CIDRAP

Center for Infectious Disease Research & Policy

University of Minnesota

MCEIRS AVIAN INFLUENZA TRAINING

Individual Study Guide

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Avian Influenza: Detection in Wild Birds - Field Collection of Samples

Minnesota Center of Excellence for Influenza Research and Surveillance

CONTENTS

INTRODUCTION

Avian Influenza: Detection in Wild Birds – Field Collection of Samples

In this module, the techniques for collecting samples for the detection of avian influenza viruses in wild bird populations will be demonstrated. The selection and use of personal protective equipment, and handling and shipment of samples are among the topics addressed. The role of wild birds in the maintenance and spread of these viruses and the importance of monitoring wild birds for the presence of avian influenza viruses will also be discussed.

LESSON 1: OVERVIEW OF INFLUENZA VIRUSES

In this lesson, we will cover:

- Influenza A Virus
- Avian Influenza Classification
- Avian Influenza in Wild Birds
- Wild Birds as Reservoirs for Avian Influenza
- HPAI Viruses: Impact on Wild Birds

INFLUENZA A VIRUS

Avian influenza is caused by influenza A viruses. Influenza A viruses also are the main cause of influenza in humans and other mammalian species.

Influenza A virus subtypes (shown in Figure 1) are defined by two of the surface proteins that are part of the structure of the virus:

- HA - Hemagglutinin
- NA – Neuraminidase

There are 18 different HA antigens (H1 to H18) and 11 different NA antigens (N1 to N11) for influenza A. These antigens give rise to the subtype designation. Subtypes H1 to H16 and N1 to N9 are found in birds (mostly wild birds) and some of these subtypes have been found in mammals. H17N10 and H18N11 were discovered in bats in Guatemala in 2009 and in Peru in 2013, respectively. The NA genes in these influenza subtypes are highly divergent from other known influenza NAs and researchers propose that the attachment and activation of these viruses occur by a different mechanism than other influenza viruses. As of January 2017, these two subtypes appear to be unique to the bat population but have been shown to infect and replicate in other mammalian cells (such as canine cell lines).

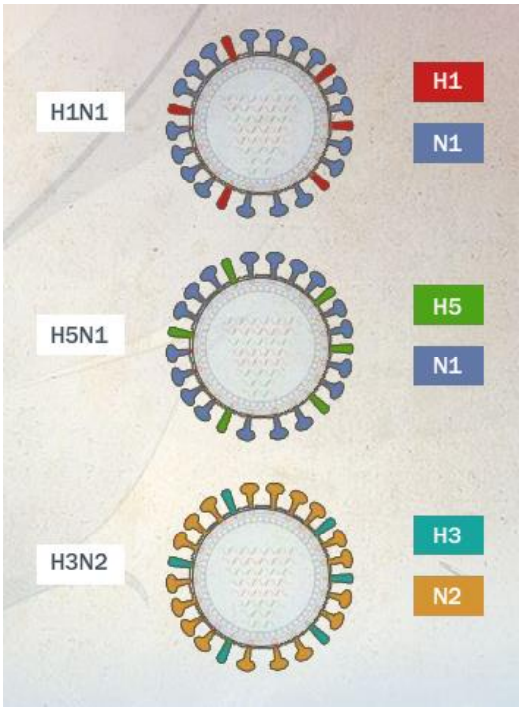

Figure 1: Viral Subtypes

Influenza viruses can mutate or re-assort to form new viruses. Sometimes the mutation or reassortment can result in a virus that is capable of infecting another species or can affect the virulence (severity) of the virus.

Current information suggests that the Influenza A virus can survive on environmental surfaces for 6 days or less. However, influenza viruses can survive in cool, moist, environments or in water for days to months.

AVIAN INFLUENZA CLASSIFICATION

Influenza A viruses can infect a variety of domestic and wild bird species. Infection can range from asymptomatic to severe, depending on the virulence of the virus, environmental factors and the susceptibility of the host.

Avian influenza (AI) viruses are classified according to the disease severity in domestic chickens, with two recognized forms: Highly Pathogenic Avian Influenza (HPAI) and Low Pathogenicity Avian Influenza (LPAI).

HPAI

Highly pathogenic avian influenza (HPAI) (previously known as fowl plague) is rare. It causes severe disease in domestic poultry and can cause mortality rates of up to 100% in affected flocks. Only certain subtypes of H5 (such as H5N1) and H7 (Such as H7N7) have been associated with HPAI. (These subtypes have also been associated with LPAI).

LPAI

Low pathogenicity avian influenza occurs more frequently than HPAI. It can cause mild upper respiratory symptoms in domestic poultry and does not typically cause death in affected poultry.

AVIAN INFLUENZA IN WILD BIRDS

H1 through H16 and N1 to N9 subtypes have been isolated from wild birds and certain groups of wild birds are the primary reservoirs for AI viruses. AI viruses have been isolated from more than 100 wild bird species representing 26 families of birds.

The most important of these are:

Anseiformes - ducks, geese and swans

Charadriformes - gulls, terns and waders

AI viruses do not generally cause disease in wild birds, but can be shed into the environment and passed from bird to bird.

WILD BIRDS AS RESERVOIRS FOR AVIAN INFLUENZA

There are several factors that affect the role of wetland species in the maintenance and possible spread of AI viruses to wild and domestic bird populations.

The migration behavior and patterns of some birds allow the virus to potentially be distributed to different areas.

Flyways are defined as "the entire range of a migratory bird species... through which it moves on an annual basis from the breeding grounds to the non-breeding areas, including intermediate resting and feeding places..."

The major global flyways overlap and thus different bird populations have opportunities to interface with each other, which can lead to further spread of AI viruses.

The social nature, or gregariousness, of some bird species influences the transmission of viruses.

Some species have adapted to humans and thus are more likely to come into contact with domesticated poultry and other domestic species.

AI viruses can survive in the water for days to weeks and thus can be spread from bird to bird in wetland environments.

HPAI VIRUSES: IMPACT ON WILD BIRDS

Historically, wild birds have remained relatively asymptomatic when infected with AI viruses. Before 2002 only one outbreak of HPAI in wild birds had been reported. That changed with HPAI H5N1.

- Since 2002, HPAI H5N1 has caused outbreaks in wild bird populations. Outbreaks in wild birds initially were recognized in Asia. Subsequently, wild bird deaths from HPAI H5N1 have been noted in other regions of the world, including Europe. The strain of highly pathogenic H5N1circulating in Asia has not been found in the Western Hemisphere; however, a novel reassortant H5N1 AI virus was found in a wild bird (green-winged teal) in Washington State in January 2015.

From 2012-14, several other HPAI H5 reassortant viruses derived from the H5N1 virus were detected in wild and domestic birds in Asia and Europe. A novel H5N8 virus appeared to have spread from overwintering grounds in Northern Asia/Alaska into China, South Korea, Japan, and Europe. In late 2014, the H5N8 HPAI virus that had been circulating in Europe and Asia was detected in the Pacific flyway. This virus then

mixed with other North American lineage viruses resulting in reassortant H5N2 and H5N1 viruses.

From December 2014 – December 2015, there were 100 detections of Eurasian HPAI H5 viruses in wild birds in the Pacific, Central and Mississippi flyways in the United States; 32 of these were due to wild bird mortality events. In addition, HPAI H5 viruses caused the death of nine captive wild birds including gyrfalcons, other falcons, and a great horned owl. In the spring of 2015, the HPAI H5N2 reassortant virus caused 219 cases of HPAI in domestic poultry in the United States, which resulted in the death or destruction of over 48 million birds.

Throughout the world HPAI H5 viruses have caused die offs (mortality events) in populations of birds in both captivity and in the wild. Some of these events have occurred in areas where outbreaks were seen in domestic poultry, but other outbreaks have occurred in areas where only wild species were affected.

During the winter of 2016-17, an H5N8 virus caused die- offs in wild birds including waterfowl, gulls, and raptors throughout Europe, the Middle East, Asia, and Africa. At least 4 other HPAI H5 subtypes, including H5N6 and H5N5, were also detected in wild birds in 2016.

Although it is generally agreed that wild birds are the major reservoirs of LPAI viruses, their role in the maintenance and spread of the HPAI viruses is less clear.

LESSON 2: SURVEILLANCE FOR AI IN WILD BIRDS

In this lesson, we will cover:

- Definition of Surveillance
- Goals of Avian Influenza Surveillance
- Methods of Surveillance
- US Surveillance Program
- Global Surveillance Efforts

DEFINITION OF SURVEILLANCE

Surveillance (for a disease) is defined as the ongoing systematic collection and analysis of data and the provision of information which leads to action being taken to prevent and control a disease.

Surveillance for AI involves taking samples from birds and sending them to a diagnostic laboratory to be tested for AI viruses.

GOALS OF AVIAN INFLUENZA SURVEILLANCE

The objectives of conducting surveillance for avian influenza can vary. The approaches differ when conducting surveillance for LPAI viruses versus surveillance programs for specific viruses such as HPAI H5 viruses.

Goals of surveillance for LPAI viruses include:

- To increase the understanding of the ecology, behavior, transmission, and spread of influenza viruses in wild bird populations
- To contribute to the development of prevention and control strategies for avian influenza viruses in domestic poultry and mammalian species

Goals of surveillance for HPAI include:

- To determine the susceptibility, clinical features, and viral shedding of HPAI viruses in different wild avian species
- To determine the role of migratory birds in the spread of HPAI viruses
- To monitor the spread of HPAI viruses into unaffected areas
- To inform decisions on biosecurity practices of domestic poultry operations

METHODS OF SURVEILLANCE

LPAI

Apparently healthy birds are important to the spread of AI viruses.

Since AI viruses are shed in the feces, cloacal samples are an important means of detecting the viruses.

Sentinel birds are tested for exposure to or infection with the virus after a period of contact with wild birds, when they are likely to be shedding virus. This approach is not often used. Examples of sentinel birds include:

- Captive ducks that are allowed to intermingle with wild ducks
- Existing domestic poultry flocks that are located near, and interact with, target species of migratory waterfowl or shore birds
- Mortality investigation in raptors or wild gallinaceous birds

Fecal samples can be tested for AI viruses. Samples from multiple birds can be pooled.

Since the feces from infected birds may be shed into the water, water samples have also been used to look for AI viruses, although standard protocols have not been validated.

These methods will test for the presence of virus but will not provide information on the number or percentage of birds that are infected.

HPAI

Live Bird Surveillance

Some AI viruses, especially the HPAI H5N1 virus are also shed in respiratory secretions, so tracheal or oral-pharyngeal samples may be taken in addition to cloacal samples.

Species likely targeted for HPAI surveillance include birds:

- With known exposure to HPAI viruses
- That migrate in or through areas where HPAI viruses have been detected
- That are in direct contact with exposed birds or birds that migrate in or through areas where HPAI viruses have been detected
- That share the same environment with exposed or potentially exposed birds
- That could potentially come into contact with or share the same environment with domestic poultry

- That are known reservoirs of AI viruses
- From which field staff can obtain specimens relatively easily

Mortality Investigations

Wild bird die offs can be an important means of identifying HPAI viruses in areas where it has not yet been detected. For example, certain species of wild birds have proven to be particularly susceptible to the H5N1 virus.

While most affected birds are just found dead, the disease may also cause neurological signs in wild birds such as swimming in circles, incoordination, or a head tilt.

Wildlife agencies, park, or refuge staff or members of the general public may be the first to notice a die off of wild birds. Such instances should be promptly reported and investigated.

Hunter killed birds

This method provides another opportunity to collect samples from apparently healthy birds that may be a source of AI viruses. Sampling is conducted on high-risk species.

Sentinel surveillance and environmental sampling may also be done for HPAI viruses.

US SURVEILLANCE PROGRAM

The U.S. Interagency Strategic Plan

The United States developed a plan in 2006 that was designed to detect the occurrence of HPAI H5N1 in wild birds. It was titled: An Early Detection System for Highly Pathogenic H5N1 Avian Influenza in Wild Migratory Birds: U.S. Interagency Strategic Plan. This plan functioned as an early warning system, if the virus were to be introduced into the United States.

Participants from various agencies were involved in the development of this plan (United States Department of Agriculture, Department of the Interior, Health and Human Services, Association of Fish and Wildlife Agencies, State of Alaska).

The surveillance plan for HPAI H5N1 described in this document ended in 2011. Over 500,000 birds were tested from 2006 to 2011 and no Eurasian H5 viruses were detected. After the detection of HPAI viruses in domestic poultry in Canadian flocks in 2014 surveillance for other HPAI viruses was increased in the Pacific Northwest region of the United States. There were 98 detections of H5 viruses in wild birds from December 2014 to June 2015.

The U.S. Interagency Strategic Plan

After the H5N2 outbreak in the Midwest United States, the Interagency Strategic Plan was updated. Ninety-five critical watersheds where ducks from different flyways intermingle and/or where AI viruses have previously been identified were targeted for surveillance in the US from July 2015 through June 2016. Of 45,000 samples collected, two Eurasian H5 viruses were detected in mallard ducks in Utah and Oregon.

The surveillance goal for July 2016 through June 2017 is 30,000 samples. As of January 2017, H5N2 viruses have been detected in mallard ducks in Alaska and Montana.

GLOBAL SURVEILLANCE EFFORTS

The Food and Agriculture Organization of the United Nations (FAO) has a system called EMPRES-i that compiles, stores and verifies animal disease outbreak data including HPAI.

The website is: http://empres-i.fao.org/empres-i/home

LESSON 3: METHODS OF CAPTURE

In this lesson, we will cover:

- General Considerations
- Rocket Netting (Cannon Netting)
- Baited Traps
- Drive Trapping
- Other Methods of Capture

GENERAL CONSIDERATIONS

There are various methods for capturing waterfowl that are targeted for sampling. Several of the common methods used for AI surveillance are described in this module. The capture method chosen will depend on the species targeted, the time of year, and the availability of equipment and of personnel. Most capture methods described should be conducted by personnel who are experienced or have been well trained in the field.

Safety of the personnel conducting the trapping and the welfare of the animals being captured is of top priority.

All necessary permits and licenses should be obtained before beginning any capture and sampling process.

ROCKET NETTING (CANNON NETTING)

Rocket netting involves the use of explosives to project a net over a group of birds. The area in front of the net is baited. When the birds approach the feed the rockets carrying the net are fired over the birds and the birds are trapped in the net.

The birds are carefully removed from the net and placed in cages for handling.

Rocket netting requires a great deal of expertise and in some areas a license is required.

BAITED TRAPS

Several types of baited traps can be used to capture water birds. The traps are usually baited with grain. Birds swim in through a funnel entrance to the trap and then are unable to easily get out. For diving ducks, the birds enter the trap by diving for the bait and emerging into the trap.

When baited traps are unattended, the traps should be monitored on a regular basis to ensure the birds have not become injured trying to escape or have become vulnerable to predators.

DRIVE TRAPPING

Drive trapping is done during a time period when waterfowl are molting and unable to fly. The birds are essentially "herded" by persons in boats or in the water, to a trap that funnels the birds into a holding area.

OTHER METHODS OF CAPTURE

Night lighting is a method of capturing birds that involves temporarily blinding the birds at night with a bright light.

Mist netting is usually used for smaller birds and involves a thin vertical net that is set up where birds will fly into it and become entangled. The birds are carefully removed from the net and sampled.

LESSON 4: HANDLING WILD BIRDS

In this lesson, we will cover:

- Handling Birds at the Capture Site
- Restraining Birds
- Identification, Banding, and Measurement
- Personal Protective Equipment (PPE)
- Additional Precautions When Handling Wild Birds

HANDLING BIRDS AT THE CAPTURE SITE

Captured birds should be handled carefully so as not to injure the handler or the bird. Depending on the species, the wings, legs, and head should be appropriately restrained.

Trapped birds should be carefully removed from the net or enclosure. A calm quiet but efficient approach is the most effective method.

Birds should be kept warm if the environment is cold and cool if the environment is hot.

Birds should be held in cages and handled for as little time as possible and released back to their original environment as soon as feasible.

A contingency plan should be in place in the event a bird is injured.

RESTRAINING BIRDS

The bird should be held firmly so it cannot struggle, but not so tight as to restrict its breathing.

Covering the birds head with a cloth or even its wing will help keep the bird calm.

Small birds can be held in one hand with the head of the bird between the index and middle finger and the wings held to the chest with the thumb and other fingers.

Medium sized birds, such as ducks, can be held with two hands, holding the wings against the body. The bird can also be held upside down in the handlers lap for short periods, for sampling.

Large birds should be held by experienced personnel. They may require two handlers or the use of physical or chemical restraints.

IDENTIFICATION, BANDING, AND MEASUREMENT

Once captured, the bird's species, approximate age, and sex are often determined and recorded.

If the bird already has a band or other identification, this should be recorded as well.

Captured birds are often banded. In the U.S. and other countries a federal banding permit is required to band birds. The bands are often made of aluminum and have a unique number as well as contact information stamped into the metal. They come in different sizes and are put around the bird's leg.

Other physiologic measurements may also be taken. The bird's weight, wing length, or other measurements may be taken. During AI surveillance, these measurements may be useful in determining the effects of a virus that might not be otherwise evident.

PERSONAL PROTECTIVE EQUIPMENT (PPE)

Personal protective equipment (PPE) should be worn when handling live or dead birds and taking samples for AI surveillance. The amount and type of PPE that is worn is dependent on the risk of being exposed to a disease that the bird may be carrying.

When conducting surveillance for an AI virus that has been shown to infect humans handlers should be protected. Although there is only one documented instance of a person contracting the Eurasian H5N1 HPAI virus from a wild bird, because of the severity of the virus, protection is important.

PPE can also help to prevent the spread of viruses between birds or to other sites where birds live.

PPE for Lower Risk Situations

In areas where zoonotic AI viruses have not yet been found, the situation may be considered lower risk. At a minimum PPE should include clean or disposable coveralls, boots that can be cleaned, and disposable gloves. This includes working with dead birds in low-risk areas. Goggles or other face coverings and N95 masks are recommended by the CDC especially if the work area is dusty or heavily contaminated with feces.

PPE in Outbreak Situations

In outbreak situations, in areas where zoonotic AI viruses have

been found, or when investigating any mortality events or die offs in these areas, more extensive PPE is recommended. This would include full coveralls, boots, or boot covers

that can be easily cleaned or disposed of, gloves, eye protection such as goggles, and a respirator such as an N95 face fitting particulate respirator.

N95 respirators need to be fitted by a specialist in order for the respirator to be effective. These respirators can be difficult to breathe in and a medical exam may be necessary before use. N95 respirators may not be able to be fitted to persons with facial hair or other limiting factors. In those situations another type of respirator such as a powered air purifying respirator may be needed.

Persons with known exposure to infected birds should monitor their health for one week after the last contact. If flu-like symptoms or conjunctivitis (eye redness or infection) occurs, a healthcare provider should be immediately contacted. The healthcare provider should be told that exposure to AI has occurred.

Follow all other recommendations from healthcare providers or public health personnel in areas where H5N1 or any other HPAI has been found.

ADDITIONAL PRECAUTIONS WHEN HANDLING WILD BIRDS

Do not handle dead or obviously sick birds without the more extensive PPE.

Do not eat, drink, or smoke while handling birds.

Avoid touching the face with gloved hands or sticking equipment in the mouth.

Wash hands well with soap and water before eating, drinking, smoking, or handling food.

Clean and disinfect the work area when finished as well as any tools or equipment used.

Bag disposable items and dispose of properly.

LESSON 5: SAMPLE COLLECTION

In this lesson, we will cover:

- General Comments
- Equipment Needed
- Cloacal Swabs
- Tracheal/Oral-Pharyngeal Swabs
- Hunter Harvest
- Mortality Investigations
- Environmental Sampling: fecal sampling, water samples

GENERAL COMMENTS

Samples taken as part of surveillance for AI include cloacal swabs and tracheal or oral-pharyngeal swabs. The swabs must be performed correctly to get a useful sample. The swabs must be stored and shipped correctly to the laboratory that will be doing the testing.

Preventing contamination of the samples is also important. The swabs must be kept sterile before the sample is taken and should not become otherwise contaminated before being placed in the vial and sealed.

Tests to look for AI viruses include reverse transcription polymerase chain reaction (RT-PCR) and virus isolation.

EQUIPMENT NEEDED

The specific equipment used to collect the samples is important. Sterile Dacron or rayon tipped swabs should be used. Cotton tipped or wood stemmed swabs should NOT be used.

The vials used to transport the samples should be appropriate to the temperatures, and the method of storage and transport that will be used (i.e., dry ice, liquid nitrogen). The vials should contain a viral transport medium (VTM) as specified by the diagnostic laboratory.

Cold packs and a cooler can be used to keep the VTM cool but not frozen before use and after the swab has been placed into the vial in the field.

Examples of other equipment that may be needed include appropriate PPE, trash bags for waste disposal, equipment for taking physiological measurements, and record keeping materials.

CLOACAL SWABS

To take a cloacal swab, first gently locate the opening to the cloaca.

Unwrap the sterile swab from the stem end, making sure not to touch the tip to any other surface.

Insert the swab into the cloaca. Swab the cloaca in a circular motion two to four times using gentle pressure.

Remove the swab, again being careful not to touch the tip to any other surface.

Insert the swab into the vial (some experts also recommend swirling it in the media).

With the swab in the vial, bend and break the stem off at the top of the vial.

Leaving the remaining swab in the vial, close the vial tightly, ensure it is properly labeled, and store until transport.

TRACHEAL/ORAL-PHARYNGEALSWABS

To take an oral-pharyngeal swab, grasping either side of the bill from the top, open the birds mouth using a firm but not too tight grip.

Unwrap the sterile swab from the stem end, making sure not to touch the tip to any other surface.

Swab the oral-pharyngeal region in a circular motion, being sure to swab along both sides at the base of the tongue, behind the tongue, and the roof of the mouth behind the choanal cleft.

Remove the swab, again being careful not to touch the tip to any other surface.

Insert the swab into the vial (some experts also recommend swirling it in the media).

With the swab in the vial, bend and break the stem off at the top of the vial.

Leaving the remaining swab in the vial, close the vial tightly, ensure it is properly labeled, and store until transport.

HUNTER HARVEST

Sampling as part of AI surveillance is also conducted on birds that have been killed by hunters. Cloacal and oral-pharyngeal samples are taken in the same manner as they are taken in live birds.

MORTALITY INVESTIGATIONS

- When die-offs of wild birds occur, samples from the dead birds should be sent to the diagnostic laboratory for testing.
- Carcasses should be fresh, as severely decomposed samples will not yield useful results.
- Often times an entire carcass is submitted for diagnostic purposes.
- Recommendations for handling and shipment of samples should be obtained from the diagnostic laboratory.
- If tissue or other samples are taken from dead birds, field personnel should follow all recommendations for the use of personal protective equipment.

ENVIRONMENTAL SAMPLING

Fecal Sampling

- Always sample fresh (moist) feces. Dried feces will not yield good samples.
- Observe the target wild bird species in resting or feeding areas.
- Approach the area to cause dispersal of the birds and identify fresh feces.
- Using the same type of swab, roll the swab in several fecal samples.
- Insert the swab into the vial (some experts also recommend swirling it in the media).
- With the swab in the vial, bend and break the stem off at the top of the vial.
- Leaving the remaining swab in the vial, close the vial tightly, ensure it is properly labeled, and store until transport.

Water Samples

- Undiluted water samples from water environments with dense populations of target species can be tested for AI virus.
- Collect samples in a sterile container and seal tightly. Keep cold. Do not freeze samples.
- Contact your diagnostic laboratory for further handling instructions.

LESSON 6: HANDLING AND SHIPMENT OF SAMPLES

In this lesson, we will cover:

- Handling of Samples at the Site
- Labeling and Record Keeping
- Sample Storage
- Packaging and Shipment of Samples

HANDLING OF SAMPLES AT THE SITE

It is important not to break the "cold chain" when handling samples in the field.

If the VTM is frozen before use be sure to thaw the media under refrigeration.

If vials are kept in a box or rack during the collection procedure, keep the container cool i.e. set the box on ice packs. Once a box or rack of vials is filled, store the samples in a cooler with ice packs.

Samples must also not be exposed to light, which can degrade the sample.

LABELING AND RECORD KEEPING

All vials must be labeled.

At a minimum the vial should be labeled with an identification number.

The identification number can refer to a written record. This record should note the date, species of bird, sample type (oral-pharyngeal or cloacal), and any other pertinent information associated with the identification number.

All of the above information could also be noted directly on the vial.

Labels must be marked with a material that will not smear or wipe off if wet.

It is advisable to note the date of sample collection on the box so that transport to the laboratory can be done in a timely fashion

SAMPLE STORAGE

After sample collection in the field, the vials are moved from the cooler and stored under refrigeration before transport to a diagnostic laboratory.

Depending on the distance, the timing or the materials available, samples can be transported cold or frozen.

A cold shipment method should only be used if the samples will arrive at the lab within 72 hours. The samples should be kept on ice at 4 degrees C after collection and through shipment. After shipment, the samples may then be frozen at the lab if necessary.

Samples may also be shipped frozen using a liquid nitrogen container. This method allows for a longer storage and transport time. The liquid nitrogen containers can typically maintain a charge for 7-12 days per the manufacturer's specifications.

PACKAGING AND SHIPMENT OF SAMPLES

Cold

- Cold samples may be transported by hand or shipped.
- Line an insulated container (or cooler) with cold packs on all sides and place the samples securely in the middle of the container.
- Cap all vials tightly to prevent leakage during transport.
- If transporting the samples in a vehicle, keep the transport container in the coolest part of the vehicle, depending on the outside temperature.
- When shipping samples, load the samples just prior to shipment and secure the container appropriately.
- Follow all applicable shipping regulations for medical or diagnostic specimens.
- Notify the laboratory that the samples are being shipped and confirm receipt.

Frozen

- The liquid nitrogen container or dry shipper should be charged before use.
- Ensure that all the liquid has been removed from the container before loading the samples.
- Use care and appropriate PPE when opening the liquid nitrogen container.
- Wearing protective gloves, extract the vial containment canister from the unit. Load the samples into the canister and gently lower the canister back into the unit.
- Close and lock the container and arrange for shipment to the laboratory.
- Notify the laboratory that the samples are being shipped and confirm receipt.

GLOSSARY

Anseriformes - An order of birds that are highly adapted to aquatic environments; anseriformes are characterized by webbed feet. There are approximately 150 species of birds in the order, including ducks, geese, swans, and screamers.

Bird Banding - The process by which birds are 'banded' or braceleted by a small metal ring on their leg that has an identifying serial number unique in the world.

Charadriiformes - A large diverse order of aquatic birds found along sea coasts and inland waters; includes shorebirds and coastal diving birds. This order includes approximately 350 species.

Choanal Cleft - The slit in palate of the bird's mouth.

Cloaca - The excretory vent of a bird.

Cloacal Swab - Samples from the common opening in birds through which intestinal, urinary, and reproductive tracts empty.

Cold Chain - A temperature-controlled supply chain. An unbroken cold chain is an uninterrupted series of storage and distribution activities which maintain a given temperature range.

Environmental Sampling - Waterfowl release AI viruses through the intestinal tract and the virus can be detected in both feces and water in which the birds swim. Environmental sampling entails testing the feces and water for AI viruses. Analysis of both water and fecal material from waterfowl habitat can provide evidence of avian influenza circulating in wild bird populations.

Feces - One of the three components of the bird's excreta (droppings). Often brown or green and shaped like a worm.

Flyway - A major route of travel for migratory birds; a flyway may cover thousands of miles.

Hemagglutinin (HA) - An important surface structure protein of the influenza virus that is an essential gene for the spread of the virus throughout the respiratory tract. This protein enables the virus to attach itself to a cell in the respiratory system and penetrate it. It is used to name influenza A subtypes and is referred to as the "H" in the influenza virus subtype (e.g., H5N1).

Host - An organism on or in which a parasite lives.

HPAI (Highly Pathogenic form of Avian Influenza) - Often fatal in chickens and turkeys. HPAI spreads more rapidly than LPAI and has a high mortality rate in domestic birds.

Infection - Invasion of the body or a part of the body with a pathogenic organism, which multiplies in the host. A person or animal with an infection may or may not exhibit symptoms. An infection without symptoms is called asymptomatic.

Liquid Nitrogen Container - An insulated container used especially to store liquefied nitrogen gas, having a double wall with a vacuum between the walls.

LPAI (Low Pathogenic form of Avian Influenza) - Naturally occurs in wild birds and can spread to domestic birds. In wild birds, LPAI strains generally do not cause signs of infection. In domestic birds, the illness is not severe and mortality rates are low. LPAI H5 and H7 strains have the potential to mutate into HPAI and are therefore closely monitored.

Morbidity - Disease; morbidity rate is the incidence or prevalence of disease in a specific population during a specified interval of time or a specific point in time.

Mutation - Any alteration in a gene from its natural state. Specific mutations and evolution in influenza viruses cannot be predicted, making it difficult if not impossible to know if or when a virus such as H5N1 might acquire the properties needed to spread easily among humans.

Mortality - Death; mortality rate is a measure of the number of deaths in a population during a specified interval of time.

Neuraminidase (NA) - An important surface structure protein of the influenza virus that is an essential enzyme for the spread of the virus throughout the respiratory tract. This protein enables the virus to escape the host cell and infect new cells. It is used to name influenza A subtypes and is referred to as the "N" in the influenza virus subtype (e.g., H5N1).

Oral-Pharyngeal Swabs - A sample from the oral cavity and pharyngeal area of the birds mouth.

Outbreak - Presence of disease in numbers in excess of normal in a specific geographic area or population.

Pathogenic - Causing disease or capable of doing so.

Personal Protective Equipment - Any devices or clothing worn by the worker to protect against hazards in the environment. Examples are respirators, gloves, and goggles.

Pharynx - The area in the back of the birds mouth before the esophagus.

Reservoir - A person or animal that serves as a host to a pathogenic agent, generally without visible symptoms of the disease or injury.

Reverse Transcription Polymerase Chain Reaction (RT-PCR) - A laboratory technique commonly used in molecular biology to generate many copies of a DNA sequence, a process termed "amplification". In RT-PCR, however, RNA strand is first reverse transcribed into its DNA complement (complementary DNA, or cDNA) using the enzyme reverse transcriptase, and the resulting cDNA is amplified using traditional or real-time PCR.

Sample Collection - Testing for the presence of AI requires that samples be taken from live birds, dead birds or the environment birds inhabit. Samples are routinely collected from wild birds, domestic flocks, live bird markets and quarantined birds.

Sentinel Surveillance - A monitoring method that employs a surrogate indicator for a public health problem,

allowing estimation of the magnitude of the problem in the general population.

Species - A group of biological beings that are similar to each other and capable of breeding with one another. A species is a subdivision of a genus.

Surveillance - The ongoing systematic collection and analysis of data and provision of information which leads to action to control or prevent a disease.

Trachea - The hollow tube that carries the air to the posterior air sacs when a bird inhales.

Tracheal Swab - Samples from the airway in birds through which air moves from the throat to the lungs.

Virulence - A pathogen's ability to invade host tissues and the severity of disease produced.

Virulent - Highly lethal; causing severe illness or death.

Virus - Any of various simple submicroscopic parasites of plants, animals, and bacteria that often cause disease and that consist essentially of a core of RNA or DNA surrounded by a protein coat. Unable to replicate without a host cell, viruses are typically not considered living organisms.

Virus Isolation - The gold standard test used to diagnose AI virus infections. The virus is isolated in embryos inside chicken eggs. A series of tests follow to specifically identify H and N subtypes of the AI virus.

Viral Transport Media (VTM) - A microbiological medium used specifically to transport or temporarily store an organism or a material from which an organism subsequently will be isolated. The medium is intended to maintain the viability and/or infectivity of the organism during the period between collection and culture of the specimen.

Zoonoses - Diseases that transfer from animals to humans.

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