



## **SURVEILLANCE FOR HIGHLY PATHOGENIC AVIAN INFLUENZA IN MINNESOTA'S WILD BIRDS IN 2016-17**

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### **SUMMARY OF FINDINGS**

Surveillance for highly pathogenic avian influenza (HPAI) virus in wild birds is a national priority in the United States. Outbreaks of HPAI continue to occur in domestic poultry, wild birds, and people in Asia and Europe, and there is concern that these viruses may be introduced into North America. The Minnesota Department of Natural Resources (MNDNR) has partnered with the United States Department of Agriculture's Wildlife Services (USDA-WS) since 2007 to conduct HPAI surveillance in wild birds, but it wasn't until 2015 that a highly pathogenic strain of H5N2 was detected in Minnesota. Since detection in a poultry facility in Pope County MN on February 27 2015, MNDNR extended our partnership with the United States Geological Survey's National Wildlife Health Center (USGS), the United States Fish and Wildlife Service (USFWS), and the University of Minnesota (UMN) to conduct surveillance for any HPAI virus subtypes in Minnesota wild birds. The H5N2 HPAI virus strain is a combination of the highly pathogenic Eurasian H5 and low pathogenic North American H2 subtypes. Since June 2015, there have been no detections of HPAI in MN poultry facilities or in wild birds sampled in MN. From May 2016 through March 2017, the MNDNR and partners collected swab samples from 1,065 dabbling ducks across six watersheds; AI viral material was detected in 17% of these samples. Only 2.2% and 0.3% of all samples contained detectable H5 and H7 viral material, respectively. The highest apparent prevalence of LP AI was in the St. Croix watershed at 44%, and the lowest was 10.4% in the Red watershed. Only three successful viral isolates were recoverable and included H2N3 (Mississippi Headwaters watershed), H10N7 (Upper Mississippi-Black Root watershed), and H4N9 (Upper Mississippi-Black Root watershed). All of these samples were collected as part of the 2016 USDA national surveillance efforts with oropharyngeal/tracheal and cloacal samples combined. No HPAI positive cases were found. In partnership with the UMN, MNDNR began a collaboration on a project to investigate avian influenza dynamics in ring-billed gulls (*Larus delawarensis*) across Minnesota; results are pending.

### **INTRODUCTION**

Avian Influenza (AI) is a viral infection that occurs naturally in wild birds, especially waterfowl, gulls, and shorebirds. It is caused by type A influenza viruses that have 2 important surface antigens, hemagglutinin (H) and neuraminidase (N), that give rise to 144 possible virus subtypes. Influenza viruses vary widely in pathogenicity and ability to spread among birds. The emergence of an Asian strain of highly pathogenic avian influenza (HPAI) H5N1 virus in 1996, and subsequent spread of the virus in Asia, Africa, and Europe, killed thousands of wild birds and millions of domestic poultry. In 1997, HPAI H5N1 became zoonotic in Hong Kong and to-date has infected at least 859 humans around the world, resulting in 453 deaths (World Health

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Organization 2017). Furthermore, since 2013 HPAI H7N9 has been confirmed in 1,552 people (mostly in eastern Asia) with 596 deaths (Food and Agriculture Organization 2017). As of 6 June 2017, there were 51 ongoing outbreaks of HPAI in wild birds in Asia and Europe; these include strains of the subtypes H5N1, H5N2, H5N5, and H5N8 (OIE 2017). Furthermore, there were 667 ongoing outbreaks of HPAI subtypes H5N1, H5N2, H5N6, H5N8, H7N3, and H7N9 in domestic poultry operations around the world (World Organisation for Animal Health 2017). There have been no HPAI outbreaks in the US since March 2017, when an American strain of H7N9 was confirmed in domestic poultry facilities in Tennessee (USDA 2017). These results highlight that HPAI viruses continue to be active around the world and pose a threat to both wild birds and domestic poultry. The diversity of active highly pathogenic subtypes, coupled with the ability of avian influenza strains to mutate quickly underscores the pandemic risk from these viruses. As such, there is an urgent need to understand transmission dynamics, host-species susceptibility, and the role of the environment in AI dynamics.

Since the first Minnesota detection of HPAI H5N2 in March 2015, the MNDNR has collected over 7,500 samples for AI testing, which is the most of any state in the Mississippi flyway.

The migratory movements of waterfowl and other shorebirds and subsequent mixing of birds from Asia and North America in the northern latitude breeding grounds likely facilitated the mixing of low pathogenicity avian influenza (LPAI) and HPAI strains (Pasick et al. 2015). Such mixing resulted in discovery of 3 reassortant highly pathogenic strains including H5N1 (World Organisation for Animal Health 2014), H5N2 (World Organisation for Animal Health 2014, Pasick et al. 2015), and H5N8 (Ip et al. 2015) in British Columbia and the western United States in 2014.

In August and December of 2016, HPAI H5N2 was again detected in wild waterfowl in Alaska and Montana, respectively. In addition, there were several detections of HPAI H7N9 (distinct from Asian strain) that affected domestic poultry facilities in the Mississippi flyway. Since July 2015, there have been over 80,000 wild waterfowl tested in the continental US and only 4 positive HPAI detections have occurred. Our efforts to detect HPAI in wild birds, if present, include live-bird and hunter-harvest sampling of waterfowl and the continued monitoring of morbidity/mortality events. These efforts permit the estimation of temporal and spatial detection limits for AI on the Minnesota landscape, which leads to development of specific hypotheses that can help us understand AI dynamics in wild birds.

## **METHODS**

We collected samples for AI testing from three sources: public- or agency-reported morbid or dead wild birds (i.e., morbidity and mortality events), live-captured and released ducks through banding programs, and hunter-harvested ducks. Dabbling ducks were primarily sampled, including mallard (*Anas platyrhynchos*), blue-winged teal (*A. discors*), American green-winged teal (*A. crecca*), American wigeon (*A. americana*), gadwall (*A. strepera*), American black duck (*A. rubripes*), northern pintail (*A. acuta*), northern shoveler (*A. clypeata*), wood duck (*Aix sponsa*), and ring-necked duck (*Aythya collaris*). Morbidity and mortality samples depended on opportunistic circumstances and public willingness to report or submit dead birds, and were collected statewide. Sampling live wild ducks and hunter-harvested ducks afforded more control over sampling design elements; both spatial and temporal dimensions were within our design control.

## **USDA National Plan Sampling**

As part of the 2016 USDA National Surveillance Plan (USDA 2016a), which called for 1,040 oropharyngeal/tracheal and cloacal swab samples from dabbling ducks in MN, the MNDNR partnered with both USFWS and UMN to achieve the sample goal between summer and winter 2016. As part of an independent winter avian influenza study, Dr. Patrick Redig and his technicians assisted in our efforts to achieve our winter sampling goals. The samples collected were broken down by watershed (Minnesota, Mississippi Headwaters, Red River, St. Croix, Upper Mississippi – Black Root, and Western Lake Superior) and season (summer, fall, and winter). The source of samples was from live waterfowl or hunter-harvested waterfowl. We collected swab samples from the oropharyngeal cavity or trachea (depending on live or dead birds) and cloacal cavities of each bird in order to test for viral shedding. Both swab samples from a bird were placed in the same brain-heart infusion (BHI) media, and kept cool in a portable cooler with ice packs or a refrigerator. Samples were shipped overnight to the US Geological Survey National Wildlife Health Center (USGS) for avian influenza virus (AIV) testing using a real time reverse transcription polymerase chain reaction (rRT-PCR) matrix test, which tests for type-A influenza virus RNA. Material from positive matrix tests were further tested with an H5 and H7 assay. If either H5 or H7 assay were positive, the remaining sample material was sent to the National Veterinary Services Laboratories in Ames, IA for confirmation and strain-typing.

## **Morbidity and Mortality Sampling**

Through outreach on the MNDNR and Minnesota Board of Animal Health websites and official press releases, we solicited the public and agency staff to report any wild birds exhibiting neurological symptoms consistent with AIV infection anywhere in the state. We investigated reports of dead ducks if circumstances of mortality were unclear and if individuals showed neurologic signs. We emphasized the need to report dead birds as soon as possible to ensure collection of viable tissue samples; generally we only collected samples from birds that were deceased for <24 hours. Depending on the resources available for staff (e.g., BHI media and swabs), we either collected whole carcasses (double-bagged and frozen) or swabs from the trachea and cloaca of dead birds. Both swab samples from a morbidity/mortality sample bird were placed in the same BHI media, and kept cool in a portable cooler with ice packs or a refrigerator. Whole carcasses were shipped overnight to the USGS National Wildlife Health Center or the University of Minnesota's Veterinary Diagnostic Laboratory for necropsy and AIV testing using real time reverse transcription polymerase chain reaction (rRT-PCR) test, which tests for AIV RNA. Swab samples were submitted to the US Department of Agriculture National Wildlife Disease Laboratory (USDA) in Fort Collins, CO. If samples tested AIV positive initially at any lab, they were forwarded to the National Veterinary Services Laboratories in Ames, IA for confirmation and strain-typing. We had no fixed sample goal for this surveillance effort due to the opportunistic nature of public discovery and reporting of sick or dead birds. We used these data as an auxiliary source of information in our surveillance efforts.

## **RESULTS AND DISCUSSION**

From May 2016 through March 2017, the MNDNR in partnership with USDA-WS and the USFWS collected 1,065 oropharyngeal/tracheal and cloacal samples from dabbling ducks across 6 watersheds of Minnesota (Figure1). As part of the USDA national surveillance plan, Minnesota was asked to provide 1,040 samples towards their goals, and we slightly exceeded that number (Table 1). While about 17% of all samples were positive for LPAI, this aligns with expectations of type-A avian influenza prevalence in waterfowl (Webster et al. 1992). Only 3 LPAI subtypes were isolated from positive samples, and they included H2N3, H4N9, and H10N7. This result underscores the difficulty of acquiring enough viral material in swab

samples to successfully identify AI subtypes. We did not detect HPAI in any samples. Of particular note is that apparent prevalence of H5 and H7 LPAI subtypes across all samples were 2.2% and 0.3% - these subtypes are typically what can become highly pathogenic.

From August 1 2016 through June 15 2017, we collected 14 morbidity and mortality samples from wild birds. Of these submissions (1 American crow, 4 common terns, 2 mallards, 2 trumpeter swans, 2 snow geese, 1 red-tailed hawk, 1 wild turkey, and 1 house finch), none tested positive for HPAI (Table 2). In June 2016, MNDNR partnered with UMN and USGS to collect and test oropharyngeal and cloacal samples (combined) from 200 common terns in a breeding colony at Interstate Island near Duluth, MN. Avian influenza was not detected in any of the samples.

Since the outbreak of HPAI began in Minnesota poultry in March 2015, through June 2017, the MNDNR and partners have collected and tested over 7,500 samples for HPAI, which included waterfowl feces (Jennelle et al. 2016), reported wild bird mortalities, hunter-harvested waterfowl, live waterfowl, and wild turkeys (Jennelle et al. 2017). To date, there has been only one confirmed HPAI H5N2 positive result in 2015, a likely spillover species - Coopers Hawk (predator of small birds) (Jennelle et al 2016). The positive hawk was only 12 miles from an infected poultry facility. The final report on the 2014-2015 HPAI outbreak in the US, the largest outbreak in the US, indicated that 7.4 million domestic turkeys and 43 million egg-layers/pullet chickens were impacted, costing nearly a billion dollars for the response, indemnity, and future preparedness actions (USDA 2016b). The report highlights poultry facility biosecurity as a major concern and likely contributor to the spread and broad impact of the outbreak (USDA 2016b).

### **Current Projects and Future Surveillance**

The MNDNR is collaborating on a newly funded Legislative-Citizen Commission on Minnesota Resources (LCCMR) project led by Dr. Marie Culhane of UMN to investigate AI prevalence, exposure, and potential health effects on ring-billed and herring gulls across Minnesota. Other partners in this effort include the USFWS, USDA-WS, and MN Turkey Growers Association. The study began in fall 2016 and will continue through 2017, with field efforts led by M.S. student Todd Froberg who is supervised by Dr. Francie Cuthbert. Field sampling efforts have focused on capture and sampling of gulls at landfills, farm fields, and gull colonies across Minnesota. Thus far the team has collected oropharyngeal, cloacal, and blood samples from over 700 ring-billed gulls across MN. The results are pending, and data collection is expected to be completed by December 2017.

As part of the USDA national surveillance plan for 2017, Minnesota has a goal of 1,140 samples from dabbling ducks for avian influenza sampling and testing. Varying sample sizes will be requested from 5 to 6 watersheds across Minnesota spanning summer, fall, and winter seasons in 2017.

MNDNR sampling and testing of morbidity and mortality events is ongoing. We have adopted a risk-based approach to AIV surveillance in wild birds designed to respond to new detection events in a rapid and efficient manner. Three triggers will initiate intensive, and spatially and temporally designed AI surveillance efforts; if HPAI virus is detected in (1) wild, migratory birds in Minnesota through ongoing morbidity and mortality surveillance, (2) wild migratory birds in the Mississippi flyway, or (3) commercial or backyard poultry in Minnesota.

## ACKNOWLEDGMENTS

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Table 1. Avian influenza swab results (n = 1,065) from Minnesota participation in the 2016 USDA National plan\*.

Watershed	n	Type-A	LP AI %	HP AI %
Minnesota	331	48	14.5	0
Mississippi Headwaters	272	52	19.1	0
Red	221	23	10.4	0
St. Croix	36	16	44.4	0
Upper Mississippi-Black Root	143	33	23.1	0
Western Lake Superior	62	7	11.3	0
TOTAL	1,065	179	16.8	0

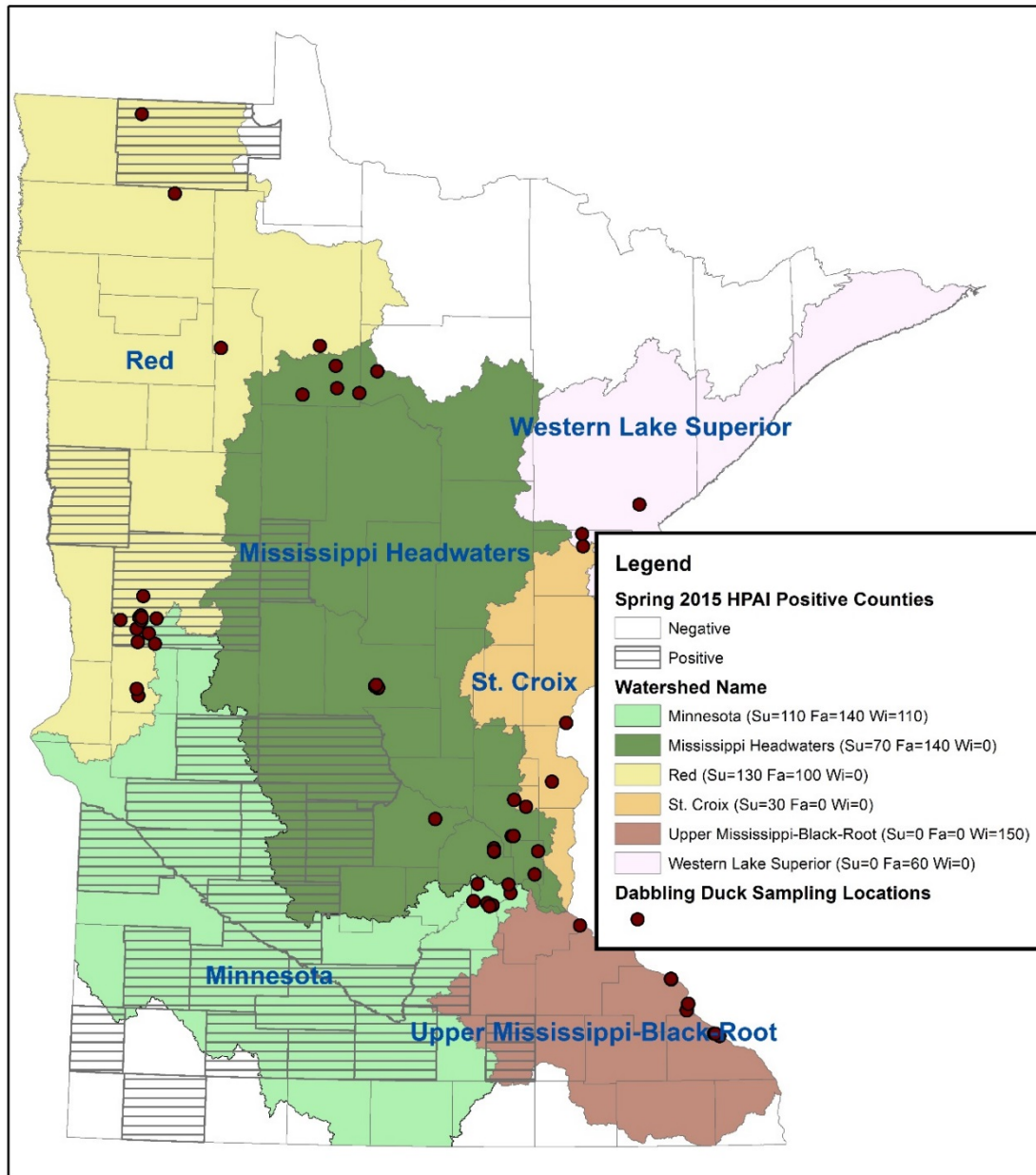
\*There were only 3 successful isolations of type-A influenza completed from these samples H2N3, H4N9, and H10N7

Table 2. Species and count of wild bird morbidity & mortality samples (n = 14) submitted by the Minnesota Department of Natural Resources for avian influenza testing from 01 August 2016 to 15 June 2017. No birds tested positive for HP AI.

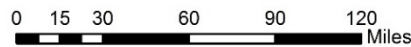
Agency	Species sampled	n*
MNDNR	American crow ( <i>Corvus brachyrhynchos</i> )	1
	Common Tern ( <i>Sterna hirundo</i> )	4
	House finch ( <i>Haemorphous mexicanus</i> )	1
	Mallard ( <i>Anas platyrhynchos</i> )	2
	Red-tailed hawk ( <i>Buteo jamaicensis</i> )	1
	Snow goose ( <i>Anser caerulescens</i> )	2
	Trumpeter swan ( <i>Cygnus buccinator</i> )	2
	Wild turkey ( <i>Meleagris gallopavo</i> )	1
	Total	14

\*Note that multiple birds may have been submitted for a given location and time.

## 2016 USDA Avian Influenza Surveillance: Summer (n=340) Fall (n=440) Winter (n=260)



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**mn** DEPARTMENT OF NATURAL RESOURCES

Figure 1. The United States Department of Agriculture (USDA) allocation of targeted Minnesota watersheds for avian influenza sampling (n=1,040) for summer, fall, and winter 2016. The 3 sample sizes noted beside watersheds in the legend are the quotas requested by USDA for summer, fall, and winter sampling, respectively. Note we exceeded our quota by 25 samples.