



## SURVEILLANCE FOR HIGHLY PATHOGENIC AVIAN INFLUENZA IN MINNESOTA'S WILD BIRDS IN 2017-18

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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. The HPAI outbreak of 2015 resulted in culling of over 50 million domestic turkeys and chickens and had an economic cost on the order of a billion dollars. Outbreaks of HPAI continue to occur in domestic poultry, wild birds, and people around the globe, and there is continued concern for the introduction of HPAI viruses into North America. 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, and in 2015 a highly pathogenic strain of H5N2 was detected in Minnesota. The H5N2 HPAI virus strain discovered in Minnesota was a combination of the highly pathogenic Eurasian H5 and low pathogenic North American H2 subtypes. Since the 2015 HPAI outbreak, which affected 110 poultry facilities and led to the euthanasia of more than 9 million turkeys and chickens in Minnesota, MNDNR extended its partnership with the United States Geological Survey's National Wildlife Health Center (USGS), the United States Fish and Wildlife Service (USFWS), the Michigan State University Veterinary Diagnostic Lab (MSU), and the University of Minnesota (UMN) to conduct surveillance for HPAI virus subtypes in Minnesota wild birds. Only one wild bird, a Cooper's hawk (*Accipiter cooperii*), was confirmed with the HPAI H5N2 strain in 2015 and was likely infected as a spillover from the poultry infections in the area. Since June 2015, there have been no detections of HPAI in MN poultry facilities or in wild birds sampled in Minnesota. As part of the USDA National Avian Influenza Surveillance plan, from May 2017 through March 2018, the MNDNR and partners collected cloacal and oropharyngeal swab samples (combined) from 1,130 dabbling ducks across 6 watersheds; AI viral material was detected in 15.4% of these samples. Only 1.5% and 0.2% of all samples contained detectable H5 and H7 viral material, respectively. The highest apparent prevalence of low pathogenicity avian influenza (LPAI) was in the St. Croix watershed at 20%, and the lowest was 1% in the Red watershed. Only 5 successful viral isolates of LPAI virus were recoverable and included H5N2 (Mississippi Headwaters watershed), H7N3 (Minnesota watershed), H3 (St. Croix watershed), N2 (St. Croix watershed), and N8 (St. Croix watershed). No HPAI positive cases were detected. In addition, the MNDNR partnered with UMN, USFWS, and USDA-WS in a study led by the UMN to investigate avian influenza dynamics in ring-billed gulls (*Larus delawarensis*) across Minnesota.

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## 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 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 860 humans around the world, resulting in 454 reported deaths (World Health Organization 2018). Another strain of HPAI, H7N9, emerged in 2013 and has been confirmed in 1,625 people (mostly in eastern Asia) with 623 deaths (Food and Agriculture Organization 2018). As of February 28 2018, there were 8 countries and territories reporting HPAI outbreaks in wild birds; these included Denmark, Hong Kong, India, Ireland, Netherlands, South Africa, Sweden, and the United Kingdom reporting strains of the subtypes H5N1, H5N6, and H5N8 (OIE 2018). There were 37 outbreaks of 6 HPAI subtypes in domestic poultry operations around the world (World Organisation for Animal Health 2018). 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, the role of wild birds in transmission, and the role of the environment in AI dynamics.

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 LPAI and HPAI strains in 2014 (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 112,734 wild waterfowl tested for avian influenza in the United States as part of national surveillance efforts and only four positive HPAI detections have occurred. Since the first Minnesota detection of HPAI H5N2 in a domestic poultry farm in March 2015, the MNDNR has collected over 8,600 samples from wild birds and the environment for AI testing with only one positive detection (Cooper's hawk with H5N2 in 2015). Our efforts to detect HPAI in wild birds, if present, have included live-bird and hunter-harvest sampling of waterfowl, environmental sampling, and the continued monitoring of morbidity and 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. From a broader perspective, our efforts also help to address the role that wild birds may play in HPAI dynamics.

## METHODS

We collected samples for AI testing from 3 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*), and wood duck (*Aix sponsa*). Morbidity and mortality samples were collected statewide but depended on opportunistic circumstances and public willingness to report or submit dead birds. 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 2017 USDA National Surveillance Plan, which called for 1,140 oropharyngeal/tracheal and cloacal swab samples from dabbling ducks in MN, the MNDNR partnered with USFWS, USDA-WS, and MSU to achieve the sample goal between summer and winter 2017. The samples collected were broken down by watershed (Minnesota, Mississippi Headwaters, Red, 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 sampling live or dead birds) and cloacal cavities of each bird in order to test for viral shedding. Both swab samples from an individual 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 MSU 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. Swab samples were submitted to the USDA National Wildlife Disease Laboratory in Fort Collins, CO. If initial screening of samples via the matrix PCR test were AIV positive at any lab, these samples 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 2017 through March 2018, the MNDNR in partnership with USDA-WS and the USFWS collected 1,130 oropharyngeal/tracheal and cloacal samples from dabbling ducks across 6 watersheds of Minnesota (Figure 1). We were short 24 samples for the St. Croix watershed, 33 samples for the Western Lake Superior watershed, and 7 samples for the Mississippi Headwaters (Table 1). However, we exceeded the watershed quota by 31 for the

Minnesota watershed, 21 for the Upper-Mississippi Black Root watershed, and 2 samples for the Red watershed (Table 1). We sampled 577 mallards, 369 wood ducks, 153 blue-winged teal, 20 American green-winged teal, 5 gadwall, 3 northern pintail, 1 American wigeon, 1 northern shoveler, and 1 mallard/black duck hybrid. While about 15.4% of all samples tested positive for LPAI, this aligns with expectations of type-A avian influenza prevalence in waterfowl (Webster et al. 1992). Only 5 LPAI subtypes were isolated (2.9% of all positive samples), and they included H5N2, H7N3, H3, N8, and N2. These results underscore the difficulty of acquiring enough viral material in swab samples to successfully identify AI subtypes. We did not detect HPAI virus in any samples. Of particular note is the apparent prevalence of H5 and H7 LPAI subtypes across all samples at 1.5% and 0.2% - these subtypes are typically considered to be at highest risk for becoming highly pathogenic.

From June 16, 2017 through June 14, 2018, we collected 16 morbidity and mortality samples from wild birds. Of these submissions (2 Canada geese, 8 mallards, 4 ring-billed gulls, and 2 trumpeter swans), none tested positive for HPAI (Table 2).

Since the outbreak of HPAI began in Minnesota poultry in March 2015, the MNDNR and partners have collected and tested over 8,600 wild bird and environmental samples for HPAI, which included waterfowl feces (Jennelle et al. 2016), reported wild bird mortalities, hunter-harvested waterfowl, live waterfowl, and hunter-harvested wild turkeys (Jennelle et al. 2017). To date, there has been only one confirmed HPAI H5N2 positive result, a likely spillover species – Cooper's hawk (predator of small birds) (Jennelle et al. 2016). The positive hawk was discovered in 2015, 12 miles from an infected poultry facility. The final report on the 2014-2015 HPAI outbreak in the U.S., the largest outbreak in the U.S. to date, 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 collaborated on a Legislative-Citizen Commission on Minnesota Resources (LCCMR) funded project, led by Dr. Marie Culhane of UMN, to investigate AI prevalence, exposure, and potential health effects on ring-billed (*Larus delawarensis*) and herring (*L. argentatus*) gulls across Minnesota. Other partners in this effort included the USFWS, USDA-WS, and MN Turkey Growers Association. The study began in fall 2016 with data collection through fall 2017 and led by Master's degree student Todd Froberg. Field sampling efforts focused on capture and sampling of ring-billed gulls at landfills and breeding colonies across MN. The research team collected oropharyngeal, cloacal, and blood samples from over 1,346 ring-billed gulls across the study area. The results suggest significant seasonal, spatial, and age-specific heterogeneity in type-A influenza apparent prevalence (Froberg 2018). Furthermore, the results show that there is significant variation in apparent prevalence between samples collected from the oropharyngeal and cloacal cavities.

At this point in time, there are no plans to continue the USDA national surveillance effort in 2018 to detect HPAI. In the future, if there is sufficient cause and available funding for AI surveillance efforts, Minnesota will participate in sampling dabbling ducks according to protocols outlined by the USDA.

The MNDNR sampling and testing of morbidity and mortality events is ongoing. We continue to apply 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 initiate intensive, 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.

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## LITERATURE CITED

- Food and Agriculture Organization. 2018. H7N9 Situation Update. [cited 2018 June 14]. [http://www.fao.org/ag/againfo/programmes/en/empres/h7n9/situation\\_update.html](http://www.fao.org/ag/againfo/programmes/en/empres/h7n9/situation_update.html).
- Froberg, T. 2018. Seasonal dynamics of avian influenza viruses in ring-billed gulls (*Larus delawarensis*) in Minnesota. Unpublished master's thesis, The University of Minnesota, St. Paul, Minnesota.
- Ip, H.S., M.K. Torchetti, R. Crespo, P. Kohrs, P. DeBruyn, K.G. Mansfield, T. Baszler, L. Badcoe, B. Bodenstein, V. Shearn-Bochsler, M.L. Killian, J.C. Pedersen, N. Hines, T. Gidlewski, T. DeLiberto, and J.M. Sleeman. 2015. Novel Eurasian highly pathogenic avian influenza A H5 viruses in wild birds, Washington, USA, 2014. *Emerging Infectious Diseases* 21: 886-890.
- Jennelle, C.S., M. Carstensen, E. Hildebrand, L. Cornicelli, P. Wolf, D.A. Grear, H.S. Ip, K.K. Vandalen, and L.A. Minicucci. 2016. Surveillance for highly pathogenic avian influenza virus in wild birds during outbreaks in domestic poultry, Minnesota, USA, 2015. *Emerging Infectious Diseases* 22:1278-1282.
- Jennelle, C.S., M. Carstensen, E. Hildebrand, P. Wolf, D.A. Grear, H.S. Ip, and L. Cornicelli. 2017. Surveillance for highly pathogenic avian influenza in wild turkeys (*Meleagris gallopavo*) of Minnesota during outbreaks in domestic poultry. *Journal of Wildlife Diseases* 53: 616-620.
- Pasick, J., Y. Berhane, T. Joseph, V. Bowes, T. Hisanaga, K. Handel, and S. Alexanderson. 2015. Reassortant highly pathogenic Influenza A H5N2 virus containing gene segments related to Eurasian H5N8 in British Columbia, Canada, 2014. *Scientific Reports* 5, 9484.
- US Department of Agriculture. 2016a. Surveillance plan for highly pathogenic avian influenza in wild migratory birds in the United States. 26p.
- US Department of Agriculture. 2016b. Final report for the 2014-2015 outbreak of highly pathogenic avian influenza (HPAI) in the United States. 47p.
- US Department of Agriculture. 2017. 2017 highly pathogenic avian influenza. [cited 2017 June 25]. <https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/avian-influenza-disease/defend-the-flock/2017-hpai>
- Webster, R.G., W.J. Bean, O.T. Gorman, T.M. Chambers, and Y. Kamaoka. 1992. Evolution and ecology of influenza A viruses. *Microbiological Review* 56:152-179.
- World Health Organization. 2018. Cumulative number of confirmed human cases of avian influenza A (H5N1) reported to WHO. [cited 2018 June 14]. [http://www.who.int/influenza/human\\_animal\\_interface/2018\\_05\\_28\\_tableH5N1.pdf?ua=1](http://www.who.int/influenza/human_animal_interface/2018_05_28_tableH5N1.pdf?ua=1)

World Organisation for Animal Health. 2014. Summary of immediate notifications and follow-ups–2014. Highly pathogenic avian influenza [cited 2016 July 15].

[http://www.oie.int/wahis\\_2/public/wahid.php/Diseaseinformation/Immsummary](http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/Immsummary)

World Organisation for Animal Health. 2018. OIE situation report for avian influenza-02/28/2018. [cited 2018 June 14].

[http://www.oie.int/fileadmin/Home/eng/Animal\\_Health\\_in\\_the\\_World/docs/pdf/OIE\\_AI\\_situation\\_report/OIE\\_SituationReport\\_AI\\_February2018.pdf](http://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/OIE_AI_situation_report/OIE_SituationReport_AI_February2018.pdf)

Table 1. Avian influenza swab results ( $n = 1,130$ ) from Minnesota participation in the 2017 USDA National Avian Influenza Surveillance plan \*.

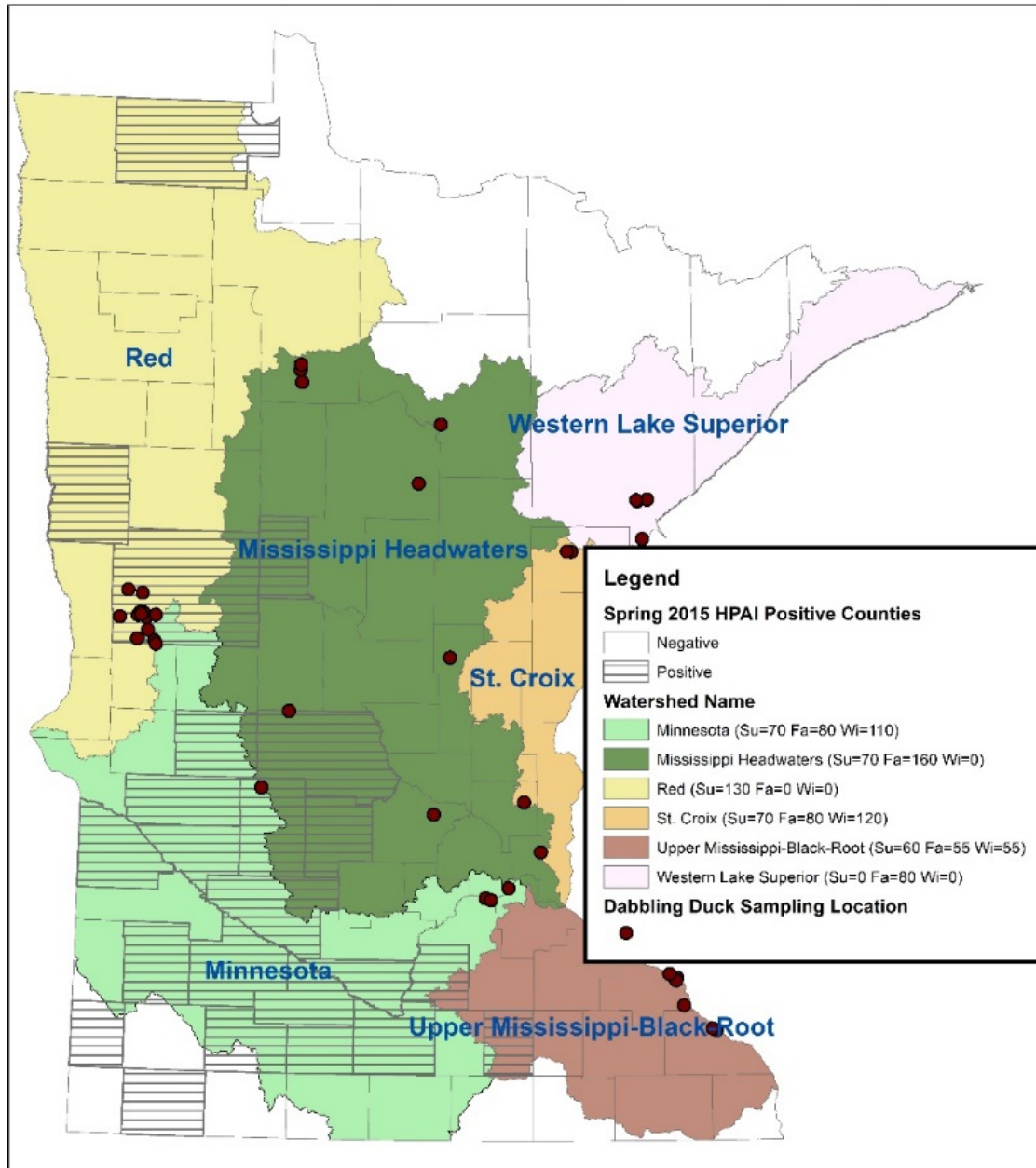
Watershed	n	Type-A	LPAI %	95% C.I.
Minnesota	291	46	15.8	11.8, 20.5
Mississippi Headwaters	223	37	16.6	12.0, 22.1
Red	132	1	0.8	0.1, 4.1
St. Croix	246	48	19.5	14.8, 25.0
Upper Mississippi-Black Root	191	35	18.3	13.1, 24.6
Western Lake Superior	47	7	14.9	6.2, 28.3
TOTAL	1,130	174	15.4	13.3, 17.6

\*There were only five successful isolations of type-A influenza completed from these samples H5N2, H7N3, H3, N2, and N8.

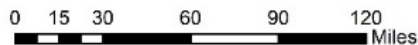
Table 2. Species and count of wild bird morbidity & mortality samples ( $n = 16$ ) submitted by the Minnesota Department of Natural Resources for avian influenza testing from June 16, 2017 to June 14, 2018. No birds tested positive for HPAI.

Agency	Species sampled	n
MNDNR	Canada goose ( <i>Branta canadensis</i> )	2
	Mallard ( <i>Anas platyrhynchos</i> )	8
	Ring-billed gull ( <i>Larus delawarensis</i> )	4
	Trumpeter swan ( <i>Cygnus buccinator</i> )	2
Total		16

## 2017 USDA Avian Influenza Surveillance: Summer (n=400) Fall (n=455) Winter (n=285)



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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=1,140) for summer, fall, and winter 2017. The three sample sizes noted beside watersheds in the legend are the quotas requested by USDA for summer, fall, and winter sampling, respectively. Points represent locations of sampling.