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## CAST Commentary

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# Avian Influenza Vaccination: A Commentary Focusing on H5N1 High Pathogenicity Avian Influenza

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## Introduction

Avian influenza (AI) is a viral disease of poultry caused by any influenza A virus.

Avian influenza (AI) is a viral disease of poultry, caused by influenzavirus A, classified in the family Orthomyxoviridae. The influenza A viruses maintained in birds are commonly referred to as avian influenza viruses (AIVs). The AIVs are antigenically, genetically, and biologically diverse; found worldwide in birds; and infect a variety of avian species.

These viruses can commonly cause asymptomatic or mild infections in birds in addition to the disease AI, leading to a complex epidemiology.

In addition to causing the disease called AI, these AIVs commonly cause asymptomatic or very mild infections that go undetected in the absence of an active laboratory-based virus surveillance program. These asymptomatic infections are important because they play a critical role in the epidemiology of AIV infections in birds and in the maintenance of AIVs in avian populations. The unique complexities associated with AI and AIVs must always be taken into account when developing or conducting AI prevention, control, and eradication programs in poultry and wild birds.

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Viruses are characterized as either high pathogenicity or low pathogenicity using standard challenge protocols in chickens.

The AIVs, along with type A influenza viruses recovered from lower mammals and humans, are further subtyped based on the antigenic properties of their surface glycoproteins: hemagglutinin (HA), of which 16 subtypes (H1-H16) have been identified, and neuraminidase (NA), of which 9 subtypes (N1-N9) have been identified (Swayne and Halvorson 2003). All 16 HA and all 9 NA subtypes have been identified among the AIVs recovered from birds. The AIVs are further characterized by genetic sequencing and pathogenicity testing of the virus in chickens and classified as either High Pathogenicity Avian Influenza (HPAI) or Low Pathogenicity Avian Influenza (LPAI), as defined by World Organization for Animal Health (OIE) *Terrestrial Animal Health Code* (OIE 2007a).

### **Asian-Lineage H5N1 High Pathogenicity Avian Influenza (HPAI)**

From 2003 to 2005, a lineage of Asian-origin H5N1 HPAI viruses infecting poultry and wild birds spread across Asia and into Europe and Africa.

From the beginning of 2003 through 2005, a lineage of Asian-origin H5N1 HPAI viruses infecting poultry and wild birds spread across Asia and into Europe and Africa. By June 2007 more than 64 countries had reported outbreaks or cases in poultry and/or wild birds. These viruses are now considered to be endemic in many areas and are expected to present serious agriculture concerns during the next few years.

Considering the large number of human exposures that have likely occurred and the 322 documented human infections, the transmission of Asian-lineage H5N1 HP AIV from poultry to people seems to be relatively uncommon.

Since 2003 there have been more than 322 human cases, including 195 deaths, reported to the World Health Organization (as of August 2007), with new bird and human cases being reported in Eurasia and less frequently in Africa (WHO 2007). Most human infections apparently resulted from close contact with affected poultry or defeathering and preparing poultry that were sick or had succumbed to infection.

Considering the large number of human exposures that have likely occurred and the 322 documented human infections, the transmission of the Asian lineage of H5N1 HP AIV from poultry to people seems to be a relatively uncommon event. Still, considering the possibility that many human cases have gone undiagnosed, as well as a small cluster of cases in Sumatra where human-to-human transmission seems to have occurred, the potential public health threat presented by the Asian-lineage H5N1 HP AIV is very concerning and must be addressed continually and vigorously.

Correct use of efficacious vaccines as part of overall control and eradication strategies in endemic areas will be critical.

One tool used for control and eradication efforts has been vaccination. This strategy has had varying degrees of long-term success, and the use of vaccine is still controversial in some circles. Yet in endemic areas, the loss of income and critical animal protein supplies and the cases of human infection have dictated, and will continue to dictate, the pragmatic use of vaccine. The correct use of efficacious vaccines as part of overall control and eradication strategies to combat Asian-lineage H5N1 HPAI virus in these areas will be critical.

### **The Science of Avian Influenza Vaccination**

Routine use of AI vaccine in industrial poultry production is rare.

Vaccination against many poultry diseases has been used for decades in the industrialized poultry production systems world wide, but routine use of AI vaccine in industrial poultry production is rare. The relatively recent global increase in infections with H5N1 HP AIV and LPAI H5, H7, H9 AIVs in poultry has increased the research, examination, and use of vaccination for these viruses in poultry populations. Vaccination has been used, in combination with other measures, as an integral part of AI eradication campaigns in Italy and Hong Kong (Capua and Marangon 2007b; Sims 2007).

## Efficiency of AI Vaccines

Current commercially available vaccines will not prevent infection completely, but studies have shown that properly used vaccines can accomplish multiple goals.

An ideal AI vaccine would provoke an immune response that protects against disease and prevents infection (Swayne and Kapczynski 2008). Current commercially available vaccines will not prevent infection completely, but experimental and field studies have shown that properly used vaccines can accomplish multiple goals: (1) protect against clinical signs and death, (2) reduce shedding of field virus if vaccinated poultry become infected, (3) prevent contact transmission of field virus, (4) protect against challenges by low to high doses of field virus, (5) protect against a changing virus, and (6) increase a bird's resistance to AI virus infection (Swayne 2006).

Protection provided by AI vaccines is dependent on vaccine quality: potency, purity, efficacy and safety. Vaccine quality control guidelines are available in the OIE's *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* and should be supplemented with national and regional requirements (OIE 2007a).

Selection of strains for inclusion in poultry AI vaccines should be based on analysis of the field challenge virus in comparison with the licensed vaccine strains.

If possible, selection of strains for inclusion in poultry AI vaccines should be based on an analysis of the field challenge virus in comparison with the licensed vaccine strains, which may require some flexibility in registration processes to allow for new products and re-formulation of existing vaccines to facilitate timely adjustments of vaccine formulation. Unlike human influenza A vaccines where antigenic drift of the field virus requires changing vaccine strains every few years, antigenic drift of poultry influenza viruses has not required a similar need to change the vaccine strain that often (Swayne and Kapczynski 2008). In one study, an H5 vaccine strain provided broad protection against diverse H5 HPAI viruses collected during 38 years and differing as much as 12% in amino acid sequence of the HA gene. The closer the HA gene sequence similarity between vaccine and field viruses, however, the greater the reduction in challenge virus replication and shedding from the respiratory tract (Swayne and Kapczynski 2008). The use of a poorly matched vaccine can result in clinical disease and increased virus shedding when vaccinated poultry are naturally infected (Suarez 2006).

## Efficiency in Domestic Ducks and Geese

Several recent studies examined AI vaccine use in domestic ducks and geese, all reporting efficacy in ducks or geese with the vaccine used.

Most studies of AI vaccine use have involved chickens and turkeys because of their high mortality rates (Swayne 2005). Determining the effectiveness of AI vaccines in other species such as ducks and geese requires additional studies. Epidemiological studies of Asian-lineage H5N1 HPAI outbreaks indicated the importance these species play in transmission, maintenance, and amplification of H5N1 HPAI in Asia (Sims 2006; Webster et al. 2006), highlighting the need for specific studies of vaccine efficacy in these species. Several recent studies examined AI vaccine use in domestic ducks and geese, all reporting efficacy in ducks or geese with the vaccine used against a specific challenge virus from the group of Asian-lineage H5N1 HP AIVs (Beato et al. 2007; Middleton et al. 2007; Qiao et al. 2006; Webster et al. 2006).

## Immunity to AI Vaccines

AI vaccines stimulate the production of antibodies against the hemagglutinin portion of the virus, the major protective mechanism against natural viral infection and replication.

AI vaccines stimulate the production of antibodies against the hemagglutinin portion of the virus, the major protective mechanism against natural viral infection and replication. Vaccines against AI will not prevent infection completely (Suarez 2005; Swayne 2006). With use of an efficacious AIV vaccine, viral replication in the respiratory and intestinal tract is significantly reduced in vaccinated birds, reflected by the inability to isolate challenge virus to the same magnitude in vaccinated chickens versus nonvaccinated chickens, with data collaborating that finding from multiple laboratories.

Duration of immunity varies based on number of doses given, bird's age at time of vaccination, antigen quantity in each dose of vaccine, and avian species.

The duration of effective immunity will vary based on the number of doses given, age of bird at time of vaccination, antigen quantity in each dose of vaccine, and avian species. Using traditional whole virus inactivated oil-emulsion vaccines in chickens, peak hemagglutination inhibition (HI) titers are observed 4-6 weeks postvaccination (Brugh, Beard, and Stone 1979), with the same work indicating a much lower seroconversion rate in turkeys. Additional studies in turkeys indicate two doses of inactivated vaccine are necessary to reduce virus shedding and replication to levels needed to reduce viral spread (Karunakaran et al. 1987).

Most published onset and duration of immunity studies are in specific-pathogen-free (SPF) birds in laboratory settings; little field evidence is presented or published. Field use of AI vaccine introduces management conditions that are usually not present in laboratory studies on vaccine efficacy. Among the things that should be considered are (1) the type of vaccine, (2) the administration of less than a full dose, (3) improper storage and handling, (4) missed birds, and (5) immune suppression and maternal immunity (Swayne 2006). Therefore, the practical immunity achieved in vaccination campaigns will be less than published research values.

### Vaccines

Two main types of vaccine are widely licensed with proven efficacy: inactivated, whole-virus vaccines and a recombinant fowl pox-vectored vaccine with an H5 insert.

Two main types of vaccine with proven efficacy are widely licensed by countries: inactivated, whole-virus vaccines from several manufacturers (FAO 2007) and a recombinant fowl pox-vectored vaccine with an H5 insert from Merial (Bublöt et al. 2006). With the epizootic of H5N1, many novel vaccines are under investigation or were recently licensed for use in some countries. No published or presented research compares these novel products available in limited countries with conventional AI vaccines.

### Vaccine Production

With multiple manufacturers, standardized protocols must be enforced to assure production of an efficacious vaccine.

Vaccine production must be standardized. If the vaccination component of a control program is to be successful, vaccines must be manufactured under the appropriate government authority to assure production of a highly efficacious vaccine. With multiple manufacturers, standardized protocols must be enforced to assure production of an efficacious vaccine. Global standards for the production of conventional inactivated AI vaccines are available in the OIE's *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, Chapter 2.7.12 (OIE 2007a).

Avian influenza vaccines should be produced from LPAI viruses, which are considered to be less dangerous than HPAI viruses in case of accidental escape during the production process (Capua, I. 2007. Personal communication). Vaccine production using HPAI viruses must be conducted in Containment Group 4 facilities (OIE 2007a), which may not exist in developing countries.

## The Art of Controlling Avian Influenza

Once the virus is in the poultry population, a successful control strategy can be aided by reducing the susceptibility and density of the poultry population.

Under conditions of high poultry density or multiple poultry establishments in one area, prevention and biosecurity may not be fully successful in preventing the entry of the virus into the poultry population. Once the virus is in that population, a successful control strategy can be aided by reducing the susceptibility and density of the poultry population. The components of a control strategy generally include five basic categories: (1) biosecurity and quarantine; (2) diagnostics and surveillance; (3) elimination of

infected poultry or controlled marketing of convalescent poultry; (4) decreasing host susceptibility to the pathogen by vaccination; and (5) education of personnel, owners, and villagers on disease transmission, prevention, and control (Swayne 2006).

Widespread use of vaccine against HPAI is being used with increasing frequency in countries experiencing large outbreaks of Asian-lineage H5N1 HPAI, especially, but not only, in nonconfined poultry populations.

In developing nations with extensive poultry production systems, AI vaccine has sometimes been used broadly for control where eradication of endemic influenza is not deemed to be achievable. Several Asian and Middle Eastern countries have reported use of H9N2 vaccine to prevent the clinical and economic effects of LPAI infection (Naaem et. al 2007). Widespread use of vaccine against HPAI is less common but is being used with increasing frequency in countries experiencing large outbreaks of Asian-lineage H5N1 HPAI, especially, but not only, in nonconfined poultry populations. In several Asian and African countries where Asian-lineage H5N1 HPAI seems to be endemic, vaccination likely will need to continue indefinitely because of production systems present in those cultures. In countries where disease is endemic, an initial approach based on control rather than eradication will be required, and eradication may take years to achieve (Sims 2007). Global agencies are predicting a 7- to 10-year time span to get many of these infected countries back to an Asian-lineage H5N1 HPAI-free status (FAO-WHO 2005).

In other countries with intensive commercial poultry enterprises, AI vaccine occasionally is used as part of a control and eradication program for LPAI and rarely as a part of preventive, control, and eradication programs for HPAI.

### **Biosecurity**

Biosecurity should be the primary component of all AI prevention, control, and eradication plans.

Biosecurity, the prevention of exposure to disease agents through management practices (isolation of infected and uninfected flocks, traffic control, and sanitation), should be the primary component of all avian influenza prevention, control, and eradication plans (Halvorson 2002). The physical and functional separation in the poultry industries accounts for much of the disease prevention success in commercial poultry production throughout the world.

Preventing the introduction of AIV by eliminating direct and indirect contact between commercial poultry and wild birds, swine farms, and live bird markets is a common and successful practice. Likewise, eliminating direct and indirect contact between poultry farms has been highly successful in preventing the spread of AI outbreaks.

Biosecurity practices have to be appropriate to the farming system.

Attempts to apply biosecurity practices that are effective in commercial poultry directly to other systems are not helpful. Instead, practices have to be appropriate to the farming system (Sims 2007). This requires a local understanding of a matrix of the sources of the virus, how the virus moves, knowledge of the poultry populations involved, cross-over points between populations, and exposure risk level (Halvorson 2008).

The production chain for most biologics companies requires a minimum of 10 to 12 weeks for production and testing of an AI vaccine.

### **Availability of AI Vaccines**

The overall effectiveness of an AI vaccination program is directly related to the time span between index case(s) and the implementation of appropriate controls, including vaccine administration (Capua and Marangon 2003) in an emergency response to introduced infection. If vaccine is used, immediate accessibility to quality AI vaccines is necessary for vaccination to be a viable option in a control plan. The production chain

Countries or groups of countries at high risk for H5 or H7 introductions should consider establishing their own AI vaccine banks.

for most biologics companies requires a minimum of 10 to 12 weeks for production and testing of an AI vaccine if the field strain is selected or a licensed master seed is used. The number of doses needed for an outbreak situation may be held at any time point by global biologics companies, but it may not be sufficient for vaccination in areas of dense poultry populations. For this reason, countries or groups of countries at high risk for H5 or H7 introductions should consider establishing their own AI vaccine banks.

### **Concerns Surrounding AI Vaccination of Poultry**

Several questionable arguments against vaccination for AI have been discussed surrounding recent Asian-lineage H5N1 HPAI outbreaks. The following scenarios have been suggested by opponents of AI vaccination use based on unavailability of sufficient scientific evidence:

- Vaccination will drive antigenic change in the viruses.
- These changes may create a virus that has increased transmissibility to humans.
- Use of vaccine in poultry will hide clinical signs and mortality in poultry, which is currently used as an alert for human health professionals to look for human cases.
- Vaccination will allow the virus to be maintained in the poultry population through inapparent infections and lead to increased pathogenicity.
- A vaccination campaign that is not managed appropriately is likely to result in endemic AI infection (Capua and Marangon 2007b).

Speculation about potential problems of vaccine use must be balanced with the real problem of outbreaks in susceptible poultry.

Like previous objections to AI vaccine use (Halvorson 2002) these objections fail to address the question of whether lack of vaccine use is in any way superior to vaccine use. Speculation about potential problems of vaccine use must be balanced with the real problem of outbreaks in susceptible poultry. No published literature compares the rate of antigenic shift, the creation of a more transmissible virus, the creation of a more pathogenic virus, or a tendency to endemicity during infections in vaccinated poultry verses nonvaccinated poultry. Experience with other influenza virus vaccines (canine, swine, equine) cannot be correlated with poultry because the AIV vaccines are very different from mammalian influenza virus vaccines (poultry vaccines are oil-adjuvanted) and poultry are shorter lived than dogs, horses, and pigs. In theory, antigenic shift will occur at a given rate regardless of the vaccination status of the susceptible avian population.

Vaccination can actually reduce the opportunity for natural mutation or reassortment by reducing the quantity of circulating virus.

“It is unlikely that vaccination of poultry will exert a selection pressure on the cell receptor of the HA protein that might lead to an increase in the transmissibility of the virus to mammals. Thus vaccination can actually reduce the opportunity for natural mutation or reassortment by reducing the quantity of circulating virus.” (FAO 2004) This objection also was made during vaccination against H5N1 in 2002 in Hong Kong, with Ellis and colleagues commenting that unvaccinated flocks will replicate increased amounts of virus and shed higher quantities of virus that will infect additional flocks and, potentially, humans (Ellis et al. 2004). Theoretically over time, the effective use of an appropriate AI vaccine will reduce the levels of AI virus shed into the environment by infected birds, thereby reducing the viral exposure to humans.

If an AI vaccine is efficacious, it will greatly reduce clinical signs and mortality, but possible circulating viruses should be detected through surveillance mechanisms implemented along with the vaccination program. Prompt isolation and characterization of the circulating viruses allows quick detection of virus changes.

### **The Decision to Use AI Vaccination**

In all cases where a decision to use AI vaccine is considered, the overall goals need to be examined. The following goals for AI vaccination programs have been suggested: (1) routine vaccination in endemic areas, (2) emergency vaccination during an epidemic, and (3) preventive or prophylactic vaccination when the risk of AI virus introduction is high (Swayne 2006).

Prophylactic AI vaccination will provide protective immunity in the target population, increasing resistance to infection, with the ultimate objective of preventing the index case.

Prophylactic vaccination can be considered when available information indicates that the country/zone/compartiment is at high risk for AI infection, with either H5 or H7 subtype or a known subtype (H5N1 in Asia, H7N2 in U.S. live bird markets). This strategy will provide protective immunity in the target population, increasing resistance to infection, with the ultimate objective of preventing the index case. In the event of introduction, prophylactic vaccination will reduce shedding of virus when biosecurity is maintained and potentially minimize the number of secondary outbreaks (Capua and Marangon 2007b).

Depending on the situation, the goal may be simply to protect against clinical signs and death, or it may be to contribute to the eradication of AI virus from a region, zone, or compartment by increasing resistance to infection and reducing viral excretion. In cases of endemic infection or high likelihood of challenge, the goal of vaccination might be to protect against disease, increase resistance to infection, reduce the number of susceptible birds, and reduce the likelihood of human exposure.

AI vaccination must be considered to be a complement to other control measures, as part of a science-based AI control strategy.

In deciding to use AI vaccines as part of a control program, several issues must be addressed including the needs of the poultry producer, availability of local resources, the poultry production system(s), the veterinary infrastructure, laboratory capacity for surveillance, biosecurity and ability to maintain a cold chain for the vaccine. Avian influenza vaccination must be considered to be a complement to other control measures. Vaccination should be available as part of a science-based AI control strategy (Halvorson 2002) that includes the following, where possible: (1) enhanced biosecurity, (2) an eradication plan, (3) controlled vaccination for flocks deemed to be at risk, (4) suitable monitoring of all flocks at risk and of all vaccinated flocks, and (5) a repopulation plan (Swayne 2006).

It has been shown that proper use of vaccine can prevent infections, and surveillance can verify that vaccinated flocks for export are not infected.

Political and economic considerations also may influence the decision to use vaccine as a tool to aid in control of AI. Historically, vaccine use has been viewed as an admission that AI is endemic in a country, and this has been used to justify embargoes of poultry exports from the affected country. More recently it has been shown that proper use of vaccine can prevent infections, and surveillance can verify that vaccinated flocks for export are not infected. The new OIE chapter on AI enables continuation of trade in the presence of vaccination (OIE 2007b). Finally, pre-emptive slaughter has caused destruction of great numbers of non-infected birds, and can be questioned from effectiveness, economical and ethical points of view (Capua and Marangon 2007b).

The decision to vaccinate or not to vaccinate should ultimately be made by official government veterinary authorities in a given country, with input from local or state



authorities and the commercial poultry industry. In the face of the spread of H5N1 HPAI, however, illegal or uncontrolled vaccination occurred and continues to occur in some countries (Jagne, J. 2007. Personal communication). Authorities must take several factors into account to inform their decision making: the disease situation in the country, structure of poultry production in the country, socio-economic impact, risk of disease, costs and benefits, availability of technology and qualified personnel, and possible impacts on markets.

Once vaccination is selected as a tool in an eradication program against an AI virus, an exit strategy must be in place to know end points of the program and define determinants of success.

Once vaccination is selected as a tool in an eradication program against an AI virus, an exit strategy must be in place to know end points of the program and define determinants of success. Monitoring tests throughout the vaccination protocol will determine when the virus circulation has been stopped and virus eliminated and vaccination can be halted. An ongoing monitoring program also must be included to continue active surveillance once vaccination has stopped. If eradication of the virus is unlikely to be achieved in the medium to long term because of the nature of the poultry industry, an exit strategy may not be applicable, but the vaccination campaign should be reviewed to assess its necessity and any changes in the target population. Ongoing surveillance is required to determine the success of the campaign. Measures of success of the campaign should be established and monitored (e.g., number of clinical cases or lack of virus isolation).

Many unknowns remain involving AI vaccine usage.

Many unknowns remain involving AI vaccine usage. The many sources of vaccine and the many species of birds mean that large gaps in knowledge exist in vaccine potency, appropriate vaccination regimen, levels of immunity to stop infection, which species to vaccinate, how to vaccinate each species, and so forth. What has been observed and reported in North America, Western Europe, Hong Kong, and other areas is that when AI vaccine has been used as part of an organized AI control program, success has been achieved according to the goals of the vaccine use (Adriatico 2005; Capua and Marangon 2007a; Ellis et al. 2003; Frame et al. 1996).

### **Surveillance in the Vaccinated Population**

Surveillance needs are dependent on the goal of the vaccination program.

Surveillance needs are dependent on the goal of the vaccination program. If AI vaccination is used only to protect against clinical disease and death, the first level of a control program, surveillance to demonstrate adequate protection following field vaccination is needed.

If vaccination is used to decrease virus shedding and stop virus transmission and spread, with advanced AI control and eradication as the goals, a robust monitoring system must be put in place by appropriate authorities. This system should allow for serological and virological monitoring of the vaccinated birds. Although AIV infection in AI-vaccinated flocks is rare, the monitoring program is necessary to promptly identify field-infected birds in the vaccinated population. Once infected, vaccinated flocks are identified, they should be quarantined and then depopulated or slaughtered by controlled marketing to reduce the risk that they act as a source of infection for other farms (Capua, I. 2007. Personal communication). Surveillance also should be conducted in vaccinated populations to identify viruses that emerge as vaccine-resistant strains and to identify problems with the vaccine protocol or vaccine quality that are providing inadequate protection (Swayne, D.E. 2007. Personal communication).

## Conclusions

Tools such as vaccination, used within a defined control program, are available to allow protection of public health, food security, and profitability, as well as disease control.

In the past, aggressive eradication programs (e.g., stamping-out or mass depopulation) were needed in exporting countries to preserve poultry export markets when HPAI or LPAI outbreaks would occur. Application of aggressive programs may not be successful in all situations. Tools such as vaccination, used within a defined control program, are available to allow protection of public health, food security, and profitability, as well as disease control.

Major concepts to be considered when examining AI vaccine use include the following:

- High-quality AI vaccines do protect poultry from disease, increase resistance to infection, and decrease excretion rate if vaccinated birds do become infected.
- Most research has been done with chickens and turkeys, so studies are needed in other species.
- Immunity and protection is largely HA based, so the vaccine strain must be same HA subtype and have adequate HA content or proprietary adjuvant.
- Vaccine should be standardized; proper handling must be explained.
- Protection from the vaccine used can be assessed through seroconversion and challenge studies.
- AI vaccines should be manufactured to be effective under a wide variety of species and conditions. Only inactivated and viral-vectored products are commercially available today.
- AI vaccine can be used in emergency, routine, or preventive programs; use should be part of a total program-vaccine alone will not eradicate AIV. Locally appropriate biosecurity practices and virus- or sero-surveillance are necessary along with identification of an exit strategy (Swayne 2008).

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For a more complete treatment of this subject, and a Comprehensive Bibliography, the reader is directed to the following publication: Council for Agricultural Science and Technology (CAST). 2007. *Avian Influenza Vaccines: Focusing on H5N1 High Pathogenicity Avian Influenza (HPAI)*. Special Publication 26. CAST, Ames, Iowa.

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