



Avian Influenza Wild Bird Surveillance Program: 2007

Nevada Department of Wildlife

Introduction

All known viruses that cause influenza in birds belong to the genus: Influenza virus A. This genus is comprised of a cluster of viral strains or subtypes that replicate as a continuous lineage and can genetically reassort with each other. These viruses are widely endemic in wild populations of waterfowl and many other species of birds, most subtypes (designated by their antigenic proteins H and N) being of little pathogenic significance. An Asian strain of the H5N1 subtype however, is classified as highly pathogenic (HPAI) and based on recent observations, has the potential to expand its range into North America. Public concern has been heightened by extensive media coverage about this virus in Asia, its spread to Europe and Africa, and the very small number of human infections—much of it includes speculation that migratory birds are a primary vector and will bring it to this continent. Apprehensions among government agencies and the public are based on a range of potential consequences that include sickness and mortality in wild bird populations, devastating impacts to the poultry industry, and potential mutation of the virus into a form that could be highly infectious and pathogenic to humans—possibly the source of the next flu pandemic. Government agencies, particularly state and federal wildlife agencies, have been called upon to develop an early detection system to determine if and when the virus arrives here.

To provide baseline information about the strains and distribution of influenza viruses in native and migratory waterfowl and to respond to the emergence of highly pathogenic avian influenza (HPAI) H5N1, the Nevada Department of Wildlife's (NDOW) wild bird influenza survey was initiated in 2005. The goals of the survey are to identify avian influenza viruses in wild waterfowl in Nevada and to detect HPAI strains early. NDOW's partners in this state-wide program are USDA APHIS-WS, Nevada Department of Agriculture, the USFWS and USGS. Federal funding in support of this program is received from the USFWS and USDA-APHIS WS.

Species

Birds considered primary candidate species in the 2007 Pacific Flyway Strategy and sampled during the Nevada surveillance efforts included tundra swan (TUSW), snow goose (SNGO) and northern pintail (NOPI). Secondary candidate species included mallard (MALL), American wigeon (AMWI), American green-winged teal (AGWT) and northern shoveler (NSHO). In Nevada, additional species tested included wood duck (WODU) (opportunistic sampling during banding operations) and gadwall (GADW). Incidental collections from cinnamon teal (CITE) and Ross's goose (ROGO) also occurred.

- TUSW: Nevada represents a portion of the winter range of the tundra swan. Birds leave breeding grounds in Alaska during the fall migration in September/October and arrive in northwestern Nevada in November/December. They return after the first spring thaw. Limited hunting for TUSW is permitted in Nevada and each year an average of 115 birds are harvested. Each harvested bird is submitted for mandatory validation by the Nevada Department of Wildlife.
- SNGO: Snow geese breed in northern Canada and northeastern Siberia and winter in the southwestern United States and beyond. SNGO are not uniformly distributed in the Nevada, are not considered common visitors and less than 1,000 birds are harvested annually.
- NOPI: Northern pintails are one of the most common visitors to northern Nevada and were found to be the second most abundant duck in the state's 2008 survey. This species is of particular interest because both North American and Asian pintails utilize the same nesting and molting areas in Russia and the species frequently migrates between Asia and North America, is abundant and often carries avian influenza viruses. In 2007, a compensatory harvest-mortality model was included in the US Fish and Wildlife Service (USFWS) Harvest Strategy for this species assuming harvest mortality to be additive to natural forms of mortality. In Nevada, therefore, harvest is currently limited to one bird per hunter and this species generally accounts for no more than 5% of the total waterfowl harvest.
- MALL: Mallards are considered the most widely distributed and abundant duck in the Northern Hemisphere. The species is similarly distributed and abundant in Nevada. Breeding records exist from all Nevada counties but it is believed that MALL generally withdraw from southern Nevada during the breeding season. Juvenile MALL are captured and banded annually allowing for live-bird sampling and both juvenile and adult MALL account for 40% of the annual waterfowl harvest in the state.



- AMWI: Wigeon are considered rare summer visitors to the Lahontan Valley area and no recent breeding records in Nevada exist. However, AMWI still constitute 6% of annual waterfowl harvest in the state allowing for adequate representation in the surveillance program.
- AGWT: Nevada represents the southern limit of the breeding range for AGWT however they are considered common visitors from early-season to freeze-up and represent 16% of harvest in Nevada. AGWT breed widely throughout north-central and northeastern Nevada.
- NSHO: Shovelers are found in major drainages and wetland systems across the northern half of Nevada and breeding has been reported only from the northern counties. They are considered very common visitors and represent 12% of waterfowl harvest in the state.

Study Area

The majority of samples were collected from two primary sites in the Lahontan Valley in western Nevada: Carson Lake Wetlands and Stillwater National Wildlife Refuge. Additional samples originated from the Mason Valley Wildlife Management Area near Yerington, NV.

With total precipitation averaging approximately 230 mm (9 inches) per year, Nevada is the most arid state in the nation. Of the precipitation that falls, only approximately 10 percent results in stream runoff and groundwater recharge; the remaining 90 percent being lost through evaporation and transpiration. Nevada's terminal lakes and wetland areas are, therefore, extremely important resources for both resident and migratory aquatic birds in this state. Opportunities for a comprehensive and representative waterfowl surveillance program for the entire state are hampered by this geographic focus but sampling efficiency can be maximized as a result. Birds congregate in these areas allowing for efficient live bird sampling during banding operations and due to limited accessibility for waterfowl hunters, harvest check stations continue to provide an effective way to gather representative statistically valid samples for analysis. The distribution pattern of waterfowl in the state also allows for highly effective morbidity and mortality surveys to be performed by agency personnel in conjunction with, or separate from, other management activities.

Sample collection and analysis

Paired cloacal and oropharyngeal swabs were collected from live birds during annual banding operations (juvenile mallards, wood ducks); from apparently healthy ducks, swans and geese presented dead at hunter check-stations and during mandatory swan validation procedures. Following collection, swabs were immediately placed in virus transport medium, refrigerated for up to 3 days, and then frozen at $<-20^{\circ}\text{C}$ until tested. All samples were tested at the Nevada Department of Agriculture's Animal Disease and Food Safety Laboratory in Reno, NV by real-time reverse transcriptase-PCR (RRT-PCR), a technique that targets a specific region of the matrix protein (M1) gene in the influenza A virus. In cases where the M1 gene sequence was detected (i.e. positive for avian influenza A virus), confirmatory testing involving follow-up RRT-PCR for H5 and H7 hemagglutinin gene segments (indicators of pathogenic virus) was performed at the USDA National Veterinary Services Laboratories (NVSL) in order to further classify the virus.

Morbidity and Mortality Surveillance

Surveys of primary surveillance sites for sick and/or dead birds occurred periodically during 2007. A total of 59 hours were spent on this activity between August, 2007 and March, 2008. Samples from dead and dying birds collected from an urban pond in Reno, NV during a suspected botulism outbreak in August 2007 yielded one Matrix (M1) positive sample derived from a cinnamon teal (CITE). Despite routine and opportunistic surveillance, no dead or dying birds were reported during the study period.

Results and Summary

According to the National HPAI Early Detection Data System (HEDDS) Update dated May 5, 2008, a total of 95,810 samples have been tested during the 2007 season across the United States. Highly pathogenic H5N1 avian influenza virus has not been detected in any of these samples. In Nevada, from a total of 1,147 samples tested, 126 samples tested positive for avian influenza A virus. Of these, only three samples were found positive for H5 and none were positive for H7. No samples tested positive for the highly pathogenic H5N1 avian influenza virus in Nevada.



RRT-PCR detected influenza A M1 gene sequence in 10.9% (n=126) and H5 gene sequence in 0.26% (n=3) of the wild bird samples collected in Nevada in the 2007 sampling period (April 1, 2007 through March 31, 2008. See Table 1 and Fig 1). No samples tested positive for the H7 gene sequence. Mallards accounted for 35% of samples, 60% of M1-positive ducks, and 33% of H5 positive ducks. 41% of the in-state origin juvenile mallards captured alive and sampled were M1-positive, accounting for 93% of M1 positive birds sampled live. Northern pintail accounted for 15% of samples and 13% of M1-positive ducks; Northern Shoveler represented 11% of samples, and 7% of M-1 positive ducks.

Despite various sampling biases considered inherent in this type of study, avian influenza viruses were commonly detected in wild waterfowl in western Nevada during the fall and winter of 2007 with M1 positive results in more than 10% of all birds tested. Based on positive M1 RRT-PCR test results, apparent prevalence of avian influenza A virus in live birds was 30%; 8% in hunter-harvested birds and 7.6% in birds found dead during morbidity/mortality investigations. The overall prevalence of H5 viruses was 0.26%, (compared to 0.4% prevalence detected in the combined Pacific Flyway samples collected in 2006). Highly pathogenic H5N1 avian influenza virus was not detected in these samples.

Table 1. Waterfowl sampled in 2007 and RRT-PCR results, Nevada*

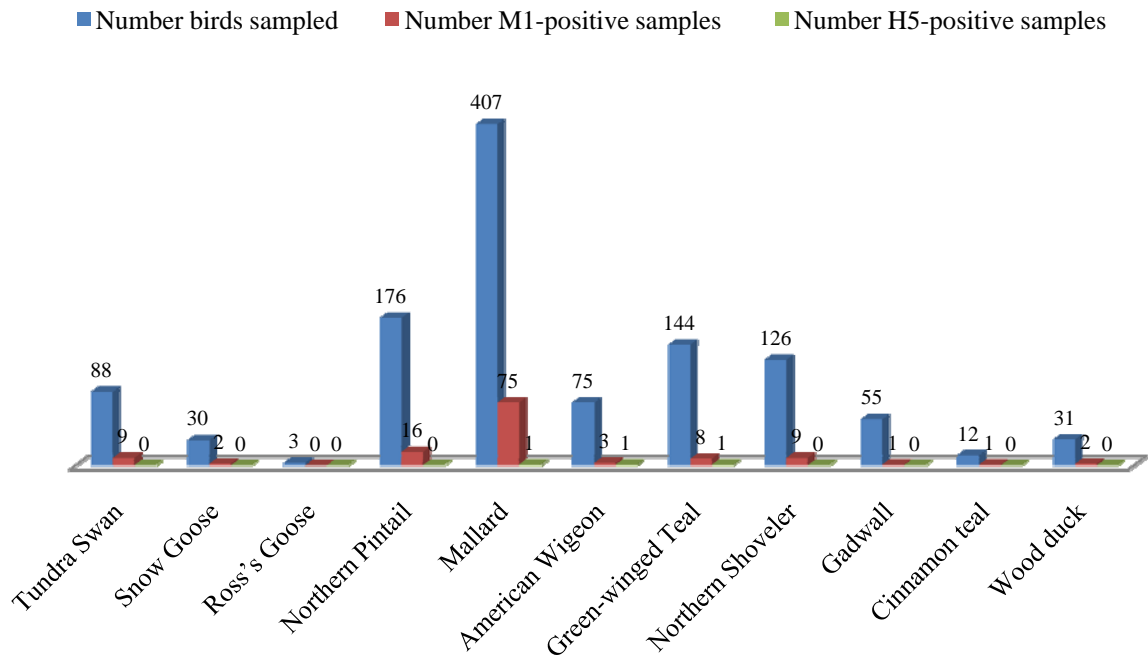
Common name	Taxonomic Name	No. sampled	No. M1-positive (%)	No. H5-positive (%)
Tundra Swan	<i>Cygnus columbianus</i>	88	9 (10)	
Snow Goose	<i>Chen caerulescens</i>	30	2 (7)	
Ross's Goose	<i>C. rossii</i>	3	0 (0)	
Northern Pintail†	<i>Anas acuta</i>	176	16 (9)	
Mallard†	<i>A. platyrhynchos</i>	407	75 (18)	1 (0.2)
American Wigeon†	<i>A. americana</i>	75	3 (4)	1 (1.3)
Green-winged Teal†	<i>A. crecca</i>	144	8 (6)	1 (0.7)
Northern Shoveler†	<i>A. clypeata</i>	126	9 (7)	
Gadwall†	<i>A. strepera</i>	55	1 (2)	
Cinnamon teal†	<i>A. cyanoptera</i>	12	1 (8)	
Wood duck‡	<i>Aix sponsa</i>	31	2 (6)	

*RRT-PCR, real-time reverse transcriptase-PCR

†Dabbling duck (Tribe Anatini)

‡Perching duck (Tribe Cairinini)

Fig 1: Waterfowl sampled and RRT-PCR results for avian influenza virus, Nevada 2007



STATE OF NEVADA HIGHLY PATHOGENIC AVIAN INFLUENZA SURVEILLANCE PLAN- 2007

Surveillance Activities Summary Date: March 31, 2008

Date of Collection:	Location:	Number										Cooperating Agency:	Date Submitted:	Testing Facility:	Findings:			Notes:		
		Pacific Flyway Strategy Candidate Spp.													RRT-PCR					
		Primary			Secondary				NV Plan Spp	Other Spp.					+ for M1:	+ for H5:	+ for H7:			
		TUSW	SNGO	NOPI	MALL Juvenile	AMWI Adult	AGWT	NSHO	WODU	GADW	CITE								ROGO	
11-Jul-07	Lahontan Valley										14							1 WODU M1 pos, H5/H7 neg		
13-Jul-07	Carson Lake				50					4	3							35 MALL, 1 GADW M1 pos, H5/H7 neg		
7-Aug-07	Mason Valley				50													6 MALL M1 Pos, H5/H7 neg		
9-Aug-07	Carson Lake										9									
2-Feb-08	Lahontan Valley										17							1 WODU M1 pos, H5/H7 neg		
Live Bird Subtotals:		0	0	0	100	0	0	0	0	4	31	12	0	0	0			44	0	0
Hunter Killed	13-Oct-07	Stillwater				14	23													
	13-Oct-07	Mason Valley			10	24														
	13-Oct-07	Carson Lake			10	40	25		20	30	21									
	13-Oct-07	Stillwater			12	4	4		21	32	21									
	11-Nov-07	Stillwater	1		3	3	1	1	20	1										
	11-Nov-07	Stillwater	4						2											
	14-Nov-07	Stillwater	1		2	10	4		7											
	15-Nov-07	Stillwater	1		1	1			2											
	16-Nov-07	Stillwater	6			11	6													
	17-Nov-07	Stillwater	8		1				1											
	21-Nov-07	Stillwater	1				1	3												
	23-Nov-07	Stillwater	2		1	1		2												
	24-Nov-07	Carson Lake			20	7	6	10	20	11	32									
	24-Nov-07	Stillwater	1																	
	24-Nov-07	Stillwater						1	3	1		1								
	25-Nov-07	Stillwater					1	1												
	25-Nov-07	Stillwater	1		7	10	3	7	17	17										
	25-Nov-07	Stillwater	5																	
	28-Nov-07	Carson Lake			3		1		20											
	29-Nov-07	Stillwater	1		8	17	3	18												
	30-Nov-07	Stillwater	8		10	3		7												
	1-Dec-07	Carson Lake	2		14	21	13													
	2-Dec-07	Carson Lake			14	13	11													
	2-Dec-07	Stillwater	1	3	18	38	10	8	7			1								
	5-Dec-07	Stillwater	2		7		6													
	7-Dec-07	Stillwater	8		1															
	7-Dec-07	Stillwater	2		5															
	8-Dec-07	Carson Lake			4															
	10-Dec-07	Stillwater	4		5															
	12-Dec-07	Carson Lake	1		6	5						2								
	15-Dec-07	Stillwater	3		5															
	15-Dec-07	Carson Lake	1		4		1													
	18-Dec-07	Stillwater	2																	
19-Dec-07	Carson Lake			5																
19-Dec-07	Stillwater			7																
21-Dec-07	Stillwater	1																		
22-Dec-07	Carson Lake			3																
24-Dec-08	Stillwater	3		1																
8-Jan-08	Lahontan Valley	3		3																
6-Jan-08	Stillwater	8	1																	
6-Jan-08	Stillwater	4																		
5-Feb-08	S-Line Res	3																		
Hunter Killed Subtotals:		88	30	176	176	130	75	144	122	0	43	0	3	0			81	0	0	
MS&M	27-Aug-07	S.Meadows				1													1 CITE M1 pos, H5/H7 neg	
	Mortality/Morbidity Subtotals:		0	0	0	1	0	0	0	0	0	0	0	0	0			1	0	0
TOTALS:		88	30	176	277	130	75	144	126	31	55	12	3	0			126	3	0	
Objectives:		Live Bird:				100				30		50		Notes: 2007sampling season runs from April 1, 2007 through March 31, 2008.						
		Hunter Killed:				75		170		300		75		100						
TOTAL Plan Objectives:						1000				TOTAL Samples Collected:		1147								