I. Introduction

The emergence of highly pathogenic H5N1 avian influenza (HPAI) in Southeast Asia in the 1990’s has evolved into one of the most significant animal health events in decades. In many countries affected by H5N1, avian vaccination has been used as one part of their response strategies. Worldwide since 2002, a recently published survey reported that 130 billion doses of H5 or H7 vaccine have been used to protect high-risk poultry against multiple outbreaks of HPAI. Additionally, H5 or H7 vaccine was used as a tool to protect over a quarter million zoo, hunting, companion or endangered birds in 20 countries (Swayne, Pavade et al. 2011)

The Association of Zoos and Aquariums, (AZA) in partnership with USDA Animal Care has have spent considerable time and effort preparing their member institutions for the possible emergence of a highly pathogenic AI that could impact their collections. These efforts include a year-long pilot surveillance project and preparation of the “United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Association of Zoos and Aquariums (AZA) Management Guidelines for Avian Influenza: Zoological Parks & Exhibitors Outbreak Management Plan” (OMP, currently under revision). Given that the AZA is a recognized authority of exotic animal health in the U.S., it is hoped that USDA will consider them important stakeholders in comprehensive AI response plans.¹

II. Purpose of this document

The purpose of this document is to present basic information regarding the use of avian influenza vaccines in zoological species during animal disease emergencies, and propose elements for incorporation into any Vaccination Plan for AZA or equivalent Institutions. It is hoped that the USDA, APHIS, Veterinary Services (VS) and State Animal Health authorities may approve the use of AI vaccines in zoos if risk assessment determines that it is appropriate. (For recommended elements in an institution specific Vaccination Plan, see Appendix C).

In addition, this document provides basic information and references on the use of vaccination in zoological specimens in other countries. The intended audiences are State and Federal Animal Health officials and zoological professionals who would be involved in decision-making in the event of a HPAI outbreak in the U.S.

The organization of this paper is loosely based on the pertinent sections of the USDA APHIS HPAI Response Plan: (The Red Book), and the National Animal Health Emergency Management System NAEHMS Guidelines: Vaccination for Contagious Diseases Appendix C: Vaccination for High Pathogenic Avian Influenza. (NAEHMS 2011). The zoological community should be included in an overall response plan, including vaccination considerations in the event of an HPAI outbreak.

III. HPAI information

Avian influenza, or fowl plague, is caused by Influenza A viruses of the Orthomyxoviridae family. Anseriformes and Charadriiformes are the natural reservoir for avian influenzas viruses (Napier and Gamble 2011). The viruses are described based on the surface proteins, hemagglutinin (H) and neuraminidase (N). As of May 2012, there are 17 known H proteins and 9 N proteins\(^2\). Influenza A is further characterized based on a strain’s potential to cause disease in poultry.

Low Pathogenicity Avian Influenza (LPAI) and HPAI are defined in this document (and in the USDA AZA Outbreak Management Plan) in accordance with OIE (World Organization for Animal Health) guidelines in the Terrestrial Animal Health Code (2012) Chapter 10.4.1\(^3\)

For the purposes of international trade, avian influenza in its notifiable form (NAI) is defined as an infection of poultry\(^4,5\) caused by any influenza A virus of the H5 or H7

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\(^2\) [http://www.cdc.gov/flu/about/viruses/bat-flu.htm](http://www.cdc.gov/flu/about/viruses/bat-flu.htm)

\(^3\) Terrestrial Animal Health Code May 2012
[http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.10.4.htm](http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.10.4.htm)

\(^4\) In the Red Book, the term ‘poultry’ does not include chickens or other birds displayed in a licensed exhibition or zoo. However, if animals other than poultry are involved in the response effort, the Incident Commander has the ability to adapt the case definition based on epidemiologic evidence. (pg. 1.1 Red Book 2012).

subtypes or by any AI virus with an intravenous pathogenicity index (IVPI) greater than 1.2 (or as an alternative at least 75% mortality) as described below. NAI viruses can be divided into highly pathogenic notifiable avian influenza (HPNAI) and low pathogenicity notifiable avian influenza (LPNAI):

a. HPNAI viruses have an IVPI in 6-week-old chickens greater than 1.2 or, as an alternative, causes at least 75% mortality in 4- to 8-week-old chickens infected intravenously. H5 and H7 viruses which do not have an IVPI of greater than 1.2 or cause less than 75% mortality in an intravenous lethality test should be sequenced to determine whether multiple basic amino acids are present at the cleavage site of the hemagglutinin molecule (HA0); if the amino acid motif is similar to that observed for other HPNAI isolates, the isolate being tested should be considered as HPNAI;

b. LPNAI are all influenza A viruses of H5 and H7 subtype that are not HPNAI viruses

The OIE uses specific language to describe ‘poultry’ as “all domesticated birds, including backyard poultry, used for the production of meat or eggs for consumption, for the production of other commercial products, for restocking supplies of game, or for breeding these categories of birds, as well as fighting cocks used for any purpose.”

The OIE goes further, and states that “birds kept in captivity for any reason other than those reasons referred to in the preceding paragraph, including those that are kept for shows, races, exhibitions, competitions or for breeding these categories of birds as well as pet birds, are not considered to be poultry.” This distinction between zoological collections and poultry is extremely important in terms of potential response to the outbreak of any HPAI in zoological specimens.

AI in the United States

Low Pathogenicity Avian influenza (LPAI) has been detected in many wild bird species\(^6\), and in the poultry sector; these strains occasionally cause mild morbidity and mortality in these species. LPAI is occasionally found in zoological specimens, but rarely causes disease in zoo birds. Given the interaction with wildlife in some exhibits, occasional detection of LPAI in zoological specimens is to be expected. As of December, 2012, the HPAI Asian H5N1 has not been detected in the poultry or wild bird compartments\(^7\), or in limited zoological surveillance (Y. Nadler, pers. communication). HPAI strains have been detected in the United States that were linked with the live bird marketing system.

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The last known outbreak of HPAI in poultry in the US occurred in 2004.  

Potential Impact of an HPAI Outbreak on Zoological Institutions

The detection of any HPAI strain in the United States in any animal compartment would have a profound impact on zoological institutions, collection specimens and conservation programs. If the HPAI strain was determined to be zoonotic, additional concerns about occupational health and safety and guest safety would be considerable. Some of these zoological community concerns include:

- **Concerns about staff and visitor safety:** Zoos will need to carefully consider with Public Health partners all appropriate protective measures for staff and visitors in the event of a zoonotic strain of HPAI. For more information, see the [USDA APHIS AZA Management Guidelines for Avian Influenza: Zoological Parks & Exhibitors Outbreak Management Plan](http://www.aphis.usda.gov/azawildlife/).  
- **Increased need for biosecurity:** An HPAI outbreak anywhere in the US in any animal compartment would likely increase biosecurity protocols for institutions, and the OMP calls for increasing levels of preparedness depending on the location of an institution relative to an outbreak.  
- **The possible culling of collection specimens in an outbreak situation:** Zoological institutions are understandably concerned about the possible euthanasia of healthy specimens in a ‘stamping out’ of HPAI (Furger, Hoop et al. 2008). This is a practice that has been employed in the poultry industry with HPAI and in some instances, LPAI. The lifespan and rapid turnover of many types of poultry (broilers, etc) make this a cost-effective method within that industry. In zoological collections, there would be intense emotional impact of the culling of healthy, long lived, beloved specimens. Breeding and conservation programs could be devastated with any culling program. The genetic value of some individuals is priceless (Gilbert and Philippa 2012). Unlike the poultry industry, zoological facilities are not eligible for indemnification for the culling of collection specimens. In some cases because of the rarity of a species, the replacement costs of zoological specimens could be astronomical: if a replacement could even be found.  
- **Animal Welfare concerns.** Not all zoos would have sufficient space to house all their susceptible birds indoors. Indoor confinement in some species would

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9 See OMP
increase stress and affect physical health of specimens (Osterhaus, Fouchier et al. 2007).

- **Unknown susceptibility of various species to different Influenza viruses:** While the National Wildlife Health Service keeps a record of reported cases of H5N1 from various animal species\textsuperscript{10}, the true number of susceptible species or species which could act as disease reservoirs is not known. This variability could increase the risk of disease in mixed species exhibits. (Redrobe 2007).

- **The effect on Conservation programs.** Collection specimens are often moved between institutions for breeding purposes. Likely, movement of birds would cease, at least temporarily, which could halt breeding efforts.

- **Economic Impact of an outbreak occurring in a Zoological Institution:** The Association of Zoos and Aquariums has an annual attendance of roughly 180 million visitors annually. In 2010, the contribution of AZA-accredited zoos and aquariums to the U.S. economy was $16 billion, generating personal earnings totaling $4.7 billion and supporting 142,436 jobs\textsuperscript{11}. These figures illustrate that zoos and aquariums contribute a significant amount to the U.S. economy. Continuity of business is an important consideration for staff, animals and local economies.

### IV. HPAI Response

Extensive documentation exists which describes a framework for the response to a Foreign Animal Disease such as HPAI. The Red Book (The response plan for HPAI in poultry) is located on the FAD PReP site which houses a number of documents that address Foreign Animal Disease preparedness and response.\textsuperscript{12} Note that the plan for dealing with HPAI as described in the Red Book adds disease-specific detail to the National Response Framework (NRF) which is the guideline for national all-hazards response. The nature of the response to HPAI may also be influenced by social and political factors on the local, State, federal and international levels.

\textsuperscript{10} [http://www.nwhc.usgs.gov/disease_information/avian_influenza/affected_species_chart.jsp](http://www.nwhc.usgs.gov/disease_information/avian_influenza/affected_species_chart.jsp)


\textsuperscript{12} Foreign Animal Disease Preparedness and Response Plan [FAD PReP website](http://www.fadprep.org)
Goals of Response

According to the Red Book, there are 3 goals of an HPAI response in poultry:\(^{13}\):

1) detect, control and contain HPAI as quickly as possible
2) eradicate HPAI using strategies that seek to protect public health and stabilize animal agriculture, the food supply and the economy and
3) provide science- and risk-based approaches and systems to facilitate continuity of business for non-infected animals and non-contaminated animal products

These same overarching goals are equally important to the animals within the zoological community. The use of science and risk based approaches to response in this industry is encouraged rather than a ‘one size fits all’ response to HPAI in any animal sector, including zoological institutions.

Critical Activities

In the event HPAI is detected in the US, the Red Book outlines critical activities for containment, control and eradication of HPAI in poultry. For more information on this list of these activities and tools, please see Red Book section 4.2.

While methods and actions may differ, these critical activities for response in poultry may be appropriate for response to a HPAI outbreak in a zoological institution. One of those critical activities or tools listed is emergency vaccination, as the response strategy indicates.

<table>
<thead>
<tr>
<th>Critical Activities and Tools for Containment and Control in Zoological Institutions*</th>
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<tbody>
<tr>
<td>• Public awareness campaign</td>
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<tr>
<td>• Swift imposition of effective quarantine and movement controls</td>
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<tr>
<td>• Rapid diagnosis and reporting</td>
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<tr>
<td>• Epidemiological investigation and tracing</td>
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<td>• Increased surveillance</td>
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<td>• Continuity of business measures for unaffected premises</td>
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<td>• Biosecurity measures</td>
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<tr>
<td>• Effective and appropriate disposal procedures</td>
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<tr>
<td>• Cleaning and disinfection measures</td>
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<tr>
<td>• Emergency vaccination (as the response strategy indicates)</td>
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</table>


\(^{13}\) Section 4.1 Redbook pg 4-1.
Factors Influencing the Response to HPAI in Zoological Institutions and Zoological Species

The OMP for HPAI in zoos is a companion document which provides recommendations to fulfill these critical activities for a zoological institution. To date, no known cases of HPAI have occurred in US zoos. There is precedence, however, of survival and release from quarantine of a psittacine species infected with a notifiable LPAI Mexican lineage H5N2. The detection of these infected individuals, which may have been smuggled into the country for the pet trade, resulted in significant morbidity in one animal, with a conspecific succumbing to the virus. The ill bird eventually recovered from infection, was kept in a home-based quarantine. The bird was eventually released from quarantine once determined to be disease-free. (Hawkins, Crossley et al. 2006).

The exact procedure to be followed for an appropriate response in the zoological community will be dictated by many factors, such as viral epidemiology, zoonotic nature of the virus, and the ability for a facility to implement adequate biosecurity/biocontainment protocols. The use of vaccination in zoological specimens may be a viable option after a thorough risk assessment. Any approved vaccination plan would only be a part of a comprehensive prevention and control program, relying heavily on biosecurity and biocontainment. See Appendix A and Appendix C for more information.

In the United States, the use of AI vaccines is strictly controlled. Approval to use AI vaccine licensed for use in poultry is needed from the USDA Chief Veterinary Officer (Deputy Administrator, Animal and Plant Health Inspection Service, Veterinary Services). Since vaccination could potentially impact the commerce of poultry and poultry products with trading partners, the vaccination of domestic poultry and other birds for AI H5 and H7 strains is currently prohibited. The Red Book does, however, state that emergency vaccination may be considered in poultry in specific circumstances. The zoological industry should be included in any risk assessment evaluating the benefits of avian influenza vaccination.14

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14 Red Book Section 5.16 Vaccination
V. Vaccination Basics

Vaccine Strategies

There are vaccination strategies that may be employed for combating HPAI. Per the NAEHMS guidelines, vaccination has never been used for HPAI viruses in the US. An excellent, expanded overview of these strategies is provided by Maragon and Busani and the NAEHMS guide.

**Routine** vaccination is a strategy that is often employed as ‘a last resort’ (Maragon, Busani et al. 2007) when it is determined that a disease cannot be eradicated in a particular area. It should be noted that vaccination alone would not be a complete response resulting in eradication of the virus. (NAEHMS 2011).

**Prophylactic or preventative** vaccination in the face of an outbreak may be an important tool to reduce morbidity and mortality in valuable species. This strategy could be considered if a zoo, currently unaffected, is near an outbreak zone. This strategy is generally considered in regions or groups of birds considered at high risk, and may be used for targeted populations, short or longer term. (NAEHMS 2011) According to the NAEHMS 2011 Guideline: “Prophylactic vaccination of extremely rare, endangered birds can begin as soon as state and federal animal health authorities have approved the request. These birds are listed in CITES Appendix I or the U.S. Fish and Wildlife Service Endangered Species List.” (Excerpt originally from USDA APHIS Vaccination Plan for AZA Zoos and Equivalents, 2006). It is hoped that the USDA and State regulatory officials will consider allowing prophylactic vaccination of certain individuals, based on risk assessment, provided that individual zoos meet stringent criteria. (Appendix B & C). Preventative programs tend to be more effective when used in high risk, high value populations (Swayne, Pavade et al. 2011).

**Emergency** vaccination is an option which may be employed if there is evidence of widespread disease or evidence of rapid spread of disease (Kapczynski and Swayne 2009). This strategy may be utilized with the declaration of an animal health emergency. Additional birds, which may include CITES Appendix II and III species in zoological collections, or non-threatened, non-endangered flight restricted birds on open ponds

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15 For more information on the USDA use of zones when managing disease outbreaks in poultry, or describing zones for the application of vaccination, see Red Book
16 NAEHMS guidelines, Pg 87.
17 NAEHMS guidelines, Pg. 87.
(Excerpt originally from USDA APHIS Vaccination Plan for AZA Zoos and Equivalents, 2006), could possibly be eligible for emergency vaccination, based on risk assessment, under these circumstances.

With any vaccination program, ‘exit strategy’ should be determined, which defines the timeframe and conditions for continuing with or halting the vaccination program, based on risk (OIE 2007).

Vaccine Types
Vaccine technology is a rapidly evolving field, and a thorough discussion of AI vaccinology is beyond the scope of this document. See the NAEHMS guidelines for more information. There are 5 main types of AI vaccines: live, inactivated, subunit, DNA, and recombinant vaccines which express AI genes. Zoos which have vaccinated for HPAI in other countries have successfully used commercially available products, licensed for use in poultry species. Oil-emulsion-inactivated vaccines are among the most commonly produced and used in the poultry industry (Swayne and Kapczynski 2008).

The type of vaccine that would be chosen for use in zoological specimens will be determined based on recommendations and permissions from USDA, State veterinarians, Incident Commanders, with additional information from vaccine manufactures and any existing avian vaccine studies. If approved for a vaccination plan, it is likely that the zoo would purchase vaccine directly from a USDA licensed veterinary biologics avian influenza vaccine manufacturer, if the USDA grants the veterinary biologics manufacturer 9 CFR 103.3 authorization to ship the product to that zoo. Information regarding manufacturers is available at the following web-site: http://www.aphis.usda.gov/animal_health/vet_biologics/publications/CurrentProdCodeBook.pdf

The Red Book and NAHEMS Guidelines stress the importance of selecting a vaccine that will adhere to the DIVA principle. DIVA is the acronym for: Differentiating Infected from Vaccinated Animals. For example, vaccines would be chosen to provide protection from the HPAI H5N1 by selecting vaccines using inactivated H5N2 or H5N9 strains. Using heterologous N vaccines allowed some immunity to develop to H5, but when testing for serologic activity after vaccination, the antibodies in the vaccinated bird would be to

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18 If the outbreak of AI is of sufficient magnitude in a State, it is possible that vaccine may be supplied by the National Veterinary Stockpile. These are resources that may be available to zoos if they are mobilized by State Animal Health officials.
either N2 or N9, not the circulating pathogenic N1. Thus, animal health officials could determine whether the titer had developed from an infection with H5N1, or via vaccination using the inactive vaccine with N2 or N9. The DIVA principle should be considered when selecting a vaccine, but this technique has not been validated for zoological species (Koch, Steensels et al. 2009). Additionally, some zoological birds may have contact with free-ranging birds which may transmit LPAI strains. These silent infections may interfere with serological interpretation, the basis of the DIVA principle (Osterhaus, Fouchier et al. 2007).

The use of the most advanced technology available at the time should be used to differentiate zoo birds which have been vaccinated from those who may have been exposed and infected by influenza strains of concern.

**VI. History of Vaccine use in Zoological Institutions**

Vaccination for HPAI in zoos has been employed in other countries to respond to the threat of HPAI to collection specimens. Their use was instituted because the application of control measures such as culling or requirements of strict indoor housing was recognized as detrimental to the welfare of birds, and could seriously compromise breeding programs (Gilbert and Philippa 2012). The outcry from the public and animal health experts would have been significant. It should be noted, however, that there are no specific vaccines that have been licensed for use in zoo birds, companion birds or endangered species (Swayne, Pavade et al. 2011). However, extra label use of several licensed poultry vaccine products show promise in a number of non-domestic species.

The European Union has enacted multiple Commission Decisions over the last 20 years to specifically deal with avian influenza outbreaks that threatened poultry and zoological specimens. In 2003, to respond to an outbreak of HPAI H7N7 that threatened Dutch Zoos, Commission Decision 2003/291/EC19, stated that Belgium and the Netherlands “may decide to carry out an emergency vaccination against avian influenza of susceptible birds in zoos.” If extremely strict requirements were met, this could prevent culling of birds, or prolonged confinement which threatened overall animal welfare. This Directive allowed vaccination of AI susceptible orders such as

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Anseriformes, Struthioniformes, Charidiiformes, Ciconiiformes, Columbiformes and other orders. (Philippa, Munster et al. 2005).

In 2005, The European Union responded to the detection of H5N1 in Turkey, Romania and Russia by drafting Commission Decision 2005/734/EC. This outlined control measures to prevent avian influenza from moving from the wild bird population to poultry and other captive birds. European Union Commission Decision 2005/744EC was specifically targeted toward birds kept in zoos in the Member States. This Directive stated that ‘vaccination of susceptible birds kept in zoos could be an appropriate preventative measure, under certain circumstances.’ The Directive was clear to point out that vaccination of zoo birds represented a special category of animals not primarily concerned by trade. Each Member state that wished to consider vaccination was required to submit a written plan to the Commission to be granted permission to vaccinate. Seventeen countries submitted plans for consideration, not every zoo or every country actually vaccinated their birds. Individual Member State plans can be accessed at: http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/eu_resp_vaccination_en.htm

The Commission:

- defined institutional eligibility requirements for participation in vaccination
- defined ‘susceptible birds’: those likely to be susceptible to AI, and those not intended for the production of animal products
- Member States had to take practical measures to reduce transmission of AI from wild birds to susceptible zoo birds
- Stated that a vaccination plan may be applied based on Risk Assessment
- Individual Member States’ plans for the vaccination of susceptible birds in zoos must be submitted to the Commission

Each individual plan included the following elements:

- Exact location of zoo (physical address) where vaccination would be carried out
- Numbers of susceptible birds, individual identification of each bird
- Vaccine type, dosing scheme and timing
- The reasons for the decision to implement the vaccination measures

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21 http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/eu_resp_vaccination_en.htm
Outcomes of AI Vaccination in European Zoos

There are several reports in the literature that describe the vaccination of birds of non-traditional poultry species as a strategy for preventing morbidity and mortality in the face of an outbreak. In a survey on the use of vaccination as a control strategy for HPAI, from 2002-2010, 20 countries used vaccination to protect zoo, hunting, companion, or endangered birds from either H5 or H7 HPAI. Vaccination of these birds utilized 0.000003% of the total amount of AI doses used on all birds in the countries which responded to this survey.

From 2005 to 2006 the 13 EU countries vaccinated over 44,000 zoological birds (Swayne, Pavade et al. 2011). Three hundred seventy four species from 19 taxonomic orders were vaccinated as part of the overall EU effort (Osterhaus, Fouchier et al. 2007). Individual Member States and institutions utilized different dosing volume, vaccine, dosage schedules and serologic laboratories. Challenge studies could not be performed. It should also be noted that protective titer levels have not been established for the zoological species (Lecu, De Langhe et al. 2009). These factors make it impossible to make scientific conclusion about efficacy (Koch, Steensels et al. 2009) however:

- Zoos utilized H5 vaccine licensed for use in chickens and turkeys. The vaccines were oil-emulsified and inactivated. The vaccines were H5N2, or H5N9 to allow differentiation from infected vs. vaccinated animals. (Swayne, Pavade et al. 2011)
- Species differences exist across taxa in vaccine response when measuring neutralizing antibodies using the hemagglutination inhibition (HI) test. (Furger, Hoop et al. 2008)
- Depending on the species, a single vaccination would illicit response, with a booster vaccination often increasing titers. Greater than 82% of Anseriformes, Ciconiformes, Falconiformes, Phoenicopteriformes, and Psittaciformes seroconverted after one dose of vaccine. (Swayne, Pavade et al. 2011)
- Certain avian orders had low sero-conversion rates; this may be due to vaccine administration, dosing, or biologic factors. (Lecu, DeLanghe et al. 2009) Generally, titers began to decrease 6 months following last vaccination. This may indicate the need for annual or more frequent boosters. (Osterhaus, Fouchier et al. 2007)
- While challenge testing was not performed, these data reported suggest that between 71%-100% mounted presumed protective titers after 2 doses of vaccine were administered (cut off points for protective titer levels varied across studies, from ≥16, ≥32 to ≥40. In poultry, protection from mortality is generally believed to occur at titers of 1:32-40 or greater. (Kapczynski and Swayne 2009)
• Reported adverse effects to vaccination was extremely low, and primarily due to the stress of handling and blood collection.

• EU Commission Report on AI vaccination in zoo birds concluded that it was not necessary to confine vaccinated zoo birds (Decision 2005/744/EC) to prevent contact with wild birds. Increased biosecurity measures (manure management, increased cleaning and disinfection) allowed the vaccinated birds to remain in their outdoor enclosures. (Osterhaus, Fouchier et al. 2007)

• The vaccination of zoo birds is recommended by the European Association of Zoos and Aquarius (EAZA) and the European Food Safety Association (EFSA) (Gilbert and Philippa 2012)

VII. Likely Consequence of Vaccine use in U.S. Zoological Institutions

The decision to vaccinate birds in zoological institutions will likely change management and business practices for the facility significantly. The risk assessment should consider the consequences for animal health and husbandry, public health, commerce and trade, and business continuity. Biological/animal health consequences of vaccination can be found in Appendix D: Risk Assessment. Additional consequences may include:

• The zoo will be required to follow all elements of their approved vaccination plan. This will likely increase staff time to fulfill requirements.
  o There will likely be an increased need for manpower to implement the vaccination program.
  o Costs of materials, labor, increased biosecurity and record-keeping may be onerous for a small number of animals in a given institution.

• The permission to vaccinate will likely include the requirement to implement, continue or increase surveillance sampling.

• Misuse or careless use of AI vaccine (including improper record-keeping) could jeopardize poultry export markets.

• Strict adherence to guidelines established by zoological, State and federal partners for the vaccination of zoological collections is vital. Failure to adhere to these guidelines may result in sanctions from foreign governments, affecting the poultry industry and other animal industries.
Movement of Vaccinated and Recovered Birds
The ability to move vaccinated or recovered birds will be dictated largely by Federal and State regulations. This may impact the ability to move animals across state lines for breeding and exhibition. The strict adherence to vaccination protocols and accurate medical records for recovered individuals may increase the likelihood that animals may be moved.

Recall that the DIVA strategy is not validated in most zoological species, thereby making it difficult to determine if the animal’s serologic response is to an infection by the pathogenic strain, or from vaccination. Therefore, specific conditions (the need for additional pre-shipment diagnostic testing, such as PCR testing, additional quarantine measures, etc.) will need to be agreed upon with the appropriate State and/or Federal animal health authorities in the receiving states or countries. It is possible that the receiving state’s Animal Health Official or foreign government could deny shipment of vaccinated or recovered individuals.

It is likely that Permits will be needed to move vaccinated and recovered animals, in addition to the Health Certificate requirements of the receiving State or zoological facility. It is possible that this ‘Permit-required’ movement will be for the life of the specimen.

VIII. Conclusions

The decision to vaccinate zoological specimens should be based upon a thorough risk assessment. Salient information from the literature can be summarized as follows:

- Vaccine is not currently approved for use in zoological species in the US.
- Previous vaccination for HPAI H5 or H7 in zoos in many countries was done due to increased risk to collections, including a small number of cases in birds housed in zoos.
- Differences in vaccine type, schedule, dosing, methodology has led to an incomplete understanding of the utility of AI vaccination in zoological species
- Current vaccine appears to protect against morbidity and mortality in some species, but does not prevent infection. The infective dose of virus required to infect vaccinates appears to be higher than dose needed to infect unvaccinated birds. Vaccinated birds appear to shed fewer virus particles than unvaccinated birds. (Philippa 2006)
- Vaccination appears to increase titers in many species; however, the level of truly ‘protective’ titers remains largely unknown as cut off points have been extrapolated from poultry. Challenge studies to determine protective titers
cannot be performed in many species.

- In many avian species, there appears to be reduced shedding of virus by vaccinated birds if they are infected. This may decrease environmental contamination, decrease disease transmission, and reduce risk for human handlers.
- The licensed H5 and H7 vaccines used in non-domestic species caused few side effects in the birds due to the vaccination itself, with minor swelling at the injection site being noted occasionally (Philippa, Munster et al. 2005).
- Mortality events were very low, and primarily due to handing (Osterhaus, Fouchier et al. 2007).
- The EU scientific Report states that bio-security should always be the most important element to protect against HPAI, but if measures cannot be implemented to protect zoo birds from exposure to wild birds, and using risk assessment, vaccination for HPAI H5 or H7 using vaccine for poultry should be used to protect zoo birds. (Osterhaus, Fouchier et al. 2007)

The following notable quotes are provided from researchers and the OIE with regards to avian influenza vaccination in zoological specimens:

- “Vaccination may be a useful strategy to save exotic and valuable birds from confinement and pre-emptive culling in view of a potential outbreak of AI.” (Furger, Hoop et al. 2008)
- “AI vaccines can be used in preventative or emergency programmes to protect zoo birds, endangered species and other valuable, non-poultry species to maintain biodiversity, and such vaccine use should not prevent trade in poultry and poultry products” (Swayne, Pavade et al. 2011)
- “In the event of an avian influenza epizootic, zoo birds would constitute a potential source of infection and transmission among wild birds and poultry, and would likely need prophylactic vaccination.” (Kapczynski and Swayne 2009)
- “...for countries to consider vaccination to protect valuable birds such as specific poultry breeds, zoo birds, pet birds, ornamental birds, parent flocks and fighting cocks when there is an increased risk of infection” (OIE 2007)
Appendix A

Biosecurity Basics

INTRODUCTION

As stated in the Vaccination Information for Accredited Zoos and the USDA AZA AI Outbreak Management Plan, biosecurity is the most important element to prevent an outbreak and control the spread of avian influenza in zoological collections. **Appropriate biosecurity protocols for a zoological facility must be tailored to each individual facility, based on risk assessment.** Absolute biosecurity is seldom attainable in animal exhibits. “Biosecurity efficiency is demanding and not always sustainable in all poultry production, notably outdoor farming, backyard and subsistence operations and even public and private zoos, wildlife parks and aviaries”. 22

AZA provides guidance to its member institutions to meet performance standards for biosecurity. AZA accreditation standards require the following 23:

11.1.2. **Training and procedures must be in place regarding zoonotic diseases.** Explanation: Diseases that can be transmitted between animals and humans (zoonotic disease, zoonoses) present a potential risk for both zoo staff and the visiting public. The institution should design facilities, develop animal care protocols and present collection animals for public contact in ways that minimize this risk. Institutions must train appropriate staff in methods to prevent zoonotic disease. The National Association of State Public Health Veterinarians (NASPHV) has prepared a Compendium of Measures to Prevent Disease Associated with Animals in Public Settings which should be followed by institutions presenting animals for public contact.


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23 AZA Accreditation Standards
[http://www.aza.org/uploadedFiles/Accreditation/Accred%20Standards%20with%20elephants%29%281%29.pdf](http://www.aza.org/uploadedFiles/Accreditation/Accred%20Standards%20with%20elephants%29%281%29.pdf)
In addition, AZA requires specific quarantine guidelines to prevent disease issues associated with the movement of animals between its collections:

Quarantine, hospital, and isolation areas should be in compliance with standards/guidelines contained within the *Guidelines for Zoo and Aquarium Veterinary Medical Programs and Veterinary Hospitals* developed by the American Association of Zoo Veterinarians (AAZV), which can be obtained at the AAZV website.

**BIOSECURITY BASICS**

The level of biosecurity and record-keeping required for each institution to comply with AZA accreditation standards or as approved by State and Federal veterinary regulatory authorities will significantly reduce the risk of acquiring an exotic animal disease. These include such requirements as:

- all birds are individually identified
- daily observation by animal care staff of each bird for clinical signs of disease and method of reporting abnormalities facilitates early veterinary investigation
- each approved facility must have either an on-staff federally accredited veterinarian or have a sustaining relationship with a federally accredited veterinarian competent to administer to avian species
- recordkeeping method should include current enclosure or location of each individual
- Historical movements of individual birds between locations within an institution should be readily available in a manner which allows identification of potential contacts in an epidemiological investigation.

For more detail on specific recommendations for Risk Reduction techniques, see Section 2 pg. 13 of the Outbreak Management Plan.

In addition, it is recommended that each facility should develop written biosecurity protocols, and train their staff on these protocols. Staff veterinarians and other animal care experts should be involved in the writing of these plans. It is recommended that the following routine practices be followed:

- **Disinfection.** The facility’s veterinarian and animal healthcare team should determine appropriate disinfection protocols for day-to-day operations to reduce the likelihood of disease transmission.
- **Carcass and Contaminated Waste Disposal.** The possibility that a serious emergency disease could occur in a zoo, particularly among avian species underlines the need for strategically located disposal facilities to be discussed with stakeholders and designated
in advance of such an event. Disposal of the carcass should be performed to minimize potential disease transmission of any infectious agent to other collection birds, or wildlife

- **Staff hygiene.**
  - Work clothes, including footwear, should be worn only at work
  - Contact between animals kept at home and zoo birds should not occur. This includes indirect contact via footwear, equipment and clothes as stated above.
  - Hand washing before and after work (minimally) is an additional risk mitigation techniques that should be mandatory
  - Close preventative health monitoring of personally owned birds; it is very important to follow-up on any suspect signs of clinical illness

- **General sanitation.** Include an effective pest management program for the facility. Minimize contamination of exhibit, holding and adjacent areas by waste materials or drainage

- **Wildlife Management.** Where possible, wild birds be discouraged from entering exhibits

### BIOSECURITY AT HEIGHTENED RISK

In the event of the facility being in a declared surveillance or infected zone during an HPAI outbreak, a number of additional practices may become mandatory. A number are outlined in the Outbreak Management Plan Section 2, but it should be noted that all biosecurity recommendations and requirements will be determined in consultation with Incident Command Post (ICP), State veterinarians, USDA and other experts on site.

- **Disinfection.** While most disinfectants used for routine cleaning are effective against AI viruses, the ICP, and State and federal agencies may provide guidance on approved products.

- **Carcass and Contaminated Waste Disposal.** Direction of such disposal will be under the direct jurisdiction of the State Veterinarian and USDA AVIC.

- **Staff hygiene.** Elevated levels of PPE (personal protective equipment) may be necessary. See Appendix 6.1 of the Outbreak Management Plan for more detail. The ICP will determine the necessary PPE that should be utilized, based on USDA protocols and where the strain is a known zoonotic agent, with guidance from Centers for Disease Control (CDC).

- **Wildlife Management.** At heightened risk levels, additional measures to prevent contact of collection specimens with wildlife to prevent disease transmission may need to be implemented.
• **Sources of food and collection specimens.** The source of feeder animals and products is considered, as well as the source of collection birds to prevent AI from being brought into the facility in this manner.

• **Isolation Areas.** An isolation area and management plan should be developed to quarantine any suspect cases in consultation with the ICP. See Section 3.1 ‘Identification and Preparation of Isolation Facilities’ in the OMP.

**BIOSECURITY FOR VACCINATED ANIMALS**

In the event that a facility has been approved, and is vaccinating its avian species, this likely means that AI is either within the facility itself, or is located in a Control Area, or potentially in an adjacent Surveillance Zone. (See Section 4 of the OMP for more information on Zones). This will necessitate a very high level of biosecurity, as current vaccination technology will reduce morbidity and mortality of many species, but it does not prevent infection and viral shedding. Strict adherence to biosecurity protocols and diligence is necessary for vaccination strategies to be successfully implemented.

Additional biosecurity protocols will be determined by ICP, State veterinarians and USDA. These additional requirements should be documented and be a part of the individual institution’s Vaccination Plan (See Appendix C). Additional requirements may include:

- Additional PPE for protection of staff health and to prevent transmission to susceptible species
- Additional staff training to recognize signs and symptoms of AI in vaccinated species
- Additional training of staff on heightened biosecurity measures for vaccinated animals
- Additional training on decontamination and disposal of infectious material
- Additional training on cleaning and disinfecting of facilities and equipment
- Additional preventative measures to reduce or eliminate contact between vaccinated birds and wild birds
- Additional restrictions on public contact
- Restricted access for vehicles, equipment and personnel with exposure to birds, poultry or poultry eggs in areas affected by AI
Appendix B

Recommended Guidelines for Participation in a USDA HPAI Vaccination Plan for AZA Institutions

NATURE OF THE ENTERPRISE

The American Zoo Association (AZA) is a professional organization which represents zoos and aquariums. Most of the 224 member institutions (June 2012) are located in the U.S. Guidelines for participation in any USDA AZA approved AI vaccination plan would be applicable to only those AZA institutions within the United States.

AZA has established high professional standards for member institutions and ensures adherence to these guidelines through the accreditation process. AZA standards either meet or exceed standards set by USDA APHIS Animal Care. Accreditation is an organized, detailed visitation and review of an institution’s operating procedures. This includes review of veterinary care and medical records, physical facility and safety records, biosecurity protocols, and financial health and oversight.

Facilities considered for accreditation are defined as “a permanent institution which owns and maintains wildlife, under the direction of a professional staff, provides its collection with appropriate care and exhibits them in an aesthetic manner to the public on a regular basis. The institution, division, or section shall further be defined as having as their primary mission the exhibition, conservation, and preservation of the earth’s fauna in an educational and scientific manner.”24 (Accreditation standards can be found at http://www.aza.org/accred-materials/)

An AZA Accredited Zoo or Aquarium has met the standards of AZA and has passed the formal accreditation process.

MINIMUM SUGGESTED REQUIREMENTS OF ZOOS REQUESTING TO PARTICIPATE IN AN APPROVED VACCINATION PLAN

The decision to allow vaccination of birds in AZA institutions will be a decision made with many stakeholders and State and Federal regulators. Vaccination would only be one element of a comprehensive prevention and control program. An increased level of biosecurity (see Appendix A) should be primary in protecting animals from AI. Based upon input from State, Federal and local stakeholders, a risk assessment should be conducted (Appendix D) to analyze

24 AZA 2012 Accreditation standards
the need for vaccination for AI. AZA believes that adherence to certain standard operating procedures, required for accreditation by their member institutions should provide assurance to USDA that these facilities could meet the requirement of an approved AI Vaccination Plan. The following protocols, based on requirements of European zoos which participated in the EU Vaccination plan, should be considered as minimum requirements by USDA and State regulatory officials for participation in any approved vaccination plan. These proposed best practices for AZA institutions are more rigorous than requirements for plans drafted in 2006 for EU member states.

Recordkeeping and management considerations:
- The zoo should perform daily observations by animal care staff (an AZA accreditation standard) of each bird or animal for clinical signs of disease. There should be a method of prompt reporting of any abnormalities.
- Current animal inventories should be available to the attending veterinarian and general curator. The origination/source of all acquired birds held by the institution should be recorded and updated on an annual basis.
- All birds must be identified at all times. The identification must be permanent and may include but not be limited to leg bands, wing bands or microchips.
- Recordkeeping method should include current enclosure or location of each individual bird.
- Detailed, historical records of movement of the birds within and among institutions should be kept according to AZA accreditation standards. This will facilitate “trace-forward” and “trace-back” investigations. Attending veterinarian and general curator should be advised of the relocation within or between institutions of any bird.

Veterinary considerations:
- State animal health authorities may conduct site inspections and inspect records prior to or after vaccination has been authorized.
- Each facility must have either an on-staff federally accredited veterinarian or have a sustaining relationship with a federally accredited veterinarian competent to administer to avian species.
- Emergency disease preparedness plans should be included in the facility’s veterinary care program.
- The attending veterinarian should be familiar with all relevant aspects of the institutions’ animal handling and management practices. This would allow more informed decision-making if an exotic animal disease is suspected.
- The veterinarian should be aware of reportable diseases and procedures for reporting suspected cases. If suspected, attending veterinarians should include HPAI in their
differential diagnoses. Suspect avian influenza cases must be reported to the State veterinarian and USDA Area-Veterinarian-in-Charge (AVIC).

- The veterinary health program should be designed so that there is a reasonable chance of detecting disease should it be present.
- All birds that are relocated from a zoo collection should have a current health assessment prior to movement. The use of pre-shipment examinations and health certificates surrounding bird movement between institutions provides an additional opportunity for disease detection.
- In accordance with AZA-accreditation standards, all collection birds that die in a facility should receive a complete necropsy. If lesions consistent with AI are present, the veterinarian should know whom to notify (State Veterinarian, Zoo Director, etc.) to determine next steps. Appropriate samples for additional diagnostic testing should be collected (i.e. affected tissue, tissue fluid, serum if possible).

**Staff training considerations:**

- The main objective of training (as it applies to these guidelines) is to prepare zoo staff for an emergency disease, including training regarding biosecurity, and transmission and recognition of clinical signs of AI. Emphasis should be placed on the need for staff to report promptly any abnormalities in birds under their care.
- The veterinarian should be involved in basic training of staff in procedures to minimize the spread of disease.
- Animal care personnel should have a basic knowledge and biology of birds, and know basics of AI species susceptibility

**Quarantine considerations:**

- Biosecurity standards for quarantine should follow those outlined in the AZA accreditation standards. (See Appendix A)
- When birds are in quarantine, an ‘all-in-all-out’ policy should be adopted (based on taxonomic and disease transmission consideration per AZA quarantine guidelines.
- During quarantine, access to birds should be limited to essential staff (veterinary and designated animal care staff only).
- The attending veterinarian should be advised of the cause of death of any quarantined or imported bird as soon as possible after the postmortem examination.
- Waste material from birds in quarantine should be treated in a manner that limits access by all other fauna (including free-ranging animals/birds)
- Biological specimens/samples from birds in quarantine should be handled, transported, and stored under conditions that will minimize the potential transmission of pathogens while preserving the integrity of the sample for diagnostic testing.
• There may be a need to consider the spatial requirements (distance) between birds in quarantine and those that have been cleared. For example, zoo quarantine areas for some species may include outdoor holding areas. Potential for aerosol or fomite transmission should be minimized using facility design, bird management procedures and spatial arrangements.

Surveillance Considerations:
• Participation in a vaccination plan would likely require that both active and passive surveillance measures are implemented.
• Active surveillance should include a strategy of maintaining and sampling of sentinel Specific Pathogen Free (SPF), unvaccinated chickens. Chickens may be exposed to the environment or environmental materials (i.e. water, excrement, etc.) of birds at highest risk of infection (i.e. free-ranging waterfowl and wading birds) or flight-restricted waterfowl on ponds exposed to wild migratory waterfowl or exposed to their excrement.
• The exact requirements for surveillance in vaccinated and unvaccinated birds in an individual zoo should be documented in the institution’s Vaccination Plan (see Appendix C). The surveillance strategy will be dictated by numerous factors, including but not limited to viral epidemiology, level of biosecurity that can be achieved by the institution, statistical considerations, location of a facility relative to an outbreak (concept of zones25) and animal husbandry considerations.

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25 For more information on zones, see Table 5-1 “Summary of Premises Designations” in the Red Book.
Appendix C

Suggested Elements for Individual Institutional Plans

The OIE receives questions about AI vaccination plans; however the detailed guidelines will depend on local situations. This will likely be true for any Institution Vaccination Plan. Each individual institutional vaccination plan should be submitted to AZA, State Veterinarian, and USDA AVIC. The AZA institution should establish a good working relationship with these animal health officials before drafting the institution-specific plan, as certain plan elements will likely be dictated by these regulatory officials. State veterinarians and USDA AVICs should evaluate the ability of a facility to properly execute their plan, and this assessment of ability may be used to determine eligibility to vaccinate.

All entities should agree in writing to abide by any standards described in a plan, and all parties should consider signing a written agreement form to abide by all elements of the plan.

RECOMMENDED ELEMENTS OF AN INSTITUTIONAL VACCINATION PLAN

1. Institutional Information
   - Formal name of institution
   - Address
     - Name of Chief Executive Officer (CEO) or Chief Operational Officer (COO), Director, etc. as applicable
     - Name(s) of attending veterinarians. Cite credentials for properly administering avian vaccine (cite any training programs, accreditations, etc)

2. An outline of or reference to the biosecurity protocols that will be necessary with the implementation of the vaccination plan. (See Appendix A)

3. A map of the facility, indicating location of collection specimens to be vaccinated

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4. **List of birds for which vaccination will be requested**, including:
   - Individual permanent identification of each bird. This identifier must be a permanent physical identification (i.e. leg band, wing band, and/or microchip) that corresponds to an individual inventory number, movement history and medical history.
   - Common name of species, Latin name, taxonomic order
   - Location of bird within institution
   - These lists will likely be provided to State veterinarian, USDA AVIC and vaccine manufacturers for approval to use in these species.
   - The vaccine manufacturer will provide a copy of this list to the Center for Veterinary Biologics (in addition to the usual documentation forms) when they request a 9 CFR 103.3 authorization.

5. **Estimates to the volume of vaccine necessary to vaccinate all approved species**
   - Anecdotally, the most effective dosing to elicit the greatest response appears to be determined by body weight. Consultation with USDA, avian veterinarians and experts, input from vaccine manufacturers and available literature will likely drive dosing recommendations.
   - The anticipated dosage and expected number of doses per bird should be provided by the zoo to the Al vaccine manufacturer.
   - It is likely that The Center for Veterinary Biological will only approve 9 CFR 103.3 authorizations to allow shipment of the minimum quantity of vaccine that will allow for the vaccination of the appropriate species at a pre-determined dosage for the expected number of priming and booster vaccinations that would be expected to be administered during a one-year period.

6. **Timeline for completion of vaccination**
   - The plan should outline the vaccination intervals that will be used, i.e. number of weeks after initial vaccine, when the boosters will be given.
   - Indicate the timeline in which all vaccination of birds will be accomplished.

7. **Include plans for blood sampling**
   - Blood samples should be taken prior to vaccination from all birds dependent on size and ease of procuring samples. Plasma or serum samples will be frozen and stored. Include in the plan any requirements for testing these samples.

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27, 28 For more on information for shipping biologicals for experimental use, see [http://cfr.vlex.com/vid/103-shipment-experimental-biological-19610479](http://cfr.vlex.com/vid/103-shipment-experimental-biological-19610479)
o Additional blood samples may be collected prior to booster vaccinations in order to monitor vaccination status, and may be required at any other interval deemed appropriate by the attending veterinarian, State veterinarian and USDA.

8. **Plan for handling the vaccine and retention of used/empty vials of vaccine until authority given to destroy**
   o Vaccine should be held under lock and key with limited access to key personnel who are pre-identified in the request to the vaccine manufacturer to receive Al vaccine. Ensure appropriate cold-chain recommendations.
   o Used and empty vials of Al vaccine should be retained until permission to destroy and dispose of the vials is granted by the appropriate animal health authority. Used and empty vials of avian influenza vaccine must be made available to state and/or federal animal health authorities during an inspection.
   o Recordkeeping should account for all vaccine used, left over, and any waste
   o Each AZA, or AZA-equivalent zoo, will be required to account for every single dose of Al vaccine. Failure to accurately account for vaccine will be considered a violation of the terms of the Agreement Form.

9. **Include plans for the management of vaccinates and any sentinel flocks**
   o Management of sentinel animals/flocks should be outlined and agreed upon by the individual institution, State veterinarian and AVIC.
   o All parties put in writing any conditions for the mixing of vaccinated with non-vaccinated collection specimens

10. **Include plans for surveillance activity in vaccinated and non-vaccinated birds**
    o Vaccinated birds should continue to be visually monitored daily
    o Provide in writing assurances that vaccinated animals will not enter the food chain
    o Since vaccination does not prevent possible infection and shedding, any required surveillance responsibilities for vaccinated and unvaccinated birds should be outlined in the Plan.

11. **Include plans for keeping records on vaccinated birds**
    o Outline how the institution will preserve and maintain vaccination records, and any individual animal medical records (ZIMS, MedARKS, etc.)
    o Vaccination records may need to be kept for 10 years or longer
Data kept on the vaccinates should include

- Dates and places of vaccination and any boosters.
- Weight of bird
- Route of vaccine administration (intra-muscular, subcutaneous). Route does not appear to affect vaccine efficacy per EU commission report, except in birds with large subcutaneous air sacs where SQ seems to be less effective\(^{29}\)
- Anatomic site of vaccination
- Vaccine dose in milliliters. Dose of vaccine, adapted to bird body weight, appears to produce the highest immune response.\(^ {30,31}\)
- Influenza vaccine name, manufacturer, bottle lot number
- Any adverse effects, including mortality, attributable to vaccination

12. Include expectations and plans for providing reports on vaccinations given, adverse effects, and serological results to USDA officials and vaccine manufacturers if requested.

13. Document communication requirements for continued reporting of any ill birds and suspect cases to appropriate animal health authorities.


- The ability to cease vaccination will also likely be determined by risk assessment, involving surveillance results in zoological, agricultural and wild bird compartments, and other factors such as the success of any decontamination and disposal plans within a zoological facility.
- Include in the Plan which agencies/individuals should be included in discussions to cease vaccination.


15. A signed Agreement Form for all parties to participate in the Plan
   - This written Agreement Form would declare the intent to abide by the standards, terms, and conditions described in the institutional Vaccination Plan.
Appendix D

Risk Assessment for use of Vaccination to control HPAI in zoological facilities

Risk assessment should be an important tool to determine the appropriateness of vaccinating zoological specimens, either prophylactically or in the face of an outbreak of avian influenza. A risk assessment would be carried out with State and federal regulatory officials, including additional expert opinion from virologists, epidemiologists and others.

The risk assessment process will help determine needs and appropriate vaccination strategies for managing AI. Many factors may be considered in a risk assessment:

VIRAL BIOLOGY, EPIDEMIOLOGY AND ECOLOGY

- Viral characteristics
  - What is the pathogenicity in zoological species (avian or otherwise)
  - Transmissibility between individual animals
  - Transmissibility between species
  - What is the rate of outbreak spread\(^\text{32}\)
  - Environmental persistence
  - Infectious dose
  - Transmission modes (respiratory/droplet transmission, fecal-oral transmission?)

- Zoonotic tendencies of the strain
  - Is it highly transmissible from animals to man, man to animals, or rarely transmissible?

NATURE OF THE OUTBREAK

- What is the probability that the disease can or cannot be rapidly contained?
- Is the outbreak localized, regional or of national scope?
  - Is the zoo already infected? Or located in an infected zone, a buffer zone or surveillance zone?\(^\text{33}\)

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\(^{33}\) The Red Book
• What animal sectors /industries are involved in the outbreak?
  o Is the outbreak in the poultry sector, wild birds or in the zoo itself?
• What species are the reservoirs, and the behavioral ecology of any reservoirs? How likely is there interaction with collection specimens in open exhibit?34

NATURE OF THE FACILITY
• Facility biosecurity
  o What is the state of robustness of biosecurity protocols? (Appendix A)
  o Potential for contact with wild birds: Many species are housed in facilities, such as pens covered with nets that prevent escape but do not eliminate indirect contact with carrier wild birds35, including migratory waterfowl.
  o Are facility biosecurity protocols sufficient to prevent viral spread from vaccinated birds to susceptible, unvaccinated collection animals?
• Adequate biosecurity must be demonstrated to prevent exposure to susceptible animals from apparently healthy but virus-shedding vaccinates.
• The ability of the zoo to divide itself into separate ‘epidemiologic units’. It may be possible to determine different risk levels based on physical barriers and biosecurity protocols36
• The location/distance of the zoo, from the outbreak or detection. (See Red Book for more information on zones)
• The location of the zoo relative to any poultry producing facilities and the relative density of poultry live bird markets, or other sites
• The proximity of the zoo to international borders
• The location of the zoo relative to wetlands, agricultural land (which may provide a food source for reservoir species, etc.) and migratory bird breeding grounds
• The location of the zoo relative to regional migratory flyways
• Exhibit design of open bodies of water37:


35 Sparrows are often uncontrollable in outdoor exhibits. Sparrows may act as intermediate hosts but is likely not implicated in extended shedding of HPAI H5N1. Starlings may play a role in maintenance and circulation of HPAI, with pigeons having a minor role. (Artois and Bicout et al.)


37 AI viruses can survive for long periods of time in surface water. The virus persists in sediments, and factors such as higher salinity, acidic conditions and warm temperatures decrease persistence of virus in the environment (Artois and Bicout 2009)
• The presence of free-ranging galliformes on grounds increases risk of AI, and the ability to remove them from grounds may be a factor in the risk assessment.
• The likelihood of spread of the virus into the institution via fomites.
  o Are vendors which supply the zoo also vendors which supply infected premises or drive thru infected areas?
  o What is the likelihood of any contaminated product intended for animal feed products?

RESERVOIR CHARACTERISTICS
• Are there established reservoirs or endemnicity of the virus?
• What are the migratory patterns of reservoir species?
  o Other factors to be considered if migratory birds are reservoir of disease
    ▪ The gregarious nature of the reservoir species and species in the collection
    ▪ Abundance/density of reservoirs, based on seasonal patterns, should also be considered.
• What are the foraging and breeding characteristics or reservoirs and collection specimens?
• Are reservoirs or collection specimens predators, or scavengers? How does this effect transmission dynamics?

ZOOLOGICAL SPECIES CHARACTERISTICS
It is highly recommended that zoos determine a list of susceptible avian species (Appendix C) that will serve as a guide when conducting the risk assessment and developing the individual vaccination plan.
• All avian species are considered at risk of infection by AI. However some species may be at less risk than others in consideration of their innate resistance.
  o some zoological species may be at greater risk due to receptor presence
• What species in a facility are capable of contracting, amplifying and disseminating the virus across the zoo?
• What is the age of zoological specimens of concern
• The vast majority of species vaccinated in European plans needed 2 vaccinations to produce titers hypothesized to be protective. Will there be difficulty in applying 2 doses of vaccine\(^ {38} \) (i.e. waterfowl on open ponds)? Can the species targeted for vaccination be handled twice in 3-4 weeks time for vaccination and blood sampling?

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• The behavioral ecology of zoological specimens: are these solitary, or likely to interact with potential wild disease reservoir species?
• What are the husbandry requirements of outdoor species: not all bird species may be housed humanely indoors. It may be more humane to vaccinate rather than attempt to house indoors.
• How are rare or valuable are the genetic stocks housed in the institution? Loss of these species to HPAI could have a global impact on species survivability.

PROTECTION OF PUBLIC HEALTH AND SAFETY AND TRADE CONSIDERATIONS
• Does the zoo have a robust PPE policy to protect employees? Will protocols reasonably prevent the spread of the virus from vaccinated, but infected and shedding animals to staff and or guests?
• Societal concerns and community impacts will likely be considered in the risk assessment process.
• Increased public health confidence: Having avian vaccination as part of our preparedness plan may increase public confidence.
• Increased protection for zoological workers
  o Public health risk to employees working with collection birds and susceptible mammals during an outbreak may be decreased in those birds that are vaccinated and mount an immune response.
  o The decreased level of viral shedding in vaccinated birds may limit environmental contamination and decrease the likelihood of transmission to susceptibles and to zoological workers
• The poultry trade implications of vaccination application in zoos may be taken into account in the risk assessment process. What are the economic implications if the outbreak cannot be controlled without vaccination?

CONSEQUENCES OF THE DECISION TO VACCINATE
Vaccination in zoological species may be extremely important in controlling morbidity and mortality in our specimens. In addition to the State, national and international implications of vaccination, (See Section VII Vaccination Information for Accredited Zoos) there may be additional considerations in a thorough risk assessment.


40 Poultry trade in EU countries was not affected by vaccination of zoological specimens.
• Vaccination program may be a significant investment in resources, for vaccine, and staff time.
• Health and safety of personnel participating in the vaccination program must be considered.
• Vaccination will not prevent infection and shedding but may reduce or prevent clinical signs, thus lead to a false sense of security and decreased biosecurity. Any lapses in biosecurity may lead to predisposition for infection.
• The time between vaccination and development of protective titers has to be considered in the management of an outbreak.
• There have been no standardized trials to assess the safety and efficacy of AI vaccines in off-label species
  o The safety and efficacy of avian influenza vaccines in off-label species is largely anecdotal, reported from HPAI vaccination programs in Europe. Significant adverse events may occur with off-label use or AI vaccines in some vaccinates in zoological collections. The institution should be prepared to provide medical care in the event of a reaction to vaccination.
  o The literature reports that response to vaccination appears to be species dependant. Some species like the pelican (Order Pelicaniformes) and owls (Order Strigiformes) may fail to respond to vaccination. Not all species may be adequately protected by vaccination.
  o Variability across species: Some species have exhibited a short duration of ‘protective’ titers. This means that boosting, serologic testing and surveillance may need to be more frequent in certain species.

In conclusion, risk assessment is an important tool that could be used to determine the appropriate application of vaccine in zoological institutions. A thorough assessment will necessitate collaboration of experts from multiple scientific disciplines and animal care professionals.

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Appendix E

Selected References and Resources


