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Occurrence of Viable Avian Influenza Viruses in Water and Bed Sediments from Selected Water Bodies along the Atlantic Flyway, February and May 2006 and January 2007

Open-File Report 2009–1161

U.S. Department of the Interior
U.S. Geological Survey

Cover. *Ardea occidentalis* (great white heron), Chinoteague National Wildlife Refuge, Accomack County, Virginia (photo by Alan M. Cressler, U.S. Geological Survey).

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Contents

Figures

Tables

Conversion Factors and Horizontal Datum

Multiply	By	To obtain
	Length	
centimeter (cm)	0.3937	inch
meter (m)	3.281	foot (ft)
meter (m)	1.094	yard (yd)
	Volume	
milliliter (mL)	0.03382	once, fluid (fl. oz)
milliliter (mL)	0.000264	gallons (gal)
liter (L)	33.82	ounce, fluid (fl. oz)
liter (L)	2.113	pint (pt)
liter (L)	1.057	quart (qt)
liter (L)	0.2642	gallon (gal)
liter (L)	61.02	cubic inch (in^3)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F = (1.8 \times °C) + 32$$

Temperature in degrees Fahrenheit (°F) may be converted to degrees Celsius (°C) as follows:

$$°C = (°F - 32) / 1.8$$

Horizontal coordinate information is referenced to the North American Datum of 1983 (NAD 83).

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm at 25 °C).

Concentrations of chemical constituents in water are given in milligrams per liter (mg/L).

Occurrence of Viable Avian Influenza Viruses in Water and Bed Sediments from Selected Water Bodies along the Atlantic Flyway, February and May 2006 and January 2007

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Abstract

Water and bed-sediment samples were collected from selected water bodies along the Atlantic Flyway and analyzed for the presence of viable avian influenza viruses. Samples were collected during February and May 2006 and January 2007 at U.S. Fish and Wildlife Service National Wildlife Refuges in Georgia, South Carolina, North Carolina, Virginia, and Maryland. Avian influenza viruses were detected in samples collected from the Savannah National Wildlife Refuge in Georgia during February 2006 and from the Santee National Wildlife Refuge in South Carolina and the Pee Dee National Wildlife Refuge in North Carolina during January 2007. Avian influenza virus was detected in water temperatures ranging from 11.8 to 12.7 degrees Celsius when birds were either present or had departed at least 10 days prior to sampling. Although the literature indicates that avian influenza virus persists in the environment more effectively at colder temperature regimes, these detections were made in a comparatively warmer climate at a time of the year when cooler water temperatures prevail.

Introduction

Three types of influenza viruses occur naturally—A, B, and C. Influenza A viruses (avian influenza) can infect humans, pigs, horses, and other mammals, in addition to birds. Avian influenza viruses (AIV) cause seasonal epidemics affecting a small percentage of the human population annually; however, AIV also cause periodic pandemics (1918, 1957, and 1968) that have resulted in large losses of human life. Highly pathogenic AIV is a major cause of substantial losses in domestic poultry populations worldwide (World Health Organization, 2003, 2006; Zhang and others, 2006).

AIV subtypes are categorized by the combination of proteins (hemagglutinin, “H” and neuraminidase, “N”) found on their surfaces and include subtypes with both high and low pathogenicity (fig. 1; Karasin and others, 2000; World Health Organization, 2006). Highly pathogenic (HP) AIV H5N1 infected domestic poultry, migratory waterfowl, and humans in Asia, Europe, and Africa in 2008. During such widespread incidents, mortality rates can be as high as 90 and 100 percent for poultry and waterfowl, respectively, and greater than

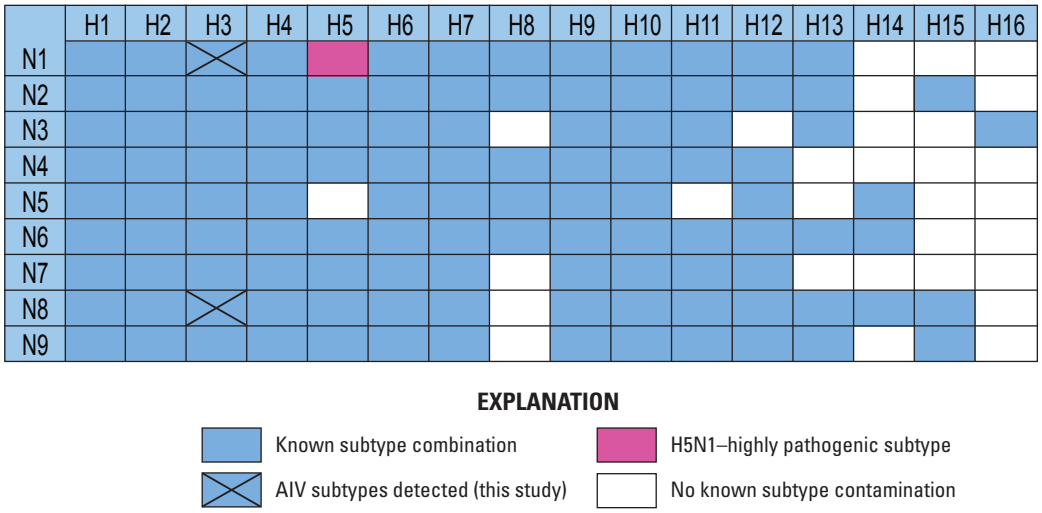

Figure 1. Matrix showing possible avian influenza virus hemagglutinin (H) and neuraminidase (N) subtypes.

50 percent for infected humans (World Health Organization 2003, 2006; U.S. Interagency Strategic Plan, 2006; U.S. Department of Health and Human Services, 2008). Most cases of HPAIV H5N1 infection in humans result from contact with infected poultry, domesticated chickens, ducks, and turkeys, or from contact with surfaces contaminated with secretions or excretions from infected birds (World Health Organization 2003; U.S. Interagency Strategic Plan, 2006; U.S. Department of Health and Human Services, 2008). Because all AIV can mutate genetically, world health agencies are concerned that in the future, HPAIV H5N1, may mutate and become capable of being transmitted efficiently from one human to another, which could result in a worldwide influenza pandemic (World Health Organization, 2003, 2006; U.S. Interagency Strategic Plan, 2006; U.S. Department of Health and Human Services, 2008). Therefore, surveillance of biotic and abiotic reservoirs for AIV (fig. 2) is critical for developing a procedure to identify sources of a future influenza pandemic and where a future pandemic could emerge. Until recently the study of these abiotic reservoirs have focused primarily on water as a transmission source (World Health Organization, 2006; Zhang and others, 2006); however, it is likely that AIV, once shed, would dilute rapidly in any body of water.

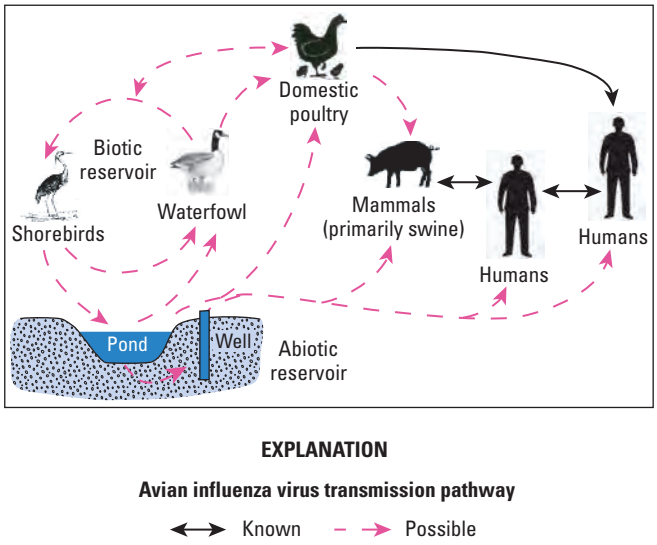

Figure 2. Diagram showing possible biotic and abiotic reservoirs and transmission pathways for avian influenza viruses (modified from Ip and Slota, 2005).

In 2006, the U.S. Geological Survey (USGS) began a reconnaissance to determine if AIV was present in the bed sediment of selected water bodies along the Atlantic Flyway. The movement of AIV between countries and continents may be the result of transport by infected migratory birds, and bed sediment may be an important substrate for AIV to spread between migratory bird populations. Investigating the presence of AIV in sediment is important because once the virus is shed from an animal it may persist longer in the sediment substrate where it is protected from conditions that can cause AIV to deteriorate.

Previous Studies

Investigators have been successful in isolating AIV in surface water when migratory ducks are present and are shedding virus in feces (Webster and others, 1978; Hinshaw and others, 1979; Halvorson and others, 1983; Kelleher and others, 1985; Sivanandan and others, 1991). What is less understood is how long the virus remains viable in the environment once shed. Does the virus persist long enough to infect the next population of migratory birds to use the contaminated water body? Does the virus remain in sufficient concentrations to infect the next host? Although previous investigators were successful in collecting water samples containing AIV, these samples were collected in cold-weather climates, such as Canada, Alaska, and Siberia (Hinshaw and others, 1979; Ito and others, 1995; Zhang and others, 2006); or in areas of concentrated bird populations, such as domestic duck production facilities (Markwell and Shortridge, 1982; Sivanandan and others, 1991).

Few AIV studies have been conducted to evaluate the persistence of the virus in warm-climate waters used by migratory waterfowl such as water bodies in the southeastern United States; as a result, little is known about the persistence of AIV in these climates. However, it has been established that the persistence of AIV is dependent on the water chemistry and temperature regime of the water body. Several studies have shown the infectivity of the virus declined as temperature and salinity increased and pH decreased (Stallknecht and others, 1990; Ito and others, 1995; and World Health Organization, 2006). This study is the first AIV reconnaissance in the southeastern United States to attempt to isolate the virus in bed sediments.

Purpose and Scope

This report presents results of a reconnaissance study by the USGS Georgia Water Science Center, Atlanta, Georgia, and USGS National Wildlife Health Center, Madison, Wisconsin, to analyze water and bed-sediment samples for the presence of AIV from selected water bodies along the Atlantic Flyway during February and May 2006 and January 2007. The selected water bodies were in National Wildlife Refuges (NWRs) in Georgia, South Carolina, North Carolina, Virginia, and Maryland (table 1; fig. 3).

Table 1. Location of water and bed-sediment samples collected from National Wildlife Refuges along the Atlantic Flyway for analysis of avian influenza viruses, February and May 2006 and January 2007.

[NWIS, U.S. Geological Survey National Water Information System; NWR, National Wildlife Refuge; °, degrees; ', minutes; ", seconds; do., ditto; NA, not available; bold text indicates positive results for avian influenza type A viruses by viral isolation tests]

Sample number	NWIS site identification number	Sample date	Waterbody	Latitude	Longitude
Savannah NWR, Georgia and South Carolina					
SNWR-01	321128081045201	02/28/2006	King Fisher Pond	32°11'28.1"	81°04'51.7"
SNWR-02	321130081045301	do.	do.	32°11'30.4"	81°04'52.9"
SNWR-03	320903081063001	do.	Pond #14	32°09'02.6"	81°06'30.4"
SNWR-04	**320901081063101**	**do.**	**do.**	**32°09'01.3"**	**81°06'31.0"**
SNWR-05	320900081063201	do.	do.	32°09'00.2"	81°06'32.5"
SNWR-06	320905081062901	01/16/2007	do.	32°09'04.6"	81°06'28.8"
SNWR-07	320955081064901	do.	Pond #10	32°09'55.0"	81°06'49.0"
SNWR-08	320857081062101	do.	Pond #14	32°08'57.4"	81°06'21.4"
Santee NWR, South Carolina					
SANWR-01	333314080262801	05/26/2006	Banding Pond	33°33'13.9"	80°26'27.9"
SANWR-02	**333315080262601**	**01/17/2007**	**do.**	**33°33'14.8"**	**80°26'25.8"**
Pee Dee NWR, North Carolina					
PDNWR-01	350446080024501	05/26/2006	Arrowhead Lake	35°04'45.8"	80°02'44.6"
PDNWR-02	**350540080010601**	**01/17/2007**	**Andrews Pond**	**35°05'40.1"**	**80°01 06.2"**
Mason Neck NWR, Virginia					
MNNWR-01	383728077120601	05/24/2006	Little Marsh Creek	38°37'27.7"	77°12'05.7"
Occoquan Bay NWR, Virginia					
ONWR-01	383838077135701	05/24/2006	unnamed tidal creek	38°38'38.4"	77°13'56.8"
Blackwater NWR, Maryland					
BWNWR-01	382627076071801	05/24/2006	Pool 3b	38°26'27.2"	76°07'18.2"
BWNWR-02	382635076072401	do.	Pool 3a	38°26'35.3"	76°07'23.5"
BWNWR-03	382642076080201	do.	Pool 5b	38°26'41.8"	76°08'01.5"
Chincoteague NWR, Virginia					
CNWR-01	375432075201501	05/25/2006	Snow Goose Pool	37°54'32.0"	75°20'15.3"
CNWR-02	375406075210201	do.	Swans Cove Pool	37°54'06.1"	75°21'02.5"

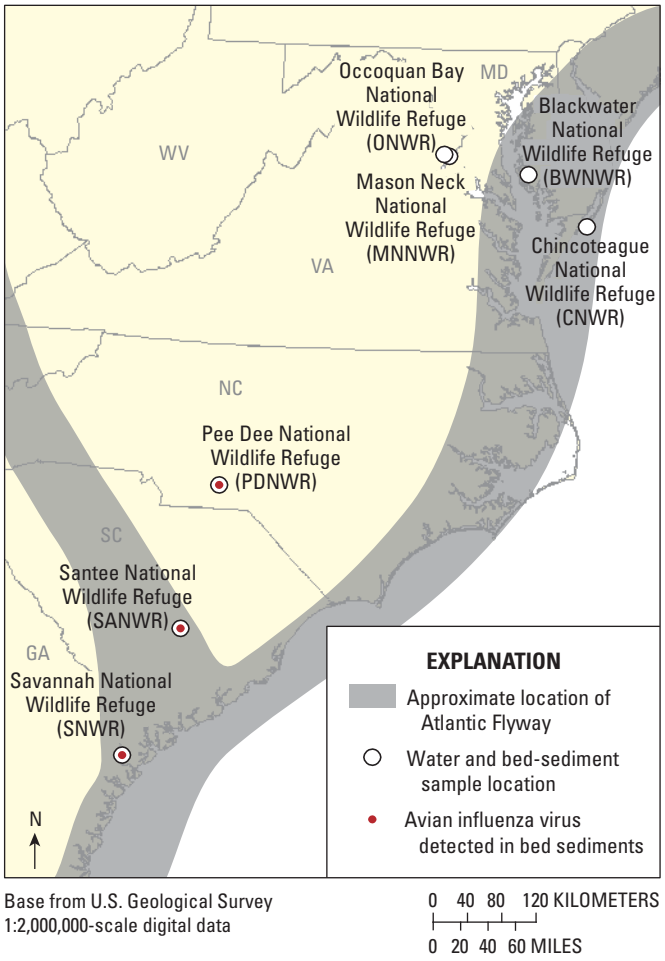

Figure 3. Water and bed-sediment samples collected from selected water bodies from National Wildlife Refuges along the Atlantic Flyway for analysis of avian influenza viruses, February and May 2006 and January 2007.

Sample Collection and Analysis

Feces shed from waterfowl settles in a water body and mixes with other organic material, such as detritus and aquatic vegetation, to form an organic layer that may be an important substrate for AIV to persist, protected from conditions that cause it to deteriorate. The methods used during this reconnaissance are preliminary in nature but constitute the first-ever successful detection of AIV from the benthic regions in bodies of water. The objective was to develop a method to collect samples from bed sediments and analyze the mixture for detection of AIV in a water body.

Sampling Method

For this reconnaissance, the term "sampling site" generally refers to an area within a water body where large populations of waterfowl were observed during regular feeding and migratory patterns, by a U.S. Fish and Wildlife Service biologist at each NWR (table 2). The term "water body" refers to a pond or managed impoundment at a selected NWR. A single water body was divided into multiple sampling sites if migratory waterfowl were observed to congregate in different areas of the water body. At each sampling site, three to five subsamples of bed sediments were collected and composited for processing.

All sampling, collection, and processing equipment were cleaned and sterilized using a Barnstead/Thermolyne, Corp., Sterilemax tabletop steam sterilizer on liquid cycle at 121 degrees Celsius (°C) for 15 minutes prior to field activities to control cross contamination of equipment. To avoid cross contamination in the field, dedicated cleaned sample bottles and tubing were assigned to each sampling site. Plastic bins and coolers used to transport sampling equipment were washed with soapy water, rinsed with tap water, and lined prior to being filled with equipment.

Water and bed-sediment samples in February and May 2006 were collected using a 1-liter plastic bottle attached with duct tape to a galvanized steel rod. The bottle was dredged along the bottom of the water body collecting as much sediment and organic matter as possible along with additional water that was removed after the samples were allowed to settle. Samples in January 2007 were collected similarly; however, the 1-liter plastic bottle was equipped with a gate valve that could be opened and closed remotely when near the bottom of the water body to reduce possible contamination from surface water. At each site, enough bed-sediment material was composited to fill a 3-liter Teflon bottle. After returning to the laboratory and allowing the bed material to settle to the bottom of the Teflon bottle, the remaining water was removed using a peristaltic pump. Once all the excess water was removed, bed-sediment material was collected in an autoclaved 250-milliliter plastic bottle and analyzed for the presence of AIV. For each sample, an aliquot of the decanted water was also analyzed for AIV.

Field measurements of pH, specific conductance, temperature, and dissolved oxygen were made at each sampling site (table 3). Laboratory molecular and virus culture techniques were performed at the USGS National Wildlife Health Center in Madison, Wisconsin.

Laboratory Analysis

Prior to analysis, the samples were centrifuged at 800 times gravitational acceleration (9.81 meters per second squared) for 15 minutes to produce a supernatant that was analyzed for AIV. Molecular detection of AIV and the determination of the presence of H5 and H7 subtypes were performed according to methods described in the U.S. Interagency Strategic Plan (2006). Analysis for AIV included recovering possible viral ribonucleic acid (RNA) from 50-microliter aliquots of the whole or filtered water or supernatant using the Ambion® MagMax™ viral RNA isolation kit. A 5-microliter sample of recovered RNA was analyzed using an AIV universal matrix-gene real-time reverse transcription polymerase-chain reaction (RT-PCR) test. The RT-PCR tests were done using a Stratagene Corporation MX3005P™ real-time PCR system with reference to samples with known AIV RNA concentrations (Spackman and others, 2002). All matrix-gene RT-PCR-positive samples were further subtyped using H5 and H7 RT-PCR tests, as these subtypes are of particular human and veterinary concern.

Analysis for AIV also was done by viral isolation. The supernatant was filtered through a 0.2-micrometer filter. A small volume (0.2 milliliter) of the filtered sample then was inoculated into three 8-day-old specific pathogen-free embryonated chicken eggs. Following a 3-day incubation period at 7 °C, a sample of allantoic fluid was collected from the eggs and analyzed for the presence of AIV using a hemagglutination assay with rooster and turkey red-blood cells. The presence of AIV was confirmed by recovering RNA from the allantoic fluid using the Ambion®MagMax™ viral RNA isolation kit and subjecting it to an AIV universal-virus matrix-gene RT-PCR test. Hemagglutination assay-negative samples were retested by inoculation into embryonated eggs a second time before the sample was considered negative.

Hemagglutination assay and matrix-gene RT-PCR-positive samples were subtyped at the U.S. Department of Agriculture National Veterinary Services Laboratory in Ames, Iowa, against a panel of reference antisera. Antisera antibodies were developed using reference strains of each H and N subtype of AIV and were used to identify specific viral subtypes present in samples.

Table 2. Sampling-site conditions for water and bed-sediment samples collected from National Wildlife Refuges along the Atlantic Flyway for analysis of avian influenza viruses, February and May 2006 and January 2007.

[NWR, National Wildlife Refuge; do., ditto; bold text indicates positive results for avian influenza type A viruses by viral isolation tests]

Sample number	Sample date	Description	Sample site condition	Estimated water-body depth (meters)	Migratory bird species common to this site (scientific name)	Estimated total population	Migratory bird activity
Savannah NWR, Georgia and South Carolina							
SNWR-01	02/28/2006	Freshwater pond	Full pool[1]	1	Ring-necked duck (*Aythya collaris*)[1]	[1]About 200	Departed 10 days prior to sample collection after over-wintering[1]
SNWR-02	do.	Freshwater pond, very loose fine sediments	do.	1	do.	do.	do.
SNWR-03	do.	Managed freshwater impoundment	Full pool, rice-stalk mat covering bottom[1]	1	do.	[1]5,000 to 10,000	do.
SNWR-04	**do.**	do.	do.	**1**	**do.**	**do.**	**do.**
SNWR-05	do.	do.	do.	1	do.	do.	do.
SNWR-06	01/16/2007	do.	Less than full pool[1]	0.5	do.	[1]About 8,000	Present at time of sampling[1]
SNWR-07	do.	do.	do.	0.1	do.	do.	do.
SNWR-08	do.	do.	do.	0.1	do.	do.	do.
Santee NWR, South Carolina							
SANWR-01	05/26/2006	Managed freshwater impoundment	Full pool[2]	1	Ring-necked duck (*Aythya collaris*), green-winged teal (*Anas carolinensis*)[2]	[2]2,000 to 6,000	Departed about 6 weeks prior to sample collection after over-wintering[2]
SANWR-02	**01/17/2007**	do.	do.	**1**	**Ring-necked duck *(Aythya collaris)*[2]**	**[2]About 6,000**	**Present at time of sampling[2]**
Pee Dee NWR, North Carolina							
PDNWR-01	05/26/2006	Freshwater pond	Full pool[3]	1	Wood duck (*Aix sponsa*), mallard (*Anas platyrhynchos*), green-winged teal (*Anas carolinensis*), Canada goose (*Branta canadensis*), northern pintail (*Anas acuta*)[3]	[3]5,000 to 10,000	Departed about 6 weeks prior to sample collection after over-wintering[3]
PDNWR-02	**01/17/2007**	do.	do.	**1**	***Ring-necked duck (Aythya collaris), green-winged teal (Anas carolinensis), Canada goose (Branta canadensis)*[3]**	**do.**	**Present at time of sampling[3]**
Mason Neck NWR, Virginia							
MNNWR-01	05/24/2006	Managed freshwater impoundment	Full pool[4]	2	American black duck (*Anas rubripes)*, wood duck (*Aix sponsa*), hooded merganser (*Lophodytes cucullatus*)[4]	[4]500 to 1,000	Departed about 6 weeks prior to sample collection after over-wintering[4]

Table 2. Sampling-site conditions for water and bed-sediment samples collected from National Wildlife Refuges along the Atlantic Flyway for analysis of avian influenza viruses, February and May 2006 and January 2007.—Continued

[NWR, National Wildlife Refuge; do., ditto; bold text indicates positive results for avian influenza type A viruses by viral isolation tests]

Sample number	Sample date	Description	Sample site condition	Estimated water-body depth (meters)	Migratory bird species common to this site (scientific name)	Estimated total population	Migratory bird activity
				Occoquan Bay NWR, Virginia			
ONWR-01	05/24/2006	Main channel of marsh stream[4]	Low tide[4]	1	American black duck (*Anas rubripes)*, wood duck (*Aix sponsa*), hooded merganser (*Lophodytes cucullatus*)[6]	[4]500 to 1,000	Departed about 6 weeks prior to sample collection after over-wintering[4]
				Blackwater NWR, Maryland			
BWNWR-01	05/24/2006	Managed freshwater impoundment[5]	Dry except slough bordering impoundment, sampled slough[5]	1	American black duck (*Anas rubripes*), wood duck (*Aix sponsa*), mallard (*Anas platyrhynchos*), green-winged teal (*Anas carolinensis*), Canada goose (*Branta canadensis*), northern pintail (*Anas acuta*)[5]	[5] 5,000 to 10,000	Departed about 6 weeks prior to sample collection after over-wintering[5]
BWNWR-02	do.	do.	Dry except isolated pools[5]	0.1	do.	do.	do.
BWNWR-03	do.	do.	do.	0.1	do.	do.	do.
				Chincoteague NWR, Virginia			
CNWR-01	05/25/2006	Managed freshwater impoundment[6]	Less than full pool[6]	1	American black duck (*Anas rubripes*), wood duck (*Aix sponsa*), mallard (*Anas platyrhynchos*), green-winged teal (*Anas carolinensis*), Canada goose (*Branta canadensis*), northern pintail (*Anas acuta*)[6]	[6]5,000 to 10,000	Departed about 6 weeks prior to sample collection after over-wintering[6]
CNWR-02	do.	do.	do.	1	do.	do.	do.

[1]Russell Webb, Biologist, Savannah National Wildlife Refuge, U.S. Fish and Wildlife Service, oral commun., February 28, 2006, and January 16, 2007

[2]Lawrence A. Woodward, Biologist, Santee National Wildlife Refuge, U.S. Fish and Wildlife Service, oral commun., May 26, 2006, and January 17, 2007

[3]Matthew Garner, Biologist, Pee Dee National Wildlife Refuge, U.S. Fish and Wildlife Service, oral commun., May 26, 2006 , and January 17, 2007

[4]Marty McClevey, Biologist, Mason Neck and Occoquan Bay National Wildlife Refuges, U.S. Fish and Wildlife Service, oral commun., May 24, 2006

[5]Dixie L. Birch, Biologist, Blackwater National Wildlife Refuge, U.S. Fish and Wildlife Service, oral commun., May 16, 2006

[6]William Hogland, Biologist, Chincoteague National Wildlife Refuge, U.S. Fish and Wildlife Service, oral commun., May 25, 2006

Table 3. Field measurements of water-quality properties and results of matrix RT-PCR and viral isolation tests associated with water and bed-sediment samples collected from National Wildlife Refuges along the Atlantic Flyway for analysis of avian influenza viruses, February and May 2006 and January 2007.

[mg/L, milligrams per liter; µS/cm, microsiemens per centimeter; °C, degree Celsius; RT-PCR, reverse transcriptase-polymerase chain reaction; NWR, National Wildlife Refuge; do., ditto; NA, not available; bold text indicates positive results for avian influenza type A viruses by viral isolation tests]

Sample number (see table 1 for location)	Date	Number of measure-ments	Median dissolved oxygen (percent saturation)	Median dissolved oxygen (mg/L)	Median pH	Median specific conductance (µS/cm)	Median temperature (°C)	Matrix RT-PCR test	Viral isolation through hemagglu-tination	Detected avian influenza viral isolate
Savannah NWR, Georgia and South Carolina										
SNWR-01	02/28/2006	5	43.9	4.6	5.2	34	13.6	Negative	Negative	
SNWR-02	do.	5	37.9	3.9	5.1	33	14.2	do.	do.	
SNWR-03	do.	5	51.7	5.5	6.1	827	12.9	do.	do.	
SNWR 04	**do.**	**5**	**69.1**	**7.3**	**6.3**	**910**	**12.7**	**do.**	**Positive**	**H3N1**
SNWR-05	do.	5	85.5	8.6	6.4	1,490	12.6	do.	Negative	
SNWR-06	01/16/2007	5	NA	0.2	6.4	281	16.5	do.	do.	
SNWR-07	do.	3	NA	2.3	6.3	245	18.9	do.	do.	
SNWR-08	do.	5	NA	5.8	6.5	336	19.0	do.	do.	
Santee NWR, South Carolina										
SANWR-01	05/26/2006	1	73.5	5.6	3.8	112	29.1	Negative	Negative	
SANWR-02	**01/17/2007**	**5**	**NA**	**7.2**	**8.0**	**108**	**12.5**	**do.**	**Positive**	**H3N8**
Pee Dee NWR, North Carolina										
PDNWR-01	05/26/2006	1	25.1	2.1	2.2	55	24.6	Negative	Negative	
PDNWR-02	**01/17/2007**	**5**	**NA**	**7.0**	**7.1**	**82**	**11.8**	**Positive**	**Positive**	**H3N8**
Mason Neck NWR, Virginia										
MNNWR-01	05/24/2006	2	NA	7.1	6.7	102	18.7	Negative	Negative	
Occoquan Bay NWR, Virginia										
ONWR-01	05/24/2006	1	57.0	5.6	7.2	149	16.5	Negative	Negative	
Blackwater NWR, Maryland										
BWNWR-01	05/24/2006	1	40.3	3.3	6.0	625	25.4	Negative	Negative	
BWNWR-02	do.	1	51.5	4.6	7.4	333	20.9	do.	do.	
BWNWR-03	do.	1	97.6	8.1	7.0	1,708	24.3	do.	do.	
Chincoteague NWR, Virginia										
CNWR-01	05/25/2006	1	97.6	8.1	9.7	2,930	19.3	Negative	Negative	
CNWR-02	do.	1	135.2	10.9	10.0	3,573	19.5	do.	do.	

Occurrence of Viable Avian Influenza Viruses

Water and bed-sediment samples, evaluated for the presence of AIV, were collected during February and May 2006 and January 2007 from seven NWRs (table 1) along the Atlantic Flyway (fig. 3). None of the water samples collected tested positive for the presence of AIV. Three of the 19 bed-sediment samples collected tested positive for AIV; water-chemistry data indicate that these positive samples were collected in water with temperatures ranging from 11.8 to 12.7 °C, specific conductance ranging from 82 to 910 microsiemens per centimeter (µS/cm), and pH ranging from 6.3 to 8.0 (table 3). Viral isolation and RT-PCR tests detected AIV H3N1 in a sample from the Savannah NWR in February 2006 and H3N8 in samples from both the Santee and Pee Dee NWRs in January 2007 (fig. 3; table 3). AIV was not detected in samples collected during May 2006.

Savannah National Wildlife Refuge

Eight samples were collected during February 2006 and January 2007 from the Savannah NWR, near Savannah, Georgia (table 1). Two samples, SNWR-01 and SNWR-02, were collected from Kingfisher Pond, during February 2006 (table 2). Kingfisher Pond is a man-made freshwater pond with a loose-sediment bottom. NWR personnel estimated about 200 ring-necked ducks had used the pond for over-wintering and departed about 10 days prior to sample collection. AIV was not detected in samples from Kingfisher Pond (table 3).

Three samples, SNWR-03, SNWR-04, and SNWR-05, were collected from Pond number (#) 14 during February 2006 (table 2). Pond #14 is a managed impoundment maintained by Savannah NWR personnel as a waterfowl habitat (Russell Webb, Biologist, Savannah National Wildlife Refuge, U.S. Fish and Wildlife Service, oral commun., February 28, 2006). NWR personnel estimated about 5,000 to 10,000 ring-necked ducks had used Pond #14 for 1.5 months and departed about 10 days prior to sample collection. AIV H3N1 was isolated and detected in sample SNWR-04; which was collected when median water temperature was 12.7 °C, median specific conductance was 910 µS/cm, median pH was 6.3, and median dissolved oxygen was 7.3 milligrams per liter (mg/L; table 3).

Two samples, SNWR-06 and SNWR-08, were collected from Pond #14 during January 2007 (table 1). NWR personnel estimated about 8,000 ring-necked ducks were using Pond #14 during sample collection (table 2). AIV was not detected in samples SNWR-06 and SNWR-08 (table 3).

One sample, SNWR-07, was collected from Pond #10 during January 2007. Pond #10 is a managed impoundment maintained by Savannah NWR personnel as a waterfowl habitat (Russell Webb, Biologist, Savannah National Wildlife Refuge, U.S. Fish and Wildlife Service, oral commun., January 16, 2007). NWR personnel estimated about 8,000 ring-necked ducks were using Pond #10 during sample collection; however, AIV was not detected in sample SNWR-07 (table 3).

Santee National Wildlife Refuge

Two samples were collected from the Santee NWR, near Santee, South Carolina, one each in May 2006 and January 2007 (table 1). Both samples, SANWR-01 and SANWR-02, were collected from the Banding Pond, a man-made freshwater pond with a loose sediment bottom, maintained by Santee NWR personnel as a waterfowl habitat (Lawrence Woodward, Biologist, Santee National Wildlife Refuge, U.S. Fish and Wildlife Service, oral commun., May 26, 2006). Sample SANWR-01 was collected during May 2006. NWR personnel estimated about 2,000 to 6,000 ring-necked ducks and green-winged teals had used the pond for over-wintering and departed about 6 weeks prior to sample collection (table 2). AIV was not detected in sample SANWR-01 (table 3).

Sample SANWR-02 was collected during January 2007. At the time of sample collection, NWR personnel estimated about 6,000 ring-necked ducks and green-winged teals were using the pond for over-wintering. AIV H3N8 was detected in sample SANWR-02, which was collected when median water temperature was 12.5°C, median specific conductance was 108 µS/cm, median pH was 8.0, and median dissolved oxygen was 7.2 mg/L (table 3).

Pee Dee National Wildlife Refuge

Two sediment samples were collected from the Pee Dee NWR, near Wadesboro, North Carolina, one each during May 2006 and January 2007 (table 2). Sample PDNWR-01 was collected from Arrowhead Lake, a man-made freshwater lake maintained by Pee Dee NWR personnel as a waterfowl habitat (Matthew Garner, Biologist, Pee Dee National Wildlife Refuge, U.S. Fish and Wildlife Service, oral commun., May 26, 2006). Sample PDNWR-01 was collected during May 2006. NWR personnel estimated about 5,000 to 10,000 wood ducks, Canada geese, mallard ducks, northern pintail ducks, and green-winged teals had used the pond for over-wintering and departed about 6 weeks prior to sample collection. AIV was not detected in sample PDNWR-01 (table 3).

Sample PDNWR-02 was collected during January 2007 from Andrews Pond, a man-made freshwater pond maintained by Pee Dee NWR personnel as a waterfowl habitat (Matthew Garner, Biologist, Pee Dee National Wildlife Refuge, U.S. Fish and Wildlife Service, oral commun., January 17, 2007). Sample PDNWR-02 was collected during January 2007. At the time of sample collection, NWR personnel estimated about 5,000 to 10,000 wood ducks, Canada geese, and green-winged teals were using the pond for over-wintering. AIV H3N8 was detected in sample PDNWR-02; which was collected when median water temperature was 11.8 °C, median specific conductance was 82 (µS/cm), median pH was 7.1, and median dissolved oxygen was 7.0 mg/L (table 3).

Mason Neck and Occoquan Bay National Wildlife Refuges

Mason Neck and Occoquan Bay National Wildlife Refuges near Woodbridge, Virginia, are jointly maintained by Mason Neck NWR personnel (Marty McClevey, Biologist, Mason Neck National Wildlife Refuge, U.S. Fish and Wildlife Service, oral commun., May 25, 2006). One sediment sample was collected during May 2006 from the Mason Neck NWR (table 2). Sample MNNWR-01 was collected from Little Marsh Creek, a managed freshwater impoundment maintained by Mason Neck NWR personnel as a waterfowl habitat (Marty McClevey, Biologist, Mason Neck National Wildlife Refuge, U.S. Fish and Wildlife Service, oral commun., May 24, 2006). NWR personnel estimated about 500 to 1,000 wood ducks, American black ducks, and hooded mergansers had used the impoundment for over-wintering and departed about 6 weeks prior to sample collection. AIV was not detected in sample MNNWR-01 (table 3).

One sediment sample was collected during May 2006 from the Occoquan Bay NWR (table 2). Sample ONWR-01 was collected from an unnamed tidal creek maintained by Mason Neck NWR personnel as a waterfowl habitat (Marty McClevey, Biologist, Mason Neck National Wildlife Refuge, U.S. Fish and Wildlife Service, oral commun., May 24, 2006). NWR personnel estimated about 500 to 1,000 wood ducks, American black ducks, and hooded mergansers had used the creek for over-wintering and departed about 6 weeks prior to sample collection. AIV was not detected in sample ONWR-01 (table 3).

Blackwater National Wildlife Refuge

Three sediment samples were collected during May 2006 from the Blackwater National Wildlife Refuge, near Cambridge, Maryland (table 2). Sample BWNWR-01 was collected from Pool 3b, a managed freshwater impoundment maintained by Blackwater NWR personnel as a waterfowl habitat (Dixie Birch, Biologist, Blackwater National Wildlife Refuge, U.S. Fish and Wildlife Service, oral commun., May 16, 2006). NWR personnel estimated about 5,000 to 10,000 wood ducks, American black ducks, Canada geese, mallard ducks, northern pintail ducks, and green-winged teals had used the impoundment for over-wintering and departed about 6 weeks prior to sample collection. AIV was not detected in sample BWNWR-01 (table 3).

Sample, BWNWR-02 was collected from Pool 3a, a managed freshwater impoundment maintained by Blackwater NWR personnel as a waterfowl habitat (Dixie Birch, Biologist, Blackwater National Wildlife Refuge, U.S. Fish and Wildlife Service, oral commun., May 16, 2006). NWR personnel estimated about 5,000 to 10,000 wood ducks, American black ducks, Canada geese, mallard ducks, northern pintail ducks and green-winged teals had used the pond for over-wintering and departed about 6 weeks prior to sample collection. AIV was not detected in sample BWNWR-02 (table 3).

Sample BWNWR-03 was collected from Pool 5b, a managed freshwater impoundment maintained by Blackwater NWR personnel as a waterfowl habitat (Dixie Birch, Biologist, Blackwater National Wildlife Refuge, U.S. Fish and Wildlife Service, oral commun., May 16, 2006). NWR personnel estimated about 5,000 to 10,000 wood ducks, American black ducks, Canada geese, mallard ducks, northern pintail ducks, and green-winged teals had used the impoundment for over-wintering and departed about 6 weeks prior to sample collection. AIV was not detected in sample BWNWR-03 (table 3).

Chincoteague National Wildlife Refuge

Two sediment samples were collected during May 2006 from the Chincoteague National Wildlife Refuge, near Chincoteague, Virginia (table 2). Sample CNWR-01 was collected from Snow Goose Pool, a managed freshwater impoundment maintained by Chincoteague NWR personnel as a waterfowl habitat (William Hogland, Biologist, Chincoteague National Wildlife Refuge, U.S. Fish and Wildlife Service, oral commun., May 25, 2006). NWR personnel estimated about 5,000 to 10,000 wood ducks, American black ducks, Canada geese, mallard ducks, northern pintail ducks, and green-winged teals had used the impoundment for over-wintering and departed about 6 weeks prior to sample collection. AIV was not detected in sample CNWR-01 (table 3).

Sample, CNWR-02 was collected from Swans Cove Pool, a managed freshwater impoundment maintained by Chincoteague NWR personnel as a waterfowl habitat (William Hogland, Biologist, Chincoteague National Wildlife Refuge, U.S. Fish and Wildlife Service, oral commun., May 25, 2006. NWR personnel estimated about 5,000 to 10,000 wood ducks, American black ducks, Canada geese, mallard ducks, northern pintail ducks, and green-winged teals had used the impoundment for over-wintering and departed about 6 weeks prior to sample collection. AIV was not detected in sample CNWR-02 (table 3).

Summary

During February and May 2006, and January 2007, a total of 19 water and bed-sediment samples were collected from selected water bodies from U.S. Fish and Wildlife Service National Wildlife Refuges along the Atlantic Flyway in Georgia, South Carolina, North Carolina, Virginia, and Maryland. The samples were analyzed for the presence of viable avian influenza viruses (AIV), which was detected in bed-sediment samples collected from the Savannah National Wildlife Refuge (NWR) in Georgia during February 2006 and from the Santee NWR in South Carolina and the Pee Dee NWR in North Carolina during January 2007. AIV was not detected in any of the water samples collected for the study or in any of the bed-sediment samples collected during May 2006.

These results indicate that the sampling and analytical methods used were successful in detecting AIV in the environment at three NWRs. The reconnaissance method used in this study combined the water column and bed-sediment materials into a single composited sample and thus could not differentiate the source of the AIV between the two media. However, water samples collected concurrently at each of the sampling sites were uniformly negative for AIV, suggesting that AIV resides in the solid-phase materials. Sampling-method refinements could be implemented that would allow the determination of the relative association of AIV within the water column and sediment. Additional data collection would provide a better understanding of the occurrence of AIV in relation to water-quality conditions, climate conditions, and the presence of waterfowl.

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References

Halvorson, D.A., Karunakaran, D., Hinshaw, V.S., and Newman, J., 1983, Epizootiology of avian influenza—Simultaneous monitoring of sentinel ducks and turkeys in Minnesota: Avian Diseases, v. 27, p. 77–85.

Hinshaw, V.S., Webster, R.G., and Turner, B., 1979, Water-borne transmission of avian influenza A viruses: Intervirology, v. 11, p. 66–68.

Ip, H.S., and Slota, Paul, 2005, The avian influenza H5N1 threat, current facts and concerns about highly pathogenic avian influenza H5N1: U.S. Geological Survey, Fact Sheet 2005–3146, 2 p.

Ito, T., Okazaki, K., Kawaoka, Y., Takada, A., Webster, R.G., Kida, H., 1995, Perpetuatuion of influenza viruses in Alaskan waterfowl reservoirs: Archives of Virology, v. 140, no. 7, p. 1163–1172.

Karasin, A.I., Brown, I.H., Carmen, S., and Olsen, C.W., 2000, Isolation and characterization of H4N6 avian influenza viruses from pigs with pneumonia in Canada: Journal of Virology, Oct. 2000, p. 9322–9327.

Kelleher, C.J., Halvorson, D.A., Newman, J.A., and Senne, D.A., 1985, Isolation of avian paramyxoviruses from sentinel ducks and turkeys in Minnesota: Avian Diseases, v. 29, p. 400–407.

Markwell, D.D., and Shortridge, K.F., 1982, Possible water-borne transmission and maintenance of influenza viruses in domestic ducks: American Society for Microbiology, Applied and Environmental. Microbiology, January, v. 1, p. 110–116.

Sivanandan, V., Halvorson, D.A., Laudert, E., Senne, D.A., and Kumar, M.C., 1991, Isolation of H13N2 influenza A virus from turkeys and surface water: Avian Diseases, v. 35, p. 974–977.

Spackman, E., Senne, D.A., Myers, T.J., Bulaga, L.L., Garber, L.P., Perdue, M.I., Lohman, K., Daum, L.T., and Suarez, D.L., 2002, Development of a real-time reverse transcriptase PCR assay for influenza virus and the avian H5 and H7 hemagglutinin subtypes: Journal of Clinical Microvirology, v. 40, p. 3256–3260.

Stallknecht, D.E., Shane, S.M., Kearney, M.T., and Zwank, P.J., 1990, Persistence of avian influenza viruses in water: Avian Diseases, v. 34, p. 406–411.

U.S. Department of Health and Human Services, 2008, Pandemic flu, general information; accessed March 5, 2008, at *http://www.pandemicflu.gov/general/*.

U.S. Interagency Strategic Plan, 2006, An early detection system for highly pathogenic H5N1 avian influenza in wild migratory birds; accessed November 29, 2007, at *http://www.nwhc.usgs.gov/publications/other/Final_Wild_Bird_Strategic_Plan_0322.pdf*.

Webster, R.G., Yakhno, M., Hinshaw, V.S., Bean, W.J., Jr., and Mutri, K.G., 1978, Intestinal influenza, replication and characterization of influenza viruses in ducks: Virology, v. 84, p. 268–278.

World Health Organization, 2003, Influenza, Report of the Secretariat: March 13, 2003, A556/23, Fifty-sixth World Health Assembly.

World Health Organization, 2006, Review of the latest available evidence on risks to human health through potential transmission of avian influenza (H5N1) through water and sewage: Geneva, Water, Sanitation and Health Protection of the Human Environment, March 24, 2006, p. 15.

Zhang, G., Shoham, D., Gilichinsky, D., Davydov, S., Castello, J.D., and Rogers, S.O., 2006, Evidence of avian influenza A virus in Siberian lake ice: Journal of Virology, v. 8, no. 24, p. 12229–12235.

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