

# Highly Pathogenic Avian Influenza (HPAI) Contingency Plan for Rocky Mountain Arsenal NWR

DEPARTMENT OF THE INTERIOR  
U.S. FISH AND WILDLIFE SERVICE  
ROCKY MOUNTAIN ARSENAL NATIONAL WILDLIFE REFUGE  
COMMERCE CITY, COLORADO



## BACKGROUND

### Site History

Rocky Mountain Arsenal National Wildlife Refuge (RMA NWR) is a complex and historic site. Prior to becoming a National Wildlife Refuge, the area was occupied by homesteaders who grazed cattle and raised crops. Farmers and ranchers helped shape the current landscape by building ditches and storage reservoirs for irrigation water. These irrigation structures were later enlarged and improved when the site was used for weapons and pesticide production by Army and Shell Chemical Company. In 1992 the Arsenal was legislated to become a National Wildlife Refuge following completion of cleanup under provisions of the Rocky Mountain Arsenal National Wildlife Refuge Act (CMP 1996). Currently, the reservoirs in addition to First Creek, Parkfield Wetland Havana Pond and several small storage ponds provide waterfowl habitat throughout the year.

### Avian Resources

Winter waterfowl surveys are conducted annually from mid-October to mid-April on Lake Mary, Lake Ladora, Lower Derby Lake, Havana Pond; First Creek and the wetland complex during sufficient water years. Surveys are conducted once every two weeks for a total of 1-3 surveys each month. Target species include: Canada goose, wood duck, green-winged teal, mallard, Northern pintail, blue-winged teal, cinnamon teal, Northern shoveler, gadwall, American widgeon, canvasback, redhead, ring-necked duck; greater scaup, lesser scaup, common goldeneye; bufflehead; hooded merganser, common merganser, and red-breasted merganser.

Lower Derby had the most diverse waterfowl use followed closely by Lake Ladora in 2005-2006. Fifteen different species of waterfowl were recorded in the winter and spring for Lower Derby, whereas 13 different species were observed on Lake Ladora in the winter (Table 1). Lower Derby also had the highest total estimated waterfowl use for a single survey in November with 6580 birds recorded, whereas Lake Ladora had 960 birds, Lake Mary 0, Havana 159 birds and Parkfield 1 (Table 2). Fall and spring were the highest use times with 9522 birds recorded in November and 3187 in March and April combined (Table 2). Waterfowl totals are listed in Appendix A.

## INTRODUCTION

### **Avian Influenza and Birds (Center for Disease Control Website)**

*Avian influenza is an infection caused by avian (bird) influenza (flu) viruses. These influenza viruses occur naturally among birds. Wild birds worldwide carry the viruses in their intestines, but usually do not get sick from them. However, avian influenza is very contagious among birds and can make some domesticated birds, including chickens, ducks, and turkeys, very sick and kill them.*

*Infected birds shed influenza virus in their saliva, nasal secretions, and feces. Susceptible birds become infected when they have contact with contaminated secretions or excretions or with surfaces that are contaminated with secretions or excretions from infected birds. Domesticated birds may become infected with avian influenza virus through direct contact with infected waterfowl or other infected poultry, or through contact with surfaces (such as dirt or cages) or materials (such as water or feed) that have been contaminated with the virus.*

*Infection with avian influenza viruses in domestic poultry causes two main forms of disease that are distinguished by low and high extremes of virulence. The “low pathogenic” form may go undetected and usually causes only mild symptoms (such as ruffled feathers and a drop in egg production). However, the highly pathogenic form spreads more rapidly through flocks of poultry. This form may cause disease that affects multiple internal organs and has a mortality rate that can reach 90-100% often within 48 hours (CDC 2006).*

### **Avian Influenza and Humans (Center for Disease Control Website)**

*There are many different subtypes of type A influenza viruses. These subtypes differ because of changes in certain proteins on the surface of the influenza A virus (hemagglutinin [HA] and neuraminidase [NA] proteins). There are 16 known HA subtypes and 9 known NA subtypes of influenza A viruses. Many different combinations of HA and NA proteins are possible. Each combination represents a different subtype. All known subtypes of influenza A viruses can be found in birds.*

*Usually, “avian influenza virus” refers to influenza A viruses found chiefly in birds, but infections with these viruses can occur in humans. The risk from avian influenza is generally low to most people, because the viruses do not usually infect humans. However, confirmed cases of human infection from several subtypes of avian influenza infection have been reported since 1997. Most cases of avian influenza infection in humans have resulted from contact with infected poultry (e.g., domesticated chicken, ducks, and turkeys) or surfaces contaminated with secretion/excretions from infected birds. The spread of avian influenza viruses from one ill person to another has been reported very rarely, and transmission has not been observed to continue beyond one person.*

*There are only three known A subtypes of influenza viruses (H1N1, H1N2, and H3N2) currently circulating among humans. It is likely that some genetic parts of current human influenza A viruses came from birds originally. Influenza A viruses are constantly changing, and they might adapt over time to infect and spread among humans.*

*Symptoms of avian influenza in humans have ranged from typical human influenza-like symptoms (e.g., fever, cough, sore throat, and muscle aches) to eye infections, pneumonia, severe respiratory diseases (such as acute respiratory distress), and other severe and life-threatening complications. The symptoms of avian influenza may depend on which virus caused the infection.*

*Studies done in laboratories suggest that some of the prescription medicines approved in the United States for human influenza viruses should work in treating avian influenza infection in humans. However, influenza viruses can become resistant to these drugs, so these medications may not always work. Additional studies are needed to demonstrate the effectiveness of these medicines (CDC 2006).*

## DISEASE SURVEILLANCE

### **Regulatory and Policy Compliance**

All monitoring and surveillance will be done in accordance with USFWS policy, Refuge Comprehensive Management Plan (CMP) and the Endangered Species Act. Wintering Bald Eagles which roost and nest at RMA will not be affected by this plan.

### **Surveillance and Monitoring**

Monitoring efforts will be limited to visual inspections of waterfowl during routine survey work by Refuge staff and volunteers. During surveys, a cursory inspection will be done looking for signs of disease including, lack of awareness, ruffled feathers and inability to fly. In the event a suspect bird is identified but not recovered, staff will do a follow up inspection the next day in an attempt to locate the bird.

## FIELD INVESTIGATIONS

### **Human and Wildlife Safety**

Because HPAI can infect humans, health and safety will be given top priority. All Service personnel anticipating working with potentially infected birds will be required to have a current human influenza shot. The proper personal protective equipment (PPE) will be worn at all times when handling birds suspected of infection. PPE will include latex gloves, NIOSH N95 face mask, eye protection and coveralls. Processing equipment

such as masks and gloves will be disposed of in the proper biohazard containers. Non-disposable items such as knives, forceps and lab counters will be disinfected with bleach solution. Following work with any potentially infected birds, all persons must wash hands with soap and water.

### **Specimen Collection and Testing**

Service personnel will be responsible for sample collection, preservation and delivery for testing. Whenever possible, whole birds will be shipped FedEx or driven directly to Colorado Division of Wildlife Wildlife Health Laboratory (CDOW WHL) in Ft. Collins for necropsy and sample preparation. Testing will be done by Colorado State University (CSU) free of charge. In the event a dead bird is found in advanced stages of decomposition, samples may be taken of trachea and cloacal openings using a sterile Dacron swabs preserved in either Viral Transport Medium (VTM) or brain-heart infusion broth (BHI). Samples must include an attached information sheet with species, sex, location and date of collection when sent for testing. If a sample cannot be shipped or delivered immediately, whole birds may be frozen until ready for transport.

In the event a large scale die-off occurs, CDOW WHL personnel will come on site for specimen collection and assistance. In addition, local and state health departments will be contacted for possible quarantine. Complete protocols from the 2006 Interagency Plan for sample collection, preservation and shipping are listed in Appendix B.

## **DISEASE OUTBREAK RESPONSE**

### **Public Awareness**

Rocky Mountain Arsenal is bordered on the south by Montebello, on the west, north, northwest, and northeast by residential and commercial areas of Commerce City, and on the southeast by housing/commercial developments in the city and county of Denver. Therefore the Service has a responsibility to keep the public informed on issues concerning public safety and perception. Questions concerning HPAI and RMA can be handled directly by Tri-County Health Department or by updated notices on the RMA website. In the event human testing and quarantine are necessary, it will be handled by Colorado Department of Public Health and Environment (CDPHE).

### **Public Use**

RMA NWR hosts several public programs including fishing and nature walks, which could potentially bring visitors into contact with sick or dead birds. Therefore, it will be necessary to instruct visitors to report sick or dead birds to Service personnel immediately, avoiding any contact with the bird themselves. Additionally, the public will be instructed to view wildlife from a distance, avoiding any contact with birds, droppings or feathers while visiting the Refuge. In the event a bird, staff member, volunteer or member of the public is suspected of being infected with HPAI while at the Refuge,

public health officials will be notified and all visitor programs subject to temporary suspension. In cases involving a single bird or where potential dispersal is low, visitor access programs may continue without interruption. This decision will be made by Refuge management on a case by case basis following consultation with public health officials.

### **Contact Information**

General questions and concerns related to HPAI can be addressed by Laura Dippold and Tom Butts of Tri-County Health Department. In the event a sick bird is encountered, Dr. Laurie Baeten of the CDOW WHL in Ft. Collins will be the wildlife State contact and in charge of testing. Should staff, volunteers or the general public become exposed to HPAI while at the Refuge, John Poppy of CDPHE will be contacted for possible quarantine. Complete contact information is listed in Appendix C.

## **DATA MANAGEMENT**

A record will be kept of each bird submitted for testing as well as the results of the test. Records will be stored in electronic format using an excel spreadsheet. Each record will include location, species, sex, cause of death, test results, date, time of day, and a category for comments and observations.

## **REFERENCES**

1. Department of Health and Human Services. Center for Disease Control. Avian Influenza. [www.cdc.gov/flu/avian/](http://www.cdc.gov/flu/avian/) 2006
2. Department of the Interior. An early Detection System for Highly Pathogenic H5N1 Avian Influenza in Wild Migratory Birds. U.S. Agency Interagency Plan. [www.doi.gov/issues/birdflu\\_strategicplan.pdf](http://www.doi.gov/issues/birdflu_strategicplan.pdf) 2006
3. Rocky Mountain Arsenal National Wildlife Refuge. Comprehensive Management Plan. U.S. Fish and Wildlife Service. 1996

## APPENDIX A: Tables

Table 1. Number of different species of waterfowl recorded at RMA NWR during 2005-2006 surveys

Date	Mary	Ladora	L. Derby	Parkfield	Havana
11/2/2005	2	11	14	5	7
11/21/2005	0	10	14	1	8
12/5/2005	6	11	11	0	0
12/27/2005	1	7	15	0	0
1/9/2006	0	12	13	2	6
1/23/2006	0	13	2	0	0
2/6/2006	0	9	14	1	0
2/21/2006	0	11	0	0	0
3/6/2006	2	12	15	6	5
3/22/2006	4	11	13	0	3
4/3/2006	1	9	14	9	3

Table 2. Total numbers of waterfowl recorded for each waterbody at RMA NWR during 2005-2006 surveys

Date	Mary	Ladora	L. Derby	Parkfield	Havana	Total
11/2/2005	3	809	760	113	137	1822
11/21/2005	0	960	6580	1	159	7700
12/5/2005	194	486	242	0	0	922
12/27/2005	2	23	275	0	0	300
1/9/2006	0	150	253	14	68	485
1/23/2006	0	1069	11	0	0	1080
2/6/2006	0	231	496	13	0	740
2/21/2006	0	518	0	0	0	518
3/6/2006	5	243	395	43	176	862
3/22/2006	11	403	1125	0	11	1550
4/3/2006	8	242	1309	45	33	1637

## APPENDIX B: PROTOCOLS

### **Protocol for the Collection of Tracheal and Cloacal Swab Samples**

1. Contact Laboratory to determine specific protocol to use. Laboratories may request samples be placed in tubes containing Viral Transport Medium (VTM) or brain-heart infusion broth (BHI).
2. Thaw appropriate number of pre-labeled tubes of Viral Transport Medium (VTM) or brain-heart infusion broth (BHI) at refrigerator temperature (4 °C) overnight and keep chilled with wet/blue ice packs in a cooler during the day of collection.
3. Unwrap a Dacron swab from the stem-end of the packaging.
4. Remove swab and insert the entire head of the swab into the trachea or cloaca. Use gentle pressure and in a circular motion, swab the inside circumference of the trachea/cloaca two or three times.
5. For Cloacal swabs, shake off large pieces of feces.
6. Inserting the swab into the tube containing VTM or BHI broth. With the swab in the media, swirl the stem end of the swab between fingers vigorously. Lift the swab approximately ¼” from the bottom of the vial and bend the stem over the edge of the vial to break off the stem so that the swab remains in the vial and the cap can be screwed tight. The entire swab end and a portion of the stem will be left in the tube.  
If the stems are unable to be broken (some small swabs will have metal stems) then they can be cut with scissors. Scissors should be wiped with 70% alcohol each time they are used to cut a stem.
7. Record sample tube number on banding sheet or the Sample History Sheet along with date, species, age, sex, and location data (GIS coordinates if possible).
8. Replace tube into cooler for transport back to the base camp. Samples should be kept cold (<4°C, frozen if possible) and out of direct sunlight.
9. At camp, transfer tubes into liquid nitrogen shippers or into a freezer as soon as possible. Note any exceptions to the collection or storage conditions in field sheets and note such information on the "Sample History and Packing List Form".
10. Place tubes into a hard plastic shipping container with enough frozen gel packs to keep samples cold for at least two days.
11. Notify Laboratory that samples are being shipped



# **Protocol for Fecal Sampling and Shipping Protocol**

## **Fecal Sampling Purpose**

The purpose of this standard operating procedure is to describe the essential elements of proper handling and collection of field fecal samples for surveillance of avian influenza.

## **Procedures**

1. Before collecting samples, personnel should don the appropriate personal protective equipment (PPE). These include latex or nitrile gloves and face shields, and if it is determined to be necessary, PAPRs.
2. Label plastic whirl pack bags with necessary information including date, location (GPS coordinate if possible), species (if possible), investigator and sample identification. Record all required information on data sheets.
3. Feces must be less than 24 hours old. Feces should appear moist.
4. For collection, turn a sterile Whirl-Pak inside out and pick up feces using the Whirl-Pak as a glove, then turn the bag right side in with the feces inside the closed bag. Release as much air from the inside of the bag.
5. Label the Whirl-Pak using an indelible ink marker. The sample should be labeled with the sample number, date, time, collector's name, location, and Quality Assurance number (Protocol Number). This latter information describes in detail the context, purpose, and other procedural and review information of the samples collected.
6. Place the Whirl-Paks with fecal samples into a large zip-lock bag, tape (e.g., duct tape or packaging tape) the opening and label the outer bulk bag with name, date, location, and Protocol number.
7. Place the bulk collection bag in a cooler with wet or blue ice to keep the specimen cool. This precaution is for maximizing the chances for subsequent viral isolation. Pack samples with enough ice or frozen gel packs to keep samples cold for at least two days.
8. Maintain the temperature of samples as constant as possible.
9. Change gloves if soiled or contaminated. When finished collecting, wash hands with suitable antibacterial agent.
10. Notify laboratory that samples are being shipped, the method of shipment (FEDEX is preferred), and the expect date of arrival. Packages should only be shipped on Monday, Tuesday, or Wednesday; this allows the laboratories time to

process samples during a normal work-week, or allows for tracking if the shipment is delayed.

### **Shipping Purpose**

The purpose of this standard operating procedure is to ensure diagnostic specimens are shipped safely and in compliance with governing regulations and requirements. Shippers of diagnostic specimens where a relatively low probability exists that infectious substances are present (diagnostic specimens being transported to undergo routine screening tests or for the purpose of initial diagnosis may be considered to fall under this category) must comply with the International Air Transportation Association (IATA) Dangerous Goods Regulations. The shipper must also ensure that shipments are prepared in such a manner that they arrive at their destination in good condition and that they present no hazard to persons or animals during shipment.

### **Procedures:**

1. Federal Express is the preferred carrier for the USDA/NWRC. Use next day service.
2. The inner packaging (appropriately labeled) must be comprised of a watertight primary receptacle, and must not exceed 500 ml total volume. Primary receptacles include those of glass, metal, or plastic (i.e., test tube, plastic jar, or taped zip-loc bag). Positive means of ensuring a leak-proof seal must be provided. Screw caps on primary receptacles must be reinforced with adhesive tape.
3. A secondary packaging (also water tight) must be used, but must not exceed 4 L total volume.
4. An absorbent material sufficient to absorb the entire contents of all primary receptacles must be placed between the primary receptacle and the secondary packaging.
5. The outer packaging (i.e., cardboard box or cooler) must be of adequate strength for its capacity, weight, and intended use (capable to withstand being dropped at least 1.2 meters, without leakage of the primary receptacle or significant damage to the outer packaging). 3/14/06 69
6. An itemized list of contents must be enclosed between the secondary packaging and the outer packaging. To protect against possible leakage, the list should be enclosed in a sealed plastic bag.
7. A Shippers Declaration for Dangerous Goods is not required. However, both the air waybill and the outer box must show the text "**DIAGNOSTIC SPECIMEN PACKED IN COMPLIANCE WITH IATA PACKING INSTRUCTION 650**".

# Instructions for Collection and Shipment of Avian Carcasses for Diagnostic Evaluation

The following are general guidelines for collecting and shipping wildlife carcasses to veterinary diagnostic labs to insure adequate and well preserved specimens. Field biologists should contact the specific laboratory that they will be working with well in advance of any specimen collection and shipping to receive specific instruction for specimen submissions to that lab. Labs should always be notified ahead of time when a shipment is being made to their facility. Once you have determined what equipment and supplies will be needed for specimen shipping, keep adequate numbers of shipping containers, frozen ice packs, shipping labels and packing materials available at all times. If you plan to collect animals while in the field, take along a cooler with ice packs to chill the carcasses.

1. More than one disease may be affecting the population simultaneously. Different species may have varying susceptibility to disease agents. Therefore, collect and ship specimens representative of all species and geographic areas affected. Obtain good specimens for necropsy. Carcasses that are decomposed or scavenged are unacceptable. If the carcass has an odor, is soft and mushy, has skin discoloration, feathers or skin that easily rubs off, or has maggots present, it is too decomposed for testing.

2. Collect animals under the assumption that an infectious disease or toxic substance is involved and other animals or humans may be at risk. Remember to protect yourself as some of these diseases and toxins are hazardous to humans. Guidelines for personal protection against disease exposure for individuals working with sick or dead wild animals can be obtained from the USGS National Wildlife Health Center, the Centers for Disease Control and Prevention, and OSHA websites. Always wear latex or nitrile gloves when picking up sick or dead animals. If you are dealing with a significant number of dead animals, or you suspect the presence of a zoonotic disease agent, additional protective equipment including coveralls, eye protection and N95 respiratory protection should be used. Attach a leg tag to each animal with the following information in pencil/waterproof ink:

**Species**

**Date collected**

**Location** (state, county, location name, and latitude/longitude if available)

**Found dead or euthanized**

**Collector** (name/address/phone)

**Additional history or comments on back of tag**

Place each animal in a plastic bag, tie shut, then place inside a second bag and tie shut. This system of double bagging prevents cross-contamination of individual specimens and leaking shipping containers that can contaminate vehicle surfaces and handlers during

transportation. Contact the diagnostic lab for guidance in assistance with collecting samples from animals that are too large to ship.

3. Ship animals in a sturdy hard sided plastic cooler. These coolers can be disinfected and returned to you if a pre-paid shipping label or commercial shipping company account number is provided to the diagnostic lab. Be sure to provide a street address for return of the cooler. Line the shipping cooler with a large plastic bag and pack the individually bagged animal(s) in the cooler with enough blue ice to keep carcasses cold. Disperse blue ice packs among the carcasses so that all carcasses are kept chilled. If you are shipping blood tubes, culture tubes, or other specimen containers along with the carcasses, these specimens should be placed within a sturdy cardboard or plastic box or screw cap container with padding material to prevent breakage. That container should be placed next to blue ice packs within the large cooler. Do not use bagged wet ice for shipments in order to avoid fluid leakage during shipment. Do not use dry ice unless instructed to do so by the diagnostic lab. Place crumpled newspaper or similar absorbent material in the cooler with the bagged carcasses to fill unused space, hold the ice in contact with carcasses, provide insulation, and absorb any liquids. Tape the cooler shut with sturdy strapping tape. Place a detailed history of the animal and circumstances associated with the mortality event in a paper envelope or a plastic sleeve and tape it to the outside of the cooler. A copy of this history should be faxed or e-mailed to the diagnostic lab at the time of shipment. A standard wildlife specimen history form can be found on the last page of these instructions.

4. Prior to shipping contact the diagnostic lab to inform them of the type and number of specimens being shipped. Ship specimens for next day delivery (overnight service) from Monday through Wednesday to guarantee arrival at the diagnostic lab before the weekend. If specimens are fresh and need to be shipped on Thursday or Friday contact the diagnostic lab to make special arrangements for receipt of specimens. Freezing and thawing can make isolation of some pathogens difficult and damage tissues needed for microscopic examination. Diagnostic labs prefer unfrozen specimens if 3/14/06 63 they can be sent within 24 – 48 hours of collection or death. The diagnostic lab can provide guidance on when or if to freeze samples on a case-by-case basis. If you are in the field and cannot call or ship within 24-48 hours, freeze the animal(s).

5. Prior to shipping contact the commercial shipping company to obtain guidelines for shipping diagnostic or biological specimens. Label coolers with clear, legible labels including the diagnostic lab name, street address, and telephone number. In addition to the mailing address, attach a label reading “**DIAGNOSTIC SPECIMENS – WILDLIFE**” to the side of the cooler. If dry ice was used in the shipment a standard dry ice warning label will be required. These can be obtained from the shipping company. Please make note of the tracking number in case packages are delayed.

## APPENDIX C: Contact List

**RMA Points of Contact:** Sherry Skipper and Judson Spicer

**Phone:** 303-289-0925 (ext. 5925) 303-289-0572 (ext.5572)

**Building:** 121, office # 132 & 133

**Local Contact:** Laura Dippold and Tom Butts

**Agency:** Tri-County Health Department

**Office:** 4201 East 72<sup>nd</sup>, Suite D, Commerce City

**Phone:** 303-783-7134 and 303-846-6228

**State Contact for Human Health:** John Poppy

**Agency:** Colorado Dept. of Public Health and Environment

**Section:** Disease Control and Environmental Epidemiology

**Office:** 4300 Cherry Creek Drive South, Denver, Colorado 80246-1530

**Phone:** 303-692-2700

**State Contact for Avian Testing:** Dr. Laurie Baeten

**Agency:** Colorado Division of Wildlife

**Section:** Wildlife Health Laboratory

**Office:** Wildlife Research Center, 317 W. Prospect Road, Ft. Collins, CO

**Phone:** 970-416-1516