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Review

Success Factors for Avian Influenza Vaccine Use in Poultry and Potential Impact at the Wild Bird-Agricultural Interface

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Abstract: Thirty-two epizootics of high pathogenicity avian influenza (HPAI) have been reported in poultry and other birds since 1959. The ongoing H5N1 HPAI epizootic that began in 1996 has also spilled over to infect wild birds. Traditional stamping-out programs in poultry have resulted in eradication of most HPAI epizootics. However, vaccination of poultry was added as a control tool in 1995 and has been used during five epizootics. Over 113 billion doses of AI vaccine have been used in poultry from 2002 to 2010 as oil-emulsified, inactivated whole AIV vaccines (95.5%) and live vectored vaccines (4.5%). Over 99% of the vaccine has been used in the four H5N1 HPAI enzootic countries: China including Hong Kong (91%), Egypt (4.7%), Indonesia (2.3%), and Vietnam (1.4%) where vaccination programs have been nationwide and routine to all poultry. Ten other countries used vaccine in poultry in a focused, risk-based manner but this accounted for less than 1% of the vaccine used. Most vaccine ''failures'' have resulted from problems in the vaccination process; i.e., failure to adequately administer the vaccine to at-risk poultry resulting in lack of population immunity, while fewer failures have resulted from antigenic drift of field viruses away from the vaccine viruses. It is currently not feasible to vaccinate wild birds against H5N1 HPAI, but naturally occurring infections with H5 low pathogenicity avian influenza viruses may generate cross-protective immunity against H5N1 HPAI. The most feasible method to prevent and control H5N1 HPAI in wild birds is through control of the disease in poultry with use of vaccine to reduce environmental burden of H5N1 HPAIV, and eventual eradication of the virus in domestic poultry, especially in domestic ducks which are raised in enzootic countries on range or in other outdoor systems having contact with wild aquatic and periurban terrestrial birds.

Keywords: Avian influenza, disease, poultry, vaccination, vaccines, wild birds

Introduction

Since 1959, high pathogenicity avian influenza (HPAI) has caused 32 epizootics in avian species, mostly domestic poultry, including the H5N1 HPAI panzootic that began in Guangdong China in 1996 and has spread to affect 63 countries in Asia, Africa, and Europe in the past 17 years (OIE 2012a; Swayne et al. 2013). HPAI has affected wild birds in three epizootics: H5N3 of common terns (*Sterna hirundo*) in South Africa during 1961; H5N1 in a variety of wild bird species in Asia, Africa, and Europe since 2002; and H7N3 in a few passerine and columbiforme birds in Mexico during 2012 (Becker 1967; Ellis et al. 2004; OIE

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2012c). Historically, the reservoir of all avian influenza virus (AIV) genes, including all 16 hemagglutinin and 9 neuraminidase subtypes, are found in low pathogenicity avian influenza (LPAI) viruses (LPAIV) circulating in the wild waterfowl reservoir, mainly in birds of the orders Anseriformes and Charadriiformes, although rare infections with LPAIV have been documented in other aquatic birds. On sporadic occasions, these wild bird LPAIV have been transferred to poultry within agricultural systems and, through a process of exposure and successive adaptation especially involving village, backyard, and semi-commercial poultry, have resulted in the LPAIV adapting to domestic poultry with sustained transmission within agricultural systems (Swayne 2008b). In contrast, infections by HPAI virus (HPAIV) are less common in domestic poultry than LPAIV and arise following circulation of H5 or H7 LPAIV in poultry resulting in mutation from low to high virulence (Rohm et al. 1995). These HPAIV have not been maintained in wild birds as has LPAIV (i.e., wild birds are not the reservoir for HPAIV), although HPAIV have occasionally been transferred back to wild birds, especially with the H5N1 HPAIV of Guangdong lineage, causing sporadic to epizootic deaths in some wild bird species (Feare 2010). The finding of rare infections of H5N1 HPAIV in wild birds during extensive surveys in Asia, but common infections in live poultry markets in the same geographic region, suggests that the true reservoirs of H5N1 HPAIV in Asia are domestic poultry, especially asymptomatic domestic ducks. H5N1 HPAIV is lethal to chickens; however, in domestic ducks these viruses can produce a range of clinical disease from mild infections to severe disease with mortality. The way domestic ducks are raised in many Asian countries allows them to serve as bridging species in the transmission of H5N1 HPAIV between wild waterfowl and gallinaceous poultry. Occasionally, spill-over of H5N1 HPAI has occurred into wild birds. For example, intermediate-distance migrants may have transmitted the virus from Mainland Asia to Japan and Korea (Feare 2010; Guan et al. 2009; Hulse-Post et al. 2005; Pepin et al. 2012; Sturm-Ramirez et al. 2005). Asymptomatic infected migratory ducks are also suspected of contributing to the spread of H5N1 HPAIV from Asia to other parts of the world (Cattoli et al. 2009; Keawcharoen et al. 2008; Kim et al. 2009). In addition, periurban terrestrial birds such as sparrows, pigeons, and starlings that enter agricultural housing and access feed for domestic poultry, have been infected with H5N1 HPAIV and can be either mechanical vectors or biological vectors of H5N1 HPAIV between farms or farming systems (Brown et al. 2009; Kou et al. 2005). Therefore, control of H5N1 HPAIV infection in agricultural systems will have a profound effect on reducing and eliminating HPAIV exposure and infections of diverse aquatic and terrestrial wild birds.

Since 2003, the H5N1 HPAIV has become enzootic in poultry within several countries which has necessitated two main changes for HPAI control and eradication strategies; (1) development and implementation of rapid diagnostic tests to accelerate diagnosis before the virus spreads, which permits a quicker stamping-out action leading to eradication, and (2) addition of vaccines and vaccination as a control tool to manage clinical disease, prevent human infections, and maintain food security, especially in economically disadvantaged countries.

High Pathogenicity Avian Influenza Eradication Programs

Historical Strategies

The primary goal for HPAI epizootics in agricultural systems had been rapid eradication. For 26 HPAI epizootics, this has been achieved through comprehensive, integrated control programs that utilized education, diagnostics and surveillance, enhanced biosecurity, and elimination of infected poultry (Swayne et al. 2013). This successful strategy, often termed "stamping-out" relies upon: (1) educating farmers, service personnel, and governmental officials in disease control methods including changes in high-risk behaviors that can spread the virus; (2) using rapid diagnostics and surveillance methods to identify infected flocks; (3) implementing better biosecurity through quarantining infected flocks, imposing movement controls within the outbreak zone, and employing programs that clean and disinfect premises and equipment to limit virus spread, and (4) eliminating the source of infection by culling poultry on infected farms (Swayne et al. 2013). The success of stamping-out programs to eradicate HPAI has been associated with effective and efficient governmental veterinary services, sufficient economic resources for rapid mobilization and implementation, transparency of government in reporting outbreaks and good governance (Pavade et al. 2011).

Vaccines and Vaccination as a New Control Tool

The paradigm of HPAI eradication changed in 1994–95 when epizootics of H5N2 HPAI in central Mexico and

H7N3 HPAI in Pakistan overpowered the resources of the respective governments and commercial poultry industries in stamping-out programs, requiring the addition of a fifth control tool (i.e., vaccination) to permit interim management of the clinical disease and allow continued food security until eradication was achievable in the long-term (Swayne et al. 2011). Since this initial vaccine use, vaccination has been used in HPAI control programs for poultry and captive birds in thirteen Asian, European, and African countries for H5N1 HPAI (2002–present); North Korea for H7N7 HPAI (2005); and Mexico for H7N3 HPAI (2012–present) (OIE 2012b; Swayne et al. 2011). The use of vaccination in poultry has become a valuable tool for temporary management of HPAI, supporting national food security and promoting the livelihood of rural poor, especially in underdeveloped countries (Swayne 2012a; Swayne et al. 2011). Ninety-nine percent of the vaccine used in birds against HPAI has been in the four countries where H5N1 HPAI is enzootic; i.e., China (including Hong Kong), Egypt, Indonesia, and Vietnam (Swayne et al. 2011). H5N1 HPAI was already enzootic in these four countries before vaccination was implemented, indicating that vaccination did not create enzootic infections (Swayne et al. 2011). However, routine use of vaccines and improper vaccination has delayed eradication, by contributing to complacency, and has complicated surveillance (Swayne and Spackman 2013).

Vaccines and Vaccination for High Pathogenicity Avian Influenza

Role of Vaccines and Vaccination

The role vaccines and vaccination can play in control of avian influenza has been assessed in multiple experimental studies in poultry. In the field, vaccines and vaccination have been shown to increase resistance of poultry to virus infection thereby preventing infection in a large percentage of poultry within the housing operation, and among any infected birds, prevent illness and death, and reduce the amount of virus replicating in respiratory and gastrointestinal tracts [reviewed by (Swayne 2012a)]. These data translate to reduced quantity of virus contaminating the environment (Gilbert et al. 2008; OIE 2007), which will reduce virus exposure and infections to birds (Bouma et al. 2008; Goot et al. 2003) and humans (OIE 2007; Swayne et al. 2011), and, therefore, maintaining livelihoods and food security of rural poor (OIE 2007). However, vaccines and vaccination alone will not eradicate HPAI because eradication can only be achieved through a comprehensive strategy coordinating vaccines and vaccination with the other four control components of stamping-out programs.

Assessing the Protection from Vaccines and Vaccination

The assessment of protection induced by AI vaccines is best accomplished using an in vivo challenge model and measuring quantifiable criteria that mimics protective effects in the field (Swayne 2008a) which includes prevention of clinical signs and death following HPAIV challenge (Stone 1987), prevention of egg production drops following LPAIV and HPAIV challenge (Brugh et al. 1979; Stone 1987; Swayne et al. 2012), reduction in quantity of LPAIV or HPAIV challenge virus shed from respiratory and gastrointestinal tracts (Swayne et al. 1999; Swayne et al. 1997), and prevention of contact transmission (LP and HPAIV challenge) (Swayne et al. 1997).

Experimentally, efficacious AI vaccines have been shown to have the following ideal traits: (1) protect against high environmental virus exposure or challenge dose (Swayne et al. 1997); (2) provide protection for long periods of time, usually a minimum of 6–12 months (Swayne and Spackman 2013); (3) provide reproducible protection through a defined vaccination method such as subcutaneous injection, wing web administration, coarse or fine spray, eye drop, in ovo, etc. (Swayne and Spackman 2013); (4) protect with a minimum number of vaccinations, ideally two but some species (e.g., turkeys) and long-lived birds (e.g., layers), may require three or more vaccinations (Eggert and Swayne 2010); and (5) broadly usable in multiple bird species (Swayne and Spackman 2013).

Protection in Chickens and Ducks

Antigenic matching between the vaccine and field virus is another critical factor in achieving optimal vaccine efficacy. Within the H5 and H7 subtypes, there can be enough variation that vaccines will not provide adequate protection against all challenge viruses because of poor match between vaccine and field strains (Abbas et al. 2011; Eggert et al. 2010; Grund et al. 2011; Pfeiffer et al. 2010). Therefore, selecting an initial vaccine that is a good antigenic match to the challenge virus is crucial. Antigenic drift with loss of protection has been observed in numerous cases where AIV has persisted in a population for a long time and vaccine

has been used long-term (Chen 2009; Escorcia et al. 2008; Grund et al. 2011; Lee et al. 2004). In order to maintain the most effective vaccination program, the field virus should be monitored for antigenic changes and the vaccine should be tested against new variants or at a minimum vaccines should be re-evaluated every 2–3 years for protection against current circulating field viruses.

Experimental studies in chickens and ducks evaluating several of the factors cited above have been conducted; however, very few studies have been reported for turkeys for protection from HPAI (Bublot et al. 2010; Cagle et al. 2011; Eggert and Swayne 2010; Kilany et al. 2011; Middleton et al. 2007; Pantin-Jackwood et al. 2012; Pfeiffer et al. 2010; Qiu et al. 2007; Steensels et al. 2007; Tian et al. 2005; Toffan et al. 2007; Webster et al. 2006; Yamamoto et al. 2010; Zhang et al. 2005). Few vaccines have achieved all the factors cited above, but still have been used successfully in the field. Importantly, it should be noted that vaccine studies in the laboratory cannot completely simulate field conditions and protection in the field is reported to be less effective, necessitating booster vaccinations (Eggert and Swayne 2010).

Due to the practical difficulty in evaluating the duration of immunity experimentally, a few studies have looked at the course of antibody levels in chickens in the field after vaccination with inactivated vaccines; however, general trends are difficult to establish because of numerous variables, including differences in genetic lines of chickens, number of times the vaccine was administered, vaccine dose, and different adjuvants (Boltz et al. 2009; Hwang et al. 2011; Sasaki et al. 2009). In situations where vaccination is used as an adjunct to other control methods, duration of immunity may be less critical if virus spread is controlled promptly. Although it can provide important detailed information on the performance characteristics of a vaccine, direct assessment of vaccine efficacy by in vivo testing is time consuming and expensive. A practical alternative for determining a minimal protection level is by indirect assessment using virus neutralization or, more commonly in poultry, hemagglutination inhibition tests to evaluate the antibody titers in vaccinated populations. If an adequate proportion of the flock has a minimum titer of antibody to the current field virus, they are expected to be protected. This is also why it is important to maintain adequate surveillance of vaccinated populations for exposure to the virus. This ensures that new field variants are promptly detected and can be characterized for changes which affect their antigenic traits.

Given the widespread infection of domestic ducks with H5N1 HPAIV in certain parts of the world, reducing the risk of virus infection in ducks is considered crucial for controlling the spread of H5N1 HPAI. In much of the developing world, domestic ducks are usually farmed in open fields, flooded rice paddies, or on ponds or other bodies of water, allowing direct exposure to wild waterfowl, and domestic ducks are frequently moved between fields and to live poultry markets, aiding to maintenance and spread of the virus in agricultural production systems. Since in most cases biosecurity measures are impractical or impossible to implement and enforce, vaccination is one of the few control tools available to protect domestic ducks against H5N1 HPAI. In laboratory studies with moderate to high challenge doses, vaccination has proven effective in protecting domestic ducks against clinical signs of disease; however, different species of domestic ducks respond differently to vaccination, and shedding of the virus may still occur in clinically healthy vaccinated ducks, but the titer of virus shed is reduced (Cagle et al. 2011; Steensels et al. 2007, 2009). The difficulty of adequately vaccinating sufficient number of ducks to maintain "herd immunity" is a big obstacle in the control of H5N1 HPAIV. In situations in which ducks are reared in open fields, vaccination coverage is poor, i.e., low vaccination rate in the population, and, therefore, high numbers of domestic ducks remain susceptible and serve as reservoirs and disseminators of H5N1 HPAIV.

Current vaccines and vaccination practices for the control of H5N1 HPAIV infection in domestic waterfowl should take into account different variables including susceptibility of the ducks to different circulating viruses, effect of species, and husbandry practices. Not many studies have been conducted evaluating vaccination of domestic ducks in the field. In a study examining virus transmission within infected flocks before and after vaccination, it was found that apart from issues related to the quality of protection provided by the vaccine, the overall effectiveness of the vaccination campaigns was undermined by factors that deter farmers from vaccinating their flocks and operational issues for vaccine delivery (Magalhaes et al. 2010). The authors suggested that if vaccination continues to be included as part of a sustainable disease control program, efforts should be focused on training farmers in disease prevention in addition to disease recognition, as the latter is likely to be compromised in a vaccinated population. Results from field and laboratory evaluation of vaccines against H5N1 HPAI in domestic ducks indicates that

factors such as duck species and/or breed, vaccination protocols (number of doses, age), and proper use of vaccines may significantly influence the success or failure of the H5N1 vaccination program. Other factors, including the role of maternally derived antibodies, co-infection with other pathogens, and use of adjuvants not optimized for ducks, remains to be determined. Continuous new outbreaks of H5N1 HPAI emphasize the need for a comprehensive domestic waterfowl vaccination strategy and the development of domestic waterfowl-specific efficacious vaccines.

Immunity and Protection of Wild Bird Populations

There are two viable and interrelated questions concerning protection of wild bird populations from HPAI: (1) can wild bird populations be actively vaccinated to protect from HPAIV infection and disease, and (2) will natural exposure to H5 or H7 LPAIV induce immunity and protection from HPAIV infection and disease? There is no data on capture of wild birds, individual vaccination against AIV, and release back to natural habitats. However, zoo, hunting, companion, conservation, and endangered species of birds of diverse species, including aquatic birds of the orders Anseriformes, Charadriiformes, Ciconiiformes, Pelicaniformes, and Phoenicopteriformes, on over 292 premises in 20 countries have been vaccinated with inactivated poultry vaccines against H5 and/or H7 subtypes (Bertelsen et al. 2007; Furger et al. 2008; Philippa et al. 2005, 2007). These poultry vaccines produced variable levels of H5 and/or H7 hemagglutinating antibodies with 50 and 82% of the birds seroconverting (HI titer of $\geq$1:16) following a single and booster vaccination, respectively (EFSA 2007). The presence of such HI antibodies levels has been associated with protection in chickens and turkeys, but the specific HI antibody levels needed for protection in most non-poultry species, i.e., captive ''wild'' bird species, are unknown (Koch et al. 2009). Capture of wild species from their native habitat for administration of a killed vaccine would be logistically unrealistic and likely to cause unacceptable mortality in the birds from the capture and handling process. In addition, recapture of individual birds for a second immunization would be even more unrealistic. The only practicality of vaccination of non-poultry species would be those birds already held in captivity.

The second issue would be the immunity provided by natural exposure to LPAIV and resulting protection against HPAIV. In a recent study in Alaska, 44% of 11 species of Anseriformes birds and 80–95% of Emperor geese and three eider species tested had anti-AIV antibodies, based on anti-nucleoprotein ELISA test (Wilson et al. 2013). However, the anti-AIV antibody positive rates varied with species, age, year, and season (Ely et al. 2013). Protection is not based on the broadly reactive anti-nucleoprotein antibodies but on the more specific anti-hemagglutinin or anti-neuraminidase antibodies. The prevalence of H5 antibodies is rarely reported but in one study in wild ducks in the USA, a 27% prevalence of anti-H5 antibodies was found (Nettles et al. 1985) while a study in Europe showed 49–69% anti-H5 antibody prevalence in mute swans, 64% in sacred ibis, 28% in mallards, and 27% in common pochards (Niqueux et al. 2010). These studies were spatially and geographically associated with outbreaks of H5 HPAI and may not reflect the general seroprevalence of anti-H5 antibodies of all aquatic species in all geographic regions, and seroprevalence of anti-H7 antibodies is unknown.

In one experimental study, Costa et al. (2010) using wood ducks (*Aix sponsa*) determined that exposure to LPAIV could provide protection from H5N1 HPAIV challenge, but the protection required the exposure to a H5 LPAIV and that virus must be adequately adapted to the bird species to replicate to sufficient titer to stimulate a detectible immune response based on H5 HI antibodies. They suggested that in naturally occurring outbreaks of H5N1 HPAI, birds with pre-existing immunity to homologous hemagglutinin or neuraminidase subtypes of AI virus may either survive H5N1 HPAIV infection or live longer than naive birds and, consequently, could pose a greater risk for contributing to viral transmission and dissemination, if titers of H5 and N1 antibodies are low and provide protection only from death but do not completely prevent virus replication. In addition, the ability to capture and induce protection to all susceptible wild waterfowl through timed exposure of wild birds to live LPAIV would not be acceptable because of the need for multiple individual strains adapted to individual wild bird species, which would be prohibitive and could produce unintended and unknown adverse effects. Furthermore, the presence of antibodies to H5 and H7 due to natural LPAIV infections cannot be relied upon to protect wild birds from infection and disease following HPAIV exposure, if the field virus were variants, antigenically distant from the LPAIV. For wild birds, the only realistic means to protect from HPAIV would be to prevent exposure to agricultural reservoirs, and the adjunct of controlling and eradicating the HPAIV from the agricultural system.

Vaccines in the Field for Poultry

For 2002–2010, over 113 billion doses of AIV vaccine were used in poultry within 15 different countries/special administrative regions (Swayne et al. 2011). The majority of the vaccine was used in poultry within four H5N1 HPAI enzootic countries, utilizing nationwide vaccination programs with the goal of reaching all poultry within the country (Swayne et al. 2011). China used >103 billion doses (90.99%), Egypt 5 billion doses (4.65%), Indonesia 2.6 billion doses (2.32%), and Vietnam 1.6 billion doses (1.43%). With these four countries, the vaccine use was proportional to the country's poultry production with China being the number one poultry producer and consumer in the world with a production of 14.9–16.4 billion birds per year (2004–2010). The remaining 10 countries/regions (Mongolia, Kazakhstan, France, The Netherlands, Cote d'Ivoire, Sudan, North Korea, Israel, Russia, and Pakistan) used 698 million doses of vaccines (0.6%) in poultry for targeted preventative or emergency vaccination programs; focusing to either specific geographic areas, around outbreak zones or to specific types of poultry or farming systems. In mid-2012, Mexico began a AIV vaccine program in laying chickens within the defined control zone of the state of Jalisco in response to the H7N3 HPAI epizootic (OIE 2012b). By contrast, AIV vaccine has had minimal use in non-poultry birds, with 271,690 doses being used during 2002 and 2010 in zoo, hunting, companion, conservation, or endangered birds to protect from H5 and/or H7 HPAI, which represents 0.00024% of the total AIV vaccine used in birds (Swayne et al. 2011).

The vast majority of the 113 billion doses of vaccine used have been inactivated oil-emulsified whole AIV vaccines (95.5%) which require handling and injection of individual birds, while live recombinant virus vaccines (4.5%) have had a more restricted, focused use within some poultry populations (Swayne et al. 2011). The live recombinant vaccines have included Newcastle disease virus (rNDV)-vectored vaccine with H5 influenza gene insert (rNDV-H5-AIV) which can be administered by spray respiratory application, and two fowlpox virus (rFPV)-vectored vaccines with either an H5 AIV gene insert (rFPV-H5-AIV) or an H5 and N1 AIV gene inserts which are administered only to chickens at 1 day-of-age by injection. Two new recombinant vaccines have been developed and licensed whose potential will improve application and protection; herpesvirus turkey (rHVT)-vectored vaccine with H5 AI virus gene insert for use in chickens and turkeys, and a duck virus enteritis (rDVE)-vectored vaccine with an H5 gene insert for use in domestic ducks (Liu et al. 2011; Rauw et al. 2011). Between 1998 and 2005, over two billion doses of an rFPV-H5-AIV were used in chickens in Central America to protect against H5N2 LPAIV, (Bublot et al. 2006), and its use has continued through 2013.

Since 2002, large quantities of AI vaccines have been used against H5N1 HPAI, but this tool alone has not resulted in eradication within the four enzootic countries, but has positively contributed to the eradication or prevention of HPAI in the other 11 countries/regions. Within the four enzootic countries, reports of AIV vaccine "failures" have been made, specifically reporting of clinical disease consistent with HPAI or isolation of H5N1 HPAIV in vaccinated flocks or in regions that vaccinate (Swayne 2012a). These vaccine "failures" have resulted from two categories of problems: (1) failure of the vaccine and (2) failure of vaccination. Vaccine "failures" have resulted from poor-quality vaccines with inadequate quantity of H5 antigen, or vaccine containing a seed strain that does not protect against a field virus because of antigenic drift. Vaccination "failures" have resulted from the lack of proper administration of vaccine or inability to vaccinate and produce a protective immune response in the at-risk poultry population; i.e., a failure to achieve population immunity because of inability to vaccinate poultry properly (Bouma et al. 2007; Swayne 2012b). Delivery of vaccine to billions of poultry owned by millions of people is a huge logistic problem.

Vaccine Technologies

Five different categories of vaccine technologies have been used to develop AIV vaccines in the laboratory and study their ability to protect birds: (1) inactivated whole AIV, (2) live AIV, (3) live vectors, (4) in vitro produced hemagglutinin, and (5) DNA vaccines (Table 1) (Swayne and Spackman 2013). However, application in the field through licensing and use has only been accomplished with a few technologies and products: i.e., inactivated whole AIV vaccines and live vectored vaccines (rNDV, rFPV, rHVT, and rDVE). The inactivated vaccine requires catching, handling, and injecting each individual bird as also when using rFPV, but the rNDV can be mass applied by spray administration and rHVT can be applied at 1 day-of-age to chickens in the hatchery or in ovo, saving time and labor cost. Adoption of new technologies for commercial vaccines requires satisfying multiple ideal traits for AIV

Table 1. Experimental and Licensed Vaccines for HPAI in Poultry and Other Avian Species [Modified from (Swayne and Kapczynski 2008; Swayne and Spackman 2013)].

Vaccine category	Vaccine	Species	Route	AI subtypes	HPAIV challenge tested	Licensed	Comments	Additional references
Inactivated AIV	Adjuvanted whole AIV	Chicken (layer and broiler), turkey, duck, goose, other poultry, zoo birds	SQ, IM, in ovo	H5, H7	Yes	Yes	Mostly oil-emulsified; some with aluminum hydroxide. Includes H5 and H7 LPAIV, H5 and H7 HPAIV, and H7 reverse genetic LPAIV seed strains. Requires parenteral administration	
Live AIV	Live wild-type LPAIV	Chicken	IM, IT spray	H5, H7	Yes	No	Rumors of intentional exposure with LPAIV to protect from HPAIV have been reported in H5N1 and H5N2 HPAI outbreaks in 1990s and 2000s	(Swayne, unpublished information)
	Attenuated LPAIV	Chicken	Spray	H5, H7	Yes	No	Temperature sensitive mutant or replace HA with ectodomain of NDV HN gene; risk assessment needed for reassortment potential	Park et al. (2006), Zhang et al. (2012)
Live vector	rd-Adenovirus	Chicken	SQ, IN, in ovo	H5	Yes	No	rd = Replication defective, only 1 round of replication occurs after injection. SQ and in ovo protected	Gao et al. (2006)
	Avian leukosis virus	Chicken	IM	H7	Yes	No		Hunt et al. (1988)
	Avian paramyxovirus type 1 (rNDV)	Chicken	Eye, IN	H5, H7	Yes	Yes	Licensed in Mexico and China	Ge et al. (2007), Swayne et al. (2003)
	Duck enteritis virus (rDVE)	Duck	IM	H5	Yes	No	Submitted for license in mid-2012 for ducks (China)	Liu et al. (2011)
	Fowlpox virus (rFPV)	Chicken (layer and broiler), goose, Muscovy ducks	SQ, WW	H5, N1	Yes	Yes	Licensed 1997 for chickens (USA, Mexico); used primarily in Central America against H5N2 LPAI; limited use in China	

Table 1. continued

Vaccine category	Vaccine	Species	Route	AI subtypes	HPAIV challenge tested	Licensed	Comments	Additional references
	Herpesvirus Turkey (rHVT)	Chickens	SQ	H5, N1	Yes	Yes	Licensed 2012 for chickens (USA, Egypt). Used Egypt	CEVA (2013), Rauw et al. (2011)
	Infectious laryngotracheitis virus vector	Chicken	Eye	H7, H5, N1	Yes	No	N1 did not protect	Pavlova et al. (2009), Veits et al. (2003)
	att-*Salmonella typhimurium*	Chicken	OR	H5, M2e	Yes	No	Attenuated vaccine strain. Failed to protect from HPAIV challenge with single oral immunization	Layton et al. (2009), Pan et al. (2009)
	Vaccinia	Chicken	IM, IP	H5	Yes	No	Low to no antibody response	Chambers et al. (1988)
	rd-Venezuelan Equine Encephalitis virus		SQ, in ovo	H5	Yes	No	rd = Replication defective, only 1 round of replication occurs after injection	Schultz-Cherry et al. (2000)
In vitro produced hemagglutinin	Baculovirus in insect cell culture	Chicken, Muscovy duck	SQ	H5, H7	Yes	No		Crawford et al. (1999)
	Eukaryotic systems (plants or cells cultures)	Chicken	IM	H5	Yes	No	*Nicotiana* sp.	Kalthoff et al. (2010)
DNA	Naked DNA	Chicken	IM	H5	Yes	No	Not financially viable. Improvements needed in promoters and adjuvants to decrease quantity of nucleic acid needed and reduce number doses for protection	Rao et al. (2008), Suarez and Schultz-Cherry (2000)

Eye conjunctival sac, *IM* intramuscular, *IN* intranasal, *in ovo* into embryonating egg, *IP* intraperitoneal, *IT* intratracheal, *OR* oral, *spray* fine or coarse droplet into air space, *SQ* subcutaneous, *WW* wing web.

Table 2. Properties of Ideal AIV Vaccines and Vaccination Methods for Poultry [Modified from (Swayne and Spackman 2013)].

Desired property	Current situation
Inexpensive	Current cost for inactivated AIV vaccine: $0.05–0.10/dose plus cost of administration ($0.05–0.07 per dose for individual handling and injection) (Swayne and Kapczynski 2008)
Use in multiple avian species	Most used in meat, layer, and breeder chickens, but large quantity also used in ducks; minor amounts in turkeys, geese, quail, etc. (Swayne et al. 2011)
Single dose protection	Most situations require minimum of two doses; prime-boost scenario is optimal with additional boost in long-lived birds at 6–12 month intervals (Steensels et al. 2009; Swayne 2006)
Easy, mass application	95.5% is inactivated vaccine administered by handling and injecting individual birds, with 4.5% as vectored vaccine given by mass spray vaccination (rNDV vector) (Swayne et al. 2011)
Identify infected birds in vaccinated population	Serological differentiation tests are available, but only minor use. Most vaccine applied without using a DIVA strategy (Swayne 2006)
Overcome maternal anti body block	Maternal antibody to AIV hemagglutinin or virus vector inhibits primary immune response. Initial vaccination must be timed for declining maternal antibody titers to allow optimal primary immune response (Maas et al. 2011), as decline in active immunity before giving booster vaccinations is also needed (Swayne et al. 2000)
Given at 1 day-of-age in hatchery or in ovo	Inactivated vaccines provide poor protection if given at 1 day-of-age. Vectored vaccines can be given at 1 day-of-age, but generally require a field boost with inactivated vaccine 10 days or more later
Antigenically close to field virus	The majority of inactivated whole AIV vaccine uses reverse genetic generated vaccine seed strains to antigenically match field viruses (Swayne 2012b; Swayne et al. 2011)

vaccines including practical field application to solve poultry health problems (Table 2). In addition, the reader must understand that an ideal vaccine for humans may not be ideal for poultry.

Any new vaccine technologies will only be adopted for licensing and field use if the new vaccine will provide protection in experimental trials that is equivalent to or better than the "gold standard," i.e., oil-emulsified whole AIV vaccine (Swayne and Spackman 2013). Vaccine development and field implementation in commercial poultry is driven by economics with adoption of new technologies occurring only if a financial advantage is provided such as the cost of the new vaccine is less than the loss from disease with no vaccination, or the cost differential of new vaccine over the existing vaccine is less than the savings from additional protection from disease. Historically, most AIV vaccines have been based on inactivated whole AIV with the seed virus being produced in embryonating chicken eggs. This mature, standard technology has been used successfully for over 40 years to produce trillions of doses of killed or live-attenuated vaccines to other poultry viral diseases such as Marek's disease, reoviral arthritis, Newcastle disease, infectious bronchitis, and infectious bursal disease. This low-cost technology has produced efficacious, potent vaccines without the additional cost of royalties for patents or the purchase of new manufacturing equipment which will be needed for implementation of newer vaccine technologies. However, newer technologies will be and have been utilized at the higher cost when they have addressed one or more critical traits which have made the new technologies produce a product closer to the ideal vaccine (Table 2). As an example, 66.6 of 73 billion doses (91%) of inactivated H5 AIV vaccines used from 2007 to 2009 were based on vaccine seed strains produced through reverse genetic technology (Swayne 2012b). These vaccines are closer antigenically to H5N1 viruses encountered in the field, and provide better protection than historic inactivated vaccine seed strains based on LPAIV.

In developed countries, inactivated whole AIV vaccines have been limited to use in valuable, long-lived, or specialty poultry because of the high cost of individual bird administration and long withdrawal period for oil adjuvant in any meat poultry. By contrast, in less developed and developing/transitional countries with low labor cost for vaccination and shorter withdrawal periods for oil adjuvants, inactivated vaccines have been administered to the much larger populations of meat chickens and ducks. Experimental studies have demonstrated the possibility for low-cost mechanized in ovo injection for oil-emulsified, inactivated whole AIV vaccines (Stone et al. 1997), adenovirus-vectored vaccines (Breedlove et al. 2011),

VEE-vectored vaccine (Schultz-Cherry et al. 2000), attenuated AIV vaccine (Song et al. 2007), and rNDV-vectored vaccines (Steel et al. 2008) that could be commercially viable and allow for more use of AIV vaccines in developed countries. In addition, a superior approach would be new delivery technologies for easier, mass application such as administration by spray (respiratory delivery of fine droplets) or per os (oropharyngeal and upper digestive tract delivery in feed or water).

Even with new breakthroughs in technologies, important fundamental questions must be first answered; i.e., that is whether vaccination is needed as a control tool, or if other control tools such as prevention through management biosecurity or, if immediate stamping-out is the better approach. In a recent survey (Swayne et al. 2011), most countries preferred rapid eradication of HPAI by using a stamping-out program without vaccination and indicated that they would only use vaccination if the HPAI epizootic was large and stamping-out was not effective in producing immediate eradication. Alternatively, if the threat of an epizootic was high, vaccination might be used as a preventative measure for valuable poultry, and endangered or valuable captive bird species within zoos or other collections (Swayne et al. 2011). Historically, the decision point for implementing vaccination for HPAI was reached earlier with the least developed and developing/transition countries (13 of 15 countries that vaccinated), than in developed countries where only two countries (France and The Netherlands) vaccinated and they used a small, time-limited targeted vaccination program (Pavade et al. 2011; Swayne et al. 2011).

Vaccination of Poultry: Coverage and Population Immunity

Protection in the field can only be achieved if the at-risk poultry are able to mount an effective immune response and if individual birds receive the vaccine in the proper dose, correct number of vaccinations, and administration by the correct route. Population immunity of at-risk poultry is the goal, which is only achieved if greater than 60–80% of the poultry have a protective immune response (Bouma et al. 2007; Swayne et al. 2011). If we look at an entire country conducting routine vaccination of all poultry, the goal of national population immunity is difficult to achieve because of limitations in financial and human resources. This conclusion is based on the 113 billion doses of AIV vaccine used in poultry during 2002–2010 which resulted in only a 41.9% coverage rate among the at-risk national poultry population of the 15 vaccinating countries/regions (Swayne et al. 2011). Five of the 15 countries/regions conducted routine vaccination campaigns of all poultry with national coverage rates of 47.1% for China, 86.2% for Hong Kong, 69.9% for Egypt, 14% for Indonesia, and 52.3% for Vietnam (Swayne et al. 2011). This initial data suggest that only two countries/regions achieved population immunity (Hong Kong and Egypt), but more detailed analysis using more accurate estimates of higher village poultry populations in Egypt suggests that Egypt did not achieve a national population immunity with revised vaccination coverage rates between 27.8 and 48.6% (Swayne et al. 2011). Furthermore, the use of 1 day-of-age vaccination in broilers in Egypt using inactivated oil-emulsified vaccines may have also contributed to inadequate immune responses, even further decreasing the effective immunity in the population. Therefore, cases of H5N1 HPAI in poultry continue to occur in China, Egypt, Indonesia, and Vietnam because of the lack of population immunity, but Hong Kong did achieve national population immunity, with only one farm having H5N1 HPAI in poultry during 2003–2012 (Swayne 2012a). These findings suggest that national population immunity in poultry, with its intensive financial and human resource requirements, is not realistic in most countries. Alternatively, vaccination should be targeted to poultry at the greatest risk for exposure to HPAI and/or to specific geographic regions. Decisions on which poultry and/or geographic regions to vaccinate require both ongoing field surveillance and epidemiological data and modeling in order to design and implement effective vaccination programs. The historical yearly vaccination campaigns, used more commonly in cattle and pigs for transboundary diseases, are not effective with commercial poultry because of the shorter replacement period (i.e., chickens and ducks have a 5-month generation time) which result in production of a large naïve poultry populations between the vaccination campaigns, thus providing susceptible host to maintain the virus in the population. In addition, countries with large populations of poultry produced in the semi-commercial and village sectors must develop unique programs that will reach the large number of households with low numbers of birds. Initially, expectations were high that a spray vaccination of rNDV-H5-AIV would provide single dose, uniform protection in all poultry. Although rNDV-H5-AIV by respiratory mass application in experimental studies with specific pathogen-free chickens did provide protection from HPAIV

challenge, when transferred to the field, the presence of high levels of maternal antibody to NDV and H5 AIV inhibited rNDV-H5-AIV replication and failed to provide protection with the single vaccine dose (Swayne and Spackman 2013). The rNDV-H5-AIV has best been used as a priming vaccine followed by inactivated whole AIV booster vaccination. Additional research is needed on optimizing vaccination protocols for different poultry species and ages to achieve low-cost immunity.

Conclusions

Based on the information presented and discussed, the following conclusions may be drawn:

1. Historically, infection of wild birds by HPAIV has been rare, but wild bird infections have become more common with the emergence of H5N1 HPAIV (Guangdong lineage) in China which has spread across three continents, causing notable infections and deaths in a variety of wild bird species. However, the major source and reservoir of H5N1 HPAIV is domestic poultry, especially domestic ducks.
2. Vaccines have been used as a tool in HPAIV control and eradication for poultry in five of 32 epizootics. Most of the vaccine has been used in poultry to protect against H5N1 HPAIV (Guangdong lineage) and have been used in enzootic countries/regions (China, Egypt, Indonesia, and Vietnam) as part of nationwide vaccination campaigns. Targeted vaccination, based on geography, bird type, and/or time limitations, has been practiced in another 10 countries and regions, but accounted for less than 1% of all AI vaccine used.
3. Most poultry AI vaccines have been the traditional, inactivated oil-emulsified whole AIV vaccines, with $<5\%$ of AI vaccines being live vectored vaccines. The inactivated vaccine requires labor-intensive catching and individual bird vaccination.
4. Vaccines have been used to protect some non-poultry species, but only in captive birds on 292 premises in 20 countries; i.e., mostly for zoo, hunting, companion, conservation, or endangered birds held in captivity. Vaccination of wild birds in natural habitats has not been attempted and is neither practical nor feasible.
5. LPAIV infection in wild birds can confer protection against HPAIV if the hemagglutinin and/or neuraminidase subtype of the LPAIV matches the HPAIV and if the LPAIV infects and produces a robust immune response. However, in practice, any protection against H5 and/or H7 HPAIV in wild bird populations is dependent upon the geographic area, bird species, year, and season. Predictability of any such natural protection is unknown.
6. Control and eradication of HPAIV from the domestic poultry reservoirs is the most effective means to protect wild bird populations from HPAIV.

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References

Abbas MA, Spackman E, Fouchier R, Smith D, Ahmed Z, Siddique N, Sarmento L, Naeem K, McKinley ET, Hameed A, Rehmani S, Swayne DE (2011) H7 avian influenza virus vaccines protect chickens against challenge with antigenically diverse isolates. *Vaccine* 29:7424–7429

Becker WB (1967) Experimental infection of common terns with Tern virus: influenza virus A-Tern-South Africa-1961. *Journal of Hygiene* 65:61–65

Bertelsen MF, Klausen J, Holm E, Grondahl C, Jorgensen PH (2007) Serological response to vaccination against avian influenza in zoo-birds using an inactivated H5N9 vaccine. *Vaccine* 25:4345–4349

Boltz DA, Douangngeun B, Sinthasak S, Phommachanh P, Midouangchanh P, Walker D, Keating R, Khalenkov AM, Kumar M, Webster RG (2009) Field assessment of an H5N1 inactivated vaccine in chickens and ducks in Lao PDR. *Archives of Virology* 154:939–944

Bouma A, Muljono AT, Jatikusumah A, Nell A, Mudjiartiningsih S, Dharmayanti I, Siregar E, Sawitri Claassen I, Koch G, Stegeman JA (2008) Field trial for assessment of avian influenza vaccination effectiveness in Indonesia. *Revue Scientifique et Technique Office International des Epizooties* 27:633–642

Bouma A, Tiensin T, Claassen I, Nielen M, van Boven M, Stegeman JA (2007) Estimation of the Critical Proportion of Chickens in a Flock to be Immunized to Prevent Major Outbreaks of HPAI H5N1. OIE/FAO/IZSVe Scientific Conference—

Vaccination: a tool for the control of avian influenza, 20–22 March, Verona, Italy, p 60

Breedlove C, Minc JK, Tang DC, van Santen VL, van Ginkel FW, Toro H (2011) Avian influenza adenovirus-vectored in ovo vaccination: target embryo tissues and combination with Marek's disease vaccine. *Avian Diseases* 55:667–673

Brown JD, Stallknecht DE, Berghaus RD, Swayne DE (2009) Infectious and lethal doses of H5N1 highly pathogenic avian influenza virus for house sparrows (*Passer domesticus*) and rock pigeons (*Columbia livia*). *Journal of Veterinary Diagnostic Investigation* 21:437–445

Brugh M, Beard CW, Stone HD (1979) Immunization of chickens and turkeys against avian influenza with monovalent and polyvalent oil emulsion vaccines. *American Journal of Veterinary Research* 40:165–169

Bublot M, Pritchard N, Swayne DE, Selleck P, Karaca K, Suarez DL, Audonnet JC, Mickle TR (2006) Development and use of fowlpox vectored vaccines for avian influenza. *Annals of the New York Academy of Sciences* 1081:193–201

Bublot M, Richard-Mazet A, Chanavat-Bizzini S, Le Gros FX, Duboeuf M, Stoll A, Palfi V, Niqueux E, Guionie O, Dren N (2010) Immunogenicity of poxvirus vector avian influenza vaccines in Muscovy and Pekin ducks. *Avian Diseases* 54:232–238

Cagle C, To TL, Nguyen T, Wasilenko J, Adams SC, Cardona CJ, Spackman E, Suarez DL, Pantin-Jackwood MJ (2011) Pekin and Muscovy ducks respond differently to vaccination with a H5N1 highly pathogenic avian influenza (HPAI) commercial inactivated vaccine. *Vaccine* 29:6549–6557

Cattoli G, Monne I, Fusaro A, Joannis TM, Lombin LH, Aly MM, Arafa AS, Sturm-Ramirez KM, Couacy-Hymann E, Awuni JA, Batawui KB, Awoume KA, Aplogan GL, Sow A, Ngangnou AC, El Nh I, Gamatie D, Dauphin G, Domenech JM, Capua I (2009) Highly pathogenic avian influenza virus subtype H5N1 in Africa: a comprehensive phylogenetic analysis and molecular characterization of isolates. *PLoS One* 4:e4842

CEVA (2013) Ceva develops Vectormune HVT AIV vaccine to combat avian influenza. Accessed on March 21, 2013 from http://www.ceva.com/content/download/20740/381139/file/Press_release_Vectormune_HVT_AIVLaunch_09042012.pdf

Chambers TM, Kawaoka Y, Webster RG (1988) Protection of chickens from lethal influenza infection by vaccine-expressed hemagglutinin. *Virology* 167:414–421

Chen H (2009) Avian influenza vaccination: the experience in China. *Revue Scientifique et Technique Office International des Epizooties* 28:267–274

Costa TP, Brown JD, Howerth EW, Stallknecht DE (2010) Effect of a prior exposure to a low pathogenic avian influenza virus in the outcome of a heterosubtypic low pathogenic avian influenza infection in mallards (*Anas platyrhynchos*). *Avian Diseases* 54:1286–1291

Crawford J, Wilkinson B, Vosnesensky A, Smith G, Garcia M, Stone H, Perdue ML (1999) Baculovirus-derived hemagglutinin vaccines protect against lethal influenza infections by avian H5 and H7 subtypes. *Vaccine* 17:2265–2274

EFSA (2007) Opinion of the Scientific Panel on Animal Health and Welfare (AHAW) on a request from the Commission related with the vaccination against avian influenza of H5 and H7 subtypes as a preventive measure carried out in Member States in birds kept in zoos under Community approved programmes. *The EFSA Journal* 450. Accessed on 19 July, 2011 from doi: 10.2903/j.efsa.2007.450

Eggert D, Swayne DE (2010) Single vaccination provides limited protection to ducks and geese against H5N1 high pathogenicity avian influenza virus. *Avian Diseases* 54:1224–1229

Eggert D, Thomas C, Spackman E, Pritchard N, Rojo F, Bublot M, Swayne DE (2010) Characterization and efficacy determination of commercially available central American H5N2 avian influenza vaccines for poultry. *Vaccine* 28:4609–4615

Ellis TM, Barry BR, Bissett LA, Dyrting KC, Luk GSM, Tsim ST, Sturm-Ramirez K, Webster RG, Guan Y, Peiris JSM (2004) Investigation of outbreaks of highly pathogenic H5N1 avian influenza in waterfowl and wild birds in Hong Kong in late 2002. *Avian Pathology* 33:492–505

Ely CR, Hall JS, Schmutz JA, Pearce JM, Terenzi J, Sedinger JS, Ip HS (2013) Evidence that life history characteristics of wild birds influence infection and exposure to influenza A viruses. *PLoS One* 8:e57614

Escorcia M, Vazquez L, Mendez ST, Rodriguez-Ropon A, Lucio E, Nava GM (2008) Avian influenza: genetic evolution under vaccination pressure. *Virology Journal* 5:15

Feare CJ (2010) Role of wild birds in the spread of highly pathogenic avian influenza virus H5N1 and implications for global surveillance. *Avian Diseases* 54:201–212

Furger M, Hoop R, Steinmetz H, Eulenberger U, Hatt J (2008) Humoral immune response to avian influenza vaccination over a six-month period in different species of captive wild birds. *Avian Diseases* 52:222–228

Gao W, Soloff AC, Lu X, Montecalvo A, Nguyen DC, Matsuoka Y, Robbins PD, Swayne DE, Donis RO, Katz JM, Barratt-Boyes SM, Gambotto A (2006) Protection of mice and poultry from lethal H5N1 avian influenza virus through adenovirus-based immunization. *Journal of Virology* 80:1959–1964

Ge J, Deng G, Wen Z, Tian G, Wang Y, Shi J, Wang X, Li Y, Hu S, Jiang Y, Yang C, Yu K, Bu Z, Chen H (2007) Newcastle disease virus-based live attenuated vaccine completely protects chickens and mice from lethal challenge of homologous and heterologous H5N1 avian influenza viruses. *Journal of Virology* 81:150–158

Gilbert M, Xiao X, Pfeiffer DU, Epprecht M, Boles S, Czarnecki C, Chaitaweesub P, Kalpravidh W, Minh PQ, Otte MJ, Martin V, Slingenbergh J (2008) Mapping H5N1 highly pathogenic avian influenza risk in Southeast Asia. *Proceedings of the National Academy of Sciences of the United States of America* 105:4769–4774

Goot JA, Koch G, Jong MC, Mv Boven (2003) Transmission dynamics of low- and high-pathogenicity A/Chicken/Pennsylvania/83 avian influenza viruses. *Avian Diseases* 47:939–941

Grund C, Abdelwhab E, Arafa AS, Ziller M, Hassan MK, Aly MM, Hafez HM, Harder TC, Beer M (2011) Highly pathogenic avian influenza virus H5N1 from Egypt escapes vaccine-induced immunity but confers clinical protection against a heterologous clade 2.2.1 Egyptian isolate. *Vaccine* 29:5567–5573

Guan Y, Smith GJD, Webby R, Webster RG (2009) Molecular epidemiology of H5N1 avian influenza. *Revue Scientifique et Technique Office International des Epizooties* 28:39–47

Hulse-Post DJ, Sturm-Ramirez KM, Humberd J, Seiler P, Govorkova EA, Krauss S, Scholtissek C, Puthavathana P, Buranathai C, Nguyen TD, Long HT, Naipospos TSP, Chen H, Ellis TM, Guan Y, Peiris JSM, Webster RG (2005) Role of domestic ducks in the propagation and biological evolution of highly pathogenic H5N1 influenza viruses in Asia. *Proceedings of the National Academy of Sciences of the United States of America* 102:10682–10687

Hunt LA, Brown DW, Robinson HL, Naeve CW, Webster RG (1988) Retrovirus-expressed hemagglutinin protects against lethal influenza virus infections. *Journal of Virology* 62:3014–3019

Hwang SD, Kim HS, Cho SW, Seo SH (2011) Single dose of oil-adjuvanted inactivated vaccine protects chickens from lethal infections of highly pathogenic H5N1 influenza virus. *Vaccine* 29:2178–2186

Kalthoff D, Giritch A, Geisler K, Bettmann U, Klimyuk V, Hehnen HR, Gleba Y, Beer M (2010) Immunization with plant-expressed hemagglutinin protects chickens from lethal highly pathogenic avian influenza virus H5N1 challenge infection. *Journal of Virology* 84:12002–12010

Keawcharoen J, van Riel D, van Amerongen G, Bestebroer T, Beyer WE, van Lavieren R, Osterhaus Albert DME, Fouchier Ron AM, Kuiken T (2008) Wild ducks as long-distance vectors of highly pathogenic avian influenza virus (H5NI). *Emerging Infectious Diseases* 14:600–607

Kilany WH, Abdelwhab EM, Arafa AS, Selim A, Safwat M, Nawar AA, Erfan AM, Hassan MK, Aly MM, Hafez HM (2011) Protective efficacy of H5 inactivated vaccines in meat turkey poults after challenge with Egyptian variant highly pathogenic avian influenza H5N1 virus. *Veterinary Microbiology* 150:28–34

Kim JK, Negovetich NJ, Forrest HL, Webster RG (2009) Ducks: the "Trojan horses" of H5N1 influenza. *Influenza and Other Respiratory Viruses* 3:121–128

Koch G, Steensels M, Tvd Berg (2009) Vaccination of birds other than chickens and turkeys against avian influenza. *Revue Scientifique et Technique Office International des Epizooties* 28:307–318

Kou Z, Lei FM, Yu J, Fan ZJ, Yin ZH, Jia C, X, Xiong KJ, Sun YH, Zhang XW, Wu XM, Gao XB, Li TX (2005) New genotype of avian influenza H5N1 viruses isolated from tree sparrows in China. *Journal of Virology* 79:15460–15466

Layton SL, Kapczynski DR, Higgins S, Higgins J, Wolfenden AD, Liljebjelke KA, Bottje WG, Swayne D, Berghman LR, Kwon YM, Hargis BM, Cole K (2009) Vaccination of chickens with recombinant Salmonella expressing M2e and CD154 epitopes increases protection and decreases viral shedding after low pathogenic avian influenza challenge. *Poultry Science* 88:2244–2252

Lee CW, Senne DA, Suarez DL (2004) Effect of vaccine use in the evolution of Mexican lineage H5N2 avian influenza virus. *Journal of Virology* 78:8372–8381

Liu J, Chen P, Jiang Y, Wu L, Zeng X, Tian G, Ge J, Kawaoka Y, Bu Z, Chen H (2011) A duck enteritis virus-vectored bivalent live vaccine provides fast and complete protection against H5N1 avian influenza virus infection in ducks. *Journal of Virology* 85:10989–10998

Maas R, Rosema S, Van ZD, Venema S (2011) Maternal immunity against avian influenza H5N1 in chickens: limited protection and interference with vaccine efficacy. *Avian Pathology* 40:87–92

Magalhaes RJS, Pfeiffer DU, Otte J (2010) Evaluating the control of HPAIV H5N1 in Vietnam: virus transmission within infected flocks reported before and after vaccination. *BMC Veterinary Research* 6:31

Middleton D, Bingham J, Selleck P, Lowther S, Gleeson L, Lehrbach P, Robinson S, Rodenberg J, Kumar M, Andrew M (2007) Efficacy of inactivated vaccines against H5N1 avian influenza infection in ducks. *Virology* 359:66–71

Nettles VF, Wood JM, Webster RG (1985) Wildlife surveillance associated with an outbreak of lethal H5N2 avian influenza in domestic poultry. *Avian Diseases* 29:733–741

Niqueux E, Guionie O, Schmitz A, Hars J, Jestin V (2010) Presence of serum antibodies to influenza a subtypes H5 and N1 in swans and ibises in French wetlands, irrespective of highly pathogenic H5N1 natural infection. *Avian Diseases* 54:502–508

OIE (2007) *Avian Influenza Vaccination: OIE Information Document And Verona Recommendations*. Accessed March 23, 2011 from http://www.oie.int/eng/info_ev/Other%20Files/A_Guidelines%20on%20AI%20vaccination.pdf, Paris: OIE

OIE (2012a) *Highly Pathogenic Avian Influenza, Australia (Follow-Up Report No. 1). OIE Disease Information* 25(47). Accessed 29 November, 2012 from http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?reportid=12568, Paris: OIE

OIE (2012b) *Highly Pathogenic Avian Influenza, Mexico. OIE Disease Information* 25(25). Accessed 2 August, 2012 from http://web.oie.int/wahis/reports/en_imm_0000012067_20120622_132313.pdf, Paris: OIE

OIE (2012c) *Highly Pathogenic Avian Influenza, Mexico; Follow-Up Report No. 8* (12/12/2012). *OIE Disease Information* 25(50). Accessed 21 March 2013 from http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?reportid=12466, Paris: OIE

Pan Z, Zhang X, Geng S, Cheng N, Sun L, Liu B, Huang J, Jiao X (2009) Priming with a DNA vaccine delivered by attenuated Salmonella typhimurium and boosting with a killed vaccine confers protection of chickens against infection with the H9 subtype of avian influenza virus. *Vaccine* 27:1018–1023

Pantin-Jackwood MJ, Smith DM, Wasilenko JL, Cagle C, Shepherd E, Sarmento L, Kapczynski DR, Afonso CL (2012) Effect of age on the pathogenesis and innate immune responses in Pekin ducks infected with different H5N1 highly pathogenic avian influenza viruses. *Virus Research* 167:196–206

Park MS, Steel J, Garcia-Sastre A, Swayne D, Palese P (2006) Engineered viral vaccine constructs with dual specificity: avian influenza and Newcastle disease. *Proceedings of the National Academy of Sciences of the United States of America* 103:8203–8208

Pavade G, Awada L, Hamilton K, Swayne DE (2011) Analysis of economic indicators, poultry density and performance of veterinary services for control of high pathogenicity avian influenza in poultry. *Revue Scientifique et Technique Office International des Epizooties* 30:661–671

Pavlova SP, Veits J, Mettenleiter TC, Fuchs W (2009) Live vaccination with an H5-hemagglutinin-expressing infectious laryngotracheitis virus recombinant protects chickens against different highly pathogenic avian influenza viruses of the H5 subtype. *Vaccine* 27:5085–5090

Pepin KM, Wang J, Webb CT, Smith GJ, Poss M, Hudson PJ, Hong W, Zhu H, Riley S, Guan Y (2012) Multiannual patterns of influenza A transmission in Chinese live bird market systems. *Influenza and other Respiratory Viruses* 7:97–107

Pfeiffer J, Suarez DL, Sarmento L, To TL, Nguyen T, Pantin-Jackwood MJ (2010) Efficacy of commercial vaccines in protecting chickens and ducks against H5N1 highly pathogenic avian influenza viruses from Vietnam. *Avian Diseases* 54:262–271

Philippa J, Baas C, Beyer W, Bestebroer T, Fouchier R, Smith D, Schaftenaar W, Osterhaus A (2007) Vaccination against highly pathogenic avian influenza H5N1 virus in zoos using an adjuvanted inactivated H5N2 vaccine. *Vaccine* 25:3800–3808

Philippa JD, Munster VJ, Bolhuis H, Bestebroer TM, Schaftenaar W, Beyer WE, Fouchier RA, Kuiken T, Osterhaus AD (2005) Highly pathogenic avian influenza (H7N7): vaccination of zoo

birds and transmission to non-poultry species. *Vaccine* 23:5743–5750

Qiu B, Guo Y, Liu D, Zhang Q, Qiu L, Tan Z, Fan Z, He S, Tang X, Zeng S, Peng J, Wu W, Huang Y, Liu G, Lu X, Zhang C (2007) Immunological efficacy of three kinds of avian influenza inactivated vaccines in rural backyard ducks. *China Poultry* 29:17–19

Rao S, Kong WP, Wei C-J, Yang ZY, Nason M, Styles D, DeTolla L, Sorrell E, Song H, Wan H, Ramirez-Nieto G, Perez D, Nabel G (2008) Multivalent HA DNA vaccination protects against highly pathogenic H5N1 avian influenza infection in chickens and mice. *PLoS One* 3:e2432

Rauw F, Palya V, Van BS, Welby S, Tatar-Kis T, Gardin Y, Dorsey KM, Aly MM, Hassan MK, Soliman MA, Lambrecht B, van den Berg T (2011) Further evidence of antigenic drift and protective efficacy afforded by a recombinant HVT-H5 vaccine against challenge with two antigenically divergent Egyptian clade 2.2.1 HPAI H5N1 strains. *Vaccine* 29:2590–2600

Rohm C, Horimoto T, Kawaoka Y, Suss J, Webster RG (1995) Do hemagglutinin genes of highly pathogenic avian influenza viruses constitute unique phylogenetic lineages? *Virology* 209:664–670

Sasaki T, Kokumai N, Ohgitani T, Sakamoto R, Takikawa N, Lin Z, Okamatsu M, Sakoda Y, Kida H (2009) Long lasting immunity in chickens induced by a single shot of influenza vaccine prepared from inactivated non-pathogenic H5N1 virus particles against challenge with a highly pathogenic avian influenza virus. *Vaccine* 27:5174–5177

Schultz-Cherry S, Dybing JK, Davis NL, Williamson C, Suarez DL, Johnston R, Perdue ML (2000) Influenza virus (A/HK/156/97) hemagglutinin expressed by an alphavirus replicon system protects chickens against lethal infection with Hong Kong-origin H5N1 viruses. *Virology* 278:55–59

Song H, Nieto GR, Perez DR (2007) A new generation of modified live-attenuated avian influenza viruses using a two-strategy combination as potential vaccine candidates. *Journal of Virology* 81:9238–9248

Steel J, Burmakina SV, Thomas C, Spackman E, Garcia-Sastre A, Swayne DE, Palese P (2008) A combination in ovo vaccine for avian influenza virus and Newcastle disease virus. *Vaccine* 26:522–531

Steensels M, Bublot M, Van Borm S, De Vriese J, Lambrecht B, Richard-Mazet A, Chanavat-Bizzini S, Duboeuf M, Le Gros FX, van den BT (2009) Prime-boost vaccination with a fowlpox vector and an inactivated avian influenza vaccine is highly immunogenic in Pekin ducks challenged with Asian H5N1 HPAI. *Vaccine* 27:646–654

Steensels M, Van Borm S, Lambrecht B, De Vriese J, Le Gros FX, Bublot M, van den Berg T (2007) Efficacy of an inactivated and a fowlpox-vectored vaccine in Muscovy ducks against an Asian H5N1 highly pathogenic avian influenza viral challenge. *Avian Diseases* 51:325–331

Stone H, Mitchell B, Brugh M (1997) In ovo vaccination of chicken embryos with experimental Newcastle disease and avian influenza oil-emulsion vaccines. *Avian Diseases* 41:856–863

Stone HD (1987) Efficacy of avian influenza oil-emulsion vaccines in chickens of various ages. *Avian Diseases* 31:483–490

Sturm-Ramirez KM, Hulse-Post DJ, Govorkova EA, Humberd J, Seiler P, Puthavathana P, Buranathai C, Nguyen TD, Chaisingh A, Long HT, Naipospos TSP, Chen H, Ellis TM, Guan Y, Peiris JSM, Webster RG (2005) Are ducks contributing to the endemicity of highly pathogenic H5N1 influenza virus in Asia? *Journal of Virology* 79:11269–11279

Suarez DL, Schultz-Cherry S (2000) The effect of eukaryotic expression vectors and adjuvants on DNA vaccines in chickens using an avian influenza model. *Avian Diseases* 44:861–868

Swayne DE (2006) Principles for vaccine protection in chickens and domestic waterfowl against avian influenza: emphasis on Asian H5N1 high pathogenicity avian influenza. *Annals of the New York Academy of Sciences* 1081:174–181

Swayne DE (2008) Avian influenza vaccines and therapies for poultry. *Comparative Immunology, Microbiology and Infectious Diseases* 32:351–363

Swayne DE (2008b) Epidemiology of avian influenza in agricultural and other man-made systems. In: *Avian Influenza*, Swayne DE (editor), Ames: Wylie-Blackwell Publishing, pp 59–85

Swayne DE (2012) Impact of vaccines and vaccination on global control of avian influenza. *Avian Diseases* 56:818–828

Swayne DE (2012) The role of vaccines and vaccination in high pathogenicity avian influenza control and eradication. *Expert Review of Vaccines* 11:877–880

Swayne DE, Kapczynski DR (2008) Vaccines, vaccination and immunology for avian influenza viruses in poultry. In: *Avian Influenza*, Swayne DE (editor), Ames: Blackwell Publishing, pp 407–451

Swayne DE, Spackman E (2013) Current status and future needs in diagnostics and vaccines for high pathogenicity avian influenza. *Developments in Biologicals (Basel)* 135:79–94

Swayne DE, Beck JR, Garcia M, Stone HD (1999) Influence of virus strain and antigen mass on efficacy of H5 avian influenza inactivated vaccines. *Avian Pathology* 28:245–255

Swayne DE, Beck JR, Kinney N (2000) Failure of a recombinant fowl poxvirus vaccine containing an avian influenza hemagglutinin gene to provide consistent protection against influenza in chickens preimmunized with a fowl pox vaccine. *Avian Diseases* 44:132–137

Swayne DE, Beck JR, Mickle TR (1997) Efficacy of recombinant fowl pox vaccine in protecting chickens against highly pathogenic Mexican-origin H5N2 avian influenza virus. *Avian Diseases* 41:910–922

Swayne DE, Eggert D, Beck JR (2012) Reduction of high pathogenicity avian influenza virus in eggs from chickens once or twice vaccinated with an oil-emulsified inactivated H5 avian influenza vaccine. *Vaccine* 30:4964–4970

Swayne DE, Suarez DL, Sims LD (2013) Influenza. In: *Diseases of Poultry*, Swayne DE, Glisson JR, McDougald LR, Nair V, Nolan LK, Suarez Dl (editors), Ames: Wiley-Blackwell

Swayne DE, Pavade G, Hamilton K, Vallat B, Miyagishima K (2011) Assessment of national strategies for control of high pathogenicity avian influenza and low pathogenicity notifiable avian influenza in poultry, with emphasis on vaccines and vaccination. *Revue Scientifique et Technique Office International des Epizooties* 30:839–870

Swayne DE, Suarez DL, Schultz-Cherry S, Tumpey TM, King DJ, Nakaya T, Palese P, Garcia-Sastra A (2003) Recombinant Paramyxovirus type 1-avian influenza-H7 virus as a vaccine for protection of chickens against influenza and Newcastle disease. *Avian Diseases* 47:1047–1050

Tian G, Zhang S, Li Y, Bu Z, Liu P, Zhou J, Li C, Shi J, Yu K, Chen H (2005) Protective efficacy in chickens, geese and ducks of an H5N1-inactivated vaccine developed by reverse genetics. *Virology* 341:153–162

Toffan A, Beato MS, De Nardi R, Bertoli E, Cattoli G, Terregino C, Capua I (2007) Vaccination prevents viral colonization of

muscles in experimentally infected turkeys challenged with highly pathogenic and low pathogenicity avian influenza viruses of the H7N1 subtype. OIE/FAO/IZSVe Scientific Conference—Vaccination: a tool for the control of avian influenza, 20–22 March 2007, Verona, Italy, p 64

Veits J, Luschow D, Kindermann K, Werner O, Teifke JP, Mettenleiter TC, Fuchs W (2003) Deletion of the non-essential UL0 gene of infectious laryngotracheitis (ILT) virus leads to attenuation in chickens, and UL0 mutants expressing influenza virus haemagglutinin (H7) protect against ILT and fowl plague. *Journal of General Virology* 84:3343–3352

Webster RG, Webby RJ, Hoffmann E, Rodenberg J, Kumar M, Chu HJ, Seiler P, Krauss S, Songserm T (2006) The immunogenicity and efficacy against H5N1 challenge of reverse genetics-derived H5N3 influenza vaccine in ducks and chickens. *Virology* 351:303–311

Wilson HM, Hall JS, Flint PL, Franson JC, Ely CR, Schmutz JA, Samuel MD (2013) High seroprevalence of antibodies to avian influenza viruses among wild waterfowl in Alaska: implications for surveillance. *PLoS One* 8:e58308

Yamamoto Y, Nakamura K, Yamada M, Mase M (2010) Comparative pathology of chickens and domestic ducks experimentally infected with highly pathogenic avian influenza viruses (H5N1) isolated in Japan in 2007 and 2008. *Japan Agricultural Research Quarterly* 44:73–80

Zhang P, Tang Y, Liu X, Xue F, Qiu X, Cao Y, Gao S, Wu Y, Liu X (2005) Comparison of protective efficacy of H5N2 and H5N1 subtype inactivated oil-emulsion vaccine against H5N1 avian influenza virus (AIV) in ducks and effects of maternal antibody on immune response. *China Poultry* 27:8–11

Zhang W, Tu J, Zhao Z, Chen H, Jin M (2012) The new temperature-sensitive mutation PA-F35S for developing recombinant avian live attenuated H5N1 influenza vaccine. *Virology Journal* 9:97