergence of Asian lineage H5N1 variants has made strain selection for this lineage more complex. Storing a variety of subtypes helps ensure that a suitable vaccine is available for the heterologous neuraminidase DIVA strategy. Formulated avian influenza vaccines have a relatively short shelf life, and must be replaced periodically.

Worldwide, commercially available vaccines include a variety of inactivated, oil emulsified, monovalent or bivalent H5 and H7 vaccines, and a few fowlpox-vectored, NDV-vectored and HVT-vectored H5 vaccines.

In the U.S., the USDA's Center for Veterinary Biologics (CVB) and National Veterinary Stockpile (NVS), and other agencies may be involved in purchasing vaccine antigen concentrates and/or finished routine or emergency use vaccines. NVS may also contract with manufacturers for immediate access to existing stocks of licensed emergency use vaccines. Vaccines that are currently licensed and/or may be available for emergency use in the U.S. include inactivated vaccines, the TROVAC™ fowlpox-vectored H5 vaccine, which contains H5 from A/turkey/Ireland//1378/83 (H5N8), and Vectormune HVT AI™, which contains the HA from the clade 2.2 Asian lineage H5N1 HPAI virus A/Swan/Hungary/499/2006.

The time to supply a vaccine depends on whether it is immediately available. If a new vaccine is required, it may take 4-8 months or longer from the beginning of the production process. For a vaccine to be given a full product license, the manufacturer must conduct extensive efficacy, purity and safety testing. With a full product license, developing a new avian influenza vaccine requires 2-3 years. Vaccines given a conditional biologics license and altered vaccines that have been approved as production platforms could be available sooner. A conditional biologics license allows the vaccine to be used in specific conditions, e.g., if the product will be used by or under the supervision of the USDA in emergency vaccination.

In the U.S., vaccination with an H5 or H7 vaccine, in any animal species, must be approved by both state authorities and USDA APHIS VS. If approval is granted, H5 and H7 vaccines must be employed in an official USDA avian influenza control program.

A number of experimental avian influenza vaccines have also been described. An alphavirus replicon vaccine is awaiting evaluation by the USDA, and a duck enteritis vectored vaccine is reported to be in the licensing process in China.

6.1 Vaccine Banks

Although there is sufficient worldwide production capacity to furnish avian influenza vaccines for large scale vaccination campaigns, an unexpected increase in demand might cause supply problems [496]. Vaccine banks can mitigate such concerns, and if used, should be established well before a vaccine is needed [48;496]. Avian influenza vaccines can be banked in several different ways [496]. One method is to store formulated vaccines in a government-owned vaccine bank. Such vaccines would be rapidly available in an outbreak, but formulated vaccines have a relatively short shelf life, and must be replaced periodically. A country or international organization may also purchase vaccines to be stored by the manufacturer in the country of origin. With this option, the time to ship the vaccine from the manufacturer adds to the distribution time. Other options include emergency stocks based on a rolling system, or contracts with manufacturers for vaccine production. The time to supply a vaccine depends on whether it is immediately available. If a new vaccine is required, it may take several months or more, (see section 6.3, New Vaccines from Field Viruses), in addition to licensing requirements.

Banked vaccines should be of high quality, fully tested, and manufactured according to the standards in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals [496]. A vaccine bank should contain both H5 and H7 vaccine strains, for use in HPAI outbreaks. In many cases, avian influenza vaccines can protect poultry from a variety of field strains that share the same hemagglutinin type [460;496]. This has allowed banks to be established without a prohibitively large number of vaccine strains. However, recent changes in Asian lineage H5N1 HPAI viruses, including the emergence of vaccine-resistant strains, have made the selection of seed strains more complex (see section 7.1, Vaccine Matching, for details).

The choice of vaccines to bank should be based on continuous surveillance of the antigens found in circulating viruses [48]. Storing a variety of subtypes helps ensure that a suitable vaccine is available for the heterologous neuraminidase DIVA strategy [486]. There is currently no international system to recommend poultry vaccine seed strains for AIV [45]. However, individual countries may have such systems. Japan, which is HPAI-free but banks H5 vaccines in case of need, has established a national committee to oversee strain recommendations for these viruses (and equine influenza) [534]. The initial stage of this process consists of 1) evaluating the need for vaccine updates; 2) collecting data about AIV epidemiology and field viruses; and 3) if an update is deemed necessary, requesting that research institutes or universities share candidate vaccine strains with the National Veterinary Assay Laboratory (NVAL). NVAL then stores these vaccine candidates and transfers them to private associations, which conduct tests of their suitability and submit these tests to NVAL. NVAL reviews the results, selects the most appropriate strains and transfers these viruses to companies that hold authorizations to manufacture and market influenza vaccines.

6.1.1 Global Status of Vaccine Banks

The OIE established a regional vaccine bank in 2006 to supply vaccines to countries in Africa, and a global vaccine bank in 2007 [149]. A survey of avian influenza programs in 2011 (most recent information available) found that 3 individual countries had vaccine banks containing both H5 and H7 vaccines, and 10 countries had H5 vaccine banks [8;149]. Most (8) vaccine banks were government-owned, two were held by private companies, and three were both government-held and private [149]. Final emulsified vaccines were held by 10 countries, frozen antigens by four and processed antigens by one. The small quantities held by most countries (often ≤ 3.5 million doses) would limit vaccination to targeted groups such as zoo or high value birds or high-risk poultry in a small region [149]. Most countries that established vaccine banks had concluded in 2011 that the cost/benefit ratio of maintaining government-owned banks was too high, as the perceived risks of Asian lineage H5N1 HPAI virus

introduction had declined [8]. Thus, the use of these banks was likely to be discontinued, or in some cases, shifted to contracts with manufacturers. More recent information does not seem to be available at this time.

6.1.2 Vaccines and Antigen Concentrates in the U.S.

In the U.S., the USDA's Center for Veterinary Biologics (CVB) and National Veterinary Stockpile (NVS), and other agencies may be involved in purchasing vaccine antigen concentrates and/or finished routine or emergency use vaccines [535]. NVS may also contract with manufacturers for immediate access to existing stocks of licensed emergency use vaccines. The CVB has evaluated and approved several subtypes of seed viruses for inactivated influenza vaccines [407] Vectored vaccines that have been licensed in the U.S. include TROVAC™ fowlpox-vectored H5 vaccine, which contains H5 from A/turkey/Ireland//1378/83 (H5N8) and Vectormune HVT AI™, which contains the HA from the clade 2.2 Asian lineage H5N1 HPAI virus A/Swan/Hungary/499/2006. In the U.S., H5 and H7 avian influenza vaccines may only be used in official USDA animal disease control programs.

6.2 Internationally Available Avian Influenza Vaccines

Commercially available avian influenza vaccines in 2015 include 1) inactivated vaccines containing H5 and H7 subtypes (in addition to other subtypes produced for LPAI vaccination campaigns), 2) recombinant live fowlpox-vectored H5 vaccines, 3) recombinant live or killed NDV-vectored H5 vaccines, and 4) recombinant live HTV-vectored H5 vaccines. Most avian influenza vaccines are developed and marketed for use in chickens, although some vaccines have also been used in other domesticated poultry such as turkeys, ducks, geese and quail, as well as in zoo birds and endangered species [45;342;470-477]. A list of avian influenza vaccines produced internationally is maintained at the Center for Food Security and Public Health (CFSPH) (<http://www.cfsph.iastate.edu/Vaccines/index.php>).

Worldwide, a variety of inactivated, oil emulsion avian influenza vaccines are manufactured. They include both conventionally produced vaccines based on LPAI viruses, and vaccines made using reverse genetics [45]. Both monovalent (either H5 or H7) and bivalent (both H5 and H7) vaccines are made [496]. Combination vaccines that can vaccinate birds against other diseases are also available, and may be useful when vaccination programs are prolonged [496]. Several inactivated vaccines containing Asian lineage H5N1 viruses are produced in China [498;501], and have been imported by some other countries in Asia for use in vaccination campaigns [536]. The reverse genetics Re-1 vaccine, made initially, contained the H5 and N1 genes from the clade 0 virus A/goose/Guangdong/96 [498;501]. Updated vaccines have been made as vaccine-resistant strains emerged, and have contained H5 and N1 from the clade 7 virus A/chicken/Shanxi/2/2006 (Re-4), the clade 2.3.4 virus A/duck/Anhui/1/06 (Re-5) and the clade 2.3.2.1 virus A/duck/Guangdong/S1322/2006 (Re-6) [498;501;537].

Two live recombinant fowlpox-vectored H5 vaccines are commercially available [496;498;499]. One (TROVAC™ H5) contains H5 from the Eurasian lineage virus A/turkey/Ireland//1378/83 (H5N8), and is the fowlpox-vectored vaccine licensed for emergency use in the U.S. [46;466;469]. This vaccine has been used extensively in vaccination campaigns against South American H5N2 LPAI viruses [48;407;466]. A different live recombinant fowlpox-vectored vaccine is produced in China [46;501]. This vaccine contains the HA and NA from an early Asian lineage H5N1 strain, A/goose/Guangdong/1/96 [501].

NDV-vectored H5 vaccines, which contain the HA from Asian lineage H5N1 viruses, have been licensed in China [45;47-49;94]. The original vaccine, which was first manufactured in 2006, contained the H5 from A/goose/Guangdong/1/96 in the NDV strain La Sota [501]. This vaccine is reported to protect birds against both highly pathogenic Newcastle disease and Asian lineage H5 HPAI viruses [45;49;94;342;501]. In 2008, it was replaced by an NDV-vectored vaccine that contains the HA gene

from A/duck/Anhui/1/06, which is a newer (clade 2.3.4) H5N1 virus [501]. A live NDV-vectored avian influenza vaccine (NewH5™) has also been licensed recently in Mexico. This vaccine contains H5 from the North American lineage virus A/chicken/Mexico/435/2005 (H5N2) [494].

An HVT-vectored H5 vaccine (Vectormune HVT AI™) contains the HA from the clade 2.2 Asian lineage H5N1 HPAI virus A/swan/Hungary/499/2006. This vaccine has been licensed in a small number of countries, including the U.S. [458]. It is also licensed in other countries, and has been used during Asian lineage H5N1 HPAI vaccination campaigns in Egypt since 2012 [458;497]. This vaccine is reported to help protect birds from Marek's disease as well as AIV [519;520].

6.3 New Vaccines from Field Viruses

The production of a new avian influenza vaccine from a field strain is estimated to take 4-8 months from the beginning of the production process [48;496]. The fowlpox vector can be used to produce an emergency vaccine fairly quickly from a synthetic gene, if the HA sequence of the emerging virus is known [469]. The generation of a new vaccine, using this process, has been shown to take approximately 4 months [469]. Estimates for other types of vectored HA vaccines are not available, but might be similar. If a vaccine is to receive a full product license, developing a new avian influenza vaccine or changing it requires 2-3 years [538]. Vaccines given a conditional biologics license and altered vaccines that have been approved as production platforms [539] (see section 6.4 Vaccine Licensing) could be available sooner.

6.4 Vaccine Licensing

Vaccines may be licensed and distributed with a full product license, or they may receive a conditional biologics license for use in specific conditions, e.g., if the product will be used by or under the supervision of the USDA in an emergency animal disease outbreak [535].

For a vaccine to be given a full product license, the manufacturer must conduct extensive efficacy, purity and safety testing [458;535;540]. Steps in the licensing of vaccines in the U.S. include a review of the data from the manufacturer to support the product and label claims; inspections of manufacturing processes and practices; confirmatory testing of the biological seeds, cells and product; post-licensing monitoring including inspections and random product testing; and post-marketing surveillance of product performance [535]. In standard licensing, the seed materials, product ingredients and final product must be completely characterized and tested for purity. Safety and efficacy tests must also be done, and product stability as well as duration of immunity (DOI) must be evaluated. Live recombinant vaccines require a risk analysis and environmental assessment before they can be licensed [538]. All of these steps may not be possible during an animal disease emergency. The USDA has mechanisms for expedited product approval, and can exempt products from some of the regulatory requirements for full product approval during emergencies [535]. However, every attempt is made by the CVB to establish a reasonable expectation of purity, safety, potency, and efficacy prior to the use of any vaccine. In addition to potential harm to animal, human, and environmental health, the risk of lawsuits if problems occur must be considered.

Changes in a vaccine require a new license application with the demonstration of purity, safety, efficacy and potency ([541] cited in [538]). APHIS has issued a policy memorandum with guidelines for licensing production platforms (Veterinary Services Memorandum 800.213), which may allow faster licensing of new strains in some systems [539]. This memorandum applies to products based on recombinant technology that result in non-replicating, nonviable biological products. In products that have received initial licensure, it allows the inserted gene(s) to be exchanged with gene variants of the same pathogen,

with reduced licensing requirements, i.e., without requiring additional field safety studies, reevaluation for compliance with the National Environmental Policy Act or evaluation of inactivation kinetics.

6.4.1 Regulatory Considerations in Vaccine Use

In the U.S., the use of all avian influenza vaccines, including subtypes other than H5 or H7 (for use in LPAI outbreaks), requires special approval from the state where they will be used. Vaccination with an H5 or H7 vaccine, in any animal species, must also be approved by USDA APHIS VS [49;538;542]. If approval is granted, H5 and H7 vaccines must be employed in an official USDA avian influenza control program.

6.5 Experimental Vaccines

Numerous experimental approaches have been described for influenza vaccines intended for use in poultry. They include vaccines based on AIV genes expressed in vector systems such as duck enteritis virus, human adenovirus 5, infectious laryngotracheitis virus, vaccinia virus, bacteria (e.g., *Lactococcus lactis*) and other vectors, or vaccines that contain *in vitro*-expressed hemagglutinin, with or without other AIV proteins [45;47;49;94;120;457;460;512;543-551]. Some of these vaccines are intended for oral administration or other nonparenteral routes [120]. DNA vaccines, genetically attenuated mutants (e.g., temperature sensitive mutants) as live vaccines, and universal vaccines based on conserved AIV proteins such as NP or M2e have also been described [45;47;49;51;52;94;454;457;501;525;545;548;550;552-556].

Some approaches, such as subunit vaccines, novel virus-vectored vaccines, or virus-like particles based on influenza proteins expressed *in vitro*, have been promising, but are not yet commercially available [45;49;546-548;557]. A duck enteritis virus expressing Asian lineage H5 is in the process of being licensed in China [94;458], and an alphavirus replicon H5 vaccine is awaiting evaluation by the USDA [558]. Others vaccines, including a universal vaccine based on relatively conserved AIV proteins such as M2e or NP, are in the early stages of investigation in poultry [45;49;51;52;296;550;559]. Universal vaccines based on conserved epitopes in the hemagglutinin stalk have been explored in mice, as models for potential human influenza vaccines [560;561], but there are no published reports testing these vaccines in birds. Attenuated live H5 or H7 avian influenza vaccines are not advised for poultry, due to the risk of reassortment with field viruses and the chance the vaccine strain could mutate to HPAI [49;454]; however, such vaccines might be feasible *in ovo*, where the risk of reassortment and transmission is much less [47;454]. Research into attenuated live, *in ovo* vaccines is still in the early stages [47;454], and may also face issues with interference from other vaccines routinely injected by this route (e.g., Marek's disease vaccines) [551]. Novel adjuvants have also been investigated to improve vaccine efficacy, although the cost of some may be a significant barrier in poultry vaccines [562;563].

7. VACCINE MATCHING, EFFICACY, POTENCY AND SAFETY

Summary

The efficacy of an avian influenza vaccine is mainly influenced by the amount of antigen, the antigenic relatedness between the vaccine strain and field virus, and the specific adjuvant (if any). Protection can also be affected by factors such as the host species and age, the route and site of immunization, and the dose and virulence of the challenge virus. The optimal vaccine strain may be different for different vaccination campaigns, even when the field viruses belong to the same subtype. The absence of clinical signs does not necessarily mean that a vaccine will reduce virus shedding or transmission. In some studies, different vaccines provided equivalent protection from morbidity and mortality, even when one vaccine was more effective in reducing virus excretion. Long-term vaccination campaigns have also demonstrated that a vaccine may lose its efficacy against virus shedding as the field strains change.

H5 vaccines are sometimes capable of reducing virus shedding in birds infected with a broad variety of H5 viruses. However, the closeness of the match between the HA of the vaccine strain and field virus may influence the amount of virus shed. Close matching might be particularly important when birds are exposed very soon after vaccination, before full immunity develops, or when immunity is starting to wane. The degree of homology necessary to reduce virus shedding can be influenced by the type of vaccine and its potency, the specific vaccine and field strains, and the viral lineage and agreement between specific epitopes. Despite the separation of AIV into the American and Eurasian lineages, some studies have reported good efficacy of North American lineage vaccines against Eurasian lineage field viruses, and vice versa. At one time, several North American lineage and Eurasian lineage vaccine strains were effective against Asian lineage H5N1 HPAI viruses. However, the emergence of drift variants (belonging to diverse subclades) has reduced the efficacy of some vaccines, and made vaccine selection for these viruses more complex.

Information about the degree of cross-protection between H7 viruses is limited. Studies have demonstrated good protection in several cases when the vaccine contained a virus from the same lineage as the field virus (e.g., North American or Eurasian), and sometimes when they contained viruses from other lineages. In two reports, antigenic distances between H7 viruses were not necessarily correlated with their geographic origins, and also did not always predict protection from vaccines.

Immunity to the neuraminidase may provide some protection in birds, and an NA that matches the field virus might maximize the immune response. However, immunity to this protein is relatively unimportant compared to the HA, and a vaccine with a heterologous NA may be chosen, either because a heterologous neuraminidase DIVA strategy is desired, or because no vaccine with the same subtype is available.

At present, the only reliable method of selecting an avian influenza vaccine may be to conduct vaccination and challenge studies in the target species. These studies are slow and expensive, and require the use of live virus and high security facilities. Evaluation of serological relatedness and genetic comparison of the HA can help select vaccine strains to test against the field virus. However, some vaccines that have lower (e.g., less than 95%) HA homology with the challenge strain are reported to decrease virus shedding. Conversely, vaccines with high homology may become ineffective against field viruses that have evolved during a vaccination campaign. Antigenic cartography may be useful for analyzing potential serological matches during the initial

selection of an AIV vaccine, or for evaluating changes in field viruses over the course of the vaccination campaign. Experience with this technique is still limited for poultry vaccines.

During vaccination campaigns, avian influenza vaccines should be re-evaluated against circulating field strains every 2-3 years, to ensure that protection remains adequate. This evaluation should include molecular sequencing, an assessment of antigenic cross-protection *in vitro*, and challenge studies.

In addition to HA homology, factors such as overall vaccine efficacy and potency, and the number of doses needed for full efficacy, must be considered when choosing a vaccine. Vaccine efficacy is usually evaluated in challenge studies. Avian influenza vaccines with higher antigen content generally provide better protection from clinical signs and greater decreases in virus shedding. The development of adequate HI titers is usually used as evidence of potency, provided that the challenge virus is antigenically and genetically related to the vaccine strain, and is not a drift variant. In the U.S., licensed inactivated avian influenza vaccines must induce HI titers of 32 or greater, in at least 80% of vaccinated birds. Vaccines that result in higher HI titers seem to be more likely to decrease virus shedding as well as protect birds against clinical signs. HI titers are usually determined against the homologous virus, and would be lower against a heterologous virus. Other methods to determine potency include conventional potency testing (PD₅₀ determination), or the quantification of the antigen content.

Safety assessments vary with the type of vaccine (inactivated or live, bacterial or viral), the adjuvant used, and the history of similar products in use, as well as the dose, vaccine claims, usage regimen and animal factors such as the species. Completely inactivated vaccines and subunit vaccines are generally considered to be low-risk for animal safety; however, adjuvants and other vaccine ingredients may cause local or systemic reactions in some animals. In field studies, the main risks to both poultry and zoo birds were associated with stress and trauma from handling, rather than being direct effects of the vaccine. During H5 vaccination campaigns in the E.U., all zoos reported a very low rate of adverse effects, with a higher risk of reactions in birds that had been vaccinated with very large doses. Live genetically modified organisms or vectored vaccines usually have higher-risk profiles than inactivated vaccines, and additional risks (e.g., reversion to virulence) must be evaluated during licensing.

Safety considerations should include the risks to people who administer or contact avian influenza vaccines. Local reactions from oil adjuvants or other ingredients should be addressed in label warnings for inactivated vaccines.

The efficacy of a vaccine is mainly influenced by the amount of antigen [137], the antigenic relatedness between the vaccine strain and field virus, and the specific adjuvant (if any) [142;342;564]. Protection can also be affected by factors such as host species and age, the route and site of immunization, and the dose and virulence of the challenge virus [142;468]. Avian influenza vaccines have mainly been evaluated in chickens and to a lesser extent in turkeys, and care should be used when extrapolating results to other avian species [94]. The optimal vaccine strain may be different for different vaccination campaigns, even when the field viruses are of the same subtype [448].

7.1 Vaccine Matching

7.1.1 Effect of H5 Hemagglutinin Matching on Vaccine Efficacy

To be effective, an avian influenza vaccine must contain the same HA type as the field virus, but it is not necessary for these proteins to be identical [46-49;496]. Experimental studies in chickens suggest that H5 poultry vaccines are capable of providing broad protection against strains within the same hemagglutinin type, and that even vaccine strains isolated more than 20 years before the field virus may decrease virus shedding [137-142;538]. However, a number of studies also suggest that, for both inactivated and vectored HA vaccines, a more closely matched HA is more effective and may suppress virus shedding to a greater extent (provided the vaccine strains are similarly immunogenic) [73;134;136;140;141;147;211;324;368;481;502;511;512;524;538]. Close matching might be particularly important when birds are exposed soon after immunization, before full immunity develops [73;365], or when immunity has started to wane [565]. The degree of amino acid homology that reduces virus shedding may differ with the type and potency of the vaccine, the specific vaccine and field strains, and the viral lineage and agreement between specific epitopes. In some cases, inactivated vaccines with as little as 84% amino acid homology in the HA [142], and fowlpox- vectored vaccines with as little as 83% homology [136], have decreased virus shedding (see also Table 4). In other circumstances, vaccines with approximately 95% or greater homology had limited or no effect on the shedding of drift variants [132;134;136;146;147;312;467;504;522;524;566-569]. Such viral variants have been isolated during North American H5N2 LPAI and some Asian lineage H5N1 HPAI vaccination campaigns, where viruses continued to circulate for several years or more in the presence of the vaccine. Such observations and some laboratory challenge studies suggest that overall HA homology may be less important than the agreement between specific epitopes [522;538;545]. Vaccines that do not significantly reduce virus shedding may still protect birds completely from clinical signs. In some studies, different vaccines provided equivalent protection from morbidity and mortality, even when one vaccine was more effective in reducing virus excretion [73;134;135;211].

Relatively few studies have been done in species other than chickens; however, vaccines with better homology to the challenge virus were also reported to be more effective in turkeys and domesticated waterfowl, although vaccines with lower homology were sometimes effective [211;324;368;478;537].

For optimal efficacy, therefore, the hemagglutinin in the vaccine should be reasonably well-matched to the field virus [448;455;512], and vaccine efficacy should be monitored during longer vaccination campaigns, even if clinical protection is reported in the field (see Vaccine Matching in the Presence of Antigenic Drift, section 7.1.5 for further details) [46;47;135;136;142;464].

7.1.1.1 Effect of Lineage Matching on H5 Vaccine Efficacy

Cross-protection, including the ability to reduce virus shedding, has been demonstrated between the American and Eurasian lineages of H5 viruses [135;136;142;469;538]. Some studies reported that vaccines were more effective in decreasing virus shedding or preventing infection if both the challenge virus and vaccine strain belong to the same lineage [73;140;141;502;524], but other reports did not find this to be true [135;136;570].

7.1.2 Effect of H7 Hemagglutinin Matching on Vaccine Efficacy

There is limited information about the degree of cross-protection between H7 viruses. Studies that employed experimental fowlpox-vectored H7 vaccines have reported varying degrees of protection against heterologous viruses. In one experiment, a vaccine that contained the HA from an Australian virus (A/chicken/Victoria/1/85; H7N7), protected chickens from challenge by an HPAI virus from Asia (A/chicken/Pakistan/1369-CR2/95; H7N3), but not from another virus from Europe (A/turkey/Italy/4580/99; H7N1) [47]. Similarly, only a vaccine containing the H7 from

A/turkey/Italy/4580/99 was effective against challenge by the homologous virus; vaccines containing H7 from A/chicken/Victoria/1/85 or the North American lineage virus A/turkey/Virginia/66/02 were not protective [47;469]. In contrast, another group reported that experimental fowlpox-vectored vaccines containing H7 from viruses isolated either in North America (A/seal/Massachusetts/1/80; H7N7) or Australia (A/chicken/Victoria/85; H7N7) were clinically protective against the Australian HPAI virus [571].

Most vaccination campaigns [532;572] and experiments using inactivated vaccines [105;352;365;573-575] have employed vaccines from the same lineage as the challenge strain. Only a few studies reported the degree of homology between the viruses. One inactivated H7 vaccine (A/duck/Hokkaido/Vac-2/04; H7N7) was clinically protective and decreased the shedding of the HPAI virus A/turkey/Italy/99 (H7N1), which has 92.6% amino acid homology in the HA1 region [573]. In another study, a vaccine with greater homology, as well as higher antigen content, was more effective in suppressing virus shedding when birds were challenged 1 week after vaccination, but not when they were challenged at 2 weeks [365]. The vaccine strains used were A/chicken/Italy/99 (H7N1), which has HA1 homology of 98% with the challenge strain (A/chicken/Netherlands/03; H7N7), and A/chicken/Pakistan/95 (H7N3), which is 92% homologous. A successful Italian campaign used an inactivated vaccine containing A/chicken/Pakistan/95 (H7N3) to protect poultry from Eurasian lineage H7N1 viruses [532].

Two recent studies employed geographically diverse vaccine strains. In one experiment, seven North American LPAI strains, isolated between 1971 and 2006, were completely protective against morbidity and mortality and significantly reduced virus shedding in chickens challenged with the H7N3 HPAI virus, A/chicken/Jalisco/CPA-12283-12/2012 [572]. The HA amino acid homology between these strains and the challenge virus ranged from 92% to 97%. Two Eurasian, one South American and one Australian vaccine strain with 80-84% amino acid homology did not significantly reduce virus shedding, and had variable effects on clinical signs. However, the latter four strains also resulted in much lower HI titers overall (i.e., to the homologous viruses), suggesting that poor immunogenicity could also account for their lack of efficacy. Two of these viruses, A/chicken/Chile/176822/2002 (H7N3; 84% homology) and the Australian virus A/chicken/Victoria/1985 (H7N7; 80% homology) did not protect chickens from mortality, while the Eurasian lineage viruses A/turkey/Italy/4580/1999 (H7N1) and A/chicken/Pakistan/447/1995 (H7N3), which had 80-82% homology, protected 90% of the birds from death. Abbas et al. (2011) reported that two H7N3 strains from Pakistan, one H7N7 virus from Europe (A/mallard/Netherlands/9/2005 H7N7), and one H7N3 virus from South America (A/chicken/Chile/176288/2002) provided similar clinical protection against two heterologous H7N3 HPAI viruses from Pakistan, despite varying distances from the challenge virus in antigenic cartography [576]. All of the vaccine strains decreased the shedding of one challenge virus to a similar extent, and none reduced the shedding of the second challenge virus. Antigenic distances between various H7 viruses were also measured in the two studies above, and they did not appear to correlate with their geographic origins. In one study, for example, a virus from Australia grouped with North American viruses [572].

7.1.3 Effect of Neuraminidase Matching on Vaccine Efficacy

Immunity to the neuraminidase may provide some protection in birds ([55;116]; [117] cited in [42]), and an NA that matches the field virus might maximize the immune response. As with the HA protein, there is little or no vaccine-induced cross-protection between the nine antigenic types of avian NA [42]. However, immunity to this protein is relatively unimportant compared to the HA [44-46], and a vaccine with a heterologous NA may be chosen, either because a heterologous neuraminidase DIVA strategy is desired, or because no vaccine has the same subtype.

7.1.4 Practical Aspects of Matching the Vaccine to the Outbreak Strain

At present, vaccination and challenge studies in the target species may be the only reliable method to select an avian influenza vaccine [45;464;572], particularly given the emergence of vaccine escape mutants during prolonged vaccination campaigns [8;94;134;136;145-148], the influence of vaccine potency on efficacy (section 7.2, Vaccine Efficacy and Potency) and the disparate results from some studies that have examined serological relationships in the viral HA [572;576]. A disadvantage is that challenge studies require the use of live virus and high security facilities. They are also expensive and time-consuming [136], which can be a drawback when the decision to perform emergency vaccination must be made quickly.

Methods that evaluate the closeness of the relationship between the HA proteins of vaccine and field viruses can help select vaccine strains to test. One criterion could be to select strains with greater than 95% HA amino acid homology to the field virus ([577;578] cited in [45]). It should be noted that homology is usually evaluated as the overall similarity in the HA1 region, but it would be possible for a single amino acid change to result in a substantial increase in the antigenic distance ([579] cited in [324]). In addition, vaccine strains with less than 5% homology can sometimes decrease virus shedding and prevent or reduce transmission [73;135;136;138-140;142;538;580], and might also be considered.

In vitro serological assays, which can rapidly match vaccine strains to field viruses, have been standardized for some animal diseases such as foot-and-mouth disease [581], but not for avian influenza. Serological assays that could be used to help evaluate the closeness between AIV field and vaccine strains include the HI assay, which evaluates binding between the viral HA and antiserum to AIV ([577;578] cited in [45]), and serum neutralization (SN) [545]. Matching by serology requires the availability of mono-specific sera to the target strains [545]. It is also cumbersome to interpret with more than a few strains [545].

The HI assay is used to evaluate the antigenic distance between human influenza viruses, for inclusion in vaccines [448]. International recommendations for human vaccine strains are updated when the HI titer for the new strain is at least fourfold lower than the corresponding HI titer for the vaccine strain [134;582] (i.e., the titer measured using the vaccine virus and antiserum to the new virus is compared to the titer for antiserum to the vaccine strain in the same assay). Similarly, less than fourfold difference in the HI titer has been proposed as an indication of vaccine efficacy against AIV ([577;578] cited in [45]). Nevertheless, it is not certain that this relationship is valid for avian influenza vaccines, which differ from human influenza vaccines in antigen formulations and adjuvants, and are administered using different vaccination schedules [354]. For example, avian influenza vaccines are normally much less purified than human vaccines, but use oil adjuvants, which are more potent than the adjuvants employed in human vaccines.

7.1.4.1 Antigenic Cartography

Antigenic cartography is a computational technique that can be used to visualize and quantify data from serological assays such as HI or SN [448;545]. In an antigenic map, the distance represents the similarity between viruses. The positions of the antigens and antisera on the map are determined by testing each antiserum against a variety of antigens, and vice versa. The WHO uses antigenic cartography for human influenza surveillance and vaccine recommendations. This technique may also be useful for analyzing potential matches during the initial selection of an AIV vaccine, or for evaluating changes in field viruses over the course of a vaccination campaign. After selecting the most promising vaccine seed strain(s) with antigenic maps, these strains should be tested in challenge studies.

A recent study analyzed panels of Asian lineage H5N1 viruses, using antigenic cartography [448]. One small antigenic cluster contained HPAI H5 influenza viruses from outbreaks that occurred before 1996, as

well as LPAI H5 viruses from wild birds. The Asian lineage H5N1 HPAI viruses found after 1996 were antigenically distinct, and were contained in several antigenic clusters representing the various H5N1 clades. The antigenic differences between these clades represented greater than a fourfold difference in HI titers, which would cause a vaccine update with human influenza [448]. This suggested that some vaccines made from Asian lineage H5N1 viruses might not be optimally effective against field viruses from other clades. Another study investigated HI cross-reactivity between more than 50 Eurasian lineage H5 or H7 AIV for use as potential vaccine strains, and found that the H5 viruses with the broadest cross-reactivity were the LPAI viruses A/mallard/Italy/3401/2005 (H5N1) and A/duck/Italy/775/2004 (H5N3), and the HPAI viruses A/Swan/Iran/754/2006 (H5N1) and A/chicken/Nigeria/957/2006 (H5N1) [583]. Among the H7 viruses tested, A/turkey/Italy/2987/2003 (H7N3) had the broadest cross reactivity with the tested viruses. The majority of the isolates tested in this study were Italian H7 viruses and Asian lineage H5N1 viruses, but other subtypes and lineages were also represented. American lineage H5 or H7 viruses, and Australian lineage viruses were not included in this analysis.

Only a few challenge studies have examined predictions from antigenic cartography, to date. Antigenic distances between vaccine and challenge viruses did not entirely predict protection in two studies [572;576]. One group found that vaccines made from two H7N3 viruses from Pakistan, one H7N3 virus from Chile, and an H7N7 virus from the Netherlands all had similar effects on clinical signs and virus shedding, when the birds were challenged with two H7N3 HPAI viruses from Pakistan, despite varying distances from the challenge virus in antigenic cartography [576]. While some of the most effective vaccine strains in the second study were closely related to the challenge strain, with antigenic distances ranging from < 1 to 1.5 units (where 1 unit is equivalent to greater than 2 fold change in HI titer), one North American virus with antigenic distance of > 2 units provided good protection [572]. Three of the 4 least effective vaccine viruses had antigenic distances > 2 units in this experiment; however, these strains also seemed to be poorly immunogenic, and the significance of this finding is uncertain.

7.1.4.2 Additional Factors to Consider

In choosing a vaccine, factors such as overall vaccine efficacy (see section 7.2, Vaccine Efficacy and Potency) must also be considered [142;146;538]. In addition, the desirability of high homology should be balanced with the number of doses needed for full efficacy, particularly if exposure might occur soon after vaccination. In a study that tested challenge protection in chickens after one dose of vaccine, two vaccines that had good homology to the field virus (98.8% and 88.7% in the HA1), but are labeled for two doses, did not perform as well as a less well-matched vaccine (84.6%) licensed for use as a single dose [481].

7.1.5 Vaccine Matching in the Presence of Antigenic Drift

Antigenic drift allows AIV to escape immunity from previous infections or vaccination, and replicate to higher levels [135]. While long-term vaccination campaigns were not conducted, antigenic drift among these viruses was low. This was associated with good protection using vaccine strains isolated in previous decades [137;538]. Increased antigenic drift was initially recognized during a long-term vaccination campaign in Mexico against H5N2 viruses, which began in 1995 [134;136]. As less related AIV strains emerged, the vaccine used in official vaccination campaigns lost its ability to reduce virus shedding [134;136]. In one study, this vaccine had high cross-reactivity to viruses of its own sublineage; however, HI titers to viruses from other Mexican sublineages were fourfold to 16-fold lower, although nucleic acid homology (HA1) to these strains was 93.2% to 94.6% [134]. Similarly, vaccine-resistant field strains have also emerged among H5N1 HPAI viruses in Asia and Egypt, during long-term vaccination campaigns where viruses continued to circulate (see section 7.1.6, Vaccine Matching and H5N1 Viruses).

The OIE recommends that all vaccination programs for AIV have a surveillance program to assess changes in viruses that may allow them to escape immunity from vaccines [94]. Emerging variants and

representative field isolates should be examined periodically for genetic and antigenic variations. These isolates can be screened by HI, using variant field viruses and vaccine seed strains as antigens, followed by the assessment of genetic changes in viruses thought to be variants [94]. Antigenic cartography is one method that might be used to visualize the differences between viruses. The use of a vaccine seed strain should be discontinued if there are outbreaks in well-vaccinated flocks (related to the vaccine rather than other issues such as vaccine administration) or if genetic variants emerge that are not neutralized [94]. In the absence of such evidence, vaccine efficacy should be reassessed every 2-3 years. This should include the analysis of field viruses from all relevant geographical regions and production sectors to look for genetic variants that may escape immunity from vaccines [94]. Representative strains from the major circulating antigenic lineages, as well as selected antigenic variants, should be tested in challenge trials against the current vaccines.

7.1.6 Vaccine Matching and H5N1 Viruses

Matching Asian lineage H5N1 HPAI field strains to effective vaccine viruses has become increasingly complex, with the emergence of new variants that may not be readily neutralized by existing vaccine strains, despite high HA homology in some cases. Initially, vaccine strains with varying degrees of HA homology from both Eurasian and North American lineages were reported to decrease virus shedding as well as prevent clinical signs [73;135;138-142;147;469;570;580]. In some studies, vaccines were more effective in suppressing virus shedding when they were more closely related to the challenge virus and belonged to the Eurasian lineage [73;135;140;141]. However, one group reported that an inactivated vaccine containing A/turkey/ Wisconsin/68 (H5N9) outperformed a vaccine based on an Asian lineage H5N3 virus (A/duck/Singapore/F1 19/97), when evaluated by the PD₅₀ (50% protective dose) [570].

Later, variant viruses began to emerge in some countries, including China, Vietnam, Indonesia and Egypt [8;132;147;467;569;584-588]. The specific variants differed between areas (e.g., clade 2.2.1.1 variants in Egypt; clade 2.3.2.1 in Vietnam; clade 2.1.3 in Indonesia; and clade 2.3.4.4 and clade 7 variants in China). Some new variants became widespread, while others have diminished or disappeared [132;586]. Studies evaluating the efficacy of older vaccine strains against these variants have sometimes reported conflicting results; nevertheless, some previously effective vaccines are now reported to be less effective or even poorly protective against clinical signs and/or virus shedding [132;146;467;504;522;524;537;566-569;588]. In some cases, this has occurred even when the new variants have similar (including high) homology to previous field strains, suggesting that changes in specific epitopes account for the differences [545]. One study reported that boosters increased antibody titers to some, but not all, heterologous viruses [312], probably by increasing titers overall. However, boosting was unable to induce adequate immunity to some H5N1 variants.

The following tables, while not exhaustive, summarize information from a number of older and recent studies regarding the efficacy of various commercial and experimental vaccines against Asian lineage H5N1 HPAI viruses in chickens. In some cases, there may be discrepancies between studies despite using similar vaccine and challenge strains. Such discrepancies could be related to the vaccine (e.g., amount of antigen, adjuvant type), experimental conditions (e.g., challenge doses, vaccination protocols), or the specific challenge virus used.

Table 4.1 Inactivated Vaccines and Challenge by Clade 0 H5N1 Viruses in Chickens

Challenge Virus	Vaccine Virus	Amino Acid Homology	Effect	Reference
A/Hong Kong/156/97 (H5N1 clade 0)	A/turkey/ Wisconsin/68 (H5N9) North American lineage		Protective against clinical signs; Decreased virus shedding; PD ₅₀ (morbidity) = 0.32; PD ₅₀ (mortality) = 0.25	Swayne et al., 2001 (ref [570])
A/Hong Kong/156/97 (H5N1 clade 0)	A/duck/Singapore/F1 19/97 (H5N3) Eurasian lineage		Protective against clinical signs; Decreased virus shedding; PD ₅₀ (morbidity) = 0.50; PD ₅₀ (mortality) = 0.50	Swayne et al., 2001 (ref [570])
A/Hong Kong/156/97 (H5N1 clade 0)	A/turkey/Minnesota/3689-1551/81 (H5N2) North American lineage		Protective against clinical signs; Decreased virus shedding; PD ₅₀ (morbidity) = 1.43; PD ₅₀ (mortality) = 0.89	Swayne et al., 2001 (ref [570])

Table 4.2 Inactivated Vaccines and Challenge by Clade 1 H5N1 Viruses in Chickens

Challenge Virus	Vaccine Virus	Amino Acid Homology	Effect	Reference
A/chicken/Supranburi Thailand/2/04 (H5N1 clade 1)	A/turkey/Wisconsin/68 (H5N9) North American lineage	85.6%	Protective against clinical signs; Decreased virus shedding	Bublout et al., 2007 (ref [140])
A/chicken/Supranburi Thailand/2/04 (H5N1 clade 1)	A/chicken/Italy/22A/98 (H5N9) Eurasian lineage	90.2%	Protective against clinical signs; Decreased virus shedding	Bublout et al., 2007 (ref [140])
A/chicken/Supranburi Thailand/2/2004 (H5N1 clade 1.0)	A/chicken/Indonesia/7/2003 (H5N1) Eurasian lineage		Protective against clinical signs; Decreased or eliminated virus shedding	Jadhao et al., 2009 (ref [73])
A/chicken/Supranburi Thailand/2/2004 (H5N1 clade 1.0)	Reassortant H5N9 vaccine; H5 from A/chicken/Indonesia/7/2003 (H5N1); N9 from A/turkey/Wisconsin/1968 (H5N9)		Protective against clinical signs; Decreased or eliminated virus shedding	Jadhao et al., 2009 (ref [73])
A/chicken/Supranburi Thailand/2/2004 (H5N1 clade 1.0)	A/turkey/Wisconsin/1968 (H5N9) North American lineage	89%	Protective against clinical signs; Decreased virus shedding	Jadhao et al., 2009 (ref [73])
A/chicken/Supranburi Thailand/2/2004 (H5N1 clade 1.0)	A/chicken/Hidalgo/232/1994 (H5N2) North American lineage	88%	Protective against clinical signs; Decreased virus shedding	Jadhao et al., 2009 (ref [73])

Challenge Virus	Vaccine Virus	Amino Acid Homology	Effect	Reference
A/mallard duck/VN-CM/1185/06 (H5N1 clade 1)	A/goose/Guangdong/1/96 (H5N1) reassortant Eurasian lineage		Protective against clinical signs; No virus shedding detected	Tian et al. 2010 (ref [147])
A/mallard duck/VN-CM/1159/06 (H5N1 clade 1).	A/goose/Guangdong/1/96 (H5N1) reassortant Eurasian lineage		Protective against clinical signs; No virus shedding detected	Tian et al. 2010 (ref [147])
A/muscovy duck/Vietnam/OIE-559/2011 (H5N1 clade 1.1)	A/duck/Hokkaido/Vac-3/2007 (H5N1) reassortant Eurasian lineage		Protective against clinical signs; Decreased virus shedding	Shichinohe et al., 2013 (ref [589])

Table 4.3 Inactivated Vaccines and Challenge by Clade 2 H5N1 Viruses in Chickens

Challenge Virus	Vaccine Virus	Amino Acid Homology	Effect	Reference
A/chicken/Legok/2003 (H5N1 clade 2)	A/chicken/Legok/2003 (H5N1) Eurasian lineage	100%	Decreased virus shedding; Prevented transmission	Bouma et al., 2009 (ref [139])
A/chicken/Legok/2003 (H5N1 clade 2)	A/turkey/England/N28/73 (H5N2) Eurasian lineage	92%	Decreased virus shedding; Prevented transmission	Bouma et al., 2009 (ref [139])
A/chicken/Legok/2003 (H5N1 clade 2)	A/chicken/Mexico/232/94/CPA (H5N2) North American lineage	88%	Decreased virus shedding; Prevented transmission	Bouma et al., 2009 (ref [139])
A/chicken/Legok/2003 (H5N1 clade 2)	A/turkey/England/N28/73 (H5N2) Eurasian lineage	91%	Decreased virus shedding; Prevented transmission to vaccinated contacts	Bublot et al., 2007 (ref [140])
A/chicken/Indonesia/7/03 (H5N1 clade 2.1)	A/duck/Potsdam/1402/86 (H5N2) Eurasian lineage	91%	Protective against clinical signs; Decreased virus shedding; Decreased number of chickens infected	Swayne et al., 2006 (ref [142])
A/chicken/Indonesia/7/03 (H5N1 clade 2.1)	A/chicken/Mexico/232/94 (H5N2) North American lineage	84%	Protective against clinical signs; Decreased virus shedding; Decreased number of chickens infected	Swayne et al., 2006 (ref [142])

Challenge Virus	Vaccine Virus	Amino Acid Homology	Effect	Reference
A/chicken/Indonesia/7/2003 (H5N1 clade 2.1)	A/chicken/Indonesia/7/2003 (H5N1) Eurasian lineage	100%	Protective against clinical signs; Decreased or eliminated virus shedding	Jadhao et al., 2009 (ref [73])
A/chicken/Indonesia/7/2003 (H5N1 clade 2.1)	Reassortant H5N9 vaccine; H5 from A/chicken/Indonesia/7/2003 (H5N1); N9 from A/turkey/Wisconsin/1968 (H5N9)	100%	Protective against clinical signs; Decreased or eliminated virus shedding	Jadhao et al., 2009 (ref [73])
A/chicken/Indonesia/7/2003 (H5N1 clade 2.1)	A/turkey/Wisconsin/1968 (H5N9) North American lineage	89%	Protective against clinical signs; Decreased virus shedding	Jadhao et al., 2009 (ref [73])
A/chicken/Indonesia/7/2003 (H5N1 clade 2.1)	A/chicken/Hidalgo/232/1994 (H5N2) North American lineage	88%	Protective against clinical signs; Decreased virus shedding	Jadhao et al., 2009 (ref [73])
A/chicken/WestJava Sbg/29/2007 (H5N1 clade 2.1.3)	A/chicken/Mexico/232/94 (H5N2) North American lineage		Not protective against clinical signs	Kapczynski et al., 2015 (ref [524])
A/chicken/West Java/SMI-HAMD/06 (H5N1 clade 2.1.3)	A/turkey/Wisconsin/1968 (H5N9) North American lineage	88% (HA)/ 86% (HA1)	≤ 20% mortality; Decreased oral virus shedding day 2	Swayne et al., 2015 (ref [467])
A/chicken/West Java/SMI-HAMD/06 (H5N1 clade 2.1.3)	A/chicken/Mexico/232/1994 (H5N2) North American lineage	87% (HA)/ 85% (HA1)	≤ 20% mortality; Decreased oral virus shedding day 2	Swayne et al., 2015 (ref [467])
A/chicken/West Java/SMI-HAMD/06 (H5N1 clade 2.1.3)	A/turkey/England/N28/1973 (H5N2) Eurasian lineage	90% (HA)/ 90% (HA1)	> 20% mortality; Decreased oral virus shedding day 2	Swayne et al., 2015 (ref [467])
A/chicken/West Java/SMI-HAMD/06 (H5N1 clade 2.1.3)	A/chicken/Legok/2003 (H5N1) Eurasian lineage	99.8% (HA)/ 100% (HA1)	≤ 20% mortality; Decreased oral virus shedding day 2	Swayne et al., 2015 (ref [467])

Challenge Virus	Vaccine Virus	Amino Acid Homology	Effect	Reference
A/chicken/West Java/SMI-HAMD/06 (H5N1 clade 2.1.3)	A/goose/Guangdong/1/96 (H5N1) Eurasian lineage	96% (HA)/ 96% (HA1)	≤ 20% mortality; Decreased oral virus shedding day 2	Swayne et al., 2015 (ref [467])
A/chicken/West Java/SMI-HAMD/06 (H5N1 clade 2.1.3)	Reverse genetics H5N3 vaccine, HA and NA from A/duck/Vietnam/2004 Eurasian lineage	97% (HA)/ 97% (HA1)	≤ 20% mortality; Decreased oral virus shedding day 2	Swayne et al., 2015 (ref [467])
A/chicken/West Java/SMI-HAMD/06 (H5N1 clade 2.1.3)	A/chicken/West Java/PWT-WIJ/20 (H5N1) Eurasian lineage		≤ 20% mortality; Decreased oral virus shedding day 2	Swayne et al., 2015 (ref [467])
A/chicken/Papua/TA5/2006 (H5N1 clade 2.1.3.1)	A/turkey/Wisconsin/1968 (H5N9) North American lineage	88% (HA)/ 86% (HA1)	> 20% mortality; Decreased oral virus shedding day 2	Swayne et al., 2015 (ref [467])
A/chicken/Papua/TA5/2006 (H5N1 clade 2.1.3.1)	A/chicken/Mexico/232/1994 (H5N2) North American lineage	86% (HA)/ 85% (HA1)	≤ 20% mortality; Decreased oral virus shedding day 2	Swayne et al., 2015 (ref [467])
A/chicken/Papua/TA5/2006 (H5N1 clade 2.1.3.1)	A/turkey/England/N28/1973 (H5N2) Eurasian lineage	89% (HA)/ 89% (HA1)	> 20% mortality; Decreased oral virus shedding day 2	Swayne et al., 2015 (ref [467])
A/chicken/Papua/TA5/2006 (H5N1 clade 2.1.3.1)	A/chicken/Legok/2003 (H5N1) Eurasian lineage	97% (HA)/ 97% (HA1)	≤ 20% mortality; Decreased oral virus shedding day 2	Swayne et al., 2015 (ref [467])
A/chicken/Papua/TA5/2006 (H5N1 clade 2.1.3.1)	Reverse genetics H5N1 vaccine, HA and NA from A/goose/Guangdong/1/96 Eurasian lineage	94% (HA)/ 93% (HA1)	≤ 20% mortality; Decreased oral virus shedding day 2	Swayne et al., 2015 (ref [467])
A/chicken/Papua/TA5/2006 (H5N1 clade 2.1.3.1)	Reverse genetics H5N3 vaccine, HA and NA from A/duck/Vietnam/2004 Eurasian lineage	95% (HA)/ 94% (HA1)	≤ 20% mortality; Decreased oral virus shedding day 2	Swayne et al., 2015 (ref [467])
A/chicken/Papua/TA5/2006 (H5N1 clade 2.1.3.1)	A/chicken/West Java/PWT-WIJ/20 (H5N1) Eurasian lineage		≤ 20% mortality; Decreased oral virus shedding day 2	Swayne et al., 2015 (ref [467])

Challenge Virus	Vaccine Virus	Amino Acid Homology	Effect	Reference
A/chicken/West Java/PWT-WIJ/2006 (H5N1 clade 2.1.3.2)	A/turkey/Wisconsin/1968 (H5N9) North American lineage	85% (HA)/ 80% (HA1)	> 20% mortality; Decreased oral virus shedding day 2	Swayne et al., 2015 (ref [467])
A/chicken/West Java/PWT-WIJ/2006 (H5N1 clade 2.1.3.2)	A/chicken/Mexico/232/1994 (H5N2) North American lineage	83% (HA)/ 79% (HA1)	> 20% mortality; Decreased oral virus shedding day 2	Swayne et al., 2015 (ref [467])
A/chicken/West Java/PWT-WIJ/2006 (H5N1 clade 2.1.3.2)	A/turkey/England/N28/1973 (H5N2) Eurasian lineage	86% (HA)/ 84% (HA1)	> 20% mortality; Decreased oral virus shedding day 2	Swayne et al., 2015 (ref [467])
A/chicken/West Java/PWT-WIJ/2006 (H5N1 clade 2.1.3.2)	A/chicken/Legok/2003 (H5N1) Eurasian lineage	95% (HA)/ 92% (HA1)	> 20% mortality; Decreased oral virus shedding day 2	Swayne et al., 2015 (ref [467])
A/chicken/West Java/PWT-WIJ/2006 (H5N1 clade 2.1.3.2)	Reverse genetics H5N1 vaccine, HA and NA from A/goose/Guangdong/1/96 Eurasian lineage	92% (HA)/ 90% (HA1)	> 20% mortality; Decreased oral virus shedding day 2	Swayne et al., 2015 (ref [467])
A/chicken/West Java/PWT-WIJ/2006 (H5N1 clade 2.1.3.2)	Reverse genetics H5N3 vaccine, HA and NA from A/duck/Vietnam/2004 Eurasian lineage	93.5% (HA)/ 91% (HA1)	> 20% mortality; Decreased oral virus shedding day 2	Swayne et al., 2015 (ref [467])
A/chicken/West Java/PWT-WIJ/2006 (H5N1 clade 2.1.3.2)	A/chicken/West Java/PWT-WIJ/20 (H5N1) Eurasian lineage	100%	≤ 20% mortality; Decreased oral virus shedding day 2	Swayne et al., 2015 (ref [467])
A/bar-headed goose/Qinghai/3/05 (H5N1 clade 2.2)	A/goose/Guangdong/1/96 (H5N1) reassortant Eurasian lineage		Protective against clinical signs; No virus shedding detected	Tian et al. 2010 (ref [147])
A/chicken/Miyazaki/K11/200 (H5N1 clade 2.2)	Reverse genetics H5N1 vaccine; HA and NA from A/chicken/Miyazaki/K11/2007 Eurasian lineage	100%	Complete protection from death (640 and 3200 HAU vaccine doses); Dose-dependent decrease in virus shedding,	Uchida et al., 2014 (ref [459])

Challenge Virus	Vaccine Virus	Amino Acid Homology	Effect	Reference
A/chicken/Miyazaki/K11/200 (H5N1 clade 2.2)	Reverse genetics H5N3 vaccine; HA from A/chicken/Miyazaki/K11/2007; NA from A/whistling swan/Shimane/580/2002 Eurasian lineage	100%	Complete protection from death (640 and 3200 HAU vaccine doses); Dose-dependent decrease in virus shedding,	Uchida et al., 2014 (ref [459])
A/chicken/Qalubia-Egypt/1/08 (H5N1 clade 2.2.1)	A/goose/Guangdong/96 (H5N1) reassortant Eurasian lineage		Protective against clinical signs; No virus shedding detected	Kim et al., 2010 (ref. [141])
A/chicken/Qalubia-Egypt/1/08 (H5N1 clade 2.2.1)	Reassortant vaccine; A/duck/Anhui/1/06 (H5N1) Eurasian lineage		Protective against clinical signs; No virus shedding detected	Kim et al., 2010 (ref. [141])
A/chicken/Qalubia-Egypt/1/08 (H5N1 clade 2.2.1)	Reassortant H5N3 vaccine; H5 from A/chicken/Vietnam/C58/04 (H5N1); NA from A/duck/Germany/1215/73 (H2N3)		Protective against clinical signs; No virus shedding detected	Kim et al., 2010 (ref. [141])
A/chicken/Qalubia-Egypt/1/08 (H5N1 clade 2.2.1)	A/turkey/England/N-28/73 (H5N2) Eurasian lineage		Protective against clinical signs except one bird without titer; No virus shedding detected in 7/8 birds	Kim et al., 2010 (ref. [141])
A/chicken/Qalubia-Egypt/1/08 (H5N1 clade 2.2.1)	A/chicken/Mexico/232/94 (H5N2) North American lineage		Protective against clinical signs; Decreased virus shedding	Kim et al., 2010 (ref. [141])
A/chicken/Egypt/1709-6/08 (H5N1 clade 2.2.1)	A/chicken/Mexico/232/94 (H5N2) North American lineage		30-40% morbidity, 10-20% mortality; Decreased virus shedding	Terregino et al., 2010 (ref [590])

Challenge Virus	Vaccine Virus	Amino Acid Homology	Effect	Reference
A/chicken/Egypt/NLQ P-0918/2009 (H5N1 clade 2.2.1)	A/chicken/Mexico/232/94 (H5N2) North American lineage		Clinically protective; Decreased virus shedding	Grund et al., 2011 (ref [569])
A/chicken/Egypt/NLQ P-0918/2009 (H5N1 clade 2.2.1)	A/duck/Potsdam/1402-6/1986 (H5N2) Eurasian lineage		Clinically protective; Decreased virus shedding	Grund et al., 2011 (ref [569])
A/chicken/Egypt/NLQ P-0918/2009 (H5N1 clade 2.2.1)	Reverse genetics H5N1 vaccine; HA and NA from A/duck/Anhui/1/2006 (clade 2.3.4) Eurasian lineage		Clinically protective; Decreased virus shedding	Grund et al., 2011 (ref [569])
A/chicken/Egypt/NLQ P-0918/2009 (H5N1 clade 2.2.1)	A/chicken/Egypt/0879/2008 (H5N1 clade 2.2.1 variant) Eurasian lineage		Clinically protective; Decreased virus shedding	Grund et al., 2011 (ref [569])
A/chicken/Egypt/0879/2008 (H5N1 clade 2.2.1 variant)	A/chicken/Mexico/232/94 (H5N2) North American lineage		Not protected (50% mortality); Decreased virus shedding	Grund et al., 2011 (ref [569])
A/chicken/Egypt/0879/2008 (H5N1 clade 2.2.1 variant)	A/duck/Potsdam/1402-6/1986 (H5N2) Eurasian lineage		Not protected (50% mortality); Decreased virus shedding	Grund et al., 2011 (ref [569])
A/chicken/Egypt/0879/2008 (H5N1 clade 2.2.1 variant)	Reverse genetics H5N1 vaccine; HA and NA from A/duck/Anhui/1/2006 (clade 2.3.4) Eurasian lineage	100%	Clinically protective; Virus shedding not detected	Grund et al., 2011 (ref [569])
A/chicken/Egypt/0879/2008 (H5N1 clade 2.2.1 variant)	A/chicken/Egypt/0879/2008 (H5N1 clade 2.2.1 variant) Eurasian lineage		Clinically protective; Decreased virus shedding	Grund et al., 2011 (ref [569])
A/chicken/Egypt/1709-1VIR08/2007 (H5N1 clade 2.2.1)	A/chicken/Mexico/232/94 (H5N2) North American lineage	82%	Clinically protective; Decreased virus shedding	Rauw et al. (ref [522])
A/chicken/Egypt/1709-6/2008 (H5N1 clade 2.2.1 variant)	A/chicken/Mexico/232/94 (H5N2) North American lineage	79%	Little or no protection from clinical signs; Decreased virus shedding	Rauw et al. (ref [522])

Challenge Virus	Vaccine Virus	Amino Acid Homology	Effect	Reference
A/chicken/Egypt/NLQ P-0918Q/2009 (H5N1 clade 2.2.1)	A/duck/Potsdam/1402-6/1986 (H5N2) Eurasian lineage (3 doses)	89.5%	Protective against clinical signs in most birds (12% mortality); Decreased virus shedding; Did not prevent virus deposition internally in eggs	Abdelwhab et al., 2011 (ref [312])
A/chicken/Egypt/NLQ P-0879/2008 (H5N1 clade 2.2.1 variant)	A/duck/Potsdam/1402-6/1986 (H5N2) Eurasian lineage (3 doses)	84%	Little protection from clinical signs (75% mortality); Decreased virus shedding; Did not prevent virus deposition internally in egg	Abdelwhab et al., 2011 (ref [312])
Multiple Egyptian H5N1 clade 2.2.1 viruses (A/chicken/Egypt/959-NLQP/2006, A/goose/Egypt/20-NLQP/2009, A/chicken/Egypt/2d-NLQP/2010)	A/chicken/Mexico/232/94 (H5N2) North American lineage		Protective against mortality (0%); Decreased virus shedding	Spackman et al., (ref [146])
Multiple Egyptian H5N1 clade 2.2.1 viruses (A/chicken/Egypt/959-NLQP/2006, A/chicken/Egypt/13-NLQP/2008, A/goose/Egypt/20-NLQP/2009, A/chicken/Egypt/2d-NLQP/2010. A/duck/Egypt/44-NLQP/2009)	A/goose/Guangdong/1/96 (H5N1 clade 0) reassortant Eurasian lineage		Protective against mortality (0% for 4 viruses, 10% for A/chicken/Egypt/13-NLQP/2008); Decreased virus shedding (N/A for A/duck/Egypt/44-NLQP/2009)	Spackman et al., (ref [146])

Challenge Virus	Vaccine Virus	Amino Acid Homology	Effect	Reference
Multiple Egyptian H5N1 clade 2.2.1 viruses (A/chicken/Egypt/959-NLQP/2006, A/chicken/Egypt/13-NLQP/2008, A/goose/Egypt/20-NLQP/2009, A/duck/Egypt/44-NLQP/2009)	A/chicken/Egypt/13-NLQP/2008 (H5N1 clade 2.2.1) Eurasian lineage		Protective against mortality (0% for 3 viruses, 10% for A/duck/Egypt/44-NLQP/2009); Decreased virus shedding (N/A for A/duck/Egypt/44-NLQP/2009)	Spackman et al., (ref [146])
Multiple Egyptian H5N1 clade 2.2.1 viruses (A/chicken/Egypt/959-NLQP/2006, A/chicken/Egypt/13-NLQP/2008, A/goose/Egypt/20-NLQP/2009, A/duck/Egypt/44-NLQP/2009)	A/chicken/Egypt/202-NLQP/2007 (H5N1 clade 2.2.1.1) Eurasian lineage		Clinical protection varied (0% mortality for 2 viruses; 30% for A/chicken/Egypt/13-NLQP/2008; 70% for A/duck/Egypt/44-NLQP/2009); Decreased virus shedding (N/A for A/duck/Egypt/44-NLQP/2009)	Spackman et al., (ref [146])
Multiple Egyptian H5N1 clade 2.2.1.1 viruses (A/chicken/Egypt/65-NLQP/2008, A/chicken/Egypt/63-NLQP/2010)	A/chicken/Mexico/232/94 (H5N2) North American lineage		Partially protective (10% mortality for each virus); Decreased virus shedding	Spackman et al., (ref [146])
Multiple Egyptian H5N1 clade 2.2.1.1 viruses (A/chicken/Egypt/202-NLQP/2007, A/chicken/Egypt/65-NLQP/2008, A/chicken/Egypt/63-NLQP/2010)	A/goose/Guangdong/1/96 (H5N1 clade 0) reassortant Eurasian lineage		Partially protective (mortality 20-50%); Decreased virus shedding A/chicken/Egypt/65-NLQP/2008 & A/chicken/Egypt/63-NLQP/2010	Spackman et al., (ref [146])

Challenge Virus	Vaccine Virus	Amino Acid Homology	Effect	Reference
Multiple Egyptian H5N1 clade 2.2.1.1 viruses (A/chicken/Egypt/202-NLQP/2007, A/chicken/Egypt/65-NLQP/2008)	A/chicken/Egypt/13-NLQP/2008 (H5N1 clade 2.2.1) Eurasian lineage		Reduced mortality (20% for A/chicken/Egypt/202-NLQP/2007, 0% for homologous virus); Decreased virus shedding A/chicken/Egypt/65-NLQP/2008	Spackman et al., (ref [146])
Multiple Egyptian H5N1 clade 2.2.1.1 viruses (A/chicken/Egypt/202-NLQP/2007, A/chicken/Egypt/65-NLQP/2008)	A/chicken/Egypt/202-NLQP/2007 (H5N1 clade 2.2.1.1) Eurasian lineage		Reduced mortality (20% for homologous virus, 0% for A/chicken/Egypt/65-NLQP/2008); No reduction in virus shedding for A/chicken/Egypt/65-NLQP/2008	Spackman et al., (ref [146])
A/duck/Vietnam/203/05 (H5N1 clade 2.3.2)	A/duck/Vietnam/203/05 (H5N1) Eurasian lineage	100%	Protective against clinical signs; Decreased virus shedding	Pfeiffer et al., 2010 (ref [135])
A/duck/Vietnam/203/05 (H5N1 clade 2.3.2)	A/goose/Guangdong/1/96 (H5N1) reassortant Eurasian lineage	92.5%	Protective against clinical signs; Decreased virus shedding	Pfeiffer et al., 2010 (ref [135])
A/duck/Vietnam/203/05 (H5N1 clade 2.3.2)	A/turkey/England/N-28/73 (H5N2) Eurasian lineage	87%	Decreased severity of clinical signs, prevented deaths; Decreased virus shedding	Pfeiffer et al., 2010 (ref [135])

Challenge Virus	Vaccine Virus	Amino Acid Homology	Effect	Reference
A/duck/Vietnam/203/05 (H5N1 clade 2.3.2)	A/chicken/Mexico/232/94 (H5N2) North American lineage	83%	Protective against clinical signs; Decreased virus shedding	Pfeiffer et al., 2010 (ref [135])
A/chicken/Hong Kong/782/2009 (H5N1 clade 2.3.2)	A/chicken/Mexico/232-CPA/1994 (H5N2) North American lineage	82%	Protected from mortality; Decreased virus shedding	Leung et al., (ref [566])
A/chicken/Hong Kong/782/2009 (H5N1 clade 2.3.2)	Reverse genetics H5N3 vaccine; HA and NA from A/chicken/Vietnam/C58/2004 Eurasian lineage		Most chickens protected from mortality; Decreased virus shedding	Leung et al., (ref [566])
A/chicken/Hong Kong/782/2009 (H5N1 clade 2.3.2)	Reverse genetics H5N3 vaccine; HA and NA from A/duck/Anhui/1/2006 (clade 2.3.4) Eurasian lineage		Protected from mortality; Decreased virus shedding	Leung et al., (ref [566])
A/whooper swan/Hokkaido/4/2011 (H5N1 clade 2.3.2.1)	A/duck/Hokkaido/Vac-3/2007 (H5N1) reassortant Eurasian lineage		Protective against clinical signs; Decreased virus shedding	Shichinohe et al, 2013 (ref [589])
A/chicken/Shimane/1/2010 (H5N1 clade 2.3.2.1)	Reverse genetics H5N1 vaccine; HA and NA from A/chicken/Miyazaki/K11/2007 Eurasian lineage		Dose dependent protection from clinical signs (death); dose-dependent decrease in virus shedding	Uchida et al., 2014 (ref [459])
A/chicken/Shimane/1/2010 (H5N1 clade 2.3.2.1)	Reverse genetics H5N3 vaccine; HA from A/chicken/Miyazaki/K11/2007; NA from A/whistling swan/Shimane/580/2002; Eurasian lineage		Dose dependent protection from clinical signs (death); dose-dependent decrease in virus shedding	Uchida et al., 2014 (ref [459])

Challenge Virus	Vaccine Virus	Amino Acid Homology	Effect	Reference
A/chicken/Shimane/1/2010 (H5N1 clade 2.3.2.1)	Reverse genetics H5N1 vaccine; HA and NA from A/whooper swan/Akita/1/2008 Eurasian lineage		Protected from clinical signs (death) and decrease in virus shedding at high vaccine dose (3200 HAU)	Uchida et al., 2014 (ref [459])
A/whooper swan/Akita/1/2008 (H5N1 clade 2.3.2.1)	Reverse genetics H5N1 vaccine; HA and NA from A/whooper swan/Akita/1/2008 Eurasian lineage	100%	Protected from clinical signs (death) and decrease in virus shedding at high vaccine dose (3200 HAU)	Uchida et al., 2014 (ref [459])
A/duck/QuangNgai/1037/2011 (H5N1 clade 2.3.2.1b)	Reverse genetics H5N1 vaccine; HA and NA from A/Vietnam/1194/2004; Eurasian lineage	99%	Not protective against clinical signs	Tung et al. (ref [567])
A/chicken/Vietnam/2009/05 (H5N1 clade 2.3.4)	A/chicken/Vietnam/209/05 (H5N1) Eurasian lineage	100%	Protective against clinical signs (except birds without titers); Decreased virus shedding	Pfeiffer et al., 2010 (ref [135])
A/chicken/Vietnam/2009/05 (H5N1 clade 2.3.4)	A/goose/Guangdong/1/96 (H5N1) reassortant Eurasian lineage	92.5%	Protective against clinical signs (except birds without titers); Decreased virus shedding	Pfeiffer et al., 2010 (ref [135])
A/chicken/Vietnam/2009/05 (H5N1 clade 2.3.4)	A/turkey/England/N-28/73 (H5N2) Eurasian lineage	88%	Decreased severity of clinical signs; Decreased virus shedding (greater amounts shed than with homologous vaccines)	Pfeiffer et al., 2010 (ref [135])

Challenge Virus	Vaccine Virus	Amino Acid Homology	Effect	Reference
A/chicken/Vietnam/209/05 (H5N1 clade 2.3.4)	A/chicken/Mexico/232/94 (H5N2) North American lineage	85%	Protective against clinical signs; Decreased virus shedding (greater amounts shed than with homologous vaccines)	Pfeiffer et al., 2010 (ref [135])
A/duck/Fujian/31/07 (H5N1 clade 2.3.4)	A/goose/Guangdong/1/96 (H5N1) reassortant Eurasian lineage		Protective against clinical signs; No virus shedding detected	Tian et al. 2010 (ref [147])
A/mallard duck/VN-HD/46/07 (H5N1 clade 2.3.4)	A/goose/Guangdong/1/96 (H5N1) reassortant Eurasian lineage		Protective against clinical signs; No virus shedding detected	Tian et al. 2010 (ref [147])
A/chicken/Hong Kong/8825-2/2008 (H5N1 clade 2.3.4)	A/chicken/Mexico/232-CPA/1994 (H5N2) North American lineage	83%	No reduction in mortality or virus shedding	Leung et al., 2013 (ref [566])
A/chicken/Hong Kong/8825-2/2008 (H5N1 clade 2.3.4)	Reverse genetics H5N3 clade 1 vaccine; HA and NA from A/chicken/Vietnam/C58/2004 Eurasian lineage	89.5%	No reduction in mortality or virus shedding	Leung et al., 2013 (ref [566])
A/chicken/Hong Kong/8825-2/2008 (H5N1 clade 2.3.4)	Reverse genetics H5N1 clade 2.3.4 vaccine; HA and NA from A/duck/Anhui/1/2006 Eurasian lineage		Partial clinical protection; Decreased virus shedding	Leung et al., 2013 (ref [566])
A/duck/SY/2005 (H5N1 clade 2.3.4)	A/chicken/Huadong/4/2008 (H5N1) Eurasian lineage		No protection against clinical signs (100% mortality)	Li et al., (ref [588])
A/duck/SY/2005 (H5N1 clade 2.3.4)	Bivalent vaccine: A/duck/Anhui/1/06 (H5N1 clade 2) & A/chicken/Shanxi/2/2006 (H5N1 clade 7) Eurasian lineage		Protected from clinical signs; Decreased virus shedding	Li et al., (ref [588])

Challenge Virus	Vaccine Virus	Amino Acid Homology	Effect	Reference
A/peregrine falcon/Hong Kong/810/2009 (H5N1 clade 2.3.4)	A/duck/Hokkaido/Vac-3/2007 (H5N1) reassortant Eurasian lineage		Lower antigen dose: Reduced clinical signs, but some birds died; Decreased virus shedding Higher antigen dose: Protective against clinical signs; Little virus shedding	Shichinohe et al, 2013 (ref [589])
A/peregrine falcon/Hong Kong/810/2009 (H5N1 clade 2.3.4)	A/peregrine falcon/Hong Kong/810/2009 (H5N1) reassortant Eurasian lineage	100%	Protective against clinical signs; No virus shedding	Shichinohe et al, 2013 (ref [589])
A/chicken/northern China/k0602/2010 (H5N1 clade 2.3.4.5)	Bivalent vaccine: A/duck/Anhui/1/2006 (H5N1 clade 2.3.4) & A/chicken/Shanxi/2/2006 (H5N1 clade 7) Eurasian lineage		Poor protection against clinical signs or virus shedding	Gu et al., 2013 (ref [591])
A/chicken/northern China/k0602/2010 (H5N1 clade 2.3.4.5)	Reverse generics H5N1 vaccine; HA and NA from A/chicken/northern China/k0602/2010 Eurasian lineage	100%	Protective against clinical signs; No virus shedding detected	Gu et al., 2013 (ref [591])
A/chicken/Shandong/k0603/2010 (H5N1 clade 2.3.4.5)	Bivalent vaccine: A/duck/Anhui/1/2006 (H5N1 clade 2.3.4) & A/chicken/Shanxi/2/2006 (H5N1 clade 7) Eurasian lineage		Poor protection against clinical signs or virus shedding	Gu et al., 2013 (ref [591])
A/chicken/Shandong/k0603/2010 (H5N1 clade 2.3.4.5)	Reverse generics H5N1 vaccine; HA and NA from A/chicken/northern China/k0602/2010 Eurasian lineage		Protective against clinical signs; No virus shedding detected	Gu et al., 2013 (ref [591])

Table 4.4 Inactivated Vaccines and Challenge by Other H5N1 Clades in Chickens

Challenge Virus	Vaccine Virus	Amino Acid Homology	Effect	Reference
A/chicken/Shanxi/2/06 (H5N1 clade 7)	A/goose/Guangdong/1/96 (H5N1) reassortant Eurasian lineage		Decreased clinical signs; Fewer deaths; Decreased virus shedding	Tian et al. 2010 (ref [147])
A/chicken/Pyigyitagon/204/2006 (H5N1 clade 7)	Reverse genetics H5N1 vaccine; HA and NA from A/chicken/Miyazaki/K11/2007 Eurasian lineage		Partial protection from clinical signs at vaccine dose of 640 HAU	Uchida et al., 2014 (ref [459])
A/chicken/Pyigyitagon/204/2006 (H5N1 clade 7)	Reverse genetics H5N3 vaccine; HA from A/chicken/Miyazaki/K11/2007; NA from A/whistling swan/Shimane/580/2002 Eurasian lineage		Partial protection from clinical signs at vaccine dose of 640 HAU	Uchida et al., 2014 (ref [459])
A/chicken/Huadong/4/2008 (H5N1 clade 7.2)	A/chicken/Huadong/4/2008 (H5N1) Eurasian lineage	100%	Protective against clinical signs; Decreased virus shedding	Li et al, 2013 (ref [588])
A/chicken/Huadong/4/2008 (H5N1 clade 7.2)	Bivalent vaccine: A/duck/Anhui/1/2006 (H5N1 clade 2.3.4) & A/chicken/Shanxi/2/2006 (H5N1 clade 7) Eurasian lineage		Decreased clinical signs (20% mortality); Decreased virus shedding	Li et al, 2013 (ref [588])
A/chicken/Hong Kong/86.3/2002 (H5N1 clade 8)	A/chicken/Mexico/232/94 (H5N2) North American lineage	89.3%	Decreased virus shedding; Decreased transmission	Liu et al., 2003 (ref [580])

7.2 Vaccine Efficacy and Potency

7.2.1 Vaccine Efficacy

The efficacy of a vaccine is mainly influenced by the amount of antigen, the antigenic relatedness of the vaccine strain with the field/ challenge virus (section 7.1), and the adjuvant if applicable [137;142;342;564]. Vaccine efficacy is defined as a characteristic of the product, and must be demonstrated according to the label claims before product licensing [535]. It is usually evaluated in challenge studies (required during the initial licensing in the U.S.) [458;464;535]. Prevention of mortality is the most commonly measured clinical parameter when HPAI viruses are used for challenge [458]. The OIE suggests a minimum standard of 80% protection from mortality, whether an avian influenza vaccine is used for control/ eradication programs or in animal production [94]. Other parameters that could be evaluated include the prevention of morbidity/clinical signs (including decreased egg production with LPAI challenge), and the suppression of virus shedding [458;464]. The effect of vaccination on virus transmission might also be tested; however, techniques vary, and a standardized assay method does not exist [458;464]. Protection is also influenced by factors such as host species and age, the route and site of immunization, and the dose and virulence of the challenge virus [142;468]. Most avian influenza vaccines are licensed for use in chickens and/or turkeys.

7.2.2 Effect of Antigen Quantity on Vaccine Effectiveness

Sufficient antigen is critical for a potent vaccine [137;142]. In experiments, avian influenza vaccines that contain more antigen generally provide better protection from clinical signs and greater decreases in virus shedding [137;209;437;459;564;570;574;592], and may also be more effective against heterologous viruses [459;589]. Increased antigen levels have been linked to higher HI titers [209;437;564;574;592]. However, the relationship between the antigen load and protection is not straightforward for AIV, because the degree of homology between the vaccine and challenge virus also affects efficacy [564]. Swayne et al. (1999) reported that there was a 25-fold difference in the antigen levels needed for clinical protection, using different North American lineage strains as vaccines [137]. Varying levels of antigen were also reported to be protective in other studies, depending on the specific vaccine, species of birds and other factors [209;365;437;564;570;574;580]. In some studies, inactivated vaccines used in Anseriformes have required twice the antigenic load necessary in chicken vaccines and/or a strong adjuvant ([211] cited in [49]; [331;336] cited in [342]). There have been proposals to create international standards for the antigen content of avian influenza vaccines, similar to those for human vaccines; however, the variety of adjuvants used in avian vaccines would complicate establishing such standards [209].

7.2.3 Vaccine Potency

Potency is a measurement of relative strength [535]. Each batch of the vaccine must be evaluated, to demonstrate that it is at least as potent as the reference serial(s) [535]. Assays to demonstrate potency include serology and *in vitro* tests [464;535]. The microbiological count or virus titration can also be used for live products such as some vectored vaccines [464;535].

7.2.3.1 HI Titers

The development of adequate HI titers is usually used as evidence of potency for avian influenza vaccines, provided that the challenge virus is antigenically and genetically related to the vaccine strain, and is not a drift variant [94;136;458;464]. Titers reported to be protective can vary between experiments and laboratories. In some experiments, chickens have been protected from clinical signs and death by HI titers of 16 ([208;592]; [25] cited in [471]) when challenged with HPAI viruses. This titer also decreased the transmission of one virus [365]. Titers of 32 prevented most virus shedding in chickens challenged with H5N2 viruses [134] and protected chickens from Asian lineage H5N1 HPAI viruses [142]. Other studies have reported that antibody titers as low as 8-10 could protect chickens from death, when they

were challenged with an Asian lineage H5N1 HPAI virus or a North American (Mexican) H5N2 HPAI virus [137;456;570]. In the U.S., licensed inactivated avian influenza vaccines must induce HI titers of 32 or greater, in at least 80% of vaccinated birds [593]. Such standards ensure potency that is expected to protect birds in the field against a closely related virus.

7.2.3.2 HI Titers and Virus Shedding

Some laboratories have observed that, in chickens, vaccines inducing higher HI titers are more likely to be protective against virus shedding, as well as clinical signs [142;456;467]. In one H5N1 HPAI challenge study, an experimental, inactivated H5N3 vaccine prevented death but not virus shedding in chickens with titers between 10 and 40, and decreased virus shedding in birds with titers greater than 40 [456]. Another group found that a titer of 128 was correlated with little or no shedding of an H5N1 HPAI virus from the cloaca or respiratory tract [142].

7.2.3.3 HI Titers and Protection from Heterologous Challenge

HI titers are usually determined against the homologous virus, but would be lower when tested against a heterologous virus [73;337;480;522;565;566;568;588;590]. While the presence of HI titers against the heterologous challenge virus has predicted protection in some studies, some birds with low or no titers were also protected [467;566]. In one study, three vaccines were poorly protective against clinical signs and virus shedding when titers to a heterologous challenge virus were low (≤ 10); however, they provided good protection against another virus despite similar heterologous titers (i.e., 11, 15 and 49) [566].

7.2.3.4 HI Titers and Vectored Vaccines or Species other than Chickens

Titers expected to be protective have mainly been investigated in chickens immunized with inactivated vaccines. They may not be similar in other species, including waterfowl, which are sometimes protected even when titers are low or undetectable [354]. Likewise, the relationship between protection and titers induced by vectored HA vaccines is still unclear. Although these vaccines may result in significant HI titers, birds without detectable titers can also be protected from challenge, and the titers may be overall lower than with inactivated vaccines [344;466;469;481;494;497;497;502;515;520;522;524;525].

7.2.3.5 Other Methods of Potency Testing

Conventional potency testing (PD₅₀ determination), as conducted for Newcastle disease vaccines, can also be used to evaluate the potency of avian influenza vaccines [94]. In conventional potency testing, birds are immunized with dilutions of the vaccine, followed by challenge with virulent virus ([94;464]; [594] cited in [136]). The PD₅₀ is calculated from the results based on mortality, morbidity, virus shedding or serological responses. A minimum PD₅₀ of 50 has been suggested by some authors for avian influenza vaccines [94]. Quantification of the antigen content could also be used to evaluate the potency of each batch [94;464]. In general, a PD₅₀ of 50 is reported to be equivalent to 0.3–7.8 µg of hemagglutinin protein per dose; however, this varies with the immunogenicity of the vaccine strains [458].

7.3 Vaccine Safety

Safety assessments for vaccines vary with the type of vaccine (inactivated or live, bacterial or viral), the adjuvants used, and the history of similar products in use, as well as the dose, vaccine claims, usage regimen and animal factors such as the species [595]. The ‘worst case’ scenario is usually assessed even if it is unlikely, assuming that the product will be used at its maximum potency and quantity, in animals of the highest sensitivity. Safety concerns include both manufacturing errors and user errors that could cause problems. For example, viruses in an incompletely inactivated vaccine could harm the animal or spread to other animals [595]. Contamination of vaccines by extraneous pathogens could also cause morbidity or

mortality [595]. This hazard is controlled by quality assurance steps during vaccine production. The possibility of interactions with other vaccines should also be considered [595].

7.3.1 Inactivated Vaccines and Subunit Vaccines

Completely inactivated vaccines and subunit vaccines are generally considered to be low-risk for animal safety; however, adjuvants and other vaccine ingredients may cause local or systemic reactions in some birds [595]. Granulomas, abscesses, inflammation and necrosis or fibrosis may occur at the injection site. Fever, lethargy, anorexia, arthritis, soreness and allergic reactions are possible. Quail have been reported to have more severe reactions to oil adjuvants than some other poultry [553].

In field tests using inactivated avian influenza vaccines in Germany, the main risks to chickens, ducks and geese were associated with vaccine administration rather than being side effects of the vaccine [327;468]. In a commercial, free range, layer chicken flock, 16.25% of the birds that received three doses of an inactivated vaccine, and 14.75% of the birds given four doses were lost [468]. For comparison, 8.25% of the nonvaccinated chickens were lost over the study period. Deaths in vaccinated birds were attributed to injuries during capture, uncontrollable bleeding after blood sampling, and oviduct-peritonitis. Similarly, an inactivated vaccine did not cause adverse effects in commercial, free range ducks and geese; however, losses occurred from injuries during capture (vaccination and sampling), uncontrolled bleeding from sampling sites, or oviduct-peritonitis in laying geese, which was probably induced or aggravated by capture [327].

7.3.2 Vectored Vaccines

Live genetically modified organisms or vectored vaccines usually have higher-risk profiles than inactivated vaccines [595]. Additional safety considerations unique to such vaccines, such as the potential for generating replication-competent viruses, reversion to virulence, and shedding from vaccinated birds, must be demonstrated before licensing. Some risks associated with inactivated vaccines, such as adjuvant-associated reactions, may not be relevant to vectored vaccines. Other types of side effects are still possible.

7.3.3 Vaccine Safety in Zoo Birds

(See also section 19.4.4 - Adverse Effects Associated with Vaccination - for details)

Avian influenza vaccines are not labeled for use in zoo birds, and manufacturers have not assessed safety in these species. However, adverse effects have been evaluated in several published reports of prophylactic vaccination campaigns in zoos. During H5 vaccination campaigns in the E.U., zoos reported a very low rate of adverse effects, with local reactions in 0.04% of birds and systemic side effects in 0.015% [477]. Most reactions occurred in ostriches and nandus that were given large doses and developed injection site reactions. The greatest risk of mortality to zoo birds is reported to be from the stress and trauma of restraint ([596] cited in [476]; [475;477]). On average, deaths from handling or stress occurred in 0.5% of the birds that were restrained two or three times (vaccinations plus blood collection) [477]. Skilled handling is important in reducing these losses [477].

7.3.4 Risks to Humans

Risks to people who administer or contact avian influenza vaccines should also be considered. For inactivated vaccines, local reactions from oil adjuvants or other ingredients should be addressed in label warnings [94;595]. The TROVAC™ H5 vaccine, which contains a live vector, has been evaluated for possible risks in mammals. The wild type fowlpox virus is not known to replicate in mammalian cells [331], and cats and mice injected with this vaccine did not develop any adverse reactions [469]. In addition, fowlpox virus could not be isolated from the injection site or internal organs of mice [469].

8. VACCINE WITHDRAWAL TIMES

The withdrawal period for inactivated vaccines is 42 days before slaughter. In a field trial in Germany, remnants of oil were found in the breast muscles of fattening ducks for as long as 50 days after intramuscular vaccination, and the authors concluded that subcutaneous vaccination would be desirable in these birds [327]. The withdrawal period for the HVT-vectored [519] and fowlpox-vectored H5 vaccines licensed in the U.S. is 21 days.

9. EFFECTS OF VACCINATION ON VIRUS SHEDDING AND TRANSMISSION

Summary

The ideal vaccine and vaccination protocol would completely prevent infection. However, this goal is very difficult or impossible to achieve, particularly in the field. More realistic aims are to increase resistance to infection, and reduce virus replication and excretion if infection occurs. Most studies have been conducted in chickens and turkeys, but some studies have assessed the effect of vaccination in ducks, and a few challenge studies have been conducted in other birds.

Within-flock transmission can be quantified with the reproduction ratio/ reproduction number (R), an estimate of the average number of secondary cases from each infected bird in a completely susceptible flock. If vaccination lowers R to less than 1, the epidemic is expected to eventually die out, with only minor outbreaks until that time. Some transmission is still expected to occur until the epidemic ends. If R remains higher than 1, there can be major outbreaks and the epidemic may continue to grow. The level of flock immunity needed to stop transmission varies with its composition and size, and the concentration of susceptible birds in the affected area. Some individual birds do not develop good immunity after vaccination, even under controlled conditions. If R (measured in nonvaccinated birds) is high enough, the presence of nonresponders to a vaccine may result in virus transmission in the vaccinated flock.

Vaccination with inactivated conventional vaccines (including some vaccines made by reverse genetics), fowlpox-vectored vaccines, HVT-vectored vaccines and NDV-vectored vaccines has been shown to decrease virus shedding in chickens. The amount of suppression varies, and in some laboratory experiments, AIV was undetectable in the oropharyngeal fluids and/or feces of vaccinated birds after challenge. Individual chickens may still shed large amounts of virus even when the mean virus titer is greatly decreased in the group. Vaccination can also decrease the number of chickens infected. Several studies have reported that vaccination decreases AIV transmission between experimentally infected chickens in the field. In some experimentally infected birds, vaccination may reduce R to < 1 if an efficacious vaccine is used and there is adequate time to develop immunity before challenge.

Two studies reported that vaccination could decrease transmission and/ or virus shedding in turkeys challenged with HPAI viruses, and one group found that vaccination prevented viremia (and virus localization in muscles), Additional studies reporting decreased shedding after challenge with LPAI viruses. The minimum infective dose was demonstrated to be higher in vaccinated than nonvaccinated turkeys, using an HPAI (H7) challenge virus.

Inactivated vaccines can reduce HPAI virus shedding in Muscovy ducks, Pekin ducks, sheldrake ducks, Khaki Campbell ducks, ringed teals and geese. Vaccination decreased virus shedding in ducks that ordinarily develop few or no clinical signs, as well as in birds challenged with strains that cause more severe illness. Vaccination decreased the transmission of HPAI viruses in

experimentally infected ducks; in some cases, it reduced R to < 1 . Two studies reported that NDV-vectored vaccines also reduced virus shedding in ducks.

Very few challenge studies have been conducted in species other than chickens, turkeys or waterfowl. A single dose of an inactivated vaccine was unable to suppress HPAIV transmission in golden pheasants, although it prevented severe clinical signs and mortality. The same vaccine prevented transmission among teals in this experiment. Vaccination was also reported to decrease virus shedding in domesticated rock pigeons, falcons and Chinese painted quail.

The ideal vaccine and vaccination protocol would completely prevent infection. However, this goal is very difficult or impossible to achieve [564]. More realistic aims are to increase resistance to infection, and reduce virus replication and excretion if infection occurs [49;496;499]. While a number of studies have reported that vaccination can reduce virus excretion in the laboratory, it may be difficult to assess its effect on virus spread unless shedding is eliminated. Some authors have noted that vaccination may concurrently prolong virus shedding by allowing infected birds to live longer, and this could contribute to increased transmission opportunities [312;537]. Furthermore, it can be difficult to substantiate claims that a vaccine completely suppresses virus shedding unless the amount of virus is measured on more than one or two occasions. It is possible for vaccination to delay virus shedding, as demonstrated by delayed infections in some birds exposed to vaccinated chickens [140]. Transmission studies can help resolve such uncertainties.

Within-flock transmission can be quantified with the reproduction ratio/ reproduction number (R), an estimate of the average number of secondary cases from each infected bird in a completely susceptible flock. R and the transmission rate (β) are usually estimated from studies in small numbers of experimentally infected birds. No one overall R exists for a disease; this value is specific to a population [597], and transmission parameters can vary with the species [200;365;367]. If vaccination lowers R to less than 1, the epidemic is expected to eventually die out, with only minor outbreaks. Some transmission is still expected until the epidemic ends. If R remains higher than 1, there can be major outbreaks and the epidemic may continue to grow. Reproduction ratios can be estimated within the flock (R_0) and between flocks (R_h). Because movement controls and quarantines decrease transmission between farms, R_h would generally be expected to be less than R_0 .

The level of flock immunity needed to stop transmission is affected by the flock's composition and size, and by the concentration of susceptible birds in the affected area [35]. Some individual birds do not develop good immunity after vaccination, even under controlled conditions. If R (measured in nonvaccinated birds) is high enough, the presence of nonresponders to a vaccine may result in virus transmission in the vaccinated flock [324]. Theoretically, the critical fraction of the population that must be fully protected for solid herd immunity is $1 - 1/R$, where R is calculated in nonvaccinated birds [324]. Flock immunity of 60–80% has been estimated to prevent virus transmission between well-vaccinated flocks of gallinaceous birds [49;139]. Another estimate suggests that optimal protection in poultry requires immunity in more than 80% of at-risk birds [8]. Less is known about vaccination of waterfowl. One study estimated R in nonvaccinated Pekin ducks to be 20, suggesting that at least 95% of this particular study population would need to be solidly protected by vaccination [324]. This level of protection might be difficult to reach, especially in the field [324].

Vaccines may be able to suppress transmission in one avian species but not another [200;365]. Most research has been conducted in chickens, although an increasing number of studies have examined turkeys and domesticated ducks and geese. Only a few challenge studies have been done in birds other than domesticated poultry ([200;323;598]; [599] cited in [342]).

9.1 Transmission Studies and Virus Shedding in Chickens

Vaccination with fowlpox-vectored vaccines [116;136;366;466;469;500;502;528;538;600], inactivated conventional vaccines (including some vaccines made by reverse genetics) [61;73;134-138;140-142;147;208;456;481;502;564;565;570;572-575;580;592;601;602], NDV-vectored vaccines [119;481;512;515] and HVT-vectored vaccines [497;522;524;525;603] have been shown to decrease virus shedding in chickens. The amount of suppression varies, and in some experiments, AIV could not be detected in oropharyngeal fluids and/or feces after vaccination. Individual chickens may still shed large amounts of virus even when the mean virus titer is greatly decreased in the group [564]. Vaccination with some inactivated vaccines [142] and fowlpox-vectored vaccines [366;528] has also been shown to decrease the number of chickens infected. In a study that used a fowlpox vectored H5 vaccine and challenged the birds with an Asian lineage H5N1 HPAI virus, 100,000 times more virus was needed to lethally infect vaccinated than nonvaccinated chickens [366].

Vaccination has been reported to suppress virus shedding to a greater extent when the HA of the vaccine and field strains are more closely related [73;134;136;141;147;211;324;368;502;511;512;538], but even vaccine strains from a different lineage (i.e., American or Eurasian), and vaccines isolated 20 years or more previously, can reduce the shedding of some viruses [136-142;502;528;538;600] (see also section 7.1, Vaccine Matching). In chickens, the effects of vaccination on the infection rate and virus shedding are reported to be dose-dependent [570;574]. More potent H5N1 vaccines also had a greater effect on virus shedding than vaccines with lower potency [564;592].

9.1.1 Transmission Studies in Chickens

Several studies have reported that vaccination decreases AIV transmission in experimentally infected chickens [138-140;365;528;580;600] and in the field [25]. Two groups that vaccinated chickens with a fowlpox vectored H5 vaccine (TROVAC™), and challenged the birds with a Mexican lineage HPAI virus (A/chicken/Queretaro/14588-19/95; H5N2), reported that this vaccine decreased or eliminated transmission to birds in contact [528;600]. Neither vaccinated contacts [528] nor nonvaccinated contacts [528;600] became ill or shed virus if they were exposed to vaccinated, challenged birds. Vaccinated contacts exposed to nonvaccinated, challenged birds did not become ill, but some birds shed virus [528].

In another study, two different inactivated vaccines protected chickens from clinical signs when they were challenged with an Asian lineage H5N1 virus; however, the low amounts of virus shed were able to infect nonvaccinated chickens in contact [140]. Vaccinated contacts did not develop clinical signs, and shed little or no virus. Recently, a group reported that a single dose of a commercial inactivated H5N1 vaccine prevented clinical signs in 5 vaccinated chickens inoculated directly with the virus, as well as in 10 vaccinated chickens and 4 of 5 nonvaccinated chickens housed with these birds [568]. Viral RNA was only detected in the muscles of the nonvaccinated chicken that died. Virus shedding from the trachea or cloaca was not tested. Under identical conditions, an older vaccine containing H5N2 A/Chicken/Mexico/232/94/CPA did not prevent the challenge virus from causing high mortality in all vaccinated and nonvaccinated birds.

Vaccination can decrease R_0 to less than 1 in experimentally infected birds, if an efficacious vaccine is used and there is adequate time to develop immunity before challenge [138;139;365;522]. In chickens challenged with an H7N7 HPAI virus (A/chicken/Netherlands/03), two different inactivated vaccines (A/chicken/Italy/99; H7N1 and A/chicken/Pakistan/95; H7N3) completely prevented transmission to vaccinated contacts [365]. When the birds were challenged 1 week after vaccination instead of at 2 weeks, the H7N1 vaccine was still effective, with R estimated to be 0.2 and significantly less than 1. The H7N3 vaccine, which had a lower antigen content as well as less homology with the challenge virus (92% vs. 98%) only decreased R to 1.4 (0.4–2.9) to 1.7 (0.4–4.3). Similar results were reported when

nonvaccinated contact birds were used. In another study, vaccination with an inactivated H5N2 vaccine (A/turkey/England/N28/73) completely prevented the transmission of an H5N1 virus in an Indonesian breed of native chickens [138]. R was estimated to be 12 (4.7–28.7) in nonvaccinated chickens, but there was no evidence of transmission in the vaccinated population. Vaccinated birds were also protected from infection if they were exposed to nonvaccinated, infected birds. Similarly, Bouma et al. (2009) reported a mean value of 1.6 (95% CI: 0.90–2.5) for R in nonvaccinated chickens infected with an Asian lineage H5N1 virus, and an R of 0 in chickens vaccinated with A/turkey/England/N28/73 (H5N2), A/chicken/Mexico/232/94/CPA (H5N2) or a homologous inactivated vaccine [139].

Another study examined the effect of a commercial HVT-vectored H5 vaccine, which contains the HA from an Asian lineage H5N1 virus [522]. This group found that a single dose of vaccine reduced R to less than 1 (R = 0.2) in one trial, when it was administered at one day of age and the birds were challenged 3 weeks later. In other trials from this experiment, R was lower in vaccinated than nonvaccinated birds, but nevertheless remained greater than 1. In these trials, the birds were either 1) vaccinated with the HVT-vectored vaccine and boosted with an inactivated vaccine that was poorly matched to the challenge virus (R = 1.5), or 2) vaccinated with the HVT-vectored H5 vaccine and challenged with a different H5N1 HPAI virus (R = 1.9).

Vaccination was also tested in a commercial free range flock under field conditions in Germany, with periodic challenge of vaccinated birds in the laboratory, using an H5N1 HPAI virus [468]. In this study, an inactivated heterologous vaccine significantly decreased virus shedding, although transmission to nonvaccinated or vaccinated contact birds was not entirely interrupted. Nonvaccinated contact birds developed severe clinical signs, but clinical signs were not reported in vaccinated contacts. Infectious virus was not isolated from the vaccinated contacts, although a small percentage either seroconverted or excreted viral RNA.

One field study suggests that vaccination can interrupt HPAI virus transmission on infected farms. Vaccination combined with partial depopulation was used to control an Asian lineage H5N1 virus on three farms, during an outbreak in Hong Kong [25]. On at least two of the farms, the virus spread to recently vaccinated sheds and caused low mortality between 9 and 18 days after vaccination. After 18 days, however, deaths from H5N1 avian influenza were no longer seen, and intensive monitoring of asymptomatic chickens found no evidence that the virus was being shed. The interruption of virus transmission was accompanied by the development of HI titers ≥ 16 . There was no evidence that the H5N1 virus spread to the vaccinated sheds on the third partially depopulated farm, where rapid depopulation and strict biosecurity may have minimized virus exposure.

9.2 Transmission Studies and Virus Shedding in Turkeys

Few published studies have examined transmission or virus shedding in vaccinated turkeys challenged with HPAI viruses. In one experiment, one or two doses of a commercial inactivated H7N1 vaccine reduced the excretion of an H7N7 HPAI virus to very low levels [367]. No transmission to vaccinated contacts was observed, although R could not be calculated because the inoculated, vaccinated birds did not shed virus. In nonvaccinated turkeys, R₀ was 7.8. Another study reported that an inactivated H5N1 vaccine decreased the shedding of an Asian lineage H5N1 HPAI virus in commercial turkeys, although the birds were only partially protected from clinical signs [368]. A vaccine containing A/duck/Potsdam/1402-6/1986 (H5N2) was ineffective in protecting birds from death or decreasing virus shedding in this study. These turkeys were vaccinated at a very young age (1 week), which may have contributed to the weak protection from both vaccines [368]. Another study, which did not examine virus

shedding, reported that vaccination with A/chicken/Italy/1067/1999 (H7N1) prevented viremia and virus localization in the muscles when turkeys were challenged with a related H7N1 HPAI virus, A/turkey/Italy/4580/1999 [105].

Most studies in turkeys have examined the effect of vaccination on LPAI viruses. Several experiments demonstrated that virus shedding was decreased ([352;485;604]; [605] cited in [367]). In one case, the minimum infective dose was also higher in vaccinated than nonvaccinated birds [485].

9.3 Transmission Studies and Virus Shedding in Waterfowl

Inactivated vaccines have decreased virus shedding in Muscovy ducks [331], Pekin ducks [111;135;209-211;324;327;336], sheldrake ducks [208], Khaki Campbell ducks [209], ringed teals [200] and geese [208;327]. NDV-vectored vaccines were reported to reduce virus shedding in mule ducks [341], and Muscovy ducks [344]. Although the NDV vaccine has some effect when it was used alone in the latter study, the reduction in shedding was greater when the ducks received a commercial fowlpox-vectored vaccine at one day of age, and the NDV-vectored vaccine 2 weeks later [344]. Vaccination has decreased virus shedding in ducks that ordinarily develop few or no clinical signs [208;209], as well as in birds that were challenged with strains that cause more severe illness in nonvaccinated birds.

Caution should be used when extrapolating results between waterfowl species, using a specific vaccine and challenge strain: different species may vary in their susceptibility to AIV strains and H5N1 clades, and the effects of a vaccine on virus shedding may be greater in one species than another [332;342]; ([333] cited in [333]).

9.3.1 Transmission Studies in Ducks and Geese

Vaccination is reported to decrease transmission in experimentally infected waterfowl [200;209;210;324]. Khaki Campbell ducks vaccinated once or twice with an inactivated H5N3 vaccine (H5 from A/chicken/Vietnam/C58/04) did not shed an H5N1 virus or transmit it to nonvaccinated contacts [209]. In Pekin ducks, two doses of an inactivated vaccine containing A/duck/Potsdam/1402/86 (H5N2) suppressed H5N1 virus shedding, prevented viremia, and decreased transmission to nonvaccinated or vaccinated contacts [210]. Contact birds were added 3 days after inoculation in both of these experiments [209;210], which might underestimate transmission [324]. Another study found that R was < 1 if Pekin ducks were challenged with an H5N1 virus 2 weeks after vaccination, but not when they were challenged one week after a single dose of vaccine [324]. In the latter birds, R was estimated to be > 1.5 with 95% confidence, although vaccination was partially protective against clinical signs and protected the birds from mortality [324;370]. In contrast, R was 0.6 (95% CI: 0.1–2.2) in ducks challenged 2 weeks after one dose of vaccine, and 0.2 (95% CI: 0.005–1.5) in ducks challenged 2 weeks after two doses [324]. The vaccine used in this study was A/chicken/Mexico/232/94/CPA (H5N2) and the contact birds were added one day after challenge. Vaccination also significantly decreased transmission in ringed teals vaccinated with an inactivated H7N1 vaccine (A/chicken/Italy/99) and challenged with an H7N7 HPAI virus (A/chicken/Netherlands/621557/03) [200]. R was estimated to be > 1.5 with 95% confidence in nonvaccinated teals, and less than 0.7 in vaccinated teals. The latter value was significantly less than 1.

One commercial free-range flock of geese was vaccinated (A/duck/Potsdam/1402/86; H5N2) under field conditions, and challenged periodically with an Asian lineage H5N1 HPAI virus in the laboratory [327]. Vaccination significantly decreased virus shedding, but transmission to nonvaccinated or vaccinated contact birds was not entirely interrupted. A similar study in Pekin ducks yielded equivocal results, as no clinical signs and very little shedding of live virus was reported in either vaccinated or nonvaccinated birds, and the birds became infected with an H10 field virus during the study [327].

9.4 Transmission Studies and Virus Shedding in Other Species

Very few challenge studies have been done in species other than chickens, turkeys or waterfowl. A single dose of an inactivated vaccine was unable to suppress HPAIV transmission in golden pheasants (*Chrysolophus pictus*), although it had 98% homology to the challenge virus and prevented severe clinical signs and mortality [200]. R was estimated to be > 1.5 with 95% confidence in both nonvaccinated and vaccinated birds. The same inactivated H7N1 vaccine (A/chicken/Italy/99) prevented transmission among teals in the same experiment. In domesticated rock pigeons (*Columba livia*), a reduced dose of a commercial H5 vaccine (A/duck/Potsdam/2243/84 H5N6) was clinically protective against clade 2.1.1 Indonesian H5N1 and clade 2.2 Turkish H5N1 viruses, and virus shedding was not detected ([599] cited in [342]). A single dose of a reverse genetics-derived, inactivated H5N1 (clade 1) vaccine protected Chinese painted quail (*Coturnix chinensis*) from clinical signs and reduced virus shedding when they were challenged with heterologous A/Swan/Nagybaracska/01/06 (H5N1 clade 2.2) [323]. Vaccination also decreased virus shedding in falcons challenged with an H5N1 HPAI virus [598]. (See section 19, Vaccination in Zoos and Special Collections, for details).

10. ONSET OF PROTECTIVE IMMUNITY

Summary

Although most studies have challenged birds 3 weeks after vaccination, two experiments suggest that fowlpox-vectored H5 vaccines provide some protection to chickens within the first week. Chickens immunized with some inactivated H5 or H7 vaccines may also be partially or fully protected in approximately 1-2 weeks. In a field study from Hong Kong, vaccination interrupted virus transmission on infected chicken farms after 18 days. More potent and homologous vaccines might be able to protect birds sooner than weaker and/or less well-matched vaccines.

Little is known about the onset of immunity in ducks and geese. In Pekin ducks, R was reduced to < 1 if they were challenged 2 weeks after vaccination, but not after 1 week. In geese, one vaccine was protective after 3 weeks but not at 2 weeks.

Although most studies have challenged birds 3 weeks after vaccination, a few have examined the development of immunity to AIV before this time. These studies used a variety of vaccines and challenge viruses. Most were conducted in chickens. No published studies have examined the onset of protection from HVT-vectored vaccines before 3 weeks.

10.1 Fowlpox Vectored Vaccines

One commercial fowlpox-vectored H5 vaccine, TROVAC™ H5, may provide some protection as early as 1 week after vaccination ([606] cited in [469]). In one published study, this vaccine reduced morbidity from 100% to 25%, and mortality from 86% to 0%, in chickens challenged with a Mexican HPAI H5N2 virus 1 week later ([606] cited in [469]). When these birds were challenged 7 weeks after vaccination, all were fully protected from clinical signs. A Chinese fowlpox-vectored vaccine containing H5 and N1 from A/goose/Guangdong/1/96 is also reported to protect chickens within the first week [498]. In chickens immunized with one dose of this vaccine, HI titers were detectable 1 week after vaccination and peaked at approximately 2 weeks. All vaccinated chickens were protected from clinical signs or mortality, as well as from virus-shedding, when they were challenged with a lethal dose of the homologous HPAI virus at 3 days, as well as at 1 to 40 weeks after vaccination.

10.2 Inactivated Vaccines in Chickens

Chickens immunized with some inactivated H5 or H7 vaccines may be partially or fully protected in approximately 1-2 weeks [365;459;573;592;601;607]. In one study, chickens vaccinated with a reassortant H5N1 virus and challenged with an H5N1 HPAI virus 2 to 6 days later did not develop HI antibody titers, and all infections were fatal [601]. All chickens challenged 8, 10 or 14 days after vaccination were completely protected from clinical signs, although some birds challenged at 8 days did not have detectable HI titers. In another study, the same vaccine induced HI antibody titers of at least 64 within 2 weeks, and titers ≥ 16 prevented clinical signs and reduced or eliminated virus shedding [592]. One study reported partial clinical protection of chickens vaccinated with A/turkey/Wisconsin/68 (H5N9), and challenged with an H5N2 HPAI virus (A/chicken/Pennsylvania/83) 1-2 weeks later [607]. None of the vaccinated birds were, however, protected from infection. The same vaccine prevented clinical signs in chickens challenged at 4 weeks, and fewer birds were infected. A reassortant H7N7 vaccine was fully protective against clinical signs in chickens challenged with an HPAI virus (A/turkey/Italy/99; H7N1) after 10 days, while partial clinical protection was reported on day 8 [573]. Another vaccine, which contains A/chicken/Italy/99 (H7N1), significantly decreased the transmission of the HPAI virus A/chicken/Netherlands/03 (H7N7) when chickens were challenged either one or 2 weeks later; however, a less potent vaccine with lower homology to the challenge virus was effective only after 2 weeks [365]. Both vaccines protected the birds from clinical signs, at either one or two weeks. In a field study from Hong Kong, vaccination interrupted virus transmission on infected chicken farms after 18 days [25].

10.3 Inactivated Vaccines in Waterfowl

Little is known about the onset of vaccine-induced immunity in ducks and geese. In Pekin ducks immunized with A/chicken/Mexico/232/94/CPA (H5N2), R was less than 1 if the birds were challenged with an Asian lineage H5N1 HPAI virus 2 weeks later, but not when they were challenged 1 week after a single dose [324;370]. Vaccination decreased clinical signs and protected the birds from mortality even when transmission was not significantly reduced [324;370]. In another experiment, 2 doses of the Re-1 (clade 0 Asian lineage H5N1) vaccine, given at 7 and 14 days of age, protected Pekin ducks from clinical signs and reduced virus shedding when they were challenged with clade 1.1 or clade 2.3.4.1 H5N1 HPAI viruses 16 days later [537].

In geese, a single dose of an inactivated H5N1 vaccine was completely protective against clinical signs and virus shedding, when the birds were challenged 3 weeks later with the homologous virus; however, only 2 of 5 geese survived when they were challenged at 2 weeks [208].

11. DURATION OF IMMUNITY

Summary

There is relatively little published information on the duration of immunity (DOI) for avian influenza vaccines. Under laboratory conditions, the fowlpox-vectored TROVAC™ H5 vaccine was reported to protect chickens against one heterologous challenge virus for up to 5 months. The fowlpox-vectored H5N1 vaccine made in China is reported to be protective against homologous challenge for up to 9 months. The DOI for some inactivated vaccines might be as long as 6-10 months in chickens, based on virus shedding, and up to a year based on clinical signs. Protection against some heterologous viruses may wane sooner. A single dose of one inactivated vaccine protected chickens against both homologous and heterologous challenge after one month, but only from the homologous virus after 3 months. Some inactivated vaccines may also protect ducks for up to 5-9 months in the laboratory. Antibody titers do not seem to last as long in geese;

however, the relationship between the titer and protection is poorly understood in waterfowl. A single dose of an experimental, NDV-vectored H5 vaccine did not provide long-lasting protection against a heterologous virus in day-old Muscovy ducks, with incomplete clinical protection by 12 weeks after vaccination. However, Muscovy ducks are relatively susceptible to AIV, and these vaccines might last longer in resistant species, such as Pekin ducks.

The duration of immunity under field conditions is uncertain, and could be shorter than in the laboratory. In one field test, chickens required at least one booster to be fully protected from clinical signs, and protection began to wane 9 months later. Revaccination at 6-month intervals maintained the effectiveness of the vaccine in reducing virus excretion. Two additional field studies confirmed that chickens require at least 2 doses for protection from challenge and/or the development of adequate HI titers. One group reported that approximately 50-60% of the vaccinated birds had detectable titers, 7-8 months after the booster.

Antibody titers diminished sooner in geese than chickens in one field test, but Pekin ducks maintained titers of ≥ 32 for at least 15 weeks after the initial two doses of vaccine. Ducks also responded well to 2 doses of vaccine in another study. Most of these birds still had detectable virus titers after 10 months, although the mean titer had dropped from 365 to 60. In addition, 30-40% of the ducks maintained low but detectable titers to a heterologous virus during this time.

There is limited information about the DOI for HVT-vectored vaccines. However, these vaccines can establish persistent infections in some species of birds, which may provide long-term stimulation of immunity.

There is relatively little published information on the duration of immunity (DOI) for avian influenza vaccines. Repeated boosters may be necessary in poultry with long production cycles (e.g. layers, breeders and turkeys) [46;48;327;468].

11.1 Fowlpox Vectored Vaccines

Two published studies assessed the DOI for fowlpox-vectored vaccines under laboratory conditions. The commercial vaccine TROVACT[™] H5 provided clinical protection and significantly decreased virus shedding in both feces and respiratory secretions, for up to 20 weeks (approximately 5 months) after challenge with a Mexican lineage HPAI virus [528]. Virus shedding appeared to be suppressed longer in feces than respiratory secretions (20 weeks vs. 12 weeks), although there were too few birds to determine whether the difference was statistically significant. A Chinese fowlpox-vectored vaccine, which contains Asian lineage H5 and N1, was clinically protective and decreased virus shedding, when chickens were challenged with a homologous HPAI virus up to 40 weeks (approximately 9 months) later [498]. The duration of immunity may be different for other challenge viruses.

11.2 NDV Vectored Vaccines

A single dose of an experimental, NDV-vectored H5 vaccine did not provide long-lasting protection in day-old Muscovy ducks [344]. These ducks were fully protected from clinical signs when challenged with a heterologous H5N1 HPAI virus after 6 weeks, but one of 8 ducks developed mild neurological signs (no mortality) when challenge occurred after 9 weeks, and one of 9 ducks died when they were challenged after 12 weeks. Muscovy ducks initially immunized with a commercial fowlpox-vectored H5 vaccine (European H5N8 strain) on day 1 or 2, and boosted 2 weeks later with the NDV-vectored HA vaccine, were still protected from clinical signs and shed less virus than the controls when they were challenged

after 10 weeks. Reductions in virus shedding were greatest when Muscovy ducks were challenged 4-6 weeks after vaccination, although significant decreases were also reported at later times. These results might not be applicable to other species of ducks. Muscovy ducks are reported to be more susceptible to HPAI viruses and to respond less well to vaccination, compared to more resistant species such as Pekin ducks [331;332;334;335;337].

11.3 HVT Vectored Vaccines

HVT-vectored vaccines can establish persistent infections in some species of birds, including some waterfowl, potentially resulting in long-term stimulation of immunity [526]. There is little information about the DOI for these birds. In one study, chickens challenged after 6 weeks with a homologous H5N1 virus were completely protected from clinical signs, and little virus was shed [524]. Protection was lower in birds challenged with one heterologous virus at 4 weeks. One study examined chicks vaccinated at one day of age on a commercial layer farm in Egypt, and found that 60-73% of the birds were clinically protected when challenged with an Asian lineage H5N1 HPAI virus in the laboratory at 19 weeks of age [520]. In this study, HI titers were first detected 6-8 weeks after vaccination, and increased between 6 and 19 weeks post-vaccination, which the authors attributed to the persistence of the HVT-vectored construct in these birds. However, biosecurity was reported to be “not applied rigorously and consistently” on this farm, and the possibility that other AIV might have boosted titers cannot be ruled out.

11.4 Inactivated Vaccines

Under laboratory conditions, the DOI for some inactivated vaccines is reported to be as long as 6-10 months in chickens [61;208] and 9 months in ducks [208], although protection against some heterologous viruses may not last as long [565]. An H5N2 vaccine (A/turkey/England/N-28/73) used against H5N1 HPAI viruses in China was reported to be effective for 6 months, based on unpublished results [208]. In chickens, another inactivated H5N1 vaccine (Re-1) used in China was clinically protective and decreased the shedding of a homologous HPAI virus for up to 43 weeks (10 months) after vaccination [208]. In addition to the increased homology with H5N1 viruses, this vaccine contains approximately 150% as much HA protein as the H5N2 vaccine [208]. The Re-1 vaccine also decreased virus shedding for up to 38 weeks in ducks given two doses [208]. Antibody titers declined more rapidly in geese, but geese given two boosters and challenged 17 weeks after the third dose (34 weeks after the first dose) were completely protected from clinical signs and virus shedding. A different inactivated H5N1 vaccine (A/duck/Hokkaido/Vac-1/2004) has been licensed in Japan [61]. In layer chickens challenged with an Asian lineage H5N1 virus, this vaccine was clinically protective for as long as 52 weeks (12 months) [61]. Virus isolation rates were 0-14% when the birds were challenged up to week 34, and 86% at week 52. No virus shedding or clinical signs were detected in chickens that were given a booster at week 56 and challenged up to 46 weeks later. The results were similar in layer chickens vaccinated once with an H7N7 vaccine (A/duck/Hokkaido/Vac-2/2004), and challenged with an H7N1 virus (A/turkey/Italy/4580/1999), although the virus isolation rates were higher at all time points, and reached 100% at 52 weeks. When chickens were vaccinated twice with this vaccine, no deaths were reported and no virus shedding was detected either 14 or 46 weeks later. In another study, however, protection against a heterologous virus was reported to be short-lived. In this report, an experimental, high antigen dose, H5N1 vaccine protected all chickens from clinical signs when they were challenged with the homologous virus after 3 months, and no virus shedding could be detected [565]. However, only 60% of the chickens survived a heterologous challenge at this time, although all were protected from clinical signs and virus shedding if they were challenged after 4 weeks.

HI titers, which are correlated with protection in chickens, suggest that the DOI may be 7-10 months or longer for some vaccines [61;208;592;601]. Chickens vaccinated with A/duck/Hokkaido/Vac-1/2004 (H5N1) maintained high titers for at least 7 months [601] to 23 months [592] after a single dose,

depending on the antigen content of the vaccine. Another study using this vaccine reported that the geometric mean HI titers had declined to 123 by 8 months (34 weeks), and dropped to 10 by a year (52 weeks) [61]. Serological responses induced by the inactivated Re-1 (H5N1) vaccine were maintained up to 10 months (43 weeks) after a single dose in chickens [208].

Antibody titers have also been used to estimate immunity to AIV in waterfowl, although their predictive power is still uncertain in these species [327]. In sheldrake ducks, serological responses induced by the inactivated Re-1 (H5N1) vaccine were maintained up to 9 months (38 weeks) after two doses [208]. Titers did not last as long in geese: they developed slowly after one dose, increased sharply after a high dose booster given at 4 weeks, and peaked 3 weeks later, before gradually declining to 16 over the next 10 weeks [208]. Titers rose again quickly after another high dose booster, and persisted 4 weeks longer after the second booster than the first. In Pekin ducks given two doses of an inactivated H5N2 vaccine (A/duck/Potsdam/1402/86), HI titers were still detectable 3.5 months after the second dose, but were below the threshold of detection by 5.5 months [210].

11.4.1 Field Studies of Inactivated Vaccines

The level and duration of immunity under field conditions is uncertain [496]. Field studies of vaccination have been conducted in commercial, free range geese, fattening ducks and layer chickens in Germany, which is free of HPAI viruses in poultry [327;468]. All species were vaccinated with an inactivated H5N2 (A/duck/Potsdam/1402/86) vaccine, but the vaccination schedule varied. For the purposes of the study, HI titers of at least 32 were defined as the protective level in all three species. Challenge studies were conducted in the laboratory. Layer hens required at least one booster to be fully protected from clinical signs in this study [468]. Protection began to wane 9 months (40 weeks) later, and was boosted by revaccination. Revaccination at 6 month intervals maintained the effectiveness of the vaccine in reducing virus excretion. With two doses, 92% of these chickens still had protective titers after 12 months, and 48% after 18 months. Revaccination after 6 months boosted the response rate to 100%, and this response persisted for at least 12 months. The authors concluded that layer chickens required at least two doses of this vaccine, followed by biannual revaccination for protection. Protection did not seem to last as long in geese, as measured by serological responses. Only 40% of the geese still had protective titers 6 months after the initial vaccinations [327]. Revaccination at 6-month intervals was necessary to maintain titers of at least 32 in at least 90% of these geese. However, vaccinated geese were protected from clinical signs, even if the HI titer was low. In Pekin ducks, titers of at least 32 were maintained for at least 15 weeks after the initial two doses of vaccine, at which time the experiment was ended [327].

A similar field study in Indonesia reported that a single dose of a commercial vaccine, administered on the farm at 4 weeks of age, did not induce protective titers in 66% of layer hens [479]. These birds shed virus, were not fully protected from clinical signs, and experienced 18% mortality after homologous challenge with an H5N1 HPAI virus in the laboratory.

Another field study, conducted at a vaccine production center in Lao PDR, examined antibody titers in commercial chickens and Khaki Campbell layer ducks that had free access to outdoor pens, without challenge [480]. These birds received either one or two doses (8 weeks apart) of a reverse genetics, inactivated H5N3 vaccine containing the HA from A/chicken/Vietnam/C58/04 and NA from A/duck/Germany/1215/73. Similarly to the other two studies, a single dose of this vaccine did not provide good protection in chickens. Only 20% of these birds had detectable HI titers after one dose, and the titers were low and disappeared by 3 months. Approximately 75% of the chickens seroconverted after 2 doses, with a mean titer of 66, which then declined to 35-55 by 7-8 months, at which time titers were detected in approximately 50-60% of the birds. By 10 months, 43% had titers, and the mean titer was 13. Neutralizing titers to the homologous virus were also detected for 10 months in this group of birds. However, vaccinated chickens rarely had detectable HI titers to a heterologous virus, even after a booster.

Most (71%) of the ducks in this experiment seroconverted after a single vaccine dose, with a mean titer of 61; however, this titer had dropped to 20, 3-5 months after vaccination, and only half of the birds still had detectable titers by 7-8 months (mean titers of 13-18). Titers to the heterologous virus could not be detected after 3 months. Ducks vaccinated twice had a mean titer of 352, and 88% still had titers at 10 months, with a mean titer of 60 at this time. The initial mean titer to the heterologous virus was 30, and approximately 30-40% of the ducks had low but detectable titers for at least 10 months. Neutralizing titers to the homologous virus could be detected for 10 months in ducks vaccinated either once or twice.

12. LIMITATIONS OF EXPERIMENTAL STUDIES

Protection is always expected to be lower in the field than in the laboratory [45;46;342]. The effectiveness of vaccination is influenced by factors such as proper vaccine storage and administration (e.g., the maintenance of a cold chain, good vaccination technique and administration of a full dose) [45;46;496;499]. Vaccine efficacy can also be decreased by concurrent infections or other diseases, and it may be influenced by species or breed differences, and by the birds' general health [45;46;499]. Maternally derived antibodies can suppress responses in young birds [45]. Boosters are likely to be needed for good efficacy in the field, even if a single dose of vaccine is adequate to stop AIV transmission in the laboratory [8;327;342;468;479;480].

13. MODELING STUDIES

Summary

Models have limitations, but they may provide insights into the possible impacts of vaccination approaches in specific scenarios. Two studies that evaluated the impact of control measures found that improved biosecurity, movement restrictions and the culling of infected flocks may not be sufficient to stop some epidemics. Additional measures, such as vaccination or preemptive culling, might be needed to halt transmission. Within a high poultry-density area where the virus is already circulating, epidemics might be impossible to stop after they have taken off, and the main contribution of control measures may be to prevent the virus from spreading outside the area.

One model estimated that, if infected flocks are detected by clinical signs, 90% of caged chickens would need to be protected by vaccination to decrease the probability of an outbreak by 50%. Including nonvaccinated sentinel birds was found to lower but not completely eliminate the risk of transmission. In this model, infected flocks were detected based solely on increased mortality or changes in production parameters such as decreased feed and water consumption. Serological or virological monitoring was not modeled. The authors concluded that to be successful, a vaccination program required a very effective vaccine, a highly effective delivery system for the vaccine, good biosecurity, and the rapid recognition and removal of flocks that become infected. A modeling study of H5N1 viruses suggested that, if the detection of infected flocks is based on mortality alone, the period available to decrease the infectious output of a flock would be less than 2 weeks, and reactive emergency vaccination would be unlikely to control an outbreak.

Models may provide insights into the possible impacts of vaccination approaches in specific scenarios. In outbreaks, control programs are traditionally based on experiences from previous epidemics and extrapolation from experiments [383;608]. Experiments in small groups of birds are valuable for examining the transmission characteristics of specific AIV isolates [139;367;370], or the effect of a single factor, such as vaccination, on virus transmission [383]. However, the results of these studies can be difficult to extrapolate to naturally infected populations, which are heterogeneous and influenced by

interactions between individuals [383]. Mathematical models attempt to bridge this gap. Modeling might offer insights, particularly when the conditions of the outbreak, or the combinations of control measures, are new [608]. Mathematical models range from simple to complex, and have important limitations and uncertainties. It should be kept in mind that outbreaks are unpredictable; models are a simplistic representation of the real world (and incorporate subjective decisions on how to represent aspects of disease epidemiology); the values used for important parameters may be estimated or unknown; complex human value judgments are difficult to simulate; and there are always uncertainties in the model's assumptions [383;608-611]. While models may still be useful in generating hypotheses if they are based on theoretical or incomplete information, rather than real data, their limitations in this case must also be disclosed, and the hypotheses will need to be investigated further [611]. The use of models as tools to predict the course of an ongoing outbreak is controversial. Some authors encourage this use, though some also note they should be employed in conjunction with input from other sources such as field studies, laboratory studies and past experience. Others suggest that models are best used with real data to analyze hypothetical scenarios and intervention scenarios for past epidemics, as an aid in understanding the effects of various control measures, rather than as predictive tools [612].

Models may be used to help develop surveillance programs, or to evaluate control measures [383]. Two studies that examined the effects of control measures found that improved biosecurity, movement restrictions and the culling of infected flocks might not be sufficient to stop some epidemics. In an analysis of the 2003 H7N7 HPAI epidemic in the Netherlands, R_h was estimated to be 3.1-6.5 before stamping out (not including vaccination), and 1.2 (95% CI: 0.6–1.9) afterward [613]. The estimated infectious period also decreased. Because R_h was still greater than 1, however, the authors concluded that the epidemic was probably contained because the infected areas were almost completely depopulated of poultry, rather than because the control measures sufficiently decreased transmission. This analysis suggests that additional measures, such as vaccination or preemptive culling of flocks in large areas, might be needed to halt transmission in some outbreaks. It also suggests that the main contribution of control measures may be to prevent transmission to new areas rather than to stop transmission within the infected area, especially when the density of flocks in the affected region is high. Within such high-density areas, epidemics might be impossible to stop after they have taken off [613]. Another analysis estimated R_h for HPAI outbreaks in the Netherlands (2003), Italy (1999-2000) and Canada (2004), and reported that control measures (not including vaccination) significantly decreased R_h from its pre-control value of 1.1 to 2.4 (with upper 95% bounds of 1.5–3.6), but this value remained close to 1 [614]. This study also suggested that movement restrictions, quarantines, increased biosecurity and culling of infected flocks might not be sufficient for eradication, depending on the situation, and that additional measures could be necessary [614].

One model estimated that 90% of caged birds would need to be protected by vaccination to decrease the probability of an outbreak by 50%, under conditions where the outbreak started in one cage contaminated with a small amount of virus [377]. In this model, infected flocks were detected based on clinical signs alone, e.g., increased mortality or changes in production parameters such as decreased feed and water consumption. The infectiousness of the flock peaked when the vaccine gave 80% protection: as more birds were protected, infections became fewer but were more difficult to detect. The risk of transmission was expected to be greatest at the end of the production cycle, when activities such as moving the birds and cleaning the facilities could decrease biosecurity. Including nonvaccinated sentinel birds was found to lower but not completely eliminate the risk of transmission. The authors concluded that to be successful, a vaccination program required a very effective vaccine, a highly effective delivery system for the vaccine, good biosecurity, and the rapid recognition and removal of flocks that become infected. Another model, which incorporated factors such as vaccine-induced reductions in susceptibility to infection, reductions in the infectiousness of asymptotically infected birds and the waning of immunity over time, also found that vaccination aids eradication only when vaccination coverage is sufficiently large or vaccine efficacy

is high, and that asymptomatic spread may occur under other conditions [615] Neither study evaluated the effect of monitoring flocks with virological or serological tests, in addition to clinical signs.

A modeling study of H5N1 viruses, which assumed that HPAI can be detected with high specificity if the mortality rate is at least 0.5% on two consecutive days, found that the outbreak would be detected 11-12 days after virus introduction if transmissibility is low, and 7-8 days after its introduction if transmissibility is high [139]. This model suggested that, depending on virus transmissibility, there would be a maximum of 5 to 10 days to decrease the infectious output of the flock. This implies that reactive vaccination is unlikely to control an outbreak, if detection of infected flocks is based solely on mortality. It does not preclude the possibility of controlling an outbreak with preventive vaccination, or of increasing the speed of detection by adding other clinical parameters or methods of detection [139].

14. FIELD EXPERIENCES WITH HPAI VACCINATION

Summary

Vaccination has been part of control or eradication programs for H5 or H7 LPAI or HPAI viruses in a number of countries throughout the world. Long-term vaccination programs were formerly rare; however, campaigns lasting for years have recently been conducted in Italy for H7 LPAI viruses, Mexico for H5N2 LPAI viruses, and some countries in Asia and the Middle East for H5N1 HPAI and H9N2 LPAI viruses.

Italian prophylactic and emergency vaccination campaigns, which included the use of inactivated vaccines, a heterologous neuraminidase DIVA strategy, sentinel birds, intensive monitoring, strict biosecurity and movement restrictions, successfully controlled incursions of H7 and H5 LPAI viruses in poultry. Active surveillance was the most effective technique for detecting infections, particularly in vaccinated flocks. An H7 virus spread extensively during one outbreak, despite vaccination, but other viruses affected only small numbers of flocks. One small outbreak was caused by a virus that had been maintained inapparently in a quail flock, and later infected turkey flocks that had been vaccinated less than the recommended number of times. Another virus affected birds that were close to slaughter and may have become infected after immunity waned. DIVA testing allowed the marketing of fresh meat from vaccinated, uninfected poultry during these vaccination campaigns. Other countries that have used vaccination against H7 viruses include Pakistan, the Democratic People's Republic of Korea (North Korea) and Mexico, all of which vaccinated poultry during outbreaks caused by HPAI viruses.

Long-term vaccination has been used to control HPAI and LPAI H5N2 viruses in Mexico. The HPAI viruses were successfully eradicated in a campaign that included both stamping out and vaccination. Homologous inactivated vaccines were used. LPAI viruses have continued to circulate in some flocks, despite vaccination, and antigenic drift has resulted in the emergence of vaccine-resistant variants. H5N2 viruses have also spread to other Latin American countries.

A number of countries have carried out short-term or long-term vaccination programs for Asian lineage H5N1 HPAI viruses. Several HPAI-free European countries used prophylactic vaccination in zoo birds, after H5N1 viruses were detected in wild birds and spread to poultry in some countries. The Netherlands also conducted a voluntary campaign for hobby birds and free-range layers, as an alternative to confinement during the wild bird migration season. A heterologous neuraminidase strategy was used for surveillance in hobby birds, and sentinel birds were used in vaccinated commercial flocks. Movement and marketing restrictions were placed on vaccinated birds and their products. Participation in this program was low, possibly due to the

high costs, the paperwork requirements for hobby flocks, and the trade restrictions on commercial free-range farms. The program also faced opposition from the rest of the poultry industry, which feared that vaccination might result in losing markets.

France conducted a short-term prophylactic vaccination campaign in some high-risk free-range ducks and geese that could not be maintained indoors. A heterologous (H5N2) inactivated vaccine was used to protect these birds from Asian lineage H5N1 viruses. Serological DIVA surveillance was not conducted, because many birds had been previously exposed to LPAI viruses, and because N1-based ELISAs had not been validated in ducks and geese. Nonvaccinated sentinel birds were maintained in all vaccinated flocks, and tested regularly. Clinical parameters were also monitored. HI titers in vaccinated ducks and geese were highly variable, and ducks vaccinated when they were 3 weeks old had poor immune responses.

Vaccination has also been part of the official control programs for Asian lineage H5N1 viruses in Russia, China (including Hong Kong), Indonesia, Vietnam, Pakistan, India, Egypt, Israel, Mongolia, Kazakhstan, Côte d'Ivoire and Sudan. Some countries have conducted prophylactic campaigns or emergency vaccination during outbreaks, while others carried out mass vaccination after viruses became endemic. Due to a variety of factors, some vaccination campaigns have been more successful than others. Hong Kong was able to eradicate H5N1 viruses, using vaccination as well as stamping out, and continues to employ prophylactic vaccination while the threat of virus introduction continues. Viruses were also eradicated in Israel (which vaccinated a single ostrich farm during an outbreak in poultry), Russia, Sudan and Côte d'Ivoire. Mongolia and Kazakhstan have vaccinated some types of poultry prophylactically, although outbreaks have not been reported in either country except in wild birds.

China has conducted a long-term, mass vaccination campaign since 2003, with mandatory vaccination of domesticated poultry since 2005. Vaccines used in China have been updated several times to reflect the currently circulating viruses, and new variants have continued to emerge. Most of the inactivated vaccines, as well as a fowlpox vectored vaccine and a NDV-vectored vaccine, contain both H5 and N1, and are incompatible with some DIVA strategies. Vaccine-resistant strains have also emerged in Vietnam, Indonesia and Egypt. The goal of the vaccination campaign in Vietnam has been to reduce the number of outbreaks among poultry and the number of human cases, and this has generally been accomplished. However, surveillance has documented poor vaccine coverage and/or efficacy in some poultry populations. China, Vietnam, Indonesia and Egypt face a number of obstacles in controlling endemic H5N1 viruses by vaccination (or other means), such as large numbers of backyard poultry, poor biosecurity in some small commercial flocks, the live bird trade, and various socioeconomic factors. At least one vaccine-resistant virus also caused an outbreak in Hong Kong, but it was successfully eradicated. Some of the factors likely to be important in the success of Hong Kong's vaccination campaign are its small number of poultry farms, which have strong government oversight, and a very high rate of vaccination coverage.

Vaccination has been used successfully in some eradication campaigns for LPAI outbreaks in the U.S. It has never been used during HPAI outbreaks, which were eradicated by stamping out.

Vaccination has been part of control or eradication programs for H5 or H7 LPAI or HPAI viruses in a number of countries including Mexico, Italy, China, Hong Kong, Indonesia, Vietnam, Russia, Egypt, India, Pakistan, the Democratic Republic of Korea, Côte d'Ivoire, Sudan, Mongolia, Kazakhstan and the U.S. [8;46;49;135;141;149;464;499;616;617]. Some countries have conducted prophylactic campaigns or

emergency vaccination during outbreaks, while others carried out mass vaccination after viruses became endemic. Some campaigns involved poultry, while others were conducted in zoo birds, hobby birds or other flocks. Long-term vaccination programs were formerly rare; however, campaigns lasting for years have recently been conducted in Italy for H7 LPAI viruses [1;460;531;532], in Mexico for H5N2 LPAI viruses [134;136;499;517] and in some countries in Asia and the Middle East for H5N1 HPAI and H9N2 LPAI viruses [46;94;135].

14.1 Vaccination against H7 and H5 LPAI Viruses in Italy

Beginning in 1997, a number of outbreaks involving H5 or H7 viruses occurred in Italy, mainly in a northern region where the concentration of poultry is high [1;531;532]. This area, which is referred to as the densely populated poultry area (DPPA), is at increased risk for avian influenza, most likely from the high exposure to migrating wild birds, importation of live poultry, and variety of commercially raised species in the area [532]. During the first outbreak, an H7N1 LPAI virus mutated to become an HPAI virus after circulating in commercial poultry for approximately nine months [1;532], and caused one of the most serious avian influenza epizootics ever seen in Europe [618]. An H7N1 LPAI strain re-emerged four months later from a quail farm, and affected approximately 50 turkey farms and three additional quail farms [1]. The infected farms were all depopulated [1], and over the following decade, a series of vaccination programs were conducted to control LPAI outbreaks and/or prevent these viruses from emerging [1;531].

In general, the strategy in these campaigns included strict biosecurity and the use of inactivated vaccines, combined with intensive monitoring and a heterologous neuraminidase DIVA strategy in vaccinated flocks [1;460;531;532]. In addition, 1% of the birds on each farm were confined to fenced-in areas in each poultry shed, and left unvaccinated as sentinels [531]. Sentinel birds were tested every 45 days by serology [532]. Nonvaccinated flocks inside and outside the vaccination zone were monitored by serology and/or virology, as appropriate (e.g., geese and ducks, which tend to have pre-existing antibody titers to AIV, were tested by virology) [1;460;532]. Vaccine efficacy was monitored in vaccinated birds, using HI titers, on a percentage of the farms [532]. Movement restrictions were placed on meat and live poultry from the vaccination area [532]. This overall strategy allowed poultry products from vaccinated birds to be marketed.

An evaluation of field data from the Italian monitoring system in 2000-2005 found that active surveillance was the most effective method to detect infected flocks, particularly during a vaccination program [531]. The detection rate was 61% for active surveillance, 32% for passive surveillance (surveillance after avian influenza is suspected, usually by the detection of clinical signs) and 7% for targeted surveillance (defined, in this case, as surveillance after outbreaks are confirmed). Passive surveillance was less likely to detect infections in vaccinated than nonvaccinated flocks, probably because vaccination suppresses clinical signs.

The first vaccination campaign was intended to protect poultry from H7N1 viruses, using an inactivated H7N3 vaccine (A/chicken/Pakistan/95) [532]. It was conducted from November 2000 to May 2002, in a limited region of the DPPA [1;532]. Only relatively long-lived birds such as meat turkeys, capons, layers and a limited number of breeding chickens and turkeys were immunized [1;532;618]. An H7N1 LPAI virus was detected in the early stages of the vaccination program, which was begun soon after the depopulation of affected flocks in the preceding outbreak. Between December 2000 and March 2001, this virus was found on 3 meat-type turkey farms in the vaccination zone, and 19 turkey farms and one layer farm in a nearby area outside the vaccination zone [1;532]. Only one of the affected farms in the vaccination area had been immunized, and the virus did not spread from this farm to any others. All infected flocks were depopulated by the end of March, and epidemiological evidence demonstrated that

H7N1 was no longer circulating after the first year of vaccination [1;460;532]. This allowed marketing restrictions to be lifted on fresh meat from vaccinated poultry, if monitoring including DIVA testing gave no indications of infection in the flock [460].

In October 2002, a new H7N3 LPAI strain was introduced from wild birds into poultry [1;532]. The virus spread rapidly, and emergency vaccination was used as part of the control program [1;532;618]. Only long-lived poultry were vaccinated, similarly to the previous campaign [1;532]. A DIVA strategy using an H7N1 vaccine (A/chicken/Italy/1999) was employed after this vaccine became available in January 2003 [1;532]. Until that time, an H7N3 vaccine (A/chicken/Pakistan/1995) was used to vaccinate large flocks of layers [532]. The field virus spread extensively in the initial stages of the outbreak, and affected nearly 400 poultry farms, including 88 vaccinated meat turkey flocks and 12 backyard flocks, over the next year [1;532]. Nevertheless, only sporadic outbreaks were reported in nonvaccinated flocks inside or outside the vaccination zone [1;532]. An analysis suggested that vaccination contributed to the control of virus transmission; the reproduction ratio (R) decreased from 2.9 (95% CI: 2.3 to 3.9) before vaccination to 0.6 (95% CI: 0.5 to 0.7) after vaccination [1]. The outbreak ended by October 2003 [1]. One reason this vaccination campaign might have been less successful than the previous campaign is that farms were vaccinated while the H7N3 virus was circulating, rather than after the depopulation of affected farms [532]. Other factors that could have had an effect are the delay in the vaccination campaign, and the higher poultry density and larger area affected [532]. In addition, controlled marketing was allowed in a much greater number of infected flocks, and farmers and companies had weak motivation due to the low price for poultry products [532].

Sporadic outbreaks with H5 and H7 viruses were reported in Italy over the following years, and led to additional vaccination campaigns. In February 2004, an H5N3 LPAI virus was detected in domesticated ducks and geese in a single free-range backyard flock, which was depopulated [1]. Because virus transmission could not be controlled in previous outbreaks without vaccination, a bivalent H5/H7 prophylactic vaccination program was developed for high risk areas [1]. The vaccine contained H5N9 and H7N1 or H7N4 subtypes. In addition to regular preventive vaccination, authorities made plans to boost immunity by vaccination if a field virus was detected. The poultry industry in the area with the highest poultry densities was also reorganized, and the density of turkeys was decreased short-term by a partial ban on restocking. In September 2004, an H7N3 virus re-emerged in a group of farms [1]. Affected premises included 27 vaccinated meat turkey farms and a quail farm, in which the virus had apparently been circulating for a year (serological monitoring had been inconclusive in this flock). Most of the turkeys had been vaccinated only 1-2 times, although three vaccinations were recommended in this species. Susceptible birds were given a booster, and strict control measures were implemented. The virus was quickly eradicated. An H5N2 LPAI was introduced into vaccinated turkey flocks in 2005, with virus transmission to 2 nonvaccinated and 13 vaccinated turkey farms [1]. The vaccinated farms contained adult birds that were close to slaughter and had HI titers of 4 to 16, suggesting that they had become infected after immunity waned. Only limited transmission of this virus occurred, and the virus was contained more rapidly and with fewer losses than in previous epizootics.

No H5 or H7 viruses were introduced into vaccinated poultry between April 2005 and October 2006, although H5/H7 LPAI strains and H5N1 Asian lineage HPAI viruses were detected in wild birds [1]. After a national avian influenza monitoring plan was implemented, asymptomatic H7N3 LPAIV infections were detected in a number of rural or hobby flocks in 2007 [1]. Six infected commercial meat turkey farms were also detected in the same area. All birds on affected poultry farms were culled, but 244 birds that belonged to endangered species were allowed to live. An emergency DIVA vaccination program, using an H7N1 vaccine, was conducted between October 2007 and March 2008 in areas with the highest turkey densities in the outbreak area [1;618;619]. No additional infections were detected and there was no further evidence of virus circulation [1].

14.2 Vaccination against H5N2 HPAI and LPAI Viruses in Mexico

Low pathogenic avian influenza was first suspected in Mexico in late 1993, but was not confirmed until spring 1994, by which time these viruses had spread widely among asymptomatic commercial and backyard poultry [134;136;499;517]. A control program was established, but eradication was unsuccessful, and HPAI viruses emerged from LPAI strains independently in late 1994 and early 1995 [517]. An eradication program was implemented for HPAI, and included stamping out measures, with depopulation of affected flocks, movement controls and increased biosecurity, combined with the vaccination of poultry in affected and at-risk regions [136;517]. The high density of commercial poultry farms, which made it difficult to diagnose and depopulate infected farms before the virus could spread, influenced the decision to vaccinate [517]. Another factor was that poultry exports were not important for the vaccinated farms. In laboratory tests and field trials, the chosen vaccine strain (A/chicken/Mexico/CPA/232/94; H5N2), suppressed clinical signs; however, vaccinated birds continued to transmit the challenge virus to other birds for several days [517]. Because the vaccine was homologous, a heterologous neuraminidase DIVA strategy could not have been used in this campaign. Sentinel birds were employed on farms before restocking. The HPAI viruses were eradicated after 5 months, but the H5N2 LPAI viruses were not eliminated [136;517].

Although the vaccination campaign was continued, with the goal of eradicating the H5N2 LPAI strains, these viruses have continued to circulate among commercial and backyard flocks in some parts of the country [134;136;499;517]. They have also spread from Mexico to El Salvador, Guatemala, the Dominican Republic and Haiti ([136]; [620] cited in [134]). Current components of the Mexican control program include movement controls, the establishment of farms free of H5N2 viruses, the replacement of infected flocks with uninfected birds, vaccination of LPAI virus-infected flocks, surveillance conducted every 3 months at uninfected farms, and monitoring of sentinel birds on vaccinated farms twice a year [517]. In addition to inactivated H5N2 vaccines, other vaccines that have been used in Mexico include a live, recombinant fowlpox-vectored H5 vaccine (TROVAC™ H5), and a live recombinant, NDV-vectored vaccine, which carries the HA from A/chicken/Mexico/435/2005 (H5N2). The NDV-vectored vaccine is licensed in Mexico for administration in eye drops, sprays or drinking water [517].

Antigenic drift is thought to contribute to the persistence of H5N2 LPAI viruses in Mexico [134;136]. At least two sublineages, Puebla and Jalisco, were identified among early Latin American H5N2 LPAI viruses ([621] cited in [136]), and the original vaccine seed strain is from the Jalisco sublineage [136]. The early sublineages have been replaced over time by new sublineages ([134]; [622] cited in [136]). Challenge studies found that the official inactivated vaccine seed strain reduced the shedding of Mexican lineage H5N2 LPAI and HPAI viruses isolated in 1994, but not LPAI viruses from 1998 [134], 2002 [134] or 2003 [136]. Inactivated vaccines containing strains that are more closely related to the field viruses, as well as the fowlpox vectored vaccine, significantly reduced the shedding of the 2003 isolate [136]. In addition to selection pressures from vaccination, large numbers of poultry are infected each year in Mexico, increasing the number of mutations that can be fixed in the population [134]. Maternal antibodies [134], and immunity to LPAI viruses in infected, non-vaccinated flocks [136] may also contribute to antigenic drift. Some aspects of the vaccination program, such as surveillance, have been noted to be weak [460;499].

14.3 Vaccination against H7N3 HPAI Viruses in Mexico

Mexico also used vaccination in an H7N3 HPAI outbreak, which mainly affected layer chickens, in 2012 [575]. The initial outbreaks were reported in June 2012, among commercial poultry in a poultry dense region [6]. Control measures included the establishment of a quarantine zone (later enlarged when 2 affected farms were reported outside the zone), depopulation and vaccination [6]. Vaccination was begun in July 2012, using a recent H7N3 LPAI virus isolated from wild waterfowl in Mexico (A/cinnamon teal/Mexico/2817/2006), as there were no licensed vaccines for H7 viruses in North America at this time [6;572]. At least two rounds of vaccination, with the assessment of HI titers after the first dose, were planned [6]. Initial field reports suggested that the vaccine was protective [572], and laboratory studies found that it was able to reduce virus shedding in chickens, in addition to being clinically protective [572;575]. The outbreak was reported to be controlled until January 2013, when additional cases were reported [572]. Outbreaks in commercial poultry have been reported as recently as August 2013, and vaccination was employed in their control [7]. No outbreaks were documented between December 2013 and April 2015; however, Mexico reported H7N3 HPAI viruses in backyard poultry in April 2015 [623], and in 2 wild birds as recently as May 2015 [624].

14.4 Vaccination against H7 HPAI Viruses in The Democratic People's Republic of Korea

The Democratic People's Republic of Korea (North Korea) vaccinated layer chickens with an autogenous vaccine in 2005, as a component of eradication measures during an H7N7 HPAI outbreak [149]. The virus did not become endemic.

14.5 Vaccination against H5N1 HPAI Viruses in Europe

Asian lineage H5N1 HPAI viruses, carried in wild birds, spread into Siberia, Kazakhstan and Eastern Europe in 2005, and outbreaks occurred in Romania, Turkey and the Russian Federation [616]. Most outbreaks affected backyard poultry. In early 2006, H5N1 viruses were detected in more than 700 wild birds in 14 E.U. countries, but transmission to domesticated poultry was uncommon and sporadic [616]. France, Germany, Denmark and Sweden each had one infected farm. Localized spread was reported in Hungary; approximately 30 domesticated birds, mainly geese, were affected. Control methods in Europe included enhanced biosecurity, surveillance of poultry and wild birds, and movement controls where H5N1 virus was detected. Stamping out was used to eradicate the virus in most cases.

Russia began a targeted vaccination program in early 2006, after new outbreaks were reported in domesticated flocks [616]. Vaccination with a local strain (A/duck/Novosibirsk/02/05) was conducted in several species of free-range poultry, and in other high-risk captive birds near commercial poultry or on wild bird migration routes. In Russia, the only outbreaks reported in 2006 occurred in nonvaccinated poultry. Further outbreaks were, however, reported in 2007, with one outbreak in 2008 [149]. The virus was not reported in domesticated poultry between 2009 and 2011, and is not considered to have become endemic in Russia following these vaccination campaigns [149].

E.U. legislation permits short-term emergency vaccination, as well as preventive vaccination if there is an increased risk for virus introduction [45;455]. Preventive vaccination is allowed long term under some conditions [45]. A DIVA strategy with stringent active surveillance must be used, and efficacy testing of the vaccine must be conducted [455]. Preventive vaccination plans for zoo birds were approved for 17 E.U. Member States in 2006 (see section 19, Vaccination in Zoos and Special Collections for details) [455;616]. In 2006, the Netherlands and France were also authorized to conduct prophylactic vaccination in targeted poultry populations [455;499;616].

14.5.1 Preventive Vaccination in the Netherlands

The Netherlands contains many feeding areas and bodies of water where migrating waterfowl and other birds congregate. Because migratory birds could introduce H5N1 HPAI viruses into free-range farms or hobby flocks, Dutch authorities mandated that these flocks be confined during the migration season in 2005 and 2006 [1]. This measure caused hardship to free-range farmers, because eggs cannot be labeled free-range or organic if the birds are kept in confinement for more than 12 weeks. There were also protests against confinement based on animal welfare. In addition, analyses of a previous (H7N7 HPAI) outbreak had concluded that hobby flocks played little or no role in virus transmission, and most of the hobby birds slaughtered during the eradication campaign had not been infected [1].

As a voluntary alternative to confinement, free range flocks and hobby flocks were invited to participate in a prophylactic H5 vaccination program from March 2006 to August 2006 [1]. Birds in participating flocks were given two doses of a commercial inactivated H5N9 vaccine, which was supplied by the national regulatory organization. Local veterinarians conducted the vaccination and submitted registration records, and regulatory authorities carried out random checks on vaccine use. Participating hobby flocks received unique identifying information, similar to the identification already in use for commercial flocks. Vaccinated birds were identified with leg rings, and reporting was required if these birds were transferred. Restrictions were also placed on poultry products from vaccinated birds. Eggs from vaccinated free-range birds could be handled only by designated packing stations. The export of vaccinated birds, or meat or eggs from vaccinated birds, required special approval from the receiving country. Abattoirs were not allowed to slaughter vaccinated hobby poultry.

In hobby birds, a heterologous neuraminidase strategy was used for surveillance during the vaccination campaign [1]. Birds were monitored for H5N1 HPAI viruses with a commercial competitive ELISA that detects antibody titers to the N1 neuraminidase. Positive samples were re-tested using indirect immunofluorescence. The sera from geese and other birds could not be tested with the latter assay, as appropriate reagents (fluorescein conjugated secondary antibody) were not available. Sentinel birds were also used in vaccinated commercial flocks, and were tested every 3 months. Asian lineage H5N1 viruses were not found in the Netherlands during the vaccination period, and no avian influenza viruses were detected on any vaccinated farms [1]. Sentinel birds did not become ill, and none of the sera from sentinel birds was positive in the N1 ELISA. Sera from hobby birds were occasionally positive in this ELISA; however, none of the sera reacted in the confirmatory immunofluorescence test, and extensive epidemiological investigations did not find evidence of virus circulation. No adverse effects were reported in vaccinated birds.

Participation in the program was low, possibly due to the high costs, the paperwork requirements for hobby flocks, and the trade restrictions for commercial free-range farms [1]. There was also pressure from the rest of the poultry industry, which opposed vaccination for fear of losing markets. Eight commercial flocks (approximately 19,700 birds) and 1,613 hobby flocks (22,300 birds) were vaccinated.

14.5.2 Preventive Vaccination in France

In France, provisional marketing and use authorizations were given to two inactivated vaccines and a recombinant fowlpox vectored H5 vaccine in 2005, when the possibility of vaccination was first considered [1]. A vaccine bank with 20 million doses of the authorized vaccines was established in early 2006. A preventive, targeted vaccination program was conducted in winter/spring 2006, after HPAI outbreaks were reported in Greece [1]. The goal of this campaign was to prevent H5N1 HPAI infections in migrating wild birds from reaching domesticated birds. Zoo birds, as well as free-range ducks and geese that could not be maintained indoors, were vaccinated [1;455;499;616].

Ducks and geese were given two doses of a heterologous (H5N2) inactivated vaccine that contained A/duck/Potsdam/1402/86 [1]. Nonvaccinated sentinel birds were maintained in all vaccinated flocks, with the number of birds based on the size of the flock. Vaccinated flocks were inspected by a veterinarian for clinical signs each month. Sentinels were checked for H5N1 virus shedding, using both cloacal and tracheal swabs, with matrix protein and H5-based RRT-PCR. In addition, $\geq 2\%$ mortality in one day or $\geq 0.25\%$ mortality for two successive days, and decreased food and/or water intake $\geq 50\%$ in one day or $\geq 25\%$ for three successive days, were reportable. Serological surveillance was not conducted in vaccinated waterfowl because many birds had antibody titers from exposure to LPAI viruses, and because N1-based ELISAs had not been validated in ducks and geese.

HI titers were evaluated after vaccination, by sampling 5% of the flock [1]. The titers were highly variable, and ducks vaccinated when they were 3 weeks old had poor responses. H5N1 HPAI viruses were not found in sentinel birds during the vaccination program, but unrelated H5 LPAI viruses were detected in some flocks. This program ended in June 2006, when the last vaccinated birds were slaughtered. The risk of infection from wild birds had decreased, and the direct and indirect costs of vaccination were very high. An additional motivation was the desire to regain HPAI-free status. Commercial poultry have not been vaccinated for avian influenza in France since this time, and the vaccine bank, which had been established in anticipation of the vaccination campaign, no longer exists [1].

14.6 Vaccination against H5N1 HPAI Viruses in Asia, Africa and the Middle East

Vaccination has been part of the official control programs for Asian lineage H5N1 HPAI viruses in China (including Hong Kong), Indonesia, Vietnam, Pakistan, India, Egypt, Côte d'Ivoire, Sudan, Mongolia and Kazakhstan [8;135;141;149;499;617]. The types of programs have included prophylactic, emergency and routine vaccination. Pakistan, Sudan, Cote d'Ivoire and Israel conducted emergency vaccination campaigns. Israel vaccinated a single ostrich farm in 2006, during outbreaks in gallinaceous birds which were eradicated by stamping out [8]. Pakistan has had periodic H5 and H7 outbreaks, and it has used ring vaccination around H7 or H5N1 outbreaks, as necessary [149]. Cote d'Ivoire and Sudan initiated emergency vaccination campaigns as part of eradication measures in 2006, during H5N1 outbreaks in poultry [149]. Cote d'Ivoire vaccinated commercial and village poultry, and ended the vaccination campaign after 3 months. Sudan is reported to have vaccinated 80-90% of commercial farms with high to moderate biosecurity, and ended the program after no additional cases had been reported in poultry for 15 months (16 months after vaccination was begun). Hong Kong successfully eradicated several H5N1 HPAI outbreaks in commercial poultry, using stamping out, with or without vaccination (details in section 14.6.1). It subsequently employed prophylactic vaccination, to counter the high risk of virus introduction. HPAI viruses are not considered to have become endemic after vaccination in Cote d'Ivoire, Sudan, Israel or Hong Kong [149].

Mongolia and Kazakhstan conducted preventive vaccination campaigns for H5N1 HPAI to reduce the risk of human infections, as deaths have been reported in wild waterfowl in both countries [149]. Kazakhstan vaccinated village poultry, while Mongolia vaccinated backyard flocks and flocks of small commercial (layer) poultry. There have been no reported H5N1 outbreaks among poultry in either country, and Mongolia planned to stop vaccination in 2011 [149].

Several countries in Asia, including Vietnam (section 14.6.3), Egypt, China (section 14.6.2) and Indonesia, have conducted long-term mass vaccination campaigns after H5N1 viruses became endemic in poultry [8;45;141;617]. These countries have faced a number of challenges in controlling these viruses by vaccination (or other means). One difficulty is that some nations may not have the necessary

infrastructure and other resources for intensive surveillance, biosecurity and other components of a successful vaccination campaign. Vaccination coverage can also be inadequate, especially in some poultry populations [8;50;617;625]. For example, vaccination rates among backyard poultry in Egypt are generally reported to be low, although these birds are approximately equivalent in numbers to commercial farms [141;625]. Some countries have large populations of domesticated and sometimes free-roaming ducks, which can maintain many HPAI viruses inapparently, and may either not be adequately vaccinated or not respond to the vaccine [8;50;617]. Other issues, including socioeconomic factors and various practical difficulties (e.g., in maintaining a cold chain), may also limit vaccine administration or vaccine efficacy [626;627]. In addition, repeated vaccination of breeders can result in high levels of maternal antibodies in their chicks, interfering with vaccination and resulting in poor immunity in some birds during part or all of their lifespan [141;497].

14.6.1 Hong Kong

Hong Kong has experienced repeated outbreaks with Asian lineage H5N1 viruses since the 1990s. An outbreak in 1997 was the first indication that H5N1 HPAI viruses were emerging in Asia, and the first occasion when HPAI viruses were reported to cause serious or fatal infections in people. Although the outbreak viruses were eradicated, this was followed by new introductions of H5N1 viruses between 2001 and 2003. A field trial consisting of vaccination, biosecurity and surveillance was evaluated in a high-risk area from April 2002 to March 2003 [25;628]. The vaccine was a commercial, inactivated, heterologous H5N2 vaccine that contained a North American lineage virus (A/chicken/Mexico/232-CPA/94) ([628] cited in [25]). Birds on 22 chicken farms were given two doses, 4 weeks apart [499]. Nonvaccinated sentinels were used on each farm [499]. Surveillance included serology in vaccinated flocks, as well as monitoring of sentinels and dead birds by RT-PCR [499]. The vaccine induced acceptable HI titers, and vaccinated chickens were protected from high doses of an H5N1 virus when challenged in a secure laboratory ([628] cited in [25]). In December 2002, H5N1 HPAI outbreaks among waterfowl in two recreational parks, wild water birds, and retail poultry markets led to a decision to expand the vaccination program to 53 additional farms [25;499].

An unusual situation during this outbreak provided evidence that vaccination could interrupt virus transmission in the field. H5N1 HPAI viruses were detected on five chicken farms in late December [25]. Control measures included strict quarantines, movement controls, improved biosecurity and ring vaccination of the surrounding farms. Two of the five infected farms were completely depopulated. On three farms, individual circumstances resulted in an unusual strategy, in which chicken sheds with high mortality rates or increasing mortality were depopulated, while the remaining sheds were vaccinated, and intensive monitoring was conducted on the infected and surrounding farms. On two of these farms, the virus spread to recently vaccinated sheds, but intensive monitoring found no further evidence of transmission or virus shedding after 18 days. The virus did not spread to the vaccinated sheds on the third farm.

In June 2003, a universal vaccination program was initiated for all chickens entering the live-bird market system in Hong Kong, including imported birds from China [142;499]. This vaccination program is combined with strict biosecurity and a comprehensive surveillance program. Government controls regulate the production of chickens for live poultry markets (which can only be produced by 30 registered farms), and vaccinated poultry from China are imported through only one wholesale market [149]. Vaccination coverage has been high (91%), once the vaccination program was fully implemented in 2004 [149]. Large commercial producers and village poultry are also absent from Hong Kong, which may contribute to the control program's success [149]. H5N1 viruses are normally absent from vaccinated poultry in Hong Kong, and are eradicated by movement controls, vaccination and culling, if they are introduced [149;499;629]. At least one outbreak caused by a vaccine-resistant variant has been reported

[149]. Outbreaks in wild birds, as well as introduced viruses, continue to pose a threat [629], and Hong Kong intends to continue vaccination while there is a high risk of H5N1 virus introduction [499].

14.6.2 Mainland China

A variety of AIV subtypes, including H5N1 HPAI viruses, have been found among poultry in China since the 1990s [501]. China has large numbers of backyard flocks and small farms without biosecurity, which complicates the control of this disease. Vaccination has been employed in official H5N1 control programs since 2003 [501]. An inactivated, Eurasian lineage H5N2 vaccine (A/turkey/England/N-28/73) was first used in 2003, in chickens in Guangdong Province intended for export to Hong Kong and Macao. In 2004, vaccination was implemented in districts with H5N1 outbreaks and in buffer zones [498;501]. A reverse genetics derived, inactivated vaccine (Re-1) that contains the HA and NA from an early Asian lineage H5N1 virus (A/goose/Guangdong/1/96) was licensed in China in 2004 [498;501].

Although the initial vaccination campaigns were limited, epidemiological studies conducted at this time found that all outbreaks occurred on farms that either did not vaccinate their poultry or that used unlicensed vaccines [501]. Beginning in late 2005, a mandatory vaccination program was established for domesticated poultry [501]. Vaccines are produced by eight companies approved by the government, and indemnity is provided for poultry that must be culled. Licensed vaccines include an H5 NDV-vectored vaccine (HA of A/goose/Guangdong/1/96), an H5N1 fowlpox-vectored vaccine (HA and NA genes of A/goose/Guangdong/1/96), and inactivated vaccines, including some made by reverse genetics to more closely match recently circulating strains [48;498;501]. Surveillance programs in China suggest that chickens have high levels of seroconversion after vaccination, with somewhat lower levels in ducks [617].

Asian lineage H5N1 HPAI viruses have diversified considerably in China, and some vaccines have become ineffective against certain variants [584]. In 2006, H5N1 avian influenza viruses with significant antigenic drift were detected in a chicken flock in Shanxi Province that had been vaccinated with the H5N2 inactivated vaccine [501]. The clinical signs were decreased egg production and a mortality rate of 10-20%. The flock was depopulated, but the new virus was later isolated from other flocks. Unpublished data from a challenge study suggested that the H5N2 and Re-1 (H5N1) inactivated vaccines provided only 80% protection against the new strain, although they could protect up to 100% of chickens from challenge with more closely related H5N1 viruses [501]. A new inactivated vaccine (Re-4) was created, containing the H5 and N1 genes from A/chicken/Shanxi/2/2006 [501]. The Re-4 vaccine was approved for use in 2006, for provinces affected by this strain. A combined H5N1 vaccine containing Re-1 and Re-4 was licensed in 2007, for use in a limited area of northern China where A/goose/Guangdong/1/96-like viruses and A/chicken/Shanxi/2/2006-like viruses co-circulate. In 2008, Re-1 was replaced by a vaccine strain (Re-5) that contains the HA and NA genes from A/duck/Anhui/1/06 (clade 2.3). Field strains have continued to evolve and Re-6, containing HA and NA from clade 2.3.2.1 virus A/duck/Guangdong/S1322/2010, became the major vaccine used in China as of 2012 [537;545]. Modern methods of intensive poultry production have also been introduced into some provinces in China, reducing the need to vaccinate [617].

None of the H5N1 inactivated vaccines used in China are compatible with the heterologous neuraminidase DIVA strategy. Other challenges for this program include the very large numbers of poultry, poor vaccine coverage in some birds including ducks and geese, and poor biosecurity and difficulties in conducting surveillance in backyard poultry and on small farms [501;584]. The live bird trade, which is not regulated, can spread avian influenza viruses long distances, and mix birds from different sources (e.g., backyard flocks and commercial flocks) [501;584]. Vaccination has not stopped the circulation of H5N1 HPAI viruses in China [501;584;617].

14.6.3 Vietnam

The goal of the H5N1 vaccination program in Vietnam is to reduce outbreaks among poultry and decrease the number of human cases [617]. Eradication is not considered feasible with the current poultry production systems. Clade 1 and various clade 2 viruses are endemic in Vietnam [132;536]. Clade 1 viruses have predominated in southern regions, and clade 2 in the north; however, the circulation of these viruses overlaps to some extent. Clade 7 viruses are sometimes found locally, but are thought to be imported illegally in birds brought to live poultry markets. Mass vaccination is conducted twice a year in high-risk areas, and includes small flocks of village poultry as well as commercial flocks [617]. One mass vaccination event is timed to coincide with the lunar New Year and its increased poultry trade. The vaccination strategy is regularly reviewed, and has been gradually progressing from mass vaccination toward more targeted vaccination [617]. Serological monitoring suggests that protection persists for approximately 4 months in vaccinated flocks, then rapidly declines [617]. This seems to be caused by the introduction of nonvaccinated birds into vaccinated flocks, as well as by declining titers over time.

The vaccination campaign in Vietnam has contributed to a significant reduction in the number of avian influenza outbreaks and human cases, although both continue to be reported ([617]; [629] cited in [135]). At least in some locations, however, the vaccination levels achieved have not prevented H5N1 viruses from circulating and infecting the nonvaccinated poultry that coexist with vaccinated birds [630]. In one study, only 37% of chickens or ducks reported as vaccinated were seropositive [630]. Factors that seemed to contribute to this low rate included the timing and number of doses the birds received, as well as vaccination failures (e.g., from cold chain failures). Free-roaming ducks are thought to be particularly important in maintaining viruses under these conditions [630]. In 2011-2013, a new active surveillance program detected H5N1 viruses in approximately 4% of oropharyngeal swab pools and 26% of markets [536].

Re-5, Re-6 and clade 1.1 vaccines have been used in different parts of Vietnam, and bivalent or multivalent vaccines may be needed in the future [536]. In 2011, vaccination campaigns were suspended in northern Vietnam due to the emergence of a new clade 2 subclade not neutralized by the vaccines in use [132]. These strains spread widely. Vaccination was resumed with the introduction of a vaccine (Re-5) effective against these strains. In 2013-2014, the Re-5 vaccine was recognized to be ineffective against some new clade 2.3.2.1c variants. As a result, vaccination was temporarily suspended in some northern regions until Re-6 vaccines could be employed. Variant clade 7.1 and 7.2 viruses were also been recognized locally in some markets in 2013 [536]. The clade 7.2 viruses had substantial genetic divergence from the clade 7 vaccine strain, A/chicken/Shanxi/2/2006.

14.7 Vaccination against AIV in the U.S.

Vaccination has never been used for HPAI viruses in the U.S. Past HPAI outbreaks were eradicated successfully by stamping out [631].

Vaccination has been employed in some LPAI outbreaks, but not others [631]. Vaccines were used, together with increased biosecurity and controlled marketing, in Minnesota and Utah turkey farms in the 1990s, as well as more recently in California (H6N2) and Connecticut (H7N2) [631]. In 2002, poultry producers developed a voluntary control program for an H6N2 LPAI outbreak in California [631]. The program included vaccination (using an inactivated vaccine with a heterologous NA), surveillance (targeted serological testing and daily observation of clinical signs), biosecurity, self-quarantine of infected farms, marketing/movement restrictions, cleaning and disinfection. This program successfully eliminated H6N2 viruses from commercial poultry flocks in California by 2003.

In 2003, an H7N2 LPAI outbreak was controlled partly by vaccination at a large commercial layer operation in Connecticut [499;631]. The initial economic analysis estimated that the cost of depopulation alone would be greater than US\$30 million, with a projected benefit-to-cost ratio of vaccination versus depopulation of approximately 10:1, in addition to the business and social costs of depopulating 3.5 million layers [499;631]. The eradication campaign included increased biosecurity, intensive monitoring, and the vaccination of pullets (two doses) and egg production flocks (one dose) [631]. Initially, uninfected and infected flocks were vaccinated with an inactivated H7N2 vaccine that had good homology with the field virus and was immediately available [499]. Sentinel birds were placed in the flocks and tested by serology every 2 weeks ([499]; [632] cited in [49]). Birds that died were tested by virus isolation or RRT-PCR ([499]; [632] cited in [49]). An H7N3 vaccine was employed later, allowing the use of the heterologous neuraminidase DIVA strategy ([499]; [632] cited in [49]). USDA authorization of vaccination was contingent on compliance with protocols, and the absence of any evidence that the virus was mutating to an HPAI virus or spreading to uninfected premises [631]. Approval would also have been withdrawn if significant trade restrictions were imposed on the U.S., or if there were indications of failure after 6 months. Vaccination began in April 2003, and the program successfully eradicated the virus, with quarantines lifted in September 2004 [631]. AIV was never isolated from vaccinated or sentinel birds [631], and there was no evidence that the virus continued to circulate in the flock after vaccination was begun [499]. The estimated cost was US\$5 million [631].

15. STRATEGIES FOR VACCINE USE

Summary

The goal(s) of a vaccination program (e.g., virus eradication, reduction of illness in poultry, or prevention of human disease) should be determined early. Vaccination must be part of a comprehensive avian influenza strategy if eradication is the goal. Used alone, it cannot eliminate AIV in poultry, and could contribute to the virus becoming endemic. The control program should incorporate effective biosecurity, surveillance, education, movement restrictions, quarantines of infected farms and the culling of infected poultry. Vaccinated flocks must be monitored to ensure that they do not become infected with field viruses. Prolonged and extensive vaccination programs are not usually sustainable, and an exit strategy should be planned when the program is begun.

In an eradication program, birds may be either “vaccinated to live” or “vaccinated to kill.” Vaccination-to-live has particular benefits for long-lived or valuable birds, such as breeding flocks and endangered species. Approaches to the application of vaccination include prophylactic (preventive) vaccination, emergency vaccination, routine vaccination in endemic areas, targeted vaccination, ring vaccination, barrier vaccination and mass (blanket) vaccination.

The species to be vaccinated vary with the vaccination campaign. Most avian influenza vaccines are licensed for use in chickens and/or turkeys, but some vaccines have been tested or used in a discretionary manner in ducks, geese and other birds. Some studies suggest that vaccines used in ducks might need to contain twice as much antigen and/or contain a strong adjuvant. Turkeys may need additional doses compared to chickens.

Vaccine selection is based on many factors, including matching with the field strain(s), potency, availability in sufficient quantities from a reputable source, licensing considerations and other factors. The species of birds and types of production systems should also be considered. The OIE recommends that the chosen vaccine be able to decrease virus circulation in the species

immunized, and that individual batch tests reflect this degree of efficacy. The vaccine should also be compatible with a DIVA strategy whenever possible.

Inactivated vaccines are well suited for protecting adult poultry and other birds during emergency vaccination. They have protected chickens, turkeys, geese and ducks from laboratory challenge, and have been used experimentally in zoo birds. The number of doses needed for full efficacy, depends on the vaccine and host species. In the field, even chickens appear to require 2 doses for full efficacy. Inactivated vaccines are administered by individual injection. This method may be slow and expensive in the field, especially in waterfowl. Inactivated vaccines can be given repeatedly, which may be necessary in long-lived birds. They may not provide good protection in very young birds.

Fowlpox-vectored vaccines and HVT-vectored vaccines have been labeled for use in chickens; however, preliminary reports suggest that HVT-vectored vaccines might also replicate in some waterfowl. Both vaccines are administered by individual injection, and are usually given to day-old chicks at the hatchery. This has some advantages such as the higher biosecurity at this location, the development of protective immunity while the birds are younger, and better efficiency because each hatchery serves many farms. Active immunity to the vector is expected to interfere with vaccination in older birds. Fowlpox-vectored vaccines can, however, be given to chickens of any age that are seronegative for fowlpox viruses. They cannot be used repeatedly in the same bird.

NDV-vectored vaccines have been tested and used in the field in chickens. There is little experience with these vaccines outside China and Mexico. NDV-vectored vaccines can be given by mass administration methods such as sprays or drinking water; however, published experiments have demonstrated their efficacy only by individual administration. When given individually to chicks, vaccines had similar efficacy whether administered by eye drops or orally in water.

Prime-boost regimens, with fowlpox-vectored vaccines followed by inactivated vaccines, have also been explored. This technique appears to be promising in chickens and some species of ducks.

Avian influenza vaccines should be stored, transported and administered according to the manufacturer's recommendations. If the vaccination protocol is significantly different from established technical knowledge and the manufacturers' instructions, or if a different species is vaccinated, it is best to field test the vaccine before it is used extensively. Trained personnel should perform the vaccination, using appropriate personal protective equipment (PPE). Vaccination crews must practice excellent biosecurity, to decrease the risk that field viruses might be transmitted accidentally between farms.

Maternal antibodies may result from infection or vaccination of the hen. They can persist in young poultry for up to 4 weeks after hatching, and perhaps longer in some cases. While maternal antibodies may help protect birds from some avian influenza viruses during the first few days of life, several studies suggest that they provide little or no protection against Asian lineage H5N1 HPAI viruses. Maternal antibodies can interfere with successful vaccination, especially when using inactivated vaccines. While maternal antibodies can also interfere, to some extent, with vectored HA vaccines, some of these vaccines may be effective in their presence. Vectored HA

vaccines can also prime an immune response in the presence of maternal antibodies, although one study reported some degree of interference.

15.1 General Considerations

One of the initial steps in implementing a vaccination program is to determine its goal. Some programs are intended to assist in virus eradication, but the purpose of other campaigns may be to reduce illness in poultry and/or decrease the incidence of human disease. Vaccination must be part of a comprehensive control program if eradication is the goal; vaccination alone cannot eliminate AIV ([94;496]; [633] cited in [48]). If the eradication plan is poorly executed, the virus can become endemic in poultry [94;496]. A comprehensive control program should incorporate effective biosecurity, surveillance, education, movement restrictions, quarantines of infected farms and the culling of infected poultry [46;49;455;496]. An effective and transparent disease reporting system should also be available [496].

Vaccines cannot completely prevent AIV replication or excretion in the field [1;46;47;49;455], and vaccinated flocks must be monitored to ensure that they do not become infected with field viruses. Surveillance plans may vary, depending on the structure of the poultry industry in the country. In addition to commercial flocks, such plans may need to address vaccination in smallholder and backyard flocks where a high level of coverage can sometimes be difficult [496]. Participatory, community-based approaches may be used for these flocks, under the supervision of veterinary authorities [496].

A vaccination awareness/ education program and communication strategy should be established during the vaccination campaign [496]. Issues to be addressed include public health aspects of HPAI viruses, food safety, the benefits of vaccination, the risk of inapparently infected birds, the impact on trade, and the technical and scientific basis for vaccination.

Prolonged and extensive vaccination programs are not usually sustainable, and experts have recommended that an exit strategy be planned when the program is begun [46;48;49;455;496;499]. The exit strategy should examine the most important risk factors for disease introduction and spread, establish procedures to decrease the risk, and identify the conditions under which the program will be re-examined or ended [48].

15.2 Vaccination-to-Live and Vaccination-to-Kill

In an eradication program, animals may be either “vaccinated to live” or “vaccinated to kill.” Animals that are “vaccinated to live” are allowed to live their normal lifespan [496] unless they become infected. In contrast, animals that are “vaccinated to kill” are scheduled to be killed at some point after vaccination, even if they do not become infected. Meat from these animals may or may not be allowed to enter the human food chain. One use of vaccination-to-kill is to decrease transmission while animals are waiting to be culled. Both types of vaccination decrease the short-term resources required for carcass disposal, but will require the resources to implement, manage and maintain a vaccination, movement and permitting system for the vaccinates. Vaccination-to-live has particular benefits for long-lived or valuable birds, such as breeding flocks and endangered species.

Some suggested conditions under which vaccination may be considered (combined with other control measures, including zoning and compartmentalization if appropriate), are 1) when outbreaks occur in a region that has a high density of animals, and 2) if culling infected and dangerous contact flocks is not expected to contain the outbreak [496]. If vaccination is used, infections must be detected quickly in vaccinated flocks. Uninfected zones or compartments may be able to continue trade, even when vaccinated, provided they can substantiate that the exporting flocks are not infected [35;496].

15.3 Approaches to the Application of HPAI Vaccination

Types of campaigns include emergency vaccination in the face of an epidemic, preventive/ prophylactic vaccination to prevent infections in a high risk situation, or routine vaccination in an endemic area [455;496]. Selection of a vaccination strategy is influenced by vaccine availability, economics and logistical factors [455]. DIVA strategies should be used whenever possible, and are critical in preventing trade restrictions.

15.3.1 Prophylactic Vaccination

Prophylactic (preventive) vaccination is generally considered only in regions or groups of birds at high risk for virus introduction, when other methods are not adequate for prevention [45;49;455;496;499]. This form of vaccination functions as an extra biosecurity measure that may prevent the index case, or decrease the number of infected flocks [49;499]. The vaccine and vaccination schedule should stimulate sufficient immunity to decrease transmission if a virus is introduced [499]. Birds may be revaccinated to boost the immune response if an outbreak occurs [499].

The vaccine is chosen to target the subtype(s) expected to be of concern in the region. Because H5 and H7 LPAI viruses have a significant impact on poultry trade [35], these viruses are often targeted. Preventing the introduction of both subtypes from aquatic and migratory birds would require at least a bivalent vaccine [496;499]. The emergence of newer Asian lineage H5 variants has made it more difficult to select an H5 vaccine strain that can provide broad coverage. Prophylactic vaccination may also be targeted at a specific subtype, such as Asian lineage H5N1 HPAI viruses, a LPAI subtype circulating in live bird markets, or viruses causing outbreaks in trading partners or nearby countries [496;499]. Preventive vaccination for H5 or H7 viruses can be used either short term in a targeted manner, or long term if there are adequate resources [48]. Before beginning the campaign, a risk assessment should be conducted and a clear exit strategy should be established [48]. DIVA strategies are especially important in prophylactic vaccination campaigns [48;499]. Intensive surveillance is important to detect and respond to outbreaks and prevent unjustified trade restrictions [48].

15.3.2 Emergency Vaccination

Emergency vaccination (vaccination in the face of an outbreak) is an option especially when there is the potential for widespread transmission to occur quickly in areas with high concentrations of poultry, and/or there are conditions that might favor the virus becoming endemic if it cannot be controlled [45;48;496]. It can also be used if massive stamping out will be difficult to do (e.g., where poultry are vital as food and income in poor communities), as well as under conditions specific to a particular population, such as the potential for HPAI viruses to infect endangered species, zoo and exotic birds, rare breeds, pets or sanctuary birds [45;48;496]. Emergency vaccination is usually conducted as reactive vaccination to a known strain of virus. This simplifies the choice of vaccine.

The effectiveness of emergency vaccination depends on its ability to limit virus transmission during the initial high-risk period [48]. In this form of vaccination, the decision to vaccinate must be made quickly and the vaccination program must be carried out rapidly, to allow immunity to develop before exposure [48;49]. An emergency vaccination campaign is usually short-term [45], and may be conducted as vaccination-to-live or vaccination-to-kill [496].

15.3.3 Routine Vaccination in Endemic Areas

Routine vaccination can be used as a last resort if the virus has become endemic and eradication is not feasible [45;48;455;496]. This strategy can be useful for decreasing poultry losses, and in the long term, it may decrease virus prevalence and make eradication possible [45;48;49;496]. Infected flocks may still need to be culled to decrease virus transmission [49].

Vaccination alone will not result in eradication, because it is difficult to maintain a high level of protection in large poultry populations for extended periods ([633] cited in [48]). The cost of an effective vaccination program can be significant [496], and routine vaccination is probably unsustainable when used long term in large areas [48]. Such vaccination programs should eventually be stopped, or modified and targeted to be more sustainable [48]. If the vaccination program is ended, there should be a contingency plan in the event that the virus re-emerges.

Prophylactic, emergency or routine vaccination programs can be either applied to all susceptible birds in an area (mass vaccination) or targeted to specific groups of birds (targeted vaccination) [455;496].

15.3.4 Targeted Vaccination

Targeted vaccination attempts to protect specific groups of birds, such as a species, compartment or production sector [48;496]. This form of vaccination can be used to protect uninfected birds of high value, such as poultry with particularly valuable, rare or unusual genetic backgrounds, parent flocks, zoo birds or endangered species [496]. It can also be directed at uninfected areas where there is a high density of susceptible birds, as well as at categories such as long-lived birds (e.g., layers). Targeted vaccination may also be useful when the quantities of vaccine are limited, such as in the initial stages of a vaccination campaign [496]. The use of targeted vaccination should be preceded by a risk analysis that includes the level of threat, the value of the birds and the biosecurity levels of the facilities [496].

15.3.5 Ring Vaccination

Ring vaccination is a form of targeted vaccination where birds are immunized within a defined area around infected premises or infected zones. Its purpose is to reduce or prevent virus transmission from a focal outbreak to surrounding uninfected areas. Ring vaccination is only relevant during emergency vaccination [496]. It is most likely to be successful if foci of infection can be identified rapidly, before the virus can spread. It may not be appropriate in cases where the disease is widespread or contained in widely scattered foci, if the disease is difficult to identify, where there is a significant delay between infectivity and case confirmation, or where there is a significant delay between vaccine administration and the onset of protection. Ring vaccination should be combined with the slaughter of infected flocks and other eradication measures [496].

The ring size varies with the transmission rate, and the amount of transmission during the initial high-risk period [45]. One strategy is to vaccinate or euthanize all uninfected birds within a ring around the index case, and vaccinate and monitor birds inside a secondary outer ring [49].

15.3.6 Barrier Vaccination

Barrier vaccination is very similar in principle to ring vaccination; however, the vaccination zone is used to prevent the infection from spreading from a neighboring country or region into the uninfected area, rather than to keep it from spreading outward from infected premises. Geographic and political features usually have an important influence on the shape and location of the vaccination zone.

15.3.7 Mass (Blanket) Vaccination

In mass vaccination strategies, all susceptible birds in the chosen area are vaccinated [48]. Mass vaccination can be conducted throughout an entire country or throughout an OIE-defined zone with a separate status. Mass vaccination should be chosen when the virus is unlikely to be controlled in any other way [496].

15.4 Prioritizing Vaccine Use

One recommended order for prioritizing vaccine use [46] is:

1. In high-risk situations, e.g., to suppress transmission in the outbreak area or high-risk zone, or as ring vaccination outside the outbreak zone
2. In captive, rare species, such as birds in zoos
3. In genetically valuable poultry stock, e.g., pure lines or grandparent stocks that contain valuable individual birds
4. In long-lived poultry, such as egg layers or parent breeders
5. In poultry used for meat production

15.5 Movement Restrictions and Biosecurity

A vaccination program should be part of a comprehensive control program that incorporates effective biosecurity, surveillance, education, movement restrictions, quarantines of infected farms, and the culling of infected poultry [46;49;455;496]. Vaccination alone is not expected to be effective in eradicating an outbreak ([94;496]; [633] cited in [48]).

Movement restrictions for live birds, vehicles and personnel in the vaccination zone are necessary during prophylactic vaccination as well as during outbreaks [460;499]. In a preventive vaccination program, vaccinated flocks should be determined to be AIV-free before movement. In Italian LPAI vaccination campaigns, testing of poultry before shipment included serology and clinical signs in birds intended for slaughter, and virology and serology in ready-to-lay pullets [460]. Trucks were cleaned and disinfected before and after transport. In this campaign, hatching eggs and day-old chicks were required to come from tested flocks, and all birds (except those intended for slaughter) could only be shipped to poultry houses that had been cleaned and disinfected and did not contain other birds. Poultry houses were required to be free of birds for at least 3 weeks before receiving ready-to-lay pullets. Hatching eggs and packaging materials were disinfected before shipment. Vaccinated birds could not move out of the vaccination area, except to slaughter at designated abattoirs. Table eggs could be shipped only from tested flocks, and were sent directly to packaging centers or thermal treatment plants, and packaging materials were required to be disinfected or disposable. Trucks that transported poultry were restricted from operating both inside and outside the vaccination zone on the same day. Fresh poultry meat allowed to be traded internationally was required to come from flocks that had been tested regularly using DIVA tests and monitored with sentinel birds. DIVA tests and official inspections were required before shipment.

15.6 Species to Vaccinate

The species to be vaccinated varies with the epidemiological situation and purpose of the vaccination campaign [496]. Avian influenza vaccines are usually manufactured and licensed for chickens, although most vaccines have also been validated in the field for turkeys [342]. Currently, the vaccine industry does little or no testing of these products for other species of birds [342]. There is some information in the literature addressing vaccine use in ducks [111;135;200;208-211;324;327;331;336;337;341;344;480;508;537], as well as a few challenge studies in other species such as geese [208;327], pheasants [200], quail [323], falcons [598] and pigeons ([599] cited in [342]). Vaccine use in the field has been reported in chickens, turkeys, ducks, geese and quail, as well as in exotic birds and endangered species [342] (see also Vaccination in Zoos and Special Collections, section 19). Caution should be used when extrapolating study results between waterfowl species, as different species may vary in their susceptibility to AIV strains and H5N1 clades [342].

Published challenge studies suggest that inactivated vaccines will be clinically protective in species other than chickens, if there is an adequate antigen dose and HA match with the challenge virus [342]. Inactivated vaccines might be less effective in turkeys than chickens ([634]; [635] cited in [474]), and additional doses may be needed in this species [604]. Some studies suggest that vaccines used in ducks might need a higher antigen content and/or contain a strong adjuvant ([211] cited in [49]; [331;336] cited in [342]).

15.7 Vaccine Selection

The selection of a vaccine is based on many factors, including matching with the field strain(s), potency, availability in sufficient quantities from a reputable source, licensing considerations and other factors. The species of bird(s) and type(s) of production systems should also be considered [496]. High quality vaccines produced according to OIE standards should be used [46;455;496]. Substandard or unstandardized vaccines can compromise a vaccination campaign [209]. The OIE recommends that the chosen vaccine be able to decrease virus circulation in the vaccinated species, and that individual batch tests reflect this degree of efficacy [496]. Whenever possible, the vaccine should also be compatible with a DIVA strategy [1;46;48;496].

15.7.1 Inactivated Vaccines

Inactivated vaccines are especially suitable for protecting older poultry and other birds in emergencies [469]. These vaccines may not provide good protection in very young birds [607;636]; in chickens, their efficacy is not thought to be optimal until the bird reaches 2–3 weeks of age [466;469]. However, some individual vaccines might be protective at a younger age. Two studies reported similar protection from clinical signs and reduced virus shedding, whether they administered experimental vaccines to 3-day-old or 2-3 week-old chickens [574;637]. Inactivated vaccines can be used in various poultry species including chickens, turkeys, geese and ducks [46;48]. They have also been administered safely to a wide variety of zoo birds, and stimulated HI titers expected to be protective [49;470-477]. Inactivated vaccines can be given repeatedly, which may be necessary in long-lived birds such as breeders, layers and turkeys [48;457] and zoo birds. More than one dose may be needed for full efficacy, depending on the vaccine and host species [49;327;342;477].

15.7.2 Fowlpox Vectored Vaccines

Fowlpox vectored vaccines have been licensed for use in chickens, and are ineffective if the birds have active immunity to the fowlpox virus or fowlpox-vectored vaccines [46;460;469;495]. They are effective in chicks as young as 1 day of age [45-47;466;469;496]. These vaccines are not suitable when birds must be vaccinated repeatedly; however, chickens can be vaccinated with a fowlpox-vectored vaccine and boosted with inactivated vaccines. The few studies of their experimental use in ducks suggest that fowlpox-vectored vaccines are less effective than inactivated vaccines, at least when administered alone [331;336;508]. They might be more effective in prime-boost protocols (section 15.9).

15.7.3 Newcastle Disease Vectored Vaccines

NDV-vectored vaccines have been tested and used in chickens [45;119;494;501;509-516]. They are not recommended for poultry that have been well immunized against Newcastle disease [45;49;94;481]. There is limited experimental information on the use of these vaccines in ducks. They seemed to be effective in mule ducks, when given as 2 doses [341], but protection was short-lived in Muscovy ducks that received one dose [344]. Repeated doses of one NDV-vectored vaccine were necessary for good immunity in field studies in China [45].

15.7.4 HVT-vectored Vaccines

An HVT-vectored HA vaccine is labeled for use in chickens, and has been tested experimentally and used in this species [497;519;520;522;524;525]. Studies of its use in waterfowl are in the early stages.

15.8 Vaccine Administration

Avian influenza vaccines should be stored and transported according to the manufacturer's recommendations, following the vaccination schedule and application instructions [496]. If the vaccination schedule differs significantly from established technical knowledge and the manufacturer's instructions, or if a different species is vaccinated, field testing is advisable before the vaccine is used extensively [48]. Inactivated vaccines must be stored at 2-7°C (35-45°F) and should not be frozen. Because they are viscous, they are difficult to administer when cold, and must be warmed to 18-30°C (65-85°F) immediately before use. The fowlpox vaccine is lyophilized and must be rehydrated with sterile diluent before use. Once reconstituted, it should be used within one hour. The HVT-vectored AI vaccine is fragile and requires careful handling. It is supplied in a frozen, cell-associated form, and must be shipped and stored frozen in liquid nitrogen [519]. Once it has been removed from the liquid nitrogen (using appropriate personal protective equipment), it must be thawed, mixed with diluent and used quickly. Live vectored HA vaccines may be affected if there are any chemical disinfectant residues on the vaccination equipment.

Trained personnel should perform the vaccination, using appropriate personal protective equipment [496]. The OIE recommends that vaccination records (including the date; premises, locations and categories of animals; total number of susceptible animals on the premises, vaccine name/ product and brand of vaccine, batch numbers, number of doses and names of the personnel who administered the vaccine) be maintained by the Competent Authority and the facility [496].

Inactivated vaccines and fowlpox-vectored and HVT-vectored HA vaccines are administered by individual injection [457;468;469;519]. Fowlpox-vectored vaccines are usually given at the hatchery, by subcutaneous injection into the nape of the neck with Marek's disease automatic or semiautomatic injection machines [466;469]. They can be combined with Marek's and bursal disease vaccines [469]. HVT-vectored HA vaccines are also administered to 1-day-old chicks at the hatchery [519]. Advantages to administration at this age include the higher biosecurity at the hatchery, the development of protective immunity while the birds are younger, and increased efficiency because each hatchery serves many farms [45].

In older birds, inactivated and fowlpox-vectored vaccines are injected individually. Flocks must be seronegative to fowlpox if a fowlpox-vectored vaccine is used [460]. Individual injection can be slow and expensive, especially in waterfowl, which are more difficult to handle or catch than chickens, and may require additional personnel [45;47;327;342]. Adverse effects can occur from handling and stress, as well as from side effects caused by the injection or the adjuvant [45;47]. Vaccination crews must practice excellent biosecurity, to decrease the risk that field viruses might be transmitted accidentally between farms [45-47;49;496]. Needle-free devices for vaccine delivery have not been thoroughly investigated at this time. They were found to induce good titers to AIV in at least one study, without apparent side effects in chickens; however, vaccine delivery failures were noted in some birds [638].

Inactivated vaccines may be labeled for use in chickens as one or two doses. In most other species of birds, two doses induce good antibody titers if the vaccine quantity is adjusted for body weight as necessary [342]. The timing of the doses may also affect efficacy. In one challenge experiment, young Pekin and Muscovy ducks responded best to an inactivated vaccine when they were vaccinated at 7 and 21 days of age [337]. A single vaccination at 14 days of age, or two doses at 1 and 14 days, were more

likely to result in clinical signs. The latter two protocols were equivalent in protection. Field studies have suggested that at least one booster is needed for good protection, even in chickens [8;327;468;479;480].

There is little experience with NDV-vectored avian influenza vaccines outside China and Mexico [45;49]. NDV-vectored vaccines could be given by mass administration methods such as sprays or drinking water [45;47;481;501;512;517]. However, these vaccines have been administered individually to birds in all published experiments to date [45]. Eye drops were used in most experiments, but one study demonstrated that efficacy was similar in chickens whether individual doses were administered by eye drops or orally in water [515].

15.8.1 Prime-Boost Regimens

Prime-boost regimens, using fowlpox-vectored H5 or H7 vaccines followed by inactivated vaccines, have been effective experimentally in chickens, Pekin ducks and Muscovy ducks [45;336;467;500;505;506]. A prime-boost protocol in Pekin ducks resulted in a broader antibody response against different H5N1 viral clades, compared to two doses of an inactivated H5N9 vaccine [336]. Similar broadening of the response has been reported in chickens immunized with H5 (Bublout et al. cited in [45]) and H7 viruses [500]. In one study, a prime boost protocol was clinically protective and reduced virus shedding in chickens challenged with a recently isolated, variant H5N1 HPAI virus, when a single dose of the inactivated vaccine was not clinically protective alone [467]. In another study, this strategy was at least as immunogenic as a 2-dose regimen of an inactivated vaccine, given 3 weeks apart, in Pekin ducks or Muscovy ducks [506]. One group tested a commercial fowlpox-vectored H5 vaccine, followed by an experimental NDV-vectored H5 vaccine, in Muscovy ducks [344]. It appeared to be promising against challenge with a heterologous HPAI virus. The use of two different inactivated vaccines has also been suggested as a way to improve the immune response [500].

Prime-boost strategies have also been examined with HVT-vectored H5 vaccines. Three studies reported that these vaccines, used alone, protected most (80-95%) chickens challenged with heterologous H5N1 HPAI viruses, and reduced virus shedding [522;524;525]. Boosting with an inactivated vaccine after 7-12 days seemed to provide either limited or no increase in protection. Two studies suggested that boosting might improve responses to a small degree in chicks with maternal antibodies [497;603]; however, this may depend on the maternal antibody titer (section 15.9).

15.8.2 Experimental Vaccines for Mass Administration

Mass administration methods, which are faster, more efficient and less labor intensive, would be desirable in an outbreak. However, they should be demonstrated to be effective before use. Factors to consider include both the efficacy of the vaccine by the chosen route, and the effect of any variations in vaccine dose (e.g., if birds drink different amounts of water or inhale different amounts of aerosolized vaccine). In addition to NDV vaccines, some experimental vectors such as adenoviruses and infectious laryngotracheitis herpesvirus may be suitable for administration in sprays or drinking water to birds in the field [45;47;639]. Adenovirus-vectored vaccines, given orally, have been promising in field trials, with one company reporting that 66% of the birds were protected [640]. However, some adenovirus-vectored avian influenza vaccines were less effective when they were administered by the mucosal (intranasal) route than by subcutaneous administration [47]. The use of aerosolized, inactivated avian influenza viruses has also been investigated [641;642]. In addition, various vaccines are in development for *in ovo* use at the hatchery [45;47;454;551;640;643;644].

15.9 Maternal Antibodies

Maternal antibodies may result from infection or vaccination of the hen. In birds, these antibodies are deposited in the yolk sac, and transferred to the embryo during its development in the egg and shortly after hatching ([645] cited in [636]; [646;647] cited in [504]). They are generally reported to persist in poultry for up to 4 weeks [35;525], although one study of chickens with high levels of maternal antibodies reported that complete waning took 35 days [497]. Maternal antibodies to AIV were found for up to 4 weeks in the chicks of vaccinated rheas (*Rhea americana*) and scarlet macaws (*Ara macao*), during an avian influenza vaccination campaign in zoo birds [476].

Antibodies from AIV vaccination may be clinically protective in some young birds for a short time after hatching, when they are exposed to closely related viruses. In one study, maternal antibodies to an inactivated H5N2 (A/duck/Potsdam/1402-6/1986) vaccine protected chicks from clinical signs up to the age of 10 days, when they were challenged with a related H5N2 HPAI virus (A/chicken/Italy/8/98) [637]. Mortality was 0% in chicks challenged on day 3 of life, and 10% in chicks challenged on day 10. Virus shedding was not significantly suppressed at either time. The same maternal antibodies were not protective against a recent clade 2.2.1 variant Asian lineage H5N1 HPAI virus (A/chicken/Egypt/NLQP-0879/2008) [637]. Maternal antibodies appeared to have a small protective effect against an H5N1 virus in another experiment, if chicks were challenged on the first day of life; however, most of these birds (81%) also died [648]. In two other studies, maternal antibodies induced by three different inactivated H5 vaccines were not protective against Asian lineage H5N1 viruses in most 11- or 14-day-old, nonvaccinated chickens: these antibodies were only able to delay, but not prevent, clinical signs and death [636;649].

Maternal antibodies may interfere with successful vaccination in birds, as they do in mammals [141;143;504;636;637;648;649]. The degree of interference can be influenced by a number of factors [504]. In the breeding birds, this may include the type(s) and frequency of vaccination, and the interval between immunization and the collection of the eggs. Some additional sources of variability are the type of birds, their growth rate, the specific MDA levels transferred to the egg/chick (levels can be heterogeneous in a bird's offspring), the vaccination protocols and vaccine type(s) used, and the timing of the exposure to the challenge virus. It may be possible to overcome the effects of maternal antibodies with HVT-vectored or fowlpox-vectored vaccines, more effective vaccines, prime-boost protocols, repeated boosters, or a combination of methods [494;497;504;516;525;603]. One group suggested that the use of different vaccines and vaccination protocols in breeders and their offspring might also be helpful [504].

15.9.1 Fowlpox-vectored Vaccines

Some reports have suggested that live fowlpox-vectored H5 vaccines may be able to overcome maternal antibody titers to AIV in one-day-old chicks [469;504;505]. In one study, chicks with varying levels of antibodies responded well to one vaccine ([505] cited in [94]). More recently, maternal antibodies to H5 viruses were shown to interfere somewhat with fowlpox-vectored vaccines in day-old chicks [504]. This effect could not be overcome even when the vaccine doses were increased. Nevertheless, priming appeared to be effective. When the birds were boosted with a heterologous inactivated H5 vaccine at 21 days, the resulting HI titers were significantly higher than when birds were vaccinated with 2 doses of the inactivated vaccine [504]. Another group administered hyperimmune serum to H5 as a proxy for maternal antibodies in 8-day-old chicks [516]. These antibodies decreased responses to a fowlpox-vectored vaccine, and also reduced survival after virus challenge. However, vaccination also appeared to have primed the immune response in this study. The OIE notes that boosting with an inactivated vaccine may be required after 2-3 weeks, in the presence of very high maternal antibody titers [94].

15.9.2 NDV-vectored Vaccines

One study reported that a live NDV-vectored H5 vaccine (NewH5™), given at 10 days of age, was effective in chicks with high antibody titers to AIV (and NDV) [494]. However, this was based on the prevention of clinical signs when the chicks were challenged with an HPAI virus from the same (Mexican) lineage; virus shedding was not measured. Another group administered hyperimmune serum to H5 as a proxy for maternal antibodies in 8-day-old chicks [516]. These antibodies decreased responses to NDV-vectored vaccines, and also reduced survival after virus challenge.

15.9.3 HVT-vectored Vaccines

HVT-vectored vaccines seem to be able to overcome maternal antibodies in day-old chicks. In the laboratory, they have provided clinical protection (against H5N1 HPAI viruses) in 70-95% of chicks with maternal antibodies, and also reduced virus shedding [497;525;603]. One study reported that there was no improvement in protection, when chicks with moderate antibody titers were boosted with an inactivated vaccine, 7-10 days later [525]. Two other studies, one in birds with high levels of maternal antibodies, found that protection seemed to be improved by boosting, although the effects were relatively small [497;603]. In one of these studies, the closeness of the match between the inactivated vaccine and challenge virus also influenced the results [603].

15.9.4 Inactivated Vaccines

Several studies have confirmed that maternal antibodies can interfere with AIV vaccination when inactivated vaccines are used in young chickens [141;143;504;636;637;648;649]. However, the magnitude of their effect might differ, depending on the source of the antibodies and the challenge virus. In one experiment, chicks with maternal antibodies to an H5N2 vaccine were clinically protected and had reduced virus shedding, when they were vaccinated at 10 days of age, and challenged with an Asian lineage H5N1 virus 24 days later [636]. However, HI titers were lower and virus shedding greater, compared to chicks without maternal antibodies. In another study, maternal antibodies to an H5N2 virus (A/duck/Potsdam/1402-6/1986), did not seem to interfere significantly with an Asian lineage H5N1 vaccine, when the birds were vaccinated once at 3 or 14 days of age, and challenged at 35 days of age with the homologous H5N1 virus [637]. These birds were clinically protected, and shed less virus than nonvaccinated birds, with no significant effect of maternal antibodies or age noted on virus shedding. In contrast, vaccination at 3 days of age interfered with the immune response if these chicks received the same H5N2 vaccine as the hens. In this case, vaccinated chicks without maternal antibodies were partially protected from clinical signs, when challenged with the H5N1 HPAI virus, while vaccinated chicks with moderate levels of maternal antibodies all died. Another group reported that maternal antibodies interfered with two inactivated vaccines, in birds challenged with an Asian lineage H5N1 HPAI virus, even when the titers were below the levels detected by the HI assay [649]. In an additional study, a single dose of an H5N1 vaccine had minimal effect on clinical signs, and no effect on any transmission parameters, when the birds were the offspring of multiply-vaccinated breeders and were vaccinated on the first or 10th day of life, and challenged at 28 days of age with an Asian lineage H5N1 HPAI virus [648].

15.9.5 Maternal Antibodies to the Vector

Maternal antibodies to the vector might also interfere with vaccination when using a vectored HA vaccine. For information about these antibodies, see sections 5.4.1, 5.4.2 and 5.4.3 for fowlpox-vectored, NDV-vectored and HVT-vectored vaccines, respectively.

16. LIMITATIONS OF VACCINATION

Individual responses to vaccination are variable, and some birds may not mount a good immune response. The level of immunity in each bird, and suppression of virus shedding, is influenced by vaccine factors including potency and the closeness of the match with the field virus, as well as the effectiveness of vaccine administration (e.g., the maintenance of an effective cold chain and proper administration), and possibly the route and site of immunization [73;134;136;137;141;142;147;211;324;342;368;468;502;511;512;538;564]. Protection can also be affected by host factors such as species and age [142;468], as well as general health and immunosuppression (parasitism, poor nutrition, stress, etc.). Even solid immunity can be overwhelmed by a high challenge dose, and some birds may be exposed before they have time to develop immunity.

Although laboratory studies suggest that vaccination can sometimes eliminate virus shedding or reduce it to undetectable levels [116;138;141;481;498;574], vaccines cannot completely prevent virus shedding in the field [46;49;455]. By suppressing the clinical signs that allow infected flocks to be detected, vaccination could increase the risk that AIV will be maintained in the population, unless it is part of a comprehensive program that includes good surveillance.

16.1 Monitoring for Vaccination Coverage and Efficacy

The effectiveness of the vaccination program should be monitored in the field to ensure that the vaccine and administration methods are effective [48;455]. Coverage can be evaluated by assessing compliance (e.g., by checking for leg bands placed at the time of vaccination), and the efficacy of inactivated vaccines can be monitored with antibody titers [496]. Currently, chickens and turkeys are the only species where antibody titers are known to correlate with protection [342;496]. The OIE suggests that “a large part” of the population should seroconvert, and that antibody titers should consistently reach at least the established threshold values for protection [496]. A minimum HI titer of 32-40 has been recommended [46], and in the U.S., HI titers of 32 or greater are used to gauge protection during vaccine licensing [593]. Higher titers (e.g., 128) suggest that the vaccine may be more likely to significantly reduce virus shedding [142]. Nevertheless, titers as low as 16 may protect birds against clinical signs ([208;592]; [25] cited in [471]) and have significantly reduced virus shedding in some laboratory experiments [134]. Individual chickens are occasionally protected even if they do not have detectable titers [137]. In some flocks (e.g., free range birds), previous exposures to other AIV could influence the magnitude of the titer [626].

Chickens immunized with vectored HA vaccines can develop high titers in the HI test, but they may be protected from heterologous field strains even if they do not produce significant titers to these viruses, or if the titers are lower [344;466;469;481;494;497;502;515;520;522;524;525]. Tests that can consistently predict protection from vectored vaccines against both homologous and heterologous strains would be desirable [466], but are not yet available.

The predictive power of antibodies is still uncertain in waterfowl [327;354]. Some studies report that ducks can be protected from clinical signs and virus shedding even when the vaccine-induced antibody titer, measured by HI and/or virus neutralization, is low or undetectable [111;135;209;211;354].

17. IDENTIFICATION OF VACCINATED ANIMALS

Vaccinated animals should be permanently identified, using a tamper-resistant system. Accurate vaccination records must be maintained as directed by USDA APHIS VS, and shared with other regulatory authorities as required.

18. LOGISTICAL AND ECONOMIC CONSIDERATIONS IN THE DECISION TO VACCINATE

Summary

The technical feasibility of vaccination and funding for a vaccination campaign should be evaluated before deciding to vaccinate. The assessment should include the availability of sufficient supplies of an effective, safe, and acceptably well-matched vaccine with the same HA type; the availability of DIVA tests and their validation in the species to be vaccinated; the logistics of vaccine administration; and the resources and technologies needed for associated activities including animal identification, tracing, movement permitting and surveillance to prove freedom from disease. The impact of vaccination on other eradication activities should also be determined.

The effectiveness and form of a vaccination campaign can vary with the epidemiology of the outbreak. Before prophylactic vaccination, the likelihood of HPAI virus introduction should be evaluated with a risk analysis. An analysis of the outbreak should be produced for emergency vaccination. In some outbreaks, wild birds must be taken into consideration. Because HPAI viruses have occasionally affected people, the implications for human health should also be assessed.

The pros and cons of vaccination compared to pre-emptive culling should be considered. This includes effects on trade and exports, market shocks, potential restrictions on marketing products from vaccinated birds, the types of stakeholders affected (e.g., small-scale operators with limited safety nets vs. large-scale operators), the extent of the outbreak and other factors such as the disruption of tourism or impacts on local economies.

Consideration should be given to whether poultry with rare or unusual genetic backgrounds, parent flocks, endangered species, zoo birds and other valuable birds can be successfully protected with biosecurity measures, and whether vaccination would be beneficial. Their degree of isolation from commercial poultry should be part of this analysis.

The presence of any HPAI virus in poultry, including backyard poultry, affects international trade; however, the OIE definition of 'poultry' excludes birds kept as pets or for exhibition and competitions (e.g., zoo birds or racing pigeons). After an outbreak, HPAI-free status can be regained 3 months after stamping out has eradicated the virus. If the provisions in the Terrestrial Animal Health Code are followed, vaccination does not interfere with a country's, zone's or compartment's OIE status for avian influenza, and it need not interfere with international trade. In practice, vaccination for avian influenza might lead to trade barriers.

18.1 Technical Feasibility of Vaccination

To conduct an effective vaccination campaign, an effective, safe, acceptably well-matched and sufficiently potent vaccine of the same HA type must be available, and the vaccine supply must be adequate to carry out the vaccination strategy in a timely manner. The vaccine and vaccination strategy should be expected to provide immunity quickly enough to stop or slow virus transmission. Consideration should also be given to whether flocks would need to be vaccinated more than once (for increased efficacy or to protect birds from continued virus circulation), and whether the duration of immunity from the vaccine is acceptable. Boosters may be necessary in poultry that have long production cycles (e.g. layers, breeders and turkeys) [46;48] and in zoo birds. Boosters might also be needed in other birds in the field, for sufficient efficacy. If there is only enough vaccine for the initial needs, the feasibility of procuring additional supplies should be determined before beginning the campaign. Contingency plans should evaluate the number of vaccine doses needed under various scenarios. Before an outbreak, plans should also be made for vaccine distribution and administration, including the maintenance of an effective cold chain.

The availability and cost of DIVA tests and other surveillance methods, and their level of validation in the species to be vaccinated, should be assessed.

There must be adequate numbers of trained personnel to conduct vaccination and other associated activities including surveillance, animal identification, tracing and movement permitting. The ability of vaccination crews to maintain effective biosecurity is also important. Some vaccines (e.g., fowlpox-vectored and HVT-vectored H5 vaccines) are usually administered at the hatchery, which helps alleviate biosecurity concerns [466;469;496;519;520]. However, any boosters are usually administered after a minimum of 2 weeks, and would likely need to be given in the field. In emergency vaccination, consideration should be given to the effect of vaccination activities on the number of responders available for diagnosis, culling or decontamination of infected farms [650]. Conversely, vaccination may allow the culling of some flocks to be delayed, and relieve pressures on personnel and resources involved in slaughter and disposal.

18.2 Epidemiological Considerations

The effectiveness and form of a vaccination campaign can vary with the epidemiology of the outbreak [407;496]. Before prophylactic vaccination, the likelihood of HPAI virus introduction should be evaluated with a risk analysis. An analysis of the outbreak should be produced for emergency vaccination.

The type(s) of poultry production systems and their biosecurity levels influence the risk that HPAI viruses will be introduced, and also how well they will spread [407;496]. Factors to consider include the structure and organization of the poultry industry, level of biosecurity, types of farming practices (e.g., free range, industrial, rural), and market chains; the size and density of the poultry population and its geographic distribution; and the species of birds affected [48;496]. The avian influenza status of trading partners and neighboring countries should be assessed [496]. The number and location of foci, and the length of time the disease has been present in the country should also be considered in emergency vaccination. Movements of birds, people and vehicles should be evaluated to estimate whether the virus has been spread widely from any premises known to be infected. A single focus or limited outbreak is expected to be easier to control than one that has become disseminated. Under normal conditions, airborne transmission of HPAIV between farms is thought to be unimportant [360].

In some outbreaks, wild birds may need to be taken into account. Although avian influenza viruses adapted to poultry may rarely become re-established in wild populations [95], migratory wild birds have

introduced the Asian lineage of H5N1 viruses and reassortants such as H5N8 into new areas [15-19;22;30]. Whether these viruses can be maintained long-term in wild bird populations is still unclear [28-30;196;223;224].

18.3 Economic Viability of Vaccination

Economic viability plays an important role in the decision to vaccinate. There must be sufficient funding for the purchase of the vaccine, vaccine delivery and administration, and animal identification. In addition, funding must be provided for follow on traceability of the vaccinated flocks and surveillance to prove freedom from disease. The availability of indemnity can affect whether farmers are willing to report illness, and thus influences clinical surveillance [496].

The direct costs of vaccination include:

- Investment costs – e.g., vaccine development, vaccine availability and vaccine delivery infrastructure [651]
- Variable or recurrent costs including the cost of vaccines and delivery [651]
- Costs to identify vaccinated birds, permit their movement, and conduct surveillance to prove freedom from disease

There may also be some indirect costs from vaccination such as lost productivity caused by stress to the birds, disruptions of agricultural routines, and adverse reactions to the vaccine [651].

The pros and cons of vaccination compared to pre-emptive culling should be considered. Culling flocks that were never infected can cause economic losses without necessarily affecting disease spread. However, mass vaccination or inappropriately targeted vaccination is expensive [652], and there is an increased risk that infected flocks will not be detected because clinical signs are suppressed. The value of the poultry flock may influence whether to vaccinate or cull [48].

The overall impact of vaccination on international trade in poultry products, including longer-term impacts on trade, is an important consideration. Vaccination is expected to be most beneficial when the outbreak ends sooner, or when vaccination allows the most stringent disease control measures to be carried out in a limited area [651]. It is also expected to be beneficial if it impacts a production sector in an area where there will be a limited effect on exports (e.g., zoning will be possible/practical). If the outbreak can be stopped with rapid culling, there is likely to be short-term distress but little long-term effect on livelihoods, especially if indemnity can be provided [651]. However, if culling is more widespread or the disease is out of control, vaccination may save livelihoods [651].

Vaccination is likely to be beneficial to livelihoods when it can:

- Provide effective disease control with little depopulation, especially if indemnity is not available for culled flocks [651].
- Prevent national markets from being disrupted or rapidly restore them [651]
- Minimize other economically important factors such as the disruption of tourism or impacts on local economies [651]
- Reduce the time export markets are lost

Vaccination may be particularly beneficial to small-scale operators whose safety nets are limited [651]. If stamping out is used, it is possible for culling to have a minimal effect on the national economy while having a significant effect on the livelihoods of the people who are directly affected, especially

smallholders and small-scale traders who depend on regular cash flow from agriculture. Although indemnity may be available for flocks that must be destroyed, it rarely covers the cost of lost production time and cash flow [651]. The emotional impact of the destruction of apparently healthy birds should also be taken into consideration [651], especially when outbreaks include pet birds, zoo birds, high value or unique flocks.

Consideration of market shocks should be part of the economic analysis. Market shocks can result from loss of consumer confidence (decreased demand), very severe culling or the closing of markets [651]. Consideration should be given to whether meat and eggs from vaccinated poultry can be safely marketed, and to whether consumers are willing to accept these products as safe. If the latter is not possible, there may still be market shocks from consumer fear even when poultry products have no risk of containing HPAI. If export markets are affected by vaccination, domestic markets can also be affected, because products that were once exported may be sold within the country, lowering prices [651]. Producers for domestic markets can also be affected by quarantines [651]. The cost of keeping and feeding flocks through the quarantine period should be taken into consideration.

18.4 Vaccination of Poultry with Rare or Unusual Genetic Backgrounds, Zoo Birds and other Valuable Birds

Consideration should be given to whether poultry with rare or unusual genetic backgrounds, parent flocks, endangered species, zoo birds and other valuable birds can be successfully protected with biosecurity measures, and whether vaccination would be beneficial. Their degree of isolation from poultry should be part of this analysis. The Association of Zoos and Aquariums (AZA) has developed and submitted plans, including vaccination, to protect zoo birds in the event of an H5N1 HPAI outbreak [542]. Details on vaccination in zoos and special collections can be found in section 19.

18.5 Effect of Vaccination on Regaining OIE HPAI-Free Status

The presence of any HPAI virus in poultry affects international trade [35]. For the purposes of international trade, the OIE defines poultry as domesticated birds used to produce meat, eggs or other commercial products, as well as birds used to restock game birds, birds used to breed other categories included in the definition, and all fighting cocks regardless of their purpose [35]. This definition includes backyard poultry, but not captive birds kept for other reasons, including pets; birds kept for shows, races, exhibitions and competitions (e.g., zoo birds or racing pigeons); and birds kept for breeding or sale of the “non-poultry” categories. Although HPAI viruses in birds other than poultry, such as wildlife, are events of epidemiological significance and must be reported, they should not result in immediate trade bans provided poultry or poultry products are unaffected [35;455].

A country, zone or compartment may be classified as free of either 1) HPAI viruses and H5 or H7 LPAI viruses, or 2) free of HPAI viruses. To be defined as virus-free, there must have been no evidence of infection among poultry during the last 12 months [35]. After an outbreak, HPAI-free status can be regained 3 months after stamping out has eradicated the infection, provided that surveillance has demonstrated that the virus was completely absent during those three months [35].

If provisions in the Terrestrial Animal Health Code are followed, vaccination does not interfere with a country, zone or compartment’s OIE status for avian influenza, and it need not interfere with international trade [35]. The Terrestrial Code requires that surveillance be conducted in vaccinated populations to ensure that flocks are free of the virus. The vaccine used should also comply with provisions for H5 and H7 vaccines in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, and the country

must provide evidence to demonstrate that the vaccination program is effective. In practice, vaccination for avian influenza might lead to trade barriers [455].

18.6 Effect of Vaccination on Human Health

Because HPAI viruses have occasionally affected humans, the risk assessment should include potential impacts on human health [48]. See section 20.

19. VACCINATION IN ZOOS AND SPECIAL COLLECTIONS

Summary

Vaccination may be considered in zoos and special collections during outbreaks, to protect rare and valuable birds. These facilities are often unable to completely exclude wild birds, and indoor confinement during an outbreak may be problematic.

Asian lineage H5N1 HPAI viruses have infected a variety of avian species, either asymptotically or symptomatically. In some cases, the mortality rate may be high. H5N1 outbreaks have been reported in a few zoos in Asia and Egypt. Vaccination, combined with other measures, was used to help decrease mortality in a zoological collection in Hong Kong. Other subtypes of HPAI viruses have also been reported on rare occasions in wild birds, and may cause clinical signs. Asian lineage H5N8 HPAI viruses have been found in subclinically infected as well as sick or dead birds.

Serological DIVA strategies would be desirable in zoo birds, but have not been validated for these species. Sentinel birds would be used in U.S. zoos during vaccination campaigns, and sick or dead birds should be tested for AIV. Handling carries health and welfare risks in zoo species, and may limit the direct sampling of healthy birds.

There are very few challenge studies of vaccination in captive wild species, due to unacceptable risks to the birds' health. Vaccination was clinically protective and decreased H5N1 virus shedding in domesticated rock pigeons and Gyr-Saker hybrid falcons. One falcon was protected although it never developed a good HI antibody titer. Vaccination has also protected various species of ducks from challenge by H7 and/or H5 HPAI viruses. Although vaccination was clinically protective in golden pheasants, there was no effect on virus transmission.

HI titers have been reported from a number of prophylactic vaccination campaigns in zoos. Most campaigns were intended to protect the birds from Asian lineage H5N1 HPAI viruses, but one was conducted during an H7N7 HPAI outbreak among poultry in the Netherlands. All published vaccination campaigns used commercial, heterologous, inactivated vaccines made for poultry. They included one H7N1 vaccine and various H5N2 or H5N9 vaccines. Higher doses were given to larger birds, but the specific dose varied between zoos. There is evidence that some doses were inadequate for heavy birds, especially among the Struthioniformes (ostriches and their relatives). Conversely, extremely high doses at one injection site (e.g., 10 ml of one vaccine given to Struthioniformes) may inhibit the development of good HI titers. Subcutaneous and intramuscular vaccination appeared to be equally effective, but definitive conclusions could not be made. Some species, such as pelicans and cormorants, have prominent subcutaneous air sacs, which might interfere with subcutaneous vaccination.

Approximately 1-8% of zoo birds had titers to H5 viruses before vaccination campaigns in the E.U. Titers were mainly found in birds that are usually kept outdoors in enclosures with ponds. They did not seem to interfere with serological responses to vaccination.

Whether HI titers correlate with protection from challenge is not known for zoo birds. Individual zoos defined a protective titer, extrapolated from studies in poultry and/or humans, as 16, 32 or 40. Overall, 54% of the birds in E.U. zoos seroconverted with a titer of at least 16, and 49% with a titer of at least 32, after the first dose of vaccine. After a booster, the seroconversion rate was 82% for titers ≥ 16 , and 76% for titers ≥ 32 . These values differed between individual zoos.

Zoos reported that some species might be protected by a single dose of vaccine, based on the development of protective titers. Other species did not develop titers at this level, even after several doses. Because the correlation (if any) between HI titers and protection is not known, birds without “protective” titers might nevertheless have been protected by vaccination.

HI titers after vaccination differed markedly between orders, and between species within an order. Some species had weak responses with one vaccine, but responded to another vaccine or dose. Flamingos usually developed relatively high GMTs and had good seroconversion rates. Most species of Galliformes also had good responses, but there were some differences between species, and helmeted guineafowl (*Numida meleagris*) responded poorly in several studies. Most Anseriformes responded well in four individually published vaccination campaigns, with 84-89% of these birds developing protective HI titers (32 to 40). However, some species responded poorly or required a third dose for a protective response. Most Ciconiiformes responded, with 92-93% developing HI titers of at least 32, and titers persisted well in this order at one zoo. Weak responses were reported in Humboldt penguins (*Spheniscus humboldti*), cormorants and pelicans, Columbiformes and owls in some campaigns. Serological responses among psittacine birds varied with the species and zoo.

Limited data from the E.U. vaccination campaigns suggest that HI antibody titers may persist in some species or orders, but not others, for 6 months. Based on these titers, some authors have suggested that zoo birds should be re-vaccinated at least once a year, and that a 6-month booster might be useful in many orders. A disadvantage is that twice-a-year vaccination would require the birds to be handled again, and might interfere with the breeding season.

Maternal antibodies were reported in the chicks of some zoo birds, for as long as 4 weeks of age. One recommended protocol in this situation is immunization at 3–4 weeks and 9–10 weeks of age.

Under some circumstances, the USDA may allow the vaccination of birds in zoos that are members of the Association of Zoos and Aquariums (AZA) or AZA-equivalent (i.e., meet or exceed AZA standards). Plans to be followed in vaccination campaigns have been published. Prophylactic vaccination of extremely rare, endangered birds can begin as soon as state or federal animal health authorities have approved the request. Other birds would be approved for vaccination only in an outbreak. Zoos may prepare a list of species to be vaccinated, for pre-approval if the zoo falls in a Surveillance Zone during an outbreak. Vaccination of mammals in zoos is currently not allowed.

Vaccination may be considered in zoos and special collections during outbreaks. Many of these birds belong to endangered species, and are rare and valuable [542]. Some species, such as the California condor, have irreplaceable conservation value [542]. Zoos and other special collections are often unable to exclude wild birds from the facility, or to completely prevent contact between captive and wild birds through netting or cages [542]. The highest infection risk is in certain outdoor exhibits, such as outdoor ponds [477]. Indoor confinement during an outbreak can be problematic for zoos, as they may not have enough facilities to appropriately house all of the birds and susceptible mammals in their collections [477;542]. Indoor confinement of indefinite duration may also cause stress, welfare problems and increased exposure to disease agents, resulting in illnesses such as aspergillosis and bumblefoot [472;477]. An additional benefit to vaccination is that it may reduce the risk to human caretakers [542;598]. This may be a particularly important consideration in situations when there is close contact with birds, including falconry [598].

The European Food Safety Authority (EFSA) recommends biosecurity as the first line of defense in zoos, with continuous clinical monitoring of both zoo birds and wild birds that visit zoos [477]. Vaccination should be considered if a risk assessment suggests that zoo birds cannot be protected with biosecurity alone [477]. It should be noted that some mammalian species are also susceptible to Asian lineage H5N1 HPAI viruses, as well as to some other AIV [67;92;188;190;192;226-236;239;240;254;267;269;274-281;283;429;653-655].

Prophylactic zoo vaccination campaigns have been conducted in some countries when the Asian lineage H5N1 viruses were detected in wild birds. A number of these vaccination programs were in European zoos [471;474-477;656]. As of 2013, these vaccination campaigns had been discontinued due to a perceived low risk of infection [657]. Additional countries reported to have vaccinated zoo birds or captive birds other than poultry include Egypt, the United Arab Emirates, Kuwait and Singapore [149;470;473], as well as Hong Kong during an H5N1 outbreak [658]. The Netherlands conducted a vaccination campaign in zoo birds during the 2003 H7N7 HPAI outbreak in poultry [472]

19.1 Infections and Outbreaks in Zoos and Exotic Birds

Relatively little is known about the overall susceptibility of zoo birds to HPAI viruses. Many of these birds are probably susceptible to infection with Asian lineage H5N1 HPAI viruses, which have infected a number of wild or captive species and sometimes cause fatal illness [25-29;182;183;188-194;196;598]. Some reassortants belonging to this lineage can also cause serious illnesses in birds other than gallinaceous poultry. In particular, Asian lineage H5N8 viruses have been linked to clinical cases and deaths in raptors, waterfowl and other species, although they have also been found in apparently healthy wild birds, and some experimental infections (e.g., in mallard ducks) caused no clinical signs [16;20;22;23;184;350;351]. Other HPAI viruses reported on rare occasions in wild birds include an H5N3 virus, which was isolated from an outbreak among terns in the 1960s [185]; a few isolated infections among wild birds that were associated with infected poultry ([659] cited in [186]); and HPAI H5N2 viruses, which were recently detected in asymptomatic wild ducks and geese in Africa [186]. An H7N1 HPAI virus caused illness, with a high mortality rate, in a wild siskin and canaries that were accidentally exposed to the siskin [187].

H5N1 outbreaks have been reported in zoological collections in Penfold Park and Kowloon Park, Hong Kong (People's Republic of China) [658], zoos in Thailand [660] and Egypt [312], and in Ragunan Zoo, Jakarta (Indonesia) [477]. Vaccination, combined with other measures, was used to help decrease mortality at Kowloon Park [658]. Waterfowl received two doses of an inactivated H5N2 vaccine, while flamingos were given a single dose of an experimental inactivated recombinant H5N3 vaccine. Other

control measures in zoos included isolation and quarantine of infected animals, selective culling, treatment of some animals, disinfection of affected areas, and surveillance of captive and migratory birds for AIV [477;658].

19.2 DIVA Strategies in Zoo Birds

Serological DIVA strategies would be desirable in zoo birds, but have not been validated for these species. Zoo birds may also be infected with LPAI viruses during contact with wild birds, causing false positives in DIVA tests [477]. In the U.S., sentinel birds should be used in zoos during vaccination campaigns [542]. Sick or dead birds should also be tested for AIV [542]. Handling of zoo birds carries health and welfare risks, and may limit the direct sampling of healthy birds.

19.3 Challenge Studies

There are very few challenge studies of vaccination in captive wild species, due to unacceptable risks to the birds' health. Vaccination was clinically protective and decreased virus shedding in Gyr-Saker (*F. rusticolus* × *F. cherrug*) hybrid falcons, which were immunized with two doses of a commercial inactivated H5N2 vaccine (A/duck/Potsdam/1402/86), and challenged with a lethal dose of an Asian lineage H5N1 HPAI virus [598]. HI titers were detected in 90% of the vaccinated falcons after the first dose of vaccine, rose significantly after the booster, and stayed high for at least 5 months. One bird did not have a detectable HI titer after the first dose, and developed a titer of only 8 after the booster; nevertheless, it did not become ill. Vaccination has also protected various species of ducks from challenge by H7 and/or H5 HPAI viruses [111;135;200;208-211;324;327;331;336]. In domesticated rock pigeons (*Columba livia*), a reduced dose of a commercial H5 vaccine (A/duck/Potsdam/2243/84 H5N6) was clinically protective against H5N1 viruses belonging to clade 2.1.1 (Indonesian isolate) and clade 2.2 (isolate from Turkey), and virus shedding was not detected ([599] cited in [342]). Vaccination also protected golden pheasants from clinical signs, but there was no effect on virus transmission [200].

19.4 Prophylactic Vaccination Campaigns Reported in Zoos

HI titers have been reported from a number of prophylactic vaccination campaigns in zoos. Whether these titers correlate with protection from challenge is unknown. Information has been published for a vaccination campaign in the Netherlands during the 2003 H7N7 HPAI outbreak in poultry [472], and for campaigns in Singapore [470], the United Arab Emirates [473], the Netherlands [471], Denmark [474], Switzerland [475], France [476] and other European countries [477;656] against Asian lineage H5N1 HPAI viruses found in wild birds and/or poultry.

19.4.1 Titers to AIV Before Vaccination

Few birds had titers to H5 viruses before zoo vaccination campaigns in the E.U. [477]. Approximately 99% of the birds tested in France [476] and Spain [656], 98% in the Netherlands [471], 97% in Switzerland [475] and 92.5% in Denmark [474] were seronegative for H5 AIV before vaccination. In Spain, 99% of birds were also seronegative for antibodies to the viral nucleoprotein, demonstrating low exposure overall to AIV [656]. Pre-vaccination titers were mainly found in members of Anseriformes, Ciconiiformes and Phoenicopteriformes [474;477;656]. These orders contain birds that are usually kept outdoors in enclosures with ponds [477], which attract wild birds. Pre-vaccination titers did not seem to inhibit the development of HI titers from vaccination [477].

19.4.2 Vaccines Used and Dose Effects

All published vaccination campaigns used commercial, heterologous, inactivated vaccines made for poultry. A commercial H7N1 vaccine (A/chicken/Italy/473/99) was employed during the H7N7 outbreak

in the Netherlands [472], and various H5N2, H5N3 or H5N9 vaccines were used in other campaigns [471;473-477;656]. Vaccine strains included A/duck/Potsdam/1402/86 (H5N2) in the Netherlands [471] and France [476], A/chicken/Mexico/232/95/CPA (H5N2) in Switzerland [475] and A/chicken/Mexico/232/94/CPA (H5N2) in Singapore [470]. The United Arab Emirates used the Nobilis Influenza H5™ vaccine (Intervet International), which contains A/duck/Potsdam/1402/86 (H5N2) [473], and Denmark used Gallimune Flu H5N9™ (Merial, Milanofiori, Italy), which contains A/turkey/Wisconsin/68 (H5N9) [474]. Spain initially used an H5N9 vaccine (A/chicken/Italy/22A/1998) in 2006, and re-vaccinated some birds in 2007-2008 with a reverse genetics H5N3 vaccine (A/chicken/VN/C58/04) that had high efficacy in chickens and ducks in laboratory experiments [656]. The 13 E.U. member states analyzed by the EFSA were not individually identified, but used commercial vaccines containing A/turkey/Wisconsin/68 (H5N9), A/chicken/Italy/22A/98 (H5N9), A/chicken/Italy/22A/H5N9/1998 (H5N9), A/duck/Potsdam/1402/86 (H5N2) or A/chicken/Mexico/232/94/CPA (H5N2) [477].

The interval between doses varied between vaccination campaigns, and followed the manufacturer's recommendations for poultry [477]. The vaccine dose varied with body weight, and differed between campaigns. Smaller birds typically received 0.2-0.25 ml, while larger birds were given up to 0.5 ml, 1 ml or 2.5 ml, depending on the zoo and species [471;473-476;656]. A zoo in Singapore used a dose of 0.5 ml/kg body weight [470], and one European zoo administered 10 ml to ostriches and 5 ml to nandus [477].

An overall analysis of E.U. vaccination campaigns concluded that the poultry dose is suitable for most species of zoo birds, but doses adjusted for body weight induce higher titers in some larger species [477]. Some individual zoos reported that lower doses were inadequate for heavy birds, especially among the Struthioniformes (ostriches and their relatives) [472;476]. In the Dutch H7 vaccination campaign, all birds that weighed 1.5 kg or more received 0.5 ml of vaccine, and serological responses decreased as body weight increased [472]. In this campaign, only half of the rheas and emus responded with a titer of at least 40, and the single ostrich did not respond. In a later H5 vaccination campaign in the Netherlands, the vaccine dose was 0.25 ml to 2.5 ml, based on body weight, and 82% of the Struthioniformes (including 1 emu, 4 of 5 ostriches, and 13 of 16 rheas) developed titers of at least 40 [471]. In Denmark, where birds heavier than 20 kg received 1 ml of vaccine, all three ostriches and emus, as well as all rheas, seroconverted with a titer of at least 32 [474]. Struthioniformes also received 1 ml in a French vaccination campaign [476]. Rheas responded in this campaign, but ostriches failed to develop detectable titers, suggesting that the vaccine dose was too low. Ostriches later given a dose of 2.5-5 ml, using the same vaccine, developed protective titers [476]. The E.U. analysis reported that extremely high doses at one injection site (e.g., 10 ml of one vaccine given to Struthioniformes) seemed to inhibit the development of HI titers [477].

19.4.3 Routes of Inoculation in Vaccination Campaigns

Some countries used subcutaneous vaccination, while others employed the intramuscular route [470;471;473-477;656]. The available results suggest that there is no significant difference between routes; however, the route was not known in many cases, and definitive conclusions cannot be made [477]. Some species, such as pelicans and cormorants, have prominent subcutaneous air sacs, which might interfere with subcutaneous vaccination [477].

19.4.4 Adverse Effects Associated with Vaccination

(See also section 7.3, Vaccine Safety)

Vaccine manufacturers have not labeled vaccines for use in zoo birds or assessed their safety in these species; however, adverse effects were evaluated in some published reports of prophylactic vaccination campaigns. No vaccine-related side effects or deaths were reported at the Singapore Zoo or a zoo in the United Arab Emirates [470;473]. There were no indications of vaccine-related deaths during the H7 vaccination campaign in the Netherlands, as determined by necropsies of birds that died during the campaign [472]. All zoos reported a very low rate of adverse effects during H5 vaccination campaigns in the E.U., with local reactions in 0.04% of birds and systemic side effects in 0.015% [477]. Most of these reactions occurred in ostriches (8 out of 14) that had been vaccinated with a 10 ml dose, and nandus (2 out of 10) that developed injection site reactions after subcutaneous administration of 5 ml. Other reported side effects included mild to chronic lameness after intramuscular injection, and a subcutaneous abscess in one penguin, which died in spite of antibiotic treatment. In a separate report from French zoos, systemic reactions including transient weakness, apathy, and anorexia were seen in 2 Anseriformes (*Cygnus atratus*, *Branta sandvicensis*), 2 Ciconiiformes (*Threskiornis spinicolis*, *Platalea leucorodia*), 2 Falconiformes (species not reported), 10 Galliformes (*Acryllium vulturinum*), and 1 Psittaciformes (*Psittacus erithacus*) [476]. In this campaign, the route of administration (subcutaneous vs. intramuscular) did not seem to affect the incidence of side effects.

The greatest risk of mortality to zoo birds is reported to be from the stress and trauma of restraint ([596] cited in [476]). In the E.U. vaccination campaigns, deaths were mainly caused by handling, sometimes in conjunction with subclinical infections [475;477]. At least one death was caused by a hemorrhage during sampling to determine titers [475]. On average, deaths from handling or stress occurred in 0.5% of the birds that were restrained two or three times (vaccinations plus blood collection) [477]. The importance of skilled handling is emphasized by markedly different rates of adverse effects between zoos [477]. Losses may be minimized by conducting vaccination campaigns when zoo birds are handled for other reasons, such as when they are being moved indoors for the winter [471]. This timing would be expected to reduce handling stress, disrupt the breeding season less, and provide enough time for immunity to develop before wild birds begin their spring migration.

19.4.5 Protective Titers in Zoo Birds

The protective titer in zoo birds is usually unknown, as there are very few challenge studies to correlate titers with protection in these species. During vaccination campaigns, some zoos defined a protective titer, extrapolated from studies in poultry and/or humans, as 16 [470;475], 32 [474;476;656] or 40 [471;472].

19.4.6 HI Titers Achieved During Vaccination Campaigns

In an analysis of all vaccination campaigns in E.U. zoos, 54% of the birds seroconverted with a titer of at least 16, and 49% with a titer of at least 32, after the first dose of vaccine [477]. After a booster, the seroconversion rate was 82% for titers ≥ 16 , and 76% for titers ≥ 32 .

Published reports are also available from some individual zoos. During the H7 vaccination campaign in the Netherlands, the first dose of vaccine induced a protective titer (≥ 40) in 36% of the birds, and 81.5% had protective titers after the second dose [472]. The overall geometric mean titers (GMTs) for all vaccinated birds were 20 and 190, respectively, after one or two doses. Higher vaccine doses were given to large birds during the H5 vaccination campaign in the Netherlands, and the GMT after one dose was 37 [471]. Approximately 50% of the birds had protective titers (≥ 40) at this time. After the booster, the GMT was 190, and 80.5% of the birds had protective titers. These titers were all measured against the vaccine strain, A/duck/Potsdam/1402/86 (H5N2). Titers measured against a field strain of H5N1 HPAIV were lower, with a GMT of 61 and protective titers in 61% of the birds. Swiss zoos reported that the

GMT was 65 after the initial vaccination, and 78% of the Galliformes and 98% of the non-Galliformes had protective titers (≥ 16) [475]. After the booster, the GMT was 187, and protective titers occurred in 96% of the Galliformes and 100% of the non-Galliformes. If HI titers ≥ 40 are chosen as the cutoff, 85% of the birds in Swiss zoos developed protective titers after the booster [475]. In French zoos, the protective titers (≥ 32) and GMT were 45% and 273, respectively, after one dose, and 71% and 558 after the booster [476]. In Spanish zoos, 32% of birds reached a titer of 32 after one dose of H5N9 vaccine and 51% after a second dose, with GMTs of 81 and 103, respectively [656]. At the Singapore zoo, 84% of the vaccinated birds had HI titers of at least 16 after two doses [470]. Differences in the seroconversion rate and GMT between individual zoos may be influenced by the composition of species, as well as the specific vaccine and dose; all of these factors were reported to influence HI titers [470-477]

Zoos reported that some species (e.g., flamingos) might be protected by a single dose of vaccine [474;477], while other species may not develop protective titers even after several doses [470]. At the Singapore Zoo, some species of Anseriformes and Galliformes, including two species of guineafowl (*Acryllium vulturinum* and *Numida meleagris*) required two boosters before seroconversion, but others responded to the initial two doses [470]. Cormorants had a partial response, while pelicans and owls did not respond even after two boosters. In the United Arab Emirates (UAE), the initial dose of an inactivated H5N2 vaccine resulted in 100% seroconversion in two species of ducks, crowned crane (*Balearica pavonina*), spotted thick knee (*Burhinus capensis*) and wild turkeys (*Meleagris gallopavo*), although the titers were low in some birds [473]. Danish zoos reported that flamingos had high titers after a single dose, with little increase after a booster [474], but the UAE found low titers after the first dose and high titers after a booster in this species [473]. Because the correlation (if any) between HI titers and protection is not known, birds without “protective” titers might nevertheless have been protected by vaccination.

19.4.6.1 Serological Responses in Different Orders of Birds

The HI titers after vaccination differed markedly between orders, and between species within an order [471-477;656]. Overall, one or more vaccines induced good responses in 74 out of 94 species analyzed in E.U. H5N1 vaccination campaigns [477]. Weak responses occurred with one vaccine or dose in 32 species, although some of these species responded to another vaccine or dose. The vaccination interval might also affect the response [475;477].

Flamingos (order Phoenicopteriformes) usually developed relatively high GMTs and had good seroconversion rates in the various vaccination campaigns [471-477;656]. In the Spanish vaccination campaign, the response seemed to differ between vaccines [656]. Initially, 93 flamingos responded well to 2 doses of an H5N9 vaccine (protective titers in 86% and GMT of 122); however, only 30% of 91 flamingos boosted 18-24 months later with a single dose of an H5N3 vaccine developed protective titers, and the GMT was 18. None of the 4 flamingos that were vaccinated twice with the H5N3 vaccine (and did not receive the H5N9 vaccine) developed protective titers (GMT = 8).

Most species of Galliformes had good serological responses to vaccination [471;472], but there were some differences between species, and helmeted guineafowl (*Numida meleagris*) responded poorly in several studies [470-472;474]. During vaccination campaigns in the Netherlands, only 17% to 38% of this species developed protective titers (≥ 40) [471;472]. Helmeted guineafowl and vulturine guineafowl (*Acryllium vulturinum*) required two boosters before seroconversion (≥ 16) in Singapore zoos [470]. However, 2 of 3 vulturine guineafowl developed titers ≥ 32 in a Spanish vaccination campaign [656]. In this campaign, protective titers (≥ 32) were achieved in 60% of Galliformes immunized with 2 doses of an H5N9 vaccine, but only one of 8 pheasants (*Chrysolophus amherstiae*, *Chrysolophus pictus*, *Lophura nycthemera* or *Phasianus colchicus*).

Anseriformes (ducks and geese) responded well in four individually published vaccination campaigns, with 84-89% of these birds developing protective HI titers (32 to 40) [471;472;475;476]. They were also reported to have relatively high GMTs and seroconversion rates in E.U. vaccination campaigns overall [477]. However, only 57% of Anseriformes in Danish zoos and 11% (H5N3 vaccine) to 67% (H5N9 vaccine) in Spanish zoos developed a HI titer of at least 32 [474;656]. Common/ European eider (*Somateria mollissima*) responded poorly in three vaccination campaigns [471;472;474]. Some but not all species of Anseriformes tended to require a third vaccination during the H5 campaign at the Singapore Zoo [470].

Most Ciconiiformes (herons, egrets, storks, ibis and spoonbills) responded in the Danish and French vaccination campaigns, with 92-93% developing HI titers of at least 32 [474;476]. Titers also persisted well in France; 6 months after the initial vaccination, this order contained the greatest percentage of birds with protective titers [476]. In the Netherlands, 71-75% of the Ciconiiformes developed protective titers (≥ 40) in the H5 and H7 vaccination campaigns, and the GMT varied widely between species, from less than 20 to over 2000 [471;472]. In Spain, only 34% of Ciconiiformes had protective titers (≥ 32) after 2 doses of an H5N9 vaccine [656]. This number increased to 44% in birds that received a single H5N3 booster, 18-24 months later, although the GMT was lower than after the initial vaccinations. The GMT for individual species ranged from 128 to 581 in Swiss zoos, which did not report the percentage of birds that seroconverted in each species or order [475].

Cormorants and pelicans (order Pelecaniformes) had poor serological responses in several vaccination campaigns [470-472;474-477;656], regardless of the vaccine dose [476]. In Swiss zoos, Dalmatian pelicans (*Pelecanus crispus*) developed a GMT of approximately 90; this titer, although relatively low, was above the protective level [475]. Individual responses were not reported in this campaign. In Spain, 60% of 32 great white pelicans (*Pelecanus onocrotalus*) had titers ≥ 32 after 2 doses of an H5N9 vaccine, although the GMT was low (32), and 3 additional birds seroconverted with a GMT of 512 after 2 doses of an H5N3 vaccine [656]. However, no other Pelecaniformes seroconverted at this level to either vaccine, including 8 pink backed pelicans (*Pelecanus rufescens*) that received 2 doses of the H5N9 vaccine in 2006, and a single dose of H5N3 vaccine in 2007-2008. In other campaigns, significant numbers of Pelecaniformes did not have protective titers [470-472;474;476]. An exception was an unidentified E.U. zoo that vaccinated with 1 ml of an H5N2 vaccine (A/duck/Potsdam/1402/86) [477].

Humboldt penguins (*Spheniscus humboldti*) had relatively low titers in some vaccination campaigns; the GMT was 21-76 in Danish (H5), Swiss (H5), French (H5), Spanish (H5) and Dutch (H7) vaccination campaigns [472;474-476;656], with a somewhat higher GMT of 119 in a Dutch H5 vaccination campaign [471]. The percentage of birds that developed protective titers was also low (44%) in Denmark [474], but high (80%; H5 or 91%; H7) in the Netherlands [471;472] and (100%) France [476]. In France, 93% of the Jackass penguins (*Spheniscus demersus*) and 100% of the Humboldt penguins developed HI titers of at least 32; however, the GMT was 214 in Jackass penguins and only 32 in Humboldt penguins [476]. Conversely, no Jackass penguins, but 60% of Humboldt penguins had titers ≥ 32 (GMT = 21) in Spain [656]. In Swiss zoos, HI titers were undetectable in Humboldt penguins after 26 weeks, while other birds still had detectable titers [475]. The breadth of antibody responses to H5N1 clades was also reported to be low in Sphenisciformes compared to some other orders [471].

Columbiformes had weak HI responses in some campaigns [471;472;476;477;656], although vaccination protected domesticated rock doves (*Columba livia*) from H5N1 viruses in a challenge experiment ([599] cited in [342]). In the Netherlands, 3 of 5 rock doves and the single Scheepmakers' crowned pigeon (*Goura scheepmakeri sclaterii*) tested had protective titers (≥ 40) against an H7 virus [472], but only 20% of Columbiformes developed protective titers in the H5 campaign [471]. Similarly, only 3 of the 10 birds tested in French zoos [476], and 10 of 79 birds that received an H5N9 vaccine or 2 of 5 birds that received

an H5N3 vaccine in Spanish zoos [656] had HI titers of at least 32. Columbiformes were not included in some campaigns [474;475].

Some vaccination campaigns reported weak serological responses in owls. The number of birds is low, and individual variability may have played a role. In Singapore, 7 Eurasian eagle owls (*Bubo bubo*) or barn owls (*Tyto alba*) did not respond even after 2 boosters [470]. In French zoos, protective titers were reported in 1 of 2 great horned owls (*Bubo virginianus*) and all 3 tawny owls (*Strix aluco*), but no snowy owls (*Bubo scandiacus*; 2 birds) or Eurasian eagle owls (7 birds) [476]. In Spain, one of 2 little owls (*Athene noctua*), one of 7 Eurasian eagle owls and a spectacled owl (*Pulsatrix perspicillata*) developed protective titers after receiving H5N9 or H5N3 vaccines; two snowy owls and 2 barn owls all had titers below this level [656]. In Dutch zoos, a Eurasian eagle owl developed a titer of 80 in an H7 vaccination campaign, but a snowy owl had a titer of only 20 (below the cutoff) [472], and only 1 of 2 snowy owls had a protective titer in the H5 campaign [471]. In the Danish H5 vaccination campaign, 2 of 3 great grey owls (*Strix nebulosa*) had protective titers, but the overall GMT was 25, while all 3 Eurasian eagle owls developed protective titers with a GMT of 81 [474]. In Swiss zoos, the GMT was 73 after a booster in 6 Eurasian eagle owls, 161 in 4 snowy owls, and 1024 in one barn owl [475].

Serological responses among psittacine birds varied with the species and zoo. Among 113 psittacine birds vaccinated in Denmark, 88% to 100% of the Quaker parakeets (*Myiopsitta monachus*), African grey parrots (*Psittacus erithacus* spp.), kea (*Nestor notabilis*), blue and yellow macaws (*Ara ararauna*) and Amazon parrots (*Amazona* spp.) developed protective titers, with GMTs ranging from 151 to 2195; however, only 3% of the cockatiels (*Nymphicus hollandicus*) and 24% of Fischer's lovebird (*Agapornis fischeri*) had HI titers that reached 32 [474]. Protective responses were reported in 86-100% of the scarlet macaws (*Ara macao*), blue-fronted macaws (*Amazona aestiva*), hyacinth macaws (*Anodorhynchus hyacinthinus*) and Panama amazons (*Ara ochrocephala panamensis*) in the H5 campaign in the Netherlands [471]. Only 4 psittacine birds were tested in the H7 campaign: the blue-eyed cockatoo (*Cacatua ophthalmica*), Mexican military macaw (*Ara militaris mexicana*) and Mitchell's cockatoo (*Cacatua leadbeateri*) had HI titers well over 40, but the long-billed corella (*Cacatua tenuiros tris tenuirostris*) did not respond [472]. In Spain, 43% of the 177 psittacine birds immunized with an H5N9 vaccine in 2006 had protective titers, although responses were higher in some species including scarlet macaws (87%), blue and yellow macaws (67%), red fronted macaws (*Ara rubrogenys*; 54%) yellow-crowned amazons (*Amazona ochrocephala*, 67%) and Eclectus parrots (*Eclectus roratus*, 57%) [656]. All 3 great green macaws (*Ara ambigua*) and members of some other species also had titers ≥ 32 ; however, only 23% of 17 red-and-green macaws (*Ara chloroptera*), 39% of 13 military macaws, 40% of 15 African gray parrots, 17% of 6 golden parakeets (*Guarouba guarouba*) and 6 of 24 birds belonging to 6 species of *Cacatua* (cockatoos) had titers at this level. All 7 psittacine birds boosted with an H5N3 vaccine in 2007-2008 (a red-and-green macaw, 3 military macaws and 3 Eclectus parrots) developed protective titers. Only 53% of the psittacine birds developed protective titers in France, with response rates of 45% to 50% in most species including Amazon parrots (*Amazona amazonica* and *Amazona* spp.); blue and yellow macaws; chestnut-fronted macaws (*Ara severa*) and *Cacatua* spp. [476]. The GMT for psittacine birds was relatively low (81) compared to other orders in Swiss zoos [475].

19.4.7 Duration of Immunity in Zoo Birds

Limited data from E.U. vaccination campaigns suggest that HI titers may persist in some species, but not others, for 6 months [475-477]. In French zoos, protective titers were found in 42% of all birds 6 months after the initial vaccination (4.5 months after the second dose), and in 26% after 11 months [476]. By 11 months, no order contained a high percentage of seropositive birds. Among birds that could be followed individually for all samplings, all of the Ciconiiformes, Galliformes, Phoenicopteriformes and Anseriformes initially had protective titers. Among these birds, 100% of the Ciconiiformes, 85% of the Galliformes, 64% of the Phoenicopteriformes and 56% of the Anseriformes still had titers of at least 32 at

6 months. By 11 months, these percentages had dropped to 73% for the Ciconiiformes, 38% for the Galliformes, 36% for the Phoenicopteriformes and 44% for the Anseriformes. In individual Falconiformes, the percentage of birds with protective titers dropped from 60% initially, to 40% at 6 months and 0% at 11 months. These values were 53%, 27% and 20%, respectively in Psittaciformes (15 birds), and 33%, 17% and 0%, respectively, in Strigiformes (6 birds). Although 50-92% of the Sphenisciformes, Gruiformes or Pelecaniformes had protective titers initially, none had protective titers by 6 months.

At the Singapore Zoo, 100% of peafowl and bar-headed geese, 80% of guinea fowl, 67% of Egyptian geese and no black swans or spur-winged geese had titers of 16 or greater, after 6 months [470]. In the EFSA summary, one member state reported that 63% of peacocks (*Pavo cristatus*) immunized with a commercial H5N9 vaccine had titers of at 32 after 6 months; however, the GMT was only 38 [477]. In Swiss zoos, the average GMT decreased from 187 to 74 after 6 months [475]. Decreases in individual orders ranged from 21% (Psittaciformes) to 85% (Sphenisciformes); the percentage of birds re-tested in each order ranged from 25% to 100%. The proportion of birds with protective titers in Spain decreased from 51%, 6 weeks after the second dose of vaccine, to 45% at 15 weeks, with the GMT concurrently dropping from 103 to 59 [656]. Some birds responded with elevated titers to a booster administered after 18-24 months, while others responded poorly. A different vaccine was used as in this case, and it is unclear whether the species variability was caused by differences in the duration of immunity, or by the change in vaccine virus and/or formulation. In a challenge study, HI titers persisted for at least 5 months in Gyr-Saker (*F. rusticolus* × *F. cherrug*) hybrid falcons [598].

Based on HI antibody titers, some authors have suggested that zoo birds should be re-vaccinated at least once a year [475], and that a 6-month booster might be useful in many orders [476]. A disadvantage is that twice-a-year vaccination would require the birds to be handled again, and might interfere with the breeding season [476]. A 6-month booster was recommended for Struthioniformes, Strigiformes, and Falconiformes in French zoos in 2007 [476]. The AZA suggests that close monitoring of serological titers and repeated vaccination may be necessary in some zoo birds [542]. The frequency of serological monitoring must be balanced with the detrimental effects of handling.

19.4.8 Maternal Antibodies in Zoo Birds

During a vaccination campaign among zoo birds in France, maternal antibodies were reported in the chicks of vaccinated *Rhea americana* and *Ara macao*. Decreasing titers were reported in rhea chicks up to 4 weeks of age [476]. One recommended protocol in this situation is immunization at 3–4 weeks and 9–10 weeks of age [476].

19.5 Vaccination Protocols for Zoo Birds in the U.S.

Under some circumstances, the USDA may allow the vaccination of birds in zoos that are members of the Association of Zoos and Aquariums (AZA) or AZA-equivalent (i.e., meet or exceed AZA standards) [542]. Details can be found in the USDA APHIS Vaccination Plan for HPAI for AZA Zoos and AZA-Equivalent Zoos. In general, individual zoos that wish to vaccinate must [542]:

- Recognize that the use of such vaccines is experimental, and might result in side effects.
- Present a vaccination plan, and receive approval from both the USDA and the state's animal health authorities.
- Agree to site inspections and records inspections by animal health authorities.
- Take appropriate measures to reduce the risk of virus transmission from wild or feral birds.

- Submit lists of individual birds to be vaccinated, with the anticipated vaccine dose and expected number of doses per bird, to the vaccine manufacturer. Submit lists of pre-identified birds for vaccination to state animal health authorities.
- Oversee the vaccination, which must be performed by a federally accredited veterinarian, and keep accurate vaccination records, as described in the zoo's vaccination plan. Each bird must be individually identified with a leg band, wing band and/or microchip. The disposition of all vaccine doses must be recorded, and empty vials must be kept until the zoo receives permission to discard them. Adverse effects from vaccination must be recorded.
- Keep vaccines in a locked and secure space, with access limited to key personnel. Misuse of vaccines or carelessness could affect trade in poultry.
- Conduct active and passive surveillance for AIV. Sentinel SPF chickens (exposed to environmental materials or the vaccinated bird's environment) would be used for active surveillance in zoo birds. Captive waterfowl may also be employed, but infections are more difficult to detect. Passive surveillance should be conducted in dead birds and birds with clinical signs. Suspected cases of avian influenza must be reported immediately to state and federal animal health authorities.
- Limit the movement of vaccinated birds, and keep movement records for the bird's lifetime. Vaccinated birds cannot be moved within the state, out of state, or internationally unless specific conditions are met, and approval is received from both the receiving state or country, and the state of origin.

Prophylactic vaccination of extremely rare, endangered birds can begin as soon as state and federal animal health authorities have approved the request. These birds are listed in CITES Appendix A or the U.S. Fish and Wildlife Service Endangered Species List. Other birds would be approved for vaccination only in an outbreak. Zoos may prepare a list of species to be vaccinated, for pre-approval if the zoo falls in a Surveillance Zone during an outbreak. These birds may include a) CITES Appendix II and III birds, or b) Non-threatened/ non-endangered waterfowl and wading birds that are flight restricted and occur on open ponds. The curator and attending veterinarian must justify the inclusion of each family and order of birds, based on its conservation status, species susceptibility, risk of infection and potential role in amplifying the virus.

20. PUBLIC ACCEPTABILITY OF VACCINATION AS A COMPONENT OF HPAI ERADICATION

Summary

The risks of HPAIV infections in humans, as well as in birds, should be communicated during a vaccination campaign. Infections with avian influenza viruses are occasionally reported in people. The outcome varies widely and may include asymptomatic infection, conjunctivitis, mild illness that resembles human influenza, or severe and fatal disease. Most people infected with Asian lineage H5N1 HPAI viruses seem to become seriously ill, and a high proportion of these infections have been fatal. Person-to-person transmission of AIV is rare, and sustained transmission has not been reported; however, it would be possible for an avian influenza virus to become adapted to transmission in humans.

Most HPAIV infections in people result from direct contact with sick or dead poultry. The presence of viruses in raw poultry products is also a concern. In nonvaccinated poultry, HPAI viruses may be found in tissues throughout the bird including meat, blood and feather follicles. These viruses can also be found on the surface of eggs, due to fecal contamination, or inside the

yolk and albumen. HPAI viruses that have been isolated from chicken meat include Asian lineage H5N1 viruses, as well as other subtypes. HPAI viruses have also been detected in the meat of turkeys, ducks, geese and quail. AIV is heat labile, and thoroughly cooking eggs and meat will destroy the virus. Good sanitary measures should also be used during food preparation, to avoid contaminating mucous membranes.

Vaccination decreases virus shedding, and may reduce the risks to humans from contact with poultry. It must be accompanied by good surveillance, to prevent exposure to viruses shed asymptotically from infected flocks, and to prevent contaminated poultry products from entering the food chain. Although the amount of research is limited, vaccination appears to prevent HPAI viruses from localizing in meat. These viruses may still occur in the lungs, and could contaminate meat during processing. Vaccination may reduce virus deposition in or on eggs, but it does not seem to reliably prevent it. Procedures to inactivate HPAI viruses in various poultry products have been published.

There is no evidence that eating poultry vaccinated for avian influenza has any effect on human health. Withdrawal periods are recommended by some vaccine manufacturers, due to the use of adjuvants such as oil.

Consumer surveys have been conducted in the E.U., the U.S. and Taiwan concerning attitudes and knowledge about H5N1 HPAI. The level of knowledge varied with the geographic region and other factors. A significant number of participants did not realize that thorough cooking kills AIV in meat and eggs. All three surveys suggested that consumers are likely to decrease their consumption of poultry products in an outbreak, although European survey participants expected this to be a short-term change. The studies also reported that consumers had a relatively high level of concern about eating meat from poultry vaccinated for avian influenza, whether or not an outbreak is occurring. These surveys suggest that public information campaigns concerning both food safety and vaccination would be advisable before and during a vaccination campaign. This information is more likely to be effective if it comes from institutions that are trusted. In the U.S., one survey suggests that the CDC is most likely to be considered trustworthy, but the WHO, the USDA and the FDA also had relatively high rankings.

20.1 HPAI as a Zoonosis

The risks of avian influenza for humans, as well as birds, should be communicated during a vaccination campaign [496]. Infections with both HPAI and LPAI viruses are occasionally reported in people [150;188;243;426-430;661-672]. The outcome varies widely and may include asymptomatic infections, conjunctivitis, mild illness that resembles human influenza, or severe and fatal disease. The possibility of other, unrecognized infections might be suggested by the occurrence of antibodies to various subtypes, generally at a low prevalence, in people who are exposed to poultry or waterfowl [243;662;673-686].

Illnesses caused by Asian lineage H5N1 HPAI viruses are, overall, rare in people; however, these viruses have been found in poultry (including backyard flocks) for over a decade, resulting in high levels of human exposure. Between 1997 and 2015, there were more than 650 laboratory-confirmed human infections with Asian lineage H5N1 viruses, which generally occurred as the result of close contact with poultry [265]. Most patients were young and had no predisposing conditions [129]. The infection is often life-threatening, with the patient deteriorating rapidly [106;129;687]. The case fatality rate for all laboratory confirmed cases reported to WHO has consistently been about 59-60% in the last few years [264;265]. However, it differs between countries and groups of patients [266;687-691], with lower case fatality rates reported in young children and in patients with milder symptoms at the time of diagnosis

[266;691;692]. The reasons for the reduced severity in some young children is still unclear, and might include factors such as earlier diagnosis and treatment. Laboratory confirmed, asymptomatic or mild cases have been recognized, but seem to be rare [662;681;685;693-696].

Mild illnesses, with conjunctivitis and/or upper respiratory signs, have been reported in a number of people infected with various H7 LPAI or HPAI viruses, although one of these viruses (H7N7 HPAI) also caused a fatal illness in one healthy person [429;430;654;662;664-666;697-701]. Since 2013, however, an H7N9 LPAI virus has caused more than 400 human cases in China while spreading subclinically among poultry. Most of these cases have been serious, although uncomplicated, mild illnesses have also been reported, especially in children [408;433;668;669;702-707], and serological evidence of exposure has been reported in some healthy poultry workers [708;709]. Many clinical cases have occurred in older patients, and underlying conditions may have exacerbated some infections; however, serious cases and fatalities were also seen in previously healthy individuals and young to middle-aged adults [410;433;668;669;702;704;710-712]. The reported case fatality rate in hospitalized, laboratory confirmed patients was approximately 36% [408;668;712].

Person-to-person transmission of AIV is uncommon, although it is possible that an avian influenza virus could become adapted to transmission in humans. While most infected people do not seem to transmit avian viruses to others, including family members [426-429], Asian lineage H5N1 HPAI viruses are capable of person-to-person transmission in rare instances [413-416]. One H7N7 HPAI virus was also found in a few family members of poultry workers in the Netherlands [429;430]. Likewise, the H7N9 virus in China does not appear to spread readily between people, but human-to-human transmission has been suspected in a few family clusters [408;410;411;431-436]. Close, unprotected contact, seems to be necessary to transmit any of these viruses [413-416;435;436]. Sustained human transmission has not been reported with any of these viruses.

20.2 The Use of Meat and Eggs from Vaccinated and/or Infected Animals

20.2.1 Risks from Vaccinated, Uninfected Birds

There is no evidence that eating vaccinated poultry has any effect on human health [496]. Withdrawal periods are recommended due to the use of adjuvants such as oil [496].

20.2.2 HPAI Viruses in Poultry Tissues

In nonvaccinated poultry, HPAI viruses may be found throughout the bird. In chickens and turkeys, these viruses and/or their antigens have been detected in a wide variety of organs and tissues including skeletal muscle (meat), blood, feather follicles, bone marrow, upper and lower respiratory tract, other visceral organs, reproductive organs and the brain [95;100;103;105;293-304]. HPAI viruses that have been found in chicken meat, using virus isolation or antigen detection, include Asian lineage H5N1 viruses, A/chicken/Pennsylvania/1370/83 (H5N2), A/turkey/Ontario/7732/66 (H5N2), A/chicken/Victoria/ 85 (H7N7) and A/chicken/Victoria/92 (H7N3) [100;296-298;298;300]. H5N1 HPAI viruses have also been reported in meat from ducks [47;210;300;305], and experimentally infected quail and geese [47;306]. The HPAI virus A/turkey/Italy/4580/1999 (H7N1) was detected in the meat of turkeys [105].

Eggs laid by AIV-infected flocks can be contaminated by viruses in feces. They may also, in some cases, contain viruses in the albumen or yolk. Two HPAI viruses, A/chicken/Pennsylvania/83 (H5N2) [294;309] and A/FPV/Dutch/27 (H7N7) [293], were detected in the albumen or yolk of eggs from experimentally infected chickens. The H5N2 viruses were also found in the yolk and albumen of eggs from some, but not all, HPAIV-infected chicken flocks during an outbreak in Pennsylvania [308]. During this outbreak, 17-56% of the eggs collected after the onset of clinical signs contained virus inside the egg, and 9-50%

contained virus on the shells. An HPAI virus (A/turkey/Ontario/7732/66; H5N2) was reported in the internal contents of turkey eggs [304]. Asian lineage H5N1 HPAI viruses have been found in the internal contents of quail eggs [310]. These viruses were also detected in eggs from some inadequately vaccinated, symptomatic chickens in the field [311]. Laboratory studies have confirmed that several HPAI viruses (including H5N1) are capable of infecting eggs in vaccinated chickens, even in the absence of clinical signs [47;312;313].

20.2.3 Risks to Humans from Infected Poultry or Poultry Products

Most infections in people result from direct contact with sick or dead poultry [67;106]. The presence of avian viruses in raw poultry products is also a concern; tissues from infected birds could infect humans if they are handled without adequate precautions. In addition to other mucous membranes, potential routes of exposure include the eye [420-422]. Ingestion of a raw poultry product has been associated with 2-3 published human cases [417;418], of which two had no known exposure to the virus by other routes. Another recent H5N1 infection occurred in a woman who had no exposure to poultry except through the raw duck blood and chicken hearts processed in the home and sold at her husband's food stand [412]. In addition, there is both experimental and circumstantial evidence that other mammals can be infected by eating contaminated poultry tissues [234;276;278;279;285;417;425]. Thorough cooking of eggs and meat is expected to be protective: avian influenza viruses are heat labile and are readily killed by cooking methods that destroy other pathogens found in poultry (e.g., *Salmonella*) [67]. Good sanitary practices must also be followed during food preparation to prevent contamination of mucous membranes.

20.2.4 The Effect of Vaccination on the Risk of Human Infection

Because vaccination decreases virus shedding, it may decrease the risk of infection in people who have direct or indirect contact with poultry [3;47;496]. In Vietnam, a nationwide vaccination campaign for H5N1 viruses in poultry significantly decreased the number of human H5N1 cases [3;713]. Vaccination must be accompanied by good surveillance; otherwise, people might be exposed to HPAI viruses in flocks that are shedding viruses asymptomatically [3].

Although the amount of research is limited, effective vaccination appears to prevent HPAI viruses from localizing in meat and some other edible tissues of chickens (excluding eggs) [100;105;210;568]. After vaccination with one dose of either a fowlpox-vectored H5 vaccine (TROVAC™ H5) or an inactivated vaccine (A/turkey/Wisconsin/1968; H5N9), HPAIV could not be isolated from the breast or thigh meat of chickens challenged with A/chicken/Korea/ES/2003 (H5N1) [100]. Similarly, A/duck/Vietnam/12/05 (H5N1) could not be isolated from the internal organs, blood, and thigh or breast meat of Pekin ducks immunized with two doses of an inactivated H5N2 vaccine (A/duck/Potsdam/1402/86), although it was found in these organs in nonvaccinated ducks [210]. Vaccination with two doses of a commercial inactivated bivalent vaccine also prevented an H7N1 HPAI virus (A/turkey/Italy/4580/1999) from localizing in the muscles of turkeys [105]. In this study, HPAI viruses were still found in the lungs of vaccinated turkeys, although there were no clinical signs. Virus in internal organs has the potential to contaminate meat during processing. In a recent study, only a clinically protective vaccine prevented virus localization in edible tissues; chickens vaccinated with an ineffective vaccine had viral RNA in tissues in addition to clinical signs [568].

Vaccination does not seem to prevent viruses from contaminating eggs, including the internal contents, although the amount of virus may be reduced. In 2010, Asian lineage H5N1 HPAI viruses were isolated from the albumen of eggs laid by an Egyptian chicken flock that had become infected despite three doses of the Re-1 H5N1 vaccine [311]. These birds developed clinical signs, including a 20% drop in egg production, with the mortality rate reaching 27% in one chicken house and 5.4% in another house, 12 days after the onset of clinical signs. Subsequent laboratory studies reported that Asian lineage H5N1 HPAI viruses, as well as two H5N2 HPAI viruses (A/chicken/Pennsylvania/1370/1983 and

A/chicken/Italy/8/98), could be found in the internal contents of some eggs laid by chickens that were partially or fully clinically protected by vaccination [47;312;313]. In the laboratory, one or two doses of one vaccine reduced HPAI virus contamination on the eggshell, and in some cases, the internal contents of eggs from hens challenged with A/chicken/Pennsylvania/1370/1983 (H5N2) [313]. However, it was not able to completely prevent virus localization in the reproductive tract. In this study, approximately half of the eggs from nonvaccinated hens contained virus on and/or inside the egg, compared to 28% of the eggs from vaccinated hens [313]. Contaminated eggs were not laid after the fourth day post-challenge. An earlier, unpublished study also suggested that vaccination might decrease virus shedding in eggs. In chickens, immunization with an inactivated vaccine (A/turkey/Wisconsin/68; H5N9) decreased the recovery of HPAIV A/Pennsylvania/1370/83 (H5N2) in eggs from 39% to 16% [47]. The eggs from vaccinated chickens also contained less virus. Another laboratory reported that virus contamination occurred in 23% of eggs from vaccinated chickens challenged with the HPAI virus A/chicken/Italy/8/98 (H5N2), and 36% or 63% of vaccinated chickens challenged with 2 Asian lineage HPAI viruses [312]. The highest isolation rates were in H5N1 virus-infected chickens that were 90% protected from clinical signs and mortality, and these birds laid eggs up to 9 days after challenge.

20.3 Procedures for Marketing Animal Products After Emergency Vaccination

Because vaccinated birds can be infected asymptotically, there must be safeguards to ensure that poultry products from vaccinated flocks are free of live virus before entering the food chain. Monitoring of vaccinated flocks should include both active and passive surveillance. A plan to facilitate the movement of eggs and egg products from uninfected flocks during an HPAI outbreak, while protecting the health of consumers, has been published by USDA APHIS VS [714]. A description is also available for the surveillance program used in Italy, when poultry products were marketed during LPAI vaccination campaigns (see Movement Restrictions and Biosecurity, section 15.5) [460]. It should be noted that, while surveillance and marketing plans can provide a high degree of confidence that a product does not contain AIV, the complete absence of virus cannot be guaranteed.

20.3.1 Procedures to Inactivate HPAI Viruses in Poultry Products

AIV can be inactivated with heat. Inactivation temperatures (core temperature) and times reported to be effective by the OIE [35] are:

- Whole eggs: 60°C for 188 seconds
- Whole egg blends: 60°C for 188 seconds or 61.1°C for 94 seconds
- Liquid egg white: 55.6°C for 870 seconds or 56.7°C for 232 seconds
- Dried egg white: 67°C for 20 hours or 54.4°C for 513 hours
- 10% salted yolk: 62.2°C for 138 seconds
- Poultry meat: 60.0°C for 507 seconds, 65.0°C for 42 seconds, 70.0°C for 3.5 seconds, or 73.9°C for 0.51 seconds

Other times and temperatures may also be acceptable, provided that they are scientifically documented to inactivate the virus [35].

20.4 Consumer Knowledge about HPAI and Concerns about Eating Animal Products from Vaccinated or Potentially Infected Birds

Consumer surveys have been conducted in several countries, concerning attitudes and knowledge about Asian lineage H5N1 HPAI.

20.4.1 United States

In a telephone survey of adults in the United States performed approximately 10 years ago, participants correctly answered 59% of 22 objective questions specifically concerning Asian lineage H5N1 HPAI; the scope of these questions included transmission and prevention, information about the poultry industry (e.g., the relative amount of imported chicken that is sold in the U.S.), knowledge about current cases of H5N1 avian influenza, and the geographic distribution of the virus in wild birds, poultry and humans [715]. A few questions were potentially ambiguous if the survey participant was knowledgeable (e.g., the true/false question “It is easy to tell when live chickens are infected with bird flu by looking at them,” which was scored as true).

A subset of these questions was directly related to food safety and HPAI prevention. Most survey participants realized that H5N1 HPAI viruses can be acquired by contact with infected chickens (70%) or their feces (77%). More than half (64%) also realized that H5N1 HPAI viruses occur in uncooked meat from infected birds, and might infect humans who contact the raw meat (59%), while 78% answered correctly that these viruses might be acquired by eating undercooked, contaminated meat. Only 42% of the participants realized that avian influenza viruses are killed when chicken is cooked to the recommended temperature. Similarly, 40% recognized that people cannot be infected if they eat fully cooked meat from an infected bird, while 27% stated that they were uncertain of the correct answer. The same percentage of participants (40%) realized that AIV cannot be acquired by eating fully cooked eggs, while 34% did not know whether this was possible. Only 48% knew that H5N1 HPAIV could not be acquired in mosquito bites. In this survey, 13% “strongly agreed” and 12% “somewhat agreed” that HPAIV infections can be identified in raw meat during routine food safety inspections. The authors suggest that some people might ignore food safety precautions because they believe they are protected by such inspections.

The survey also investigated attitudes toward eating poultry, either routinely or during an Asian lineage H5N1 HPAI outbreak. Initially, participants were asked to rate the safety of eating chicken, based on a scale of 0 (not at all safe) to 10 (completely safe). They rated chicken that was cooked to the recommended internal temperature (7.5), “fresh chicken you cook at home” (7.4), and chicken labeled with a certification as “free from avian influenza” (7.2) as the safest. Chicken that had a familiar brand (6.9), organic chicken (6.8), cooked chicken that had been previously frozen (6.9), canned chicken soup (6.7), and chicken vaccinated for avian influenza (6.6) were considered to be somewhat less safe. Irradiated chicken (5.9) and chicken from fast food restaurants (5.9) were considered to have the highest risk.

When they were asked to rate the safety of eating chicken during an outbreak, this survey found that people were unlikely to eat any chicken. The level of trust was, however, highest for certified avian influenza-free chicken (4.4), followed by chicken cooked to the recommended internal temperature (4.0), cooked eggs (4.0), home-cooked chicken (3.9), vaccinated chickens (3.8), a familiar brand of chicken (3.4), canned chicken soup (3.4), organic chicken (3.4), cooked chicken that had been previously frozen (3.4), irradiated chicken (3.2), and fast food chicken (2.5). Participants who were less likely to eat chicken if H5N1 HPAI viruses were detected among poultry in the U.S. included those who were more concerned about human illness or saw a greater personal risk, people who did not realize that cooking kills the virus,

and respondents with less education. The likelihood of eating chicken was not, however, correlated with overall objective knowledge in this survey.

Participants were also asked about their trust in various institutions that might deliver messages about HPAI. The level of trust in advice, based on a scale of 1 (no trust) to 10 (complete trust), was highest for the CDC (7.2), the WHO (6.5), the USDA (6.4) and the FDA (6.2), with less trust in U.S. chicken farmers (5.2), chicken processors (4.6), the U.S. Dept. of Homeland Security (4.6), the news media (4.3), President Bush (4.3) and supermarkets (4.2).

20.4.2 Europe

Short surveys in Europe and Taiwan used seven questions to examine consumers' knowledge of basic AIV safety, including the safety of eating meat from vaccinated chickens [716;717].

Among European respondents, 84% of participants knew that all poultry must be destroyed on an infected farm, 76% that vaccination for seasonal influenza is not effective against avian influenza, 74% that AIV can be acquired by touching contaminated birds, 63% that cooking poultry destroys AIV, 61% that thorough cooking of eggs kills AIV, and 60% that the virus is not easily transmitted between people [716]. However, only 47% realized that it is not dangerous to eat a bird vaccinated for avian influenza. Citizens of countries that had been affected by HPAI viruses were more likely to give the correct answers. Occupation and higher education levels were correlated with increased numbers of correct answers.

This survey also assessed the level of concern about HPAI viruses. In 2005 and early 2006, H5N1 HPAI viruses had been detected in wild birds in Europe, as well as among poultry in some countries [616]. The European survey was conducted between March and May, 2006. Approximately 22% of consumers overall stated that they ate less poultry meat than 6 months earlier, 17% that they ate fewer eggs, and 15% that they ate fewer egg-based products [716]. The countries with the highest percentages of respondents eating less poultry (38-58% of responders) or eggs/ egg products (up to 53%) had been directly affected by the virus. The greatest decrease in poultry meat consumption (58%) and egg consumption (53%) occurred in Turkey, probably because it was the only country with human deaths from HPAI. Most of those who had reduced their consumption of poultry did so out of caution; only 15% were certain that the risk was real. Most participants (76%) expected the decreased consumption to be temporary. A decline in consumer confidence overall was followed by gradual recovery [716].

Trust in the information from authorities varied. While 46% of Europeans believed that the authorities disclose everything they know about avian influenza, 43% did not. Opinions on the clarity of the information varied from 45% to 80%, depending on the country. Overall, 60% thought the information they had received was clear.

20.4.3 Taiwan

In 2007, a similar survey was conducted among consumers in metropolitan areas of Taiwan, where avian influenza outbreaks have not occurred [717]. Approximately 54% of the participants knew that AIV is not easily transmitted between people, 87% that people can become infected by contact with contaminated birds, and 92% that birds on HPAI-infected farms must be culled immediately. Only 46% realized that AIV in or on eggs could be destroyed by prolonged cooking, although 55% knew that contaminated poultry meat is not a health risk if it has been cooked. As in the European survey, participants feared meat from vaccinated birds: only 35% answered correctly that meat from chickens vaccinated for avian influenza is not dangerous to eat. Approximately 34-49% of the respondents were not completely sure of their answers, with the highest percentage of uncertainty about vaccination and about the destruction of virus in eggs.

This survey found that people in Taiwan who were more knowledgeable about avian influenza, and who also had relatively high levels of risk perception, were likely to avoid birds and being in crowded places, while people who were not knowledgeable were likely to avoid eating chicken during an outbreak. Although the values varied with risk perception and knowledge, overall 37-51% of consumers were unlikely to eat any chicken during an outbreak, 33-48% would probably reduce their chicken consumption, and 13-27% did not think they would make any changes in their chicken consumption.

20.4.4 Public Education

All three surveys reported that consumers had a relatively high level of concern about eating meat from poultry vaccinated for avian influenza. Only 35% of the survey participants from Taiwan, and 47% from Europe, answered correctly that meat from chickens vaccinated for avian influenza is not dangerous to eat [716;717]. Although this specific question was not asked in the U.S., participants rated meat from vaccinated chickens as less safe than fresh chicken cooked at home, chicken with a familiar brand, organic chicken, or cooked chicken that had been previously frozen [715]. Because the U.S. survey considered each category to be an independent item, it is not certain that participants would consider cooked meat from vaccinated chickens to be more dangerous than cooked meat from nonvaccinated chickens. However, meat from vaccinated chickens was ranked slightly lower in safety than chicken with a familiar brand or organic chicken, suggesting that this might be the case. During a hypothetical outbreak, meat from vaccinated chicken improved slightly in the risk perception analysis, but consumers were unlikely to eat any chicken [715]. Combined, these surveys suggest that public information campaigns concerning the safety of meat from vaccinated, uninfected birds would be advisable before and during a vaccination campaign.

All three surveys suggested that consumers are likely to decrease their consumption of poultry products, although European survey participants expected this to be a short-term change [715-717]. Vaccination is also being considered for foot-and-mouth disease (FMD) in some countries, and certain measures have been suggested to minimize the rejection of food products from animals vaccinated for FMD [718]. Some specific recommendations that may also be applicable to HPAI are:

- Develop a vaccination policy before an outbreak, and determine the conditions under which it would be used
- Discuss the vaccination policy with all stakeholders. Remind stakeholders that vaccines are used routinely in livestock and poultry for endemic diseases.
- Obtain the support of the public for vaccination and other control policies
- License vaccines before they will be needed. If a USDA exemption from license requirements must be given to an emergency vaccine, consider its effect on consumer concerns. Provide safety information to all stakeholders about the use of such vaccines.
- Give unequivocal and authoritative assurance that vaccinated products are safe to eat. This should include statements from national and international independent bodies that consumers respect.
- Begin communication about HPAI vaccines before an outbreak and continue to communicate during the outbreak.

Food safety is an important aspect of public education campaigns during HPAI outbreaks, and affects industry sustainability [717]. In all three international surveys, knowledge about inactivating viruses in eggs and meat was weak, with a particularly low rate of correct responses in the U.S. [715-717]. Public education campaigns would be advisable. Hsu et al. (2008) suggest an emphasis on specific facts such as handling meat, outbreak preparedness and meat safety [717]. Public safety announcements and messages are more likely to be effective if they come from institutions that are trusted. In the U.S., the CDC is most likely to be considered trustworthy, but the WHO, the USDA and the FDA also had relatively high rankings [715].

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22. ACKNOWLEDGMENTS

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Glossary

Accredited Veterinarian

A veterinarian approved by USDA APHIS to perform the duties listed in 9 CFR 160-162.

American Lineage

AIV lineage found primarily in the Western Hemisphere.

Antigenic Cartography

A computational technique that can be used to visualize and quantify data from binding assays, such as the hemagglutination inhibition (HI) test.

Antigenic Drift

A process of evolution by which a virus changes continuously throughout time due to the acquisition of point mutations during replication.

Antigenic Shift

An abrupt change in the subtypes found in a host species.

Avian Influenza Viruses Reportable to the OIE

See Notifiable Avian Influenza

Biosecurity

Management practices designed to prevent the introduction of disease agents onto an animal production facility.

Bivalent Vaccine

A vaccine containing both H5 and H7 antigens, thus conferring immunity to both subtypes.

Clade

Ten AIV H5N1 clades (0-9) are currently recognized, and replace previously used geographic designations for AIV lineages (e.g., the 'Qinghai lineage' is now designated as the second order clade 2.2). AIV clades must meet specific clade definition criteria developed by the WHO/OIE/FAO H5N1 Evolution Working Group. These generally include 1) sharing a common, clade-defining node; 2) monophyletic grouping with a bootstrap value of ≥ 60 at the clade-defining node; and 3) having average percentage pairwise nucleotide distances between clades of $>1.5\%$ and within clades of $<1.5\%$. A new sublineage that emerges within a clade is designated a second, third, fourth or fifth order clade once it also meet these criteria. It receives a numerical 'address,' using a hierarchical decimal numbering system, that connects it to the original clade. For example, the third order clade 2.1.1 emerged from subclade 2.1, and subclade 2.1 emerged from clade 2. Fifth order clades are designated by a letter to the right of the fourth order clade designation (e.g., 2.3.2.1a). Only a proportion of the known subclades are actively circulating; others are considered to be extinct.

Compartmentalization

The practice of defining subpopulations of animals, by management or husbandry practices related to biosecurity, for the purpose of disease control (OIE).

Competent Authority

The official government organization (e.g., veterinary authority) responsible for ensuring or supervising the implementation of animal health and welfare measures, international veterinary certification and other standards and recommendations in the OIE Terrestrial Code.

Efficacy

Specific ability or capacity of the biological product to effect the result for which it is offered when used under the conditions recommended by the manufacturer.

Epitope

A structural component of an antigen which induces an immune response, and to which antibodies and T cell receptors bind.

Eurasian Lineage

AIV lineage found primarily in the Eastern Hemisphere.

Geometric Mean

An average value, calculated as the n th root of the product of the values.

HA0

Hemagglutinin precursor protein, split by proteases into two fragments, HA1 and HA2. HA1 contains the receptor binding domain, while HA2 is responsible for fusion of the viral and endosomal membranes, which releases the virus into the cytosol.

Hemagglutinin (HA)

Variable AIV surface glycoprotein and major target of the immune response. The HA1 component is responsible for binding the virion to the cell. HA2 is responsible for fusion of the viral and endosomal membranes, which releases the virion into the cytosol.

Heterologous Neuraminidase DIVA strategy

The use of a vaccine that contains a different neuraminidase type than the field virus, combined with a serological test that detects antibodies to the neuraminidase of the field virus.

Heterologous Vaccine

Vaccine that is not identical to the challenge or field strain.

Homologous Vaccine

Vaccine that is identical to a field/ outbreak or challenge virus.

Infected Premises

Premises where a presumptive positive case or confirmed positive case exists, based on laboratory results, compatible clinical signs, case definition, and international standards.

Intravenous Pathogenicity Index (IVPI)

A test, described by the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, that assesses the pathogenicity of an avian influenza virus after intravenous inoculation in 4-to-8-week-old SAN or SPF chickens, by quantifying both clinical signs and death. The IVPI ranges from 0.0 (no clinical signs during the 10 day observation period) to 3.0 (all birds dead within 24 hours).

Matrix 1 Protein (M1)

Internal structural protein of AIV, which forms a matrix containing viral ribonucleoproteins.

Matrix 2 Protein (M2)

AIV transmembrane protein, a proton-selective ion channel, which acidifies the viral core and allows the viral ribonucleoproteins to be released into the cytoplasm of the host cell.

Movement Controls

Control of the movement of people, animals, vehicles, and equipment so that biosecurity can be maintained during a disease outbreak.

National Veterinary Stockpile (NVS)

Established by Homeland Security Presidential Directive 9 and operational in 2006. Able to deploy large quantities of veterinary resources anywhere in the continental U.S. within 24 hours.

Neuraminidase

Variable AIV surface glycoprotein, which cleaves sialic acids from glycoproteins and glycolipids, preventing the aggregation of virions and allowing them to be released efficiently from the cell. A target of protective immunity, but less important in protection than the hemagglutinin.

NewH5™

Live NDV-vectored avian influenza H5 vaccine, licensed in Mexico, which contains the hemagglutinin from A/chicken/Mexico/435/2005 (H5N2).

Non-Structural Proteins (NSPs)

Viral proteins that are present during replication in cells, but are not packaged into the virion.

Non-Structural Protein 1 (NS1)

A multifunctional AIV protein, which has been implicated in the inhibition of interferon-mediated host defenses, and also regulates the translation and splicing of mRNA. AIV may contain either of the two major lineages of NS1 (A and B). NS1 has recently been identified in purified virions, although the amount is low. This protein has been investigated for use in DIVA tests

Non-Structural Protein 2 (NS2)

AIV protein with an important role in exporting newly made viral RNA from the nucleus. Present in very small amounts in the viral ribonucleoprotein complex, and thus not strictly a nonstructural protein. Also known as nuclear export protein (NEP).

Notifiable Avian Influenza (NAI)

Until recently, the OIE used the term “notifiable avian influenza” (NAI) for an infection of poultry caused by any HPAI virus or H5 or H7 LPAI virus. These infections must be reported to the OIE, whereas notification is not currently required for other LPAI viruses in poultry. The OIE has now abandoned the terminology “notifiable avian influenza.” In their documents, “avian influenza” has been redefined as containing only HPAI viruses and H5/H7 LPAI viruses, i.e., those viruses formerly known as NAI [35;94]. The OIE defines poultry as domesticated birds used to produce meat, eggs or other commercial products, as well as birds used to restock game birds, birds used to breed other categories included in the definition, and all fighting cocks regardless of their purpose.

[NB: In this document, “avian influenza” continues to be used in the scientific sense, i.e., for all influenza A viruses found in birds.]

Nuclear Export Protein (NEP)

AIV protein with an important role in exporting newly made viral RNA from the nucleus. Present in very small amounts in the viral ribonucleoprotein complex, and thus not strictly a nonstructural protein. Also known as nonstructural protein 2 (NS2).

Nucleoprotein

AIV structural protein associated with the viral nucleic acids.

Prime-Boost Regimen

Sequential immunization with vaccines that use different antigen-delivery systems, to generate higher levels of immunity than by administration of a single dose of vaccine or homologous boosting.

Purity

Quality of a biological product prepared to a final form relatively free of extraneous microorganisms and extraneous material (organic or inorganic), as determined by test methods or procedures established by APHIS in Standard Requirements or in the approved Outline of Production for such product, but free of extraneous microorganisms or material which in the opinion of the Administrator adversely affects the safety, potency, or efficacy of such product.

Re-1

Inactivated reverse genetics vaccine, made in China, containing H5 and N1 genes from the clade 0 virus A/goose/Guangdong/96 (H5N1).

Re-4

Inactivated reverse genetics vaccine, made in China, containing H5 and N1 genes from the clade 7 virus A/chicken/Shanxi/2/2006 (H5N1).

Re-5

Inactivated reverse genetics vaccine, made in China, containing H5 and N1 genes from the clade 2.3.4 virus A/duck/Anhui/1/06 (H5N1).

Re-6

Inactivated reverse genetics vaccine, made in China, containing H5 and N1 genes from the clade 2.3.2.1 virus A/duck/Guangdong/S1322/2006 (H5N1).

Reassortant

Containing AIV gene segments from different parent viruses.

Reverse Genetics

A method used in AIV vaccine production, which can generate an influenza virus entirely from cloned cDNAs (DNA synthesized from the viral RNA). Reverse genetics allows a vaccine strain to be produced with the HA and NA of choice, with internal proteins from an AIV strain that grows well for vaccine production (e.g., the human vaccine strain PR8).

Sentinel Bird

AIV-seronegative, nonvaccinated, susceptible bird used to detect the introduction of avian influenza viruses into a flock. Sentinel birds must be identified with a tamper-resistant system, monitored closely for clinical signs, and sampled periodically by laboratory testing.

Stamping-Out

The killing of affected animals and animals suspected of being affected in the flock, and where appropriate, in other flocks exposed to infection by direct animal-to-animal contact or by indirect contact of a kind likely to cause the transmission of the causal pathogen.

Sterility (as applied to vaccines)

Freedom from viable contaminating microorganisms as demonstrated by procedures prescribed in part 113 of Title 9 CFR, subchapter E, Standard Requirements, and approved Outlines of Production.

Subtype

Combination of hemagglutinin and neuraminidase type used to describe AIV viruses, e.g., H5N1.

Surveillance Zone

Zone outside and along the border of a Control Area. A Control Area consists of an Infected Zone, which immediately surrounds an Infected Premises, and a Buffer Zone, which surrounds an Infected Zone or a Contact Premises.

Viral Ribonucleoprotein (vRNP)

A construct within the avian influenza virion, consisting of AIV RNA wrapped around the nucleoprotein (NP). It can include small amounts of other proteins.

World Organization for Animal Health (OIE)

An intergovernmental organization created by the International Agreement of 25 January 1924. In 2014, the OIE totaled 180 Member Countries. OIE standards are recognized by the World Trade Organization as reference international sanitary rules. The purpose of the OIE is to guarantee the transparency of animal disease status world-wide.

Zoning

The practice of defining subpopulations of animals on a geographical basis, using natural, artificial, or legal boundaries, for the purpose of disease control (OIE).

Acronyms

AGID

Agarose Gel Immunodiffusion Test

AIV

Avian Influenza Virus

APHIS

Animal and Plant Health Inspection Service

AVIC

Area Veterinarian-in-Charge

AZA

Association of Zoos and Aquariums

CDC

U.S. Centers for Disease Control
and Prevention

CI

Confidence Interval

CITES

Convention on International Trade in
Endangered Species of Wild Fauna
and Flora

CMI

Cell-mediated immunity

CTL

Cytotoxic T lymphocyte

CVB

Center for Veterinary Biologics; a division
of APHIS

DIVA

Differentiating Infected from
Vaccinated Animals

DOI

Duration of Immunity

DPPA

Densely Populated Poultry Area

EFSA

European Food Safety Authority

ELD₅₀

50 percent Embryo Lethal Dose

ELISA

Enzyme-Linked Immunosorbent Assay

EMPRES

Emergency Prevention System for
Transboundary Animal and Plant Pests and
Diseases, an FAO program

FAD PReP

Foreign Animal Disease Preparedness and
Response Plan

FAO

United Nations Food and
Agriculture Organization

FDA

U.S. Food and Drug Administration

FMD

Foot-and-Mouth Disease

GMT

Geometric Mean Titer

HA

Hemagglutinin

HA1

Variable region of the AIV hemagglutinin

HI

Hemagglutination Inhibition test

HPAI

High Pathogenicity Avian Influenza

HPAIV

High pathogenicity avian influenza virus

iIFA

Indirect Immunofluorescent Antibody test

IVPI

Intravenous Pathogenicity Index

LPAI

Low Pathogenicity Avian Influenza

LPAIV

Low pathogenicity avian influenza virus

M1

Matrix 1 protein

M2

Matrix 2 protein

M2e

Extracellular domain of matrix 2 protein

NA

Neuraminidase

NAHEMS

National Animal Health Emergency
Management System

NAHLN

National Animal Health
Laboratory Network

NASBA

Nucleic Acid Sequence-Based
Amplification

NDV

Newcastle Disease Virus

NEP

Nuclear Export Protein. Synonym: NS2

NI

Neuraminidase Inhibition test

NP

Nucleoprotein

NS1

Nonstructural protein 1

NS2

Nonstructural protein 2. Synonym: NEP

NSP

Non-Structural Protein

NVS

National Veterinary Stockpile

OFFLU

OIE/FAO Net on Avian Influenza

OIE

Office International des Epizooties',
currently referred to as the World
Organization for Animal Health

PA

Polymerase Acidic Protein

PB1

Polymerase Basic Protein 1

PB2

Polymerase Basic Protein 2

PCR

Polymerase Chain Reaction

PD₅₀

50 percent animal protective dose
(potency determination)

R

Reproduction Ratio/Reproduction Number

R₀

Within-flock/herd reproduction
ratio/reproduction number

R_h

Between flock/herd reproduction ratio/reproduction number

RRT-PCR

Real-time reverse transcription polymerase chain reaction

RT-PCR

Reverse transcription polymerase chain reaction

SN

Serum Neutralization

SPF

Specific Pathogen Free

USDA

United States Department of Agriculture

vRNP

Viral Ribonucleoprotein

VS

Veterinary Services; a division of APHIS

WHO

World Health Organization