



Surveillance Plan for Highly Pathogenic Avian Influenza (HPAI) in Minnesota Wildlife

2015



Updated September, 2015

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Introduction

In 2006, the Minnesota Department of Natural Resources (DNR) developed a comprehensive surveillance and response plan for highly pathogenic H5N1 avian influenza in wild birds (Appendix A). While that plan was specific to the H5N1 virus, several sections included thorough discussions of virus etiology, transmission, epidemiology, and clinical signs. Consequently, this document should be considered additive to the 2006 plan.

With the discovery of H5N2 highly pathogenic avian influenza (HPAI) in a Minnesota turkey farm in February 2015, DNR has focused intensive efforts on wild bird surveillance. While DNR is not conducting HPAI research at this time, our focus is on monitoring and surveillance of wildlife to help aid in the detection and response to HPAI. However, we are collaborating with a number of research institutions who are trying to learn more about the transmission dynamics of this disease. Those collaborative efforts are described at the end of this document.

The DNR works in concert with other state and federal agencies to coordinate surveillance for avian influenza in wildlife, educate stakeholders on disease issues and formulate and implement wildlife disease response plans. Ultimately, the intent of surveillance is to, 1) identify the potential distribution of avian influenza viruses in both areas with outbreaks in domestic birds and areas where infections in domestic birds have not been documented, 2) detect the apparent spread of avian influenzas of interest to new areas of concern, 3) estimate and describe overall prevalence of avian influenza virus in wild waterfowl in Minnesota, and 4) determine if temporal and spatial risk factors are associated with wild birds so the public and poultry industry can modify biosecurity practices accordingly.

To paraphrase from the USDA plan, the ultimate goal for this sampling effort is to provide information to inform and improve management actions that are taken to address the multitude of issues associated with HPAIVs. This includes risks to commercial poultry, backyard poultry, game bird farms, wild birds, wild bird rehabilitation facilities, falconry birds, and captive bird collections in zoos/aviaries. The Minnesota DNR goals align with the USDA National Implementation plan, which was under development at the time of these outbreaks.

This document describes DNR's initial response, surveillance to date, and fall 2015 surveillance plans for avian influenza viruses primarily in wild waterfowl, following the late winter-spring 2015 outbreak. Collaborating agencies include the U.S. Department of Agriculture-Wildlife Services (USDA-WS) and National Wildlife Research Center; the United States Geological Survey, National Wildlife Health Center; the University of Georgia; the University of Minnesota; and the U.S. Fish and Wildlife Service.

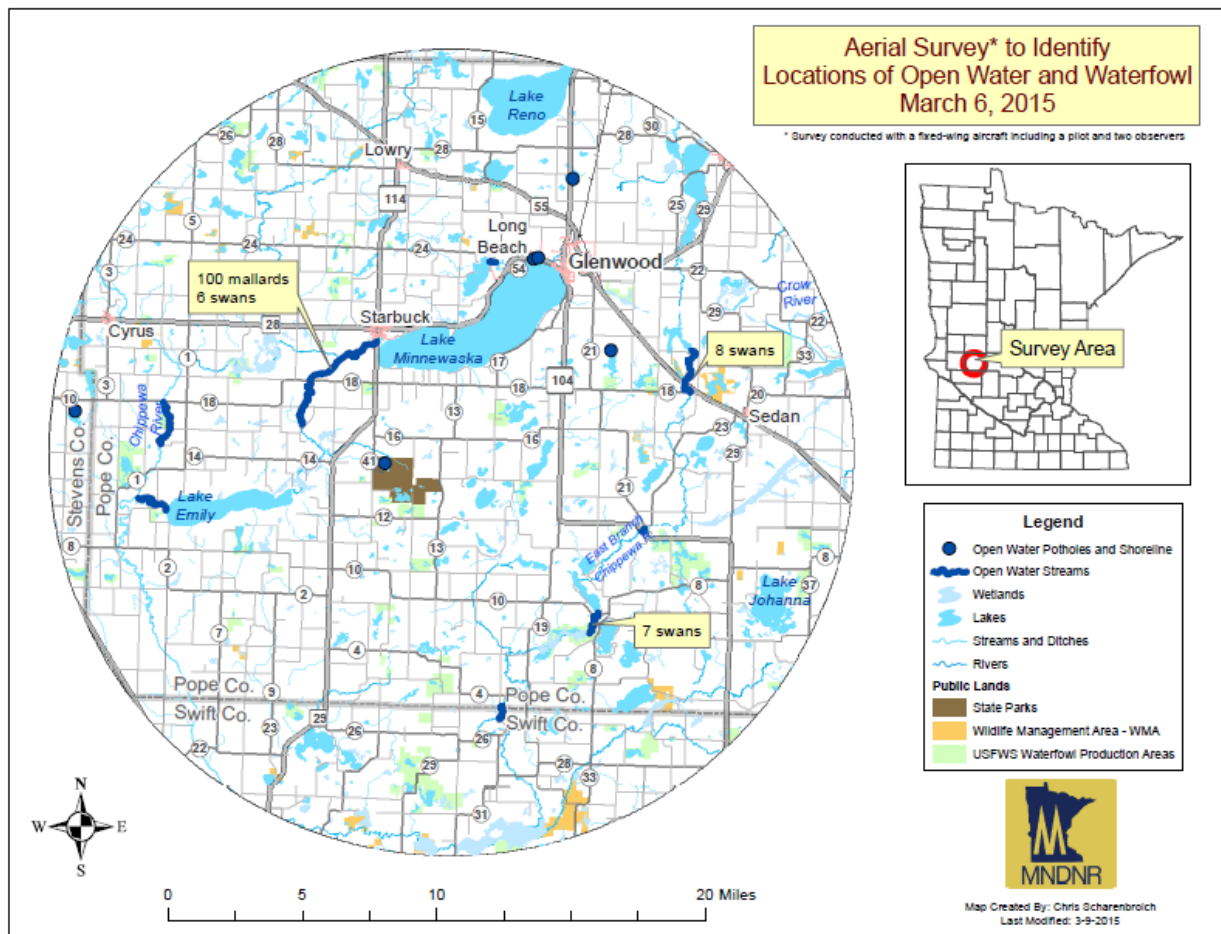
While the HPAI subtype H5N2 virus is new, DNR has conducted extensive avian influenza surveillance in the past. From the period 2006 – 2010, over 12,000 birds (9,000 as part of a DNR project, and 3,000 as part of a USDA-WS surveillance effort) were tested for HPAI H5N1 and it was not detected. A DNR research summary covering that project is available here, [Surveillance for HPAI in Minnesota's migratory birds, 2006-2010](#). Additionally, all testing described in this report was conducted at laboratories accredited through the National Animal Health Laboratory Network (NAHLN) and are approved for AI testing. Laboratories in the

NAHLN network use standardized protocols and procedures developed by USDA in accordance with the World Organization for Animal Health manual. A DNR research summary, documenting the 2015 surveillance effort, is currently under revision and will be linked to this document when it is finalized.

Initial HPAI Detection

The first infected flock reported was in a breeder hen facility in Pope County in late February, 2015. Official notification came on March 3rd and on March 6th DNR staff flew a low-level 700 mi² fixed wing survey around the infected facility to assess waterfowl populations in the area. The survey yielded very little open water and few waterfowl. In total, there were approximately 100 resident mallards contained in one group near Starbuck, 5 miles west of the farm, and 21 swans clustered in 3 groups (Figure 1). Starting on March 8, DNR, in cooperation with USDA-WS, collected 148 fecal samples from the mallards identified during the aerial survey. Six samples (4.0%) were positive for low pathogenicity avian influenza (LPAI) on the matrix test, but none were H5 or H7 positive; all remaining samples were negative.

Figure 1. Results from March 6, 2015 aerial survey around infected turkey facility. Survey results revealed very little open water and few waterfowl.



Subsequent HPAI Detections

With the discovery of HPAI in other counties starting in late March, DNR staff collected samples within Board of Animal Health (BAH) identified surveillance areas and identified additional species to test for HPAI. During these initial infections, a 3-part approach to surveillance was implemented; 1) fecal sample collection from migrating waterfowl, 2) a pilot hunter-harvested wild turkey surveillance project, and 3) publicly-reported dead bird surveillance. An information guide on the safe handling of wild birds was provided to DNR staff statewide in mid-April 2015.

Fecal sample collection - Waterfowl

With new poultry farms (and counties) becoming infected daily, the lag time between infection and DNR notification, and waterfowl moving rapidly across the landscape, it was not possible to sample live waterfowl in a timely fashion. Also, in several counties by the time DNR received notification of an infected facility, there were few waterfowl in the area to test (examples: Cottonwood, Lyons, and Watonwan counties). In order to facilitate timely sampling that better linked a poultry-infected area to exposure of infectious wild bird material, we adopted a sampling design that focused on testing waterfowl feces in counties with and without infected poultry facilities. We chose five areas where waterfowl were present and spring 2015 poultry farm infections occurred with a goal of collecting 300 fecal samples in each area (1,500 total). These treatment areas with significant waterfowl congregations were within 10km of infected farms in Stearns, Lac Qui Parle, Nobles, Meeker, and Kandiyohi counties. Five “control” sites with significant waterfowl congregations and no infection detected were also selected. Major Unit Wildlife Management Areas (WMAs) and 1 National Wildlife Refuge (NWR) were chosen as controls because they serve as major stop-overs for waterfowl migrating through Minnesota and ducks that potentially infected a facility could ultimately end up in one of these areas. Identified control sites were, 1) Carlos Avery WMA (north metro), 2) Swan Lake WMA (southcentral), 3) Thief Lake WMA (northwest), 4) Whitewater WMA (southeast), and 5) MN Valley NWR (south metro). Again, our goal was to collect 300 fecal samples (1,500 total) from each of these areas, yielding a total of 3,000 samples.

The sample goals chosen permitted us to infer with 95% probability that no more than 1% of deposited waterfowl feces contained HPAI H5N2. This statistical sampling design is consistent with the USDA National Implementation Plan, available here: [2015 USDA-WS HPAI implementation plan](#).

Testing: DNR worked with USDA-Wildlife Services for both sample collection and testing of fecal samples at the infected and control sites. Samples were sent to the USDA National Wildlife Disease Lab in Fort Collins, Colorado for initial testing using an AI matrix test that identifies whether an AI virus (all 144 strains) is present. Samples that tested positive for H5 or H7 were forwarded to the National Veterinary Services Lab (NVSL) in Ames, Iowa for confirmation and additional strain typing. All of these tests were completed at no cost to DNR.

Results: Overall, 1,738 samples were collected from areas with active infection and 1,400 were collected from the control sites. Of the 3,138 total fecal samples collected, 100 (3.2%) were positive for LPAI; no HPAI was detected (Figure 2). Of the 1,738 samples from infected sites,

from April 8 – 22, approximately 1,000 were taken from Stearns, Meeker, and Kandiyohi counties (n = 56 infected poultry farms). Consequently, these collections were completed at the time of escalating infections throughout Minnesota in the areas with significant infections (Figure 3. Epidemic curve source, [USDA-VS Epidemiologic Analysis July 2015](#)). Of the approximately 1,000 samples, 42 were LPAI positive (4.2%); however, no HPAI was detected.

Important to note is that fecal sampling is an established method of surveillance for avian influenza and DNR used methods described in the peer-reviewed literature. The fact that LPAI was detected indicates the method worked. Also, the LPAI prevalence during this surveillance effort were consistent with what has been described in the literature.

Figure 2. Collection of waterfowl fecal samples for HPAI surveillance using a strategy of collecting in poultry-infected and uninfected areas, 2015.

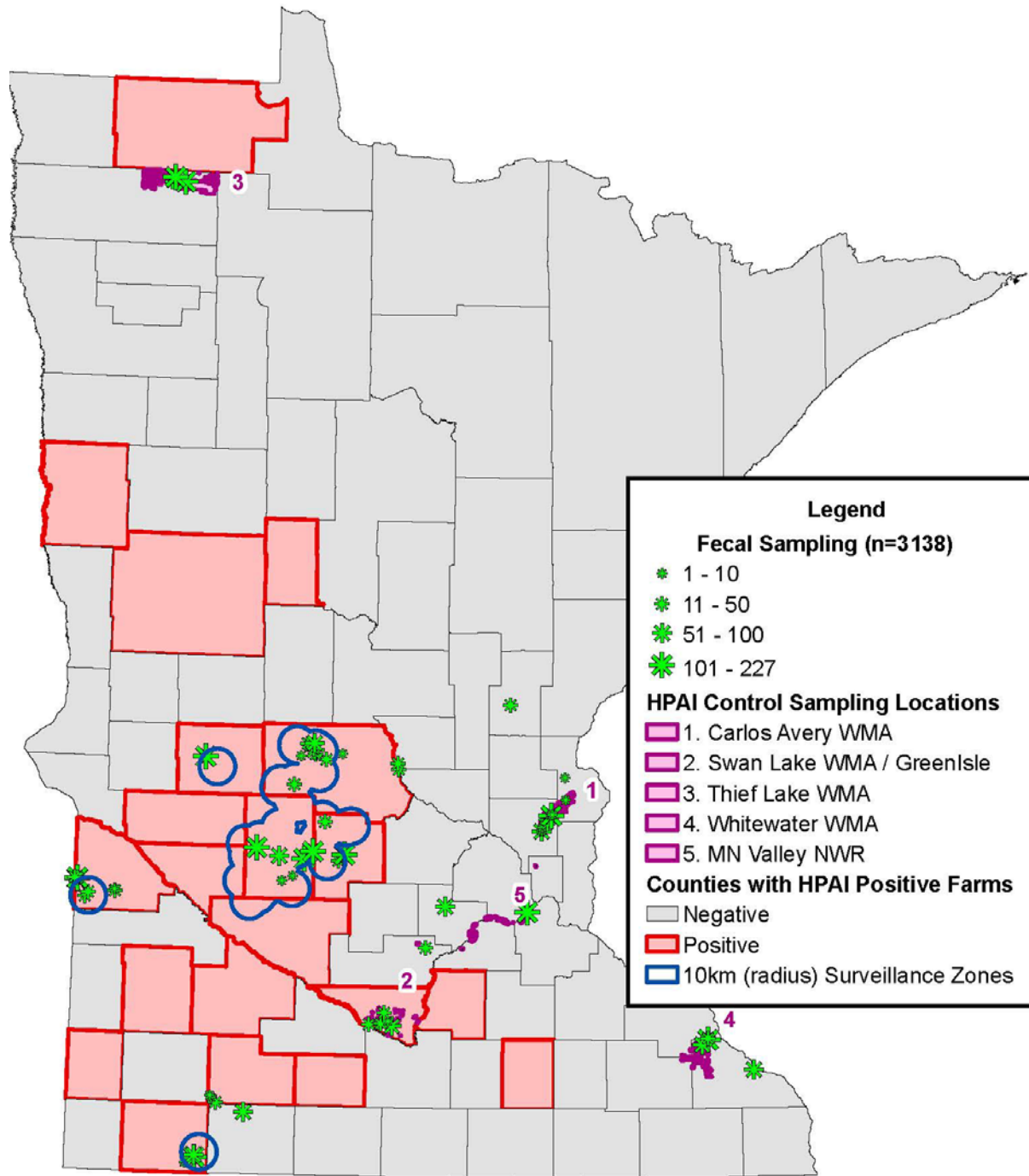
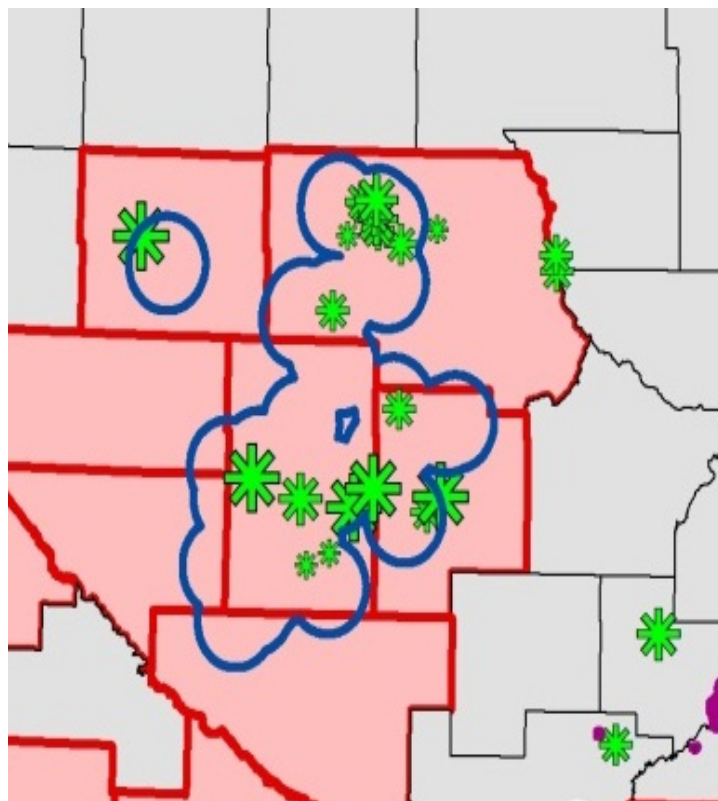
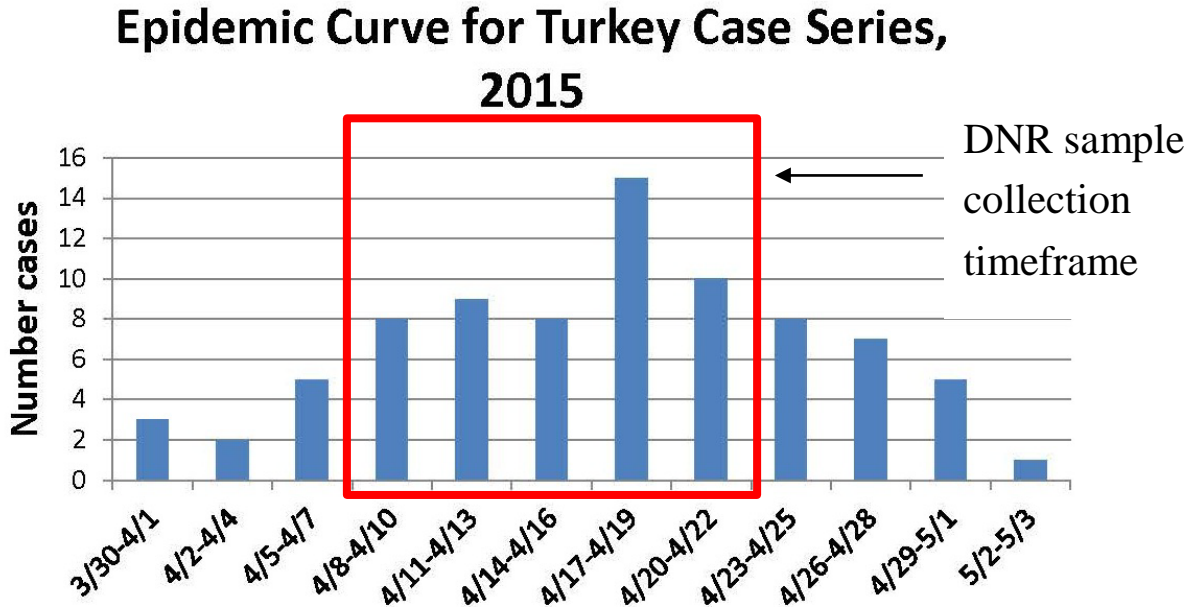


Figure 3. Epidemic curve for turkey infections in Minnesota with DNR fecal sample collection timeframe (top) and fecal sample collections (bottom) in Stearns, Meeker, and Kandiyohi counties. Blue circles are 10-km buffers around infected facilities. Green stars are locations of DNR fecal sample collections. Source for epidemic curve, [USDA-VS Epidemiologic Analysis, July 2015](#).



Hunter-harvested wild turkey surveillance

It is presumed wild turkeys may have similar susceptibility to HPAI as their domestic counterparts. Since the spring wild turkey season was opening at the time infections were intensifying, DNR initiated a pilot surveillance effort of hunter-harvested wild turkeys. In counties with active HPAI infection in commercial poultry flocks and significant wild turkey populations, DNR asked hunters to voluntarily provide a sample from their harvested bird. Hunters were asked to provide basic contact information, harvest date, and the GPS location of their harvested bird. Samples included both a cloacal (if not field dressed) and tracheal swab. Sampling supplies were provided to area offices in Swift, Stearns, Pope, Meeker and Kandiyohi counties with a goal of collecting 300 voluntary samples. A DNR news release that described the project was distributed statewide before the spring turkey season opened.

Testing: DNR worked with the USGS, National Wildlife Health Center (NWHC) in Madison, WI for testing of the swabs. The NWHC completed initial testing using the AI matrix test that identifies any AI virus (all 144 strains). Confirmation of *icA2-H5* clade would be completed in Madison with final determination done by NVSL in Ames, Iowa. All of these tests were completed at no cost to DNR.

Results: Overall, 84 wild turkeys were submitted for sampling and none tested positive; consequently, no follow-up testing was required (Figure 4).

Collection of dead birds (morbidity and mortality) reported by the public

DNR has tested dead raptors or wild turkeys reported from the public for HPAI. In addition, if other species of birds are reported dead in numbers ≥ 5 (consistent with the 2006 plan) in the same location and timeframe, specimens are collected for further diagnostics and HPAI testing. If a wild bird (single individual or < 5 total) was observed with neurologic symptoms, effort was made to collect the bird for HPAI testing. This passive collection is on-going and will continue for the foreseeable future.

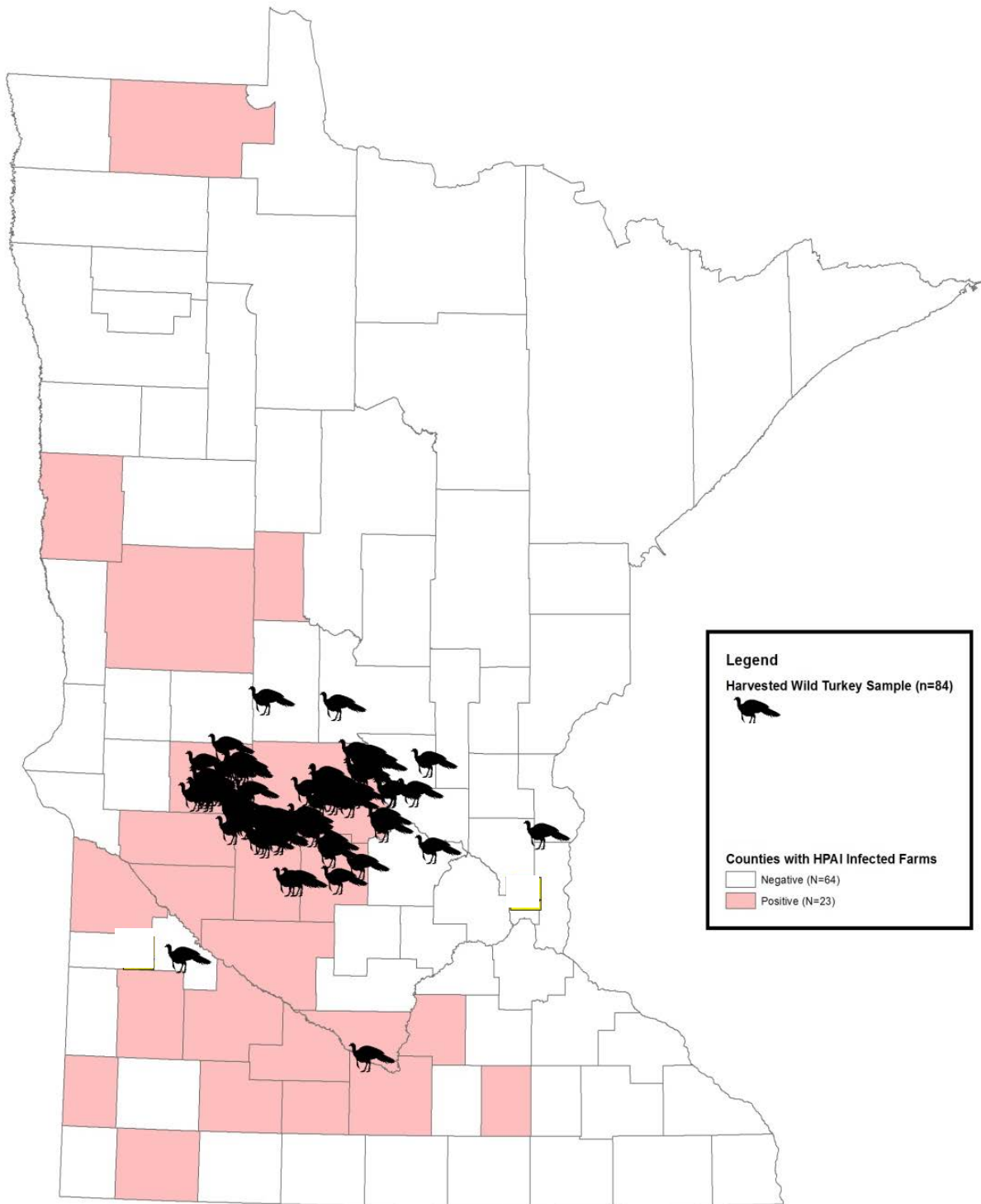
Testing: DNR worked with the USGS, National Wildlife Health Center (NWHC) in Madison, WI for testing. In most cases, whole birds were sent to the NWHC for complete necropsy, along with AI testing. In the cases of large birds (e.g., trumpeter swans), attempts were made to obtain cloacal and tracheal swabs instead. The NWHC completed initial testing using the AI matrix test that identifies any AI virus (all 144 strains). Confirmation of the *icA2-H5* clade would be completed in Madison with final determination done by NVSL in Ames, Iowa. All of these tests were completed at no cost to DNR.

Results: To date, 104 birds have been tested by DNR. An unknown number of birds were also tested by rehabilitation facilities in Minnesota. Examples of bird species tested by DNR include:

- Raptors: bald eagle, broad-winged hawk, Cooper's hawk, great horned owl, red-tailed hawk, sharp-shinned hawk
- Upland game birds: pheasant, sandhill crane, wild turkey
- Waterfowl: American coot, Canada goose, mallard, ring-billed gull, trumpeter swan, wood duck

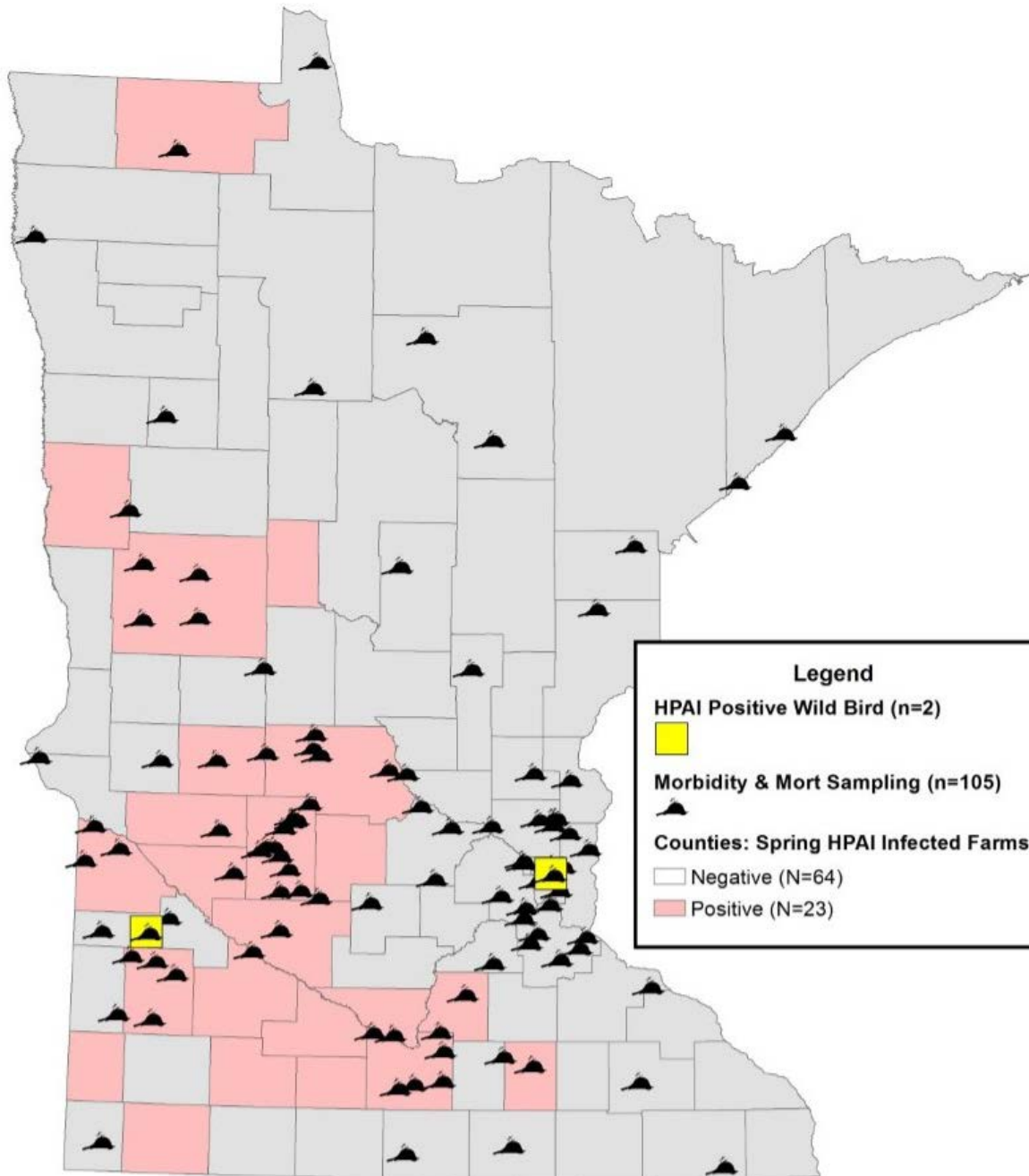
- Passerines/Other: red-winged blackbird, black-capped chickadee, house finch, mourning dove, rock doves, white pelican, house sparrow, starling, thrush, warbler

Figure 4. Locations of hunter-harvested HPAI wild turkey surveillance in Minnesota, 2015.



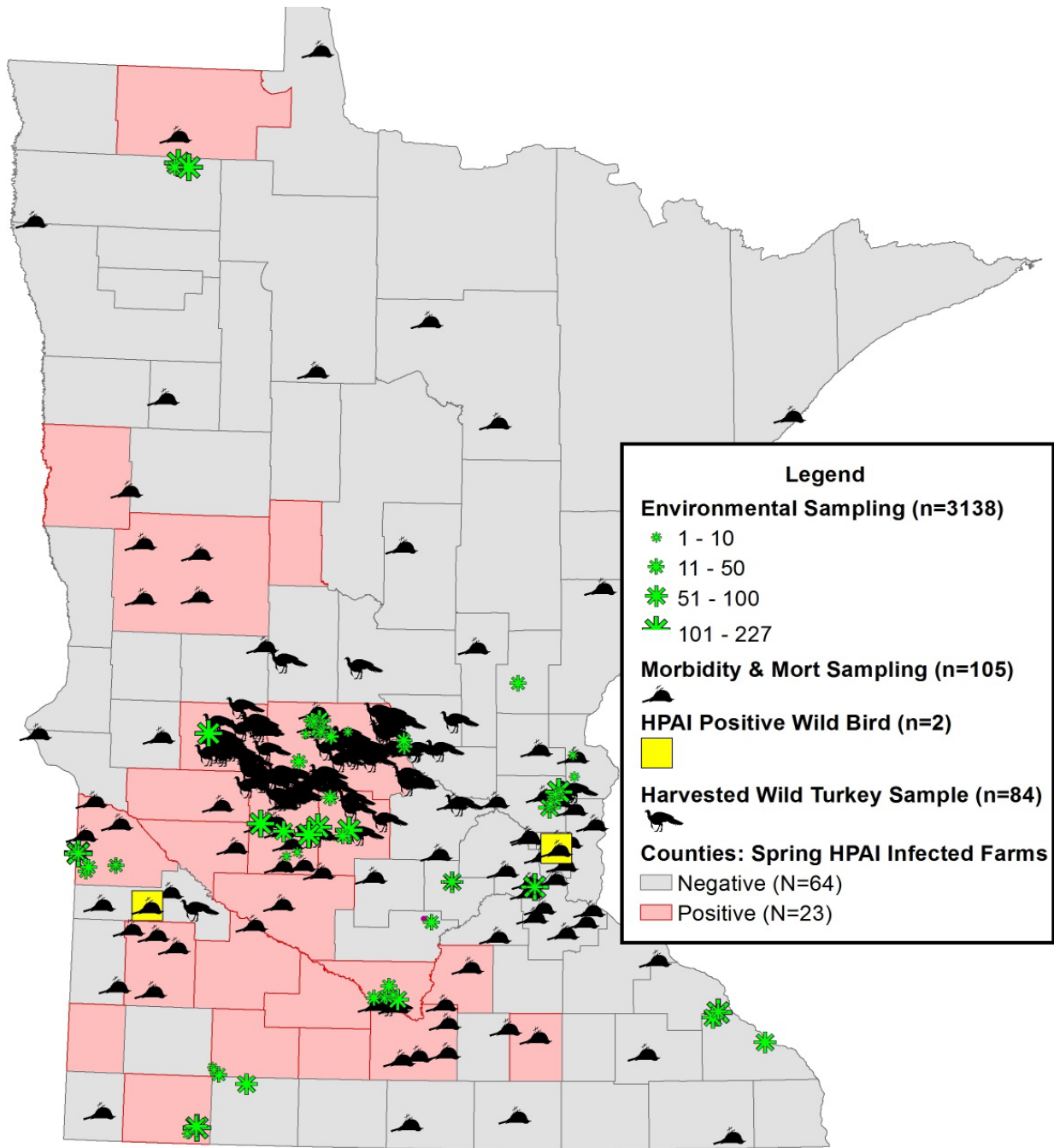
Of dead birds submitted by DNR, a Cooper's hawk was found positive in Yellow Medicine County (12.5 miles from an infected facility in Lyon County). Additionally, a black-capped chickadee that was submitted by a rehabilitation center in Ramsey County was also determined to be positive for HPAI (Figure 5).

Figure 5. Dead bird surveillance through August 31, 2015. The positive samples are represented as yellow blocks. Overall, 105 are listed in the figure because it includes the positive black-capped chickadee that was not submitted by DNR.



These two cases represent the only HPAI positive wild bird samples collected during the 3-part sampling effort covering March 8 – August 31, 2015 (n = 3,326¹; Figure 6).

Figure 6. Distribution of total samples collected (n = 3,326¹) during DNR's 3-part surveillance effort following the discovery of HPAI in domestic poultry. Data goes through August 31, 2015.



¹ The total does not include the HPAI positive chickadee from Ramsey County that was submitted by a rehabilitation center.

Canada goose surveillance

Annually, DNR bands several thousand geese throughout Minnesota. For 2015, wildlife health staff worked with DNR and USFWS banding teams and a private metro goose contractor to collect blood (extracting serum) for serology (previous exposure) and swab (cloacal/oral) samples to identify Avian Influenza Virus (AIV) RNA. The objective was to collect at least 300-400 paired blood/swab samples spatially distributed across the state within two primary zones – counties where poultry farm facilities are present both with and without infected farms. Goose banding occurs during a limited window during the summer when geese are flightless. The goals of this sampling effort are twofold:

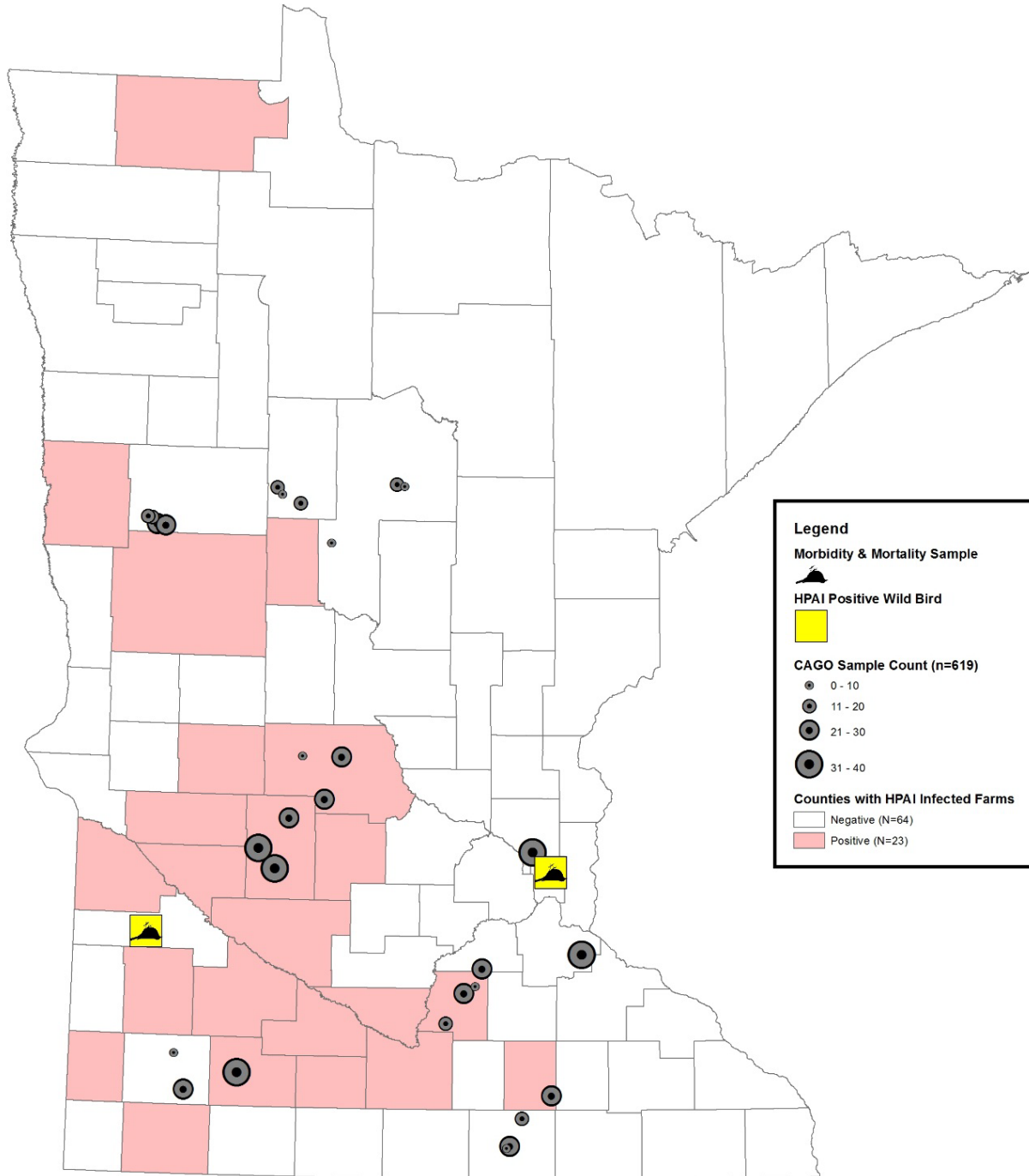
1. To provide a baseline estimate of seroprevalence of AIVs in Canada geese in MN, and establish if detectable AIV RNA is present and if possible isolate virus subtypes.
2. In collaboration with the University of Georgia, identify AIV communities in a relatively unstudied host (Canada geese), and provide data for understanding the evolution of AIV subtypes.

To allow for adequate spatial representation and sample sizes for demographic parameter estimation, if there were < 50 birds, then approximately 40% were sampled. Samples by age group (adult or hatch year) were allocated proportional to group membership size. For example if 40 birds were captured, of which 10 are adults and 30 are hatch year, then 4 adults and 12 hatch year were sampled. If >50 birds are captured, at least 30 birds were not sampled. It was not clear ahead of time how many geese will be banded in a given site, so the above rules were adhered to as closely as possible. Also, DNR typically does not band in central Minnesota, near the areas with the highest poultry infections. To facilitate testing in those areas, DNR staff located and banded geese in several new areas.

Testing: A detailed protocol was developed using datasheets/sample tracking provided by USDA-WS. A biosecurity protocol was also developed and distributed to staff. All swab samples were sent to the USDA-WS laboratory in Fort Collins, CO and were tested for AI using the procedures previously described. Serum was sent to the University of Georgia as part of a serological research project.

Results: In total, swabs were taken from 619 geese, of which 610 had a paired blood sample. Of those, 2 (0.3%) were positive on the AI-matrix test but neither was H5 or H7. Serology results regarding previous HPAI exposure are still pending.

Figure 7. Spatial distribution of Canada goose samples obtained (n = 619) during 2015. Also shown are the 2 positive samples (Cooper's hawk, black-capped chickadee) identified from previous testing.



Dabbling duck surveillance

Surveillance (and research) of dabbling ducks for HPAI is being addressed through various methods in collaboration with other influenza researchers and agencies. The DNR is partnering with the Southeast Cooperative Wildlife Disease Study (SCWDS; University of Georgia), USDA-WS, USFWS, and the USGS to collect blood samples for serology and swabs (cloacal/oral) to identify Avian Influenza Virus (AIV) in Minnesota. The goals of this work are to 1) provide estimates of HPAI shedding rates in late summer/early fall, 2) assess HPAI seroprevalence in summer dabbling ducks of Minnesota, and 3) assess HPAI shedding rates in fall hunter-harvested waterfowl. In collaboration with SCWDS, DNR will also characterize dabbling duck AIV communities in Minnesota and distinguish the various AIV subtypes that may be present.

Some of this work also involves serological research. Serology, although not yet established as a stand-alone surveillance tool, provides information about whether an animal has mounted an immune response to AIV and has circulating levels of antibodies present in its blood. This method will theoretically work; however, it has not been proven or adequately tested in the field. There are also several major limitations to serological results, including:

- Based on serologic results alone, it is not possible to determine when a bird was exposed to AIV.
- It does not indicate where or how a positive bird acquired infection
- It is unclear how long a detectable immune response will last in an exposed bird
- Cross-immunity or the immunological influence of one type of AI infection on later acquired infections generates uncertainty
- With regard to differentiating between HPAI and LPAI H5, results may not be easy to interpret— this was the case with similar work in Europe when this approach was used with HPAI H5N1
- Based on previous testing, there is a significant amount of existing antibodies to North American LPAI viruses, especially in geese

Subtyping HPAI and LPAI, while promising, is an active area of research with unestablished standard protocols. DNR's contribution to this collaborative effort is part of a national serological research project at the University of Georgia that seeks to reduce some of these uncertainties.

Specific projects are,

1. Paired serology research and virus shedding surveillance. This is work being conducted in collaboration with the University of Georgia, SCWDS; USFWS; USDA-WS; and USGS. The goal of this work is to evaluate serological results of blood samples obtained from susceptible duck species (primarily mallard and blue-winged teal). Six DNR work areas were selected for collecting paired samples (i.e., swabs and serology) from live ducks (primarily mallards and teal) in counties that contained poultry facilities that experienced spring HPAI infection and counties with poultry facilities that were not

affected (Figure 8). Sampling is currently underway with a goal of collecting 625 samples statewide.

2. 2015 USDA National Surveillance Plan. Minnesota is a full partner in this collaborative effort to obtain 41,000 dabbling duck samples throughout the United States during all 4 seasons. Minnesota is asked to submit 545 oropharyngeal and cloacal cavity swab samples of dabbling ducks for AIV testing to the USGS by the end of winter 2015. The sample requests are broken down by watershed (Mississippi Headwaters, Red River, St. Croix, Upper Mississippi – Black Root, and Western Lake Superior) and season; summer, fall, and winter. The summer quota of 30 samples for the St. Croix and 130 samples for the Red River watersheds was achieved and is in the process of analysis at the USGS. The fall quota of 40 samples for the Red River, 140 samples for the Mississippi Headwaters, 50 samples for the St. Croix, 40 samples for the Upper Mississippi – Black Root, and 60 samples for the Western Lake Superior watersheds will be forthcoming. Similarly, the DNR expects to collect a winter quota of 55 samples from the Upper Mississippi – Black Root watershed. Most of these samples are derived from sampling hunter-harvested ducks. The full USDA plan is available here, [2015 USDA-WS HPAI implementation plan](#).
3. Fall hunter-harvested surveillance. The USDA national surveillance plan was developed with watersheds as the primary area of focus. While this is a legitimate surveillance design, it omitted central Minnesota, which was the center of poultry infections. During the waterfowl hunting season starting September 26 2015, DNR is planning to sample the trachea and cloacal cavities of an additional 800 hunter-harvested dabbling ducks in central Minnesota. The sampling will take place in two types of study areas at the county scale designated by whether poultry facilities experienced HPAI infection or not (Figure 10). The sample size will be approximately split between the two types study areas for comparison. Control areas (non-infected farms) are Todd and Morrison counties. Treatment areas are Kandiyohi, Meeker, Pope, and Stearns counties. The sample size is chosen to detect HPAI viral shedding at a 1% prevalence level with at least 95% confidence.

Research and surveillance collaborations

Overview

DNR is collaborating extensively with other entities that are involved in avian influenza surveillance and research. DNR scientists do not conduct basic research and have focused efforts on enhanced surveillance and monitoring of potential infections in wildlife. DNR is interested in further collaboration with those researching this important area and would support additional funding for research institutions and agencies who are pursuing that type of work.

Currently, DNR research staff are collaborating on the following projects:

1. National serological research with the University of Georgia. The objectives of this work are to: 1) Determine if there is a clear serologic signal to these viruses that can be used with field samples from wild birds, 2) Utilize serology to follow the incursion and possible establishment of these viruses in wild bird populations in North America and Europe, and 3) Optimize and standardize the diagnostic procedures for this approach (related to both testing and sampling).
2. USDA Implementation Plan for HPAI Surveillance. The objectives of this work are to: 1) Identify the distribution of influenzas of interest by U.S. flyways and through select, high priority watersheds, 2) Detect spread of influenzas of interest to new areas of concern, and 3) Provide a flexible surveillance framework that can be modified to monitor wild waterfowl populations for re-assortments of influenzas, introductions of new viruses, and to estimate apparent prevalence of important influenzas once detected in an area of concern.
3. Rapid Ag Grant – University of Minnesota (Drs. Pat Redig and Carol Cardona). This project is titled, “Surveillance for High-consequence Poultry Diseases in Wild bird Reservoirs: Influenza and Newcastle Disease”. This is an ambitious project examining many facets of surveillance and DNR will assist with collection of data as needed.
4. USGS peri-domestic research. This project is titled, “Wild and peri-domestic species exposure to HPAIV in natural wetlands and peri-domestic habitats”. The objectives of this project are to test several hypotheses associated with the recent discovery of HPAI. Specifically, they will 1) examine the maintenance of HPAIV in natural ecosystems among wild and peri-domestic (animals associated with farms) in poultry outbreak situations, and 2) examine the potential role of other wildlife species in HPAIV transmission. A meeting was held in early August to discuss this project; in attendance were DNR, BAH [Drs. Hartmann, Lauer, and Voss], Dr. Carol Cardona (UMN), and several USGS scientists. DNR agreed to assist with the off-farm portions of the research and has issued a research permit to USGS to collect on state lands. USGS scientists are working with Dr. Cardona on the on-farm component.

Funding

During the 2015 Legislative Special Session, Chapter 4, Section 5 (d) the Legislature appropriated funds for surveillance (detect and monitor) and possible research both in an outside of control zones. Specifically, the session law reads,

\$350,000 is appropriated from the general fund in fiscal year 2016 to the commissioner of natural resources for sampling wild animals to detect and monitor the avian influenza virus. This appropriation may also be used to conduct serology sampling, in consultation with the Board of Animal Health and the University of Minnesota Pomeroy Chair in Avian Health, from birds within a control zone and outside of a control zone. This appropriation is available the day following final enactment until June 30, 2017.

Going forward, additional dialogue is necessary to identify research gaps (some of that has occurred) and determine if additional funds are needed to conduct more surveillance or answer specific research questions. As part of these collaborations, DNR would support additional requests for funding by Universities or other Agencies for work that falls outside the scope of DNR's role of surveillance and applied research.

Post-2015 plans

We have not yet devised specific plans for spring 2016. Much of that will be dependent on what is found after our extensive surveillance concludes this winter. We will continue to work with the many groups involved in response, management, and research of AIVs, and collaborators who meet frequently to work through what research is on-going and where there are gaps in knowledge. While improvements need to be made in communications among these groups, we share the common goal of documenting, explaining, and informing our constituents about the role wildlife may play in future infections. We want to help inform the discussion and answer the larger questions regarding HPAI transmission and subsequent introductions into domestic facilities.

This document will be subject to frequent revisions as new information becomes available or when surveillance results are generated.

Figure 8. Study area design for summer 2015 paired collection and testing of dabbling duck swab and blood samples in collaboration with SCWDS. A goal of 625 paired samples is established in six work areas throughout Minnesota, and sampling is currently underway.

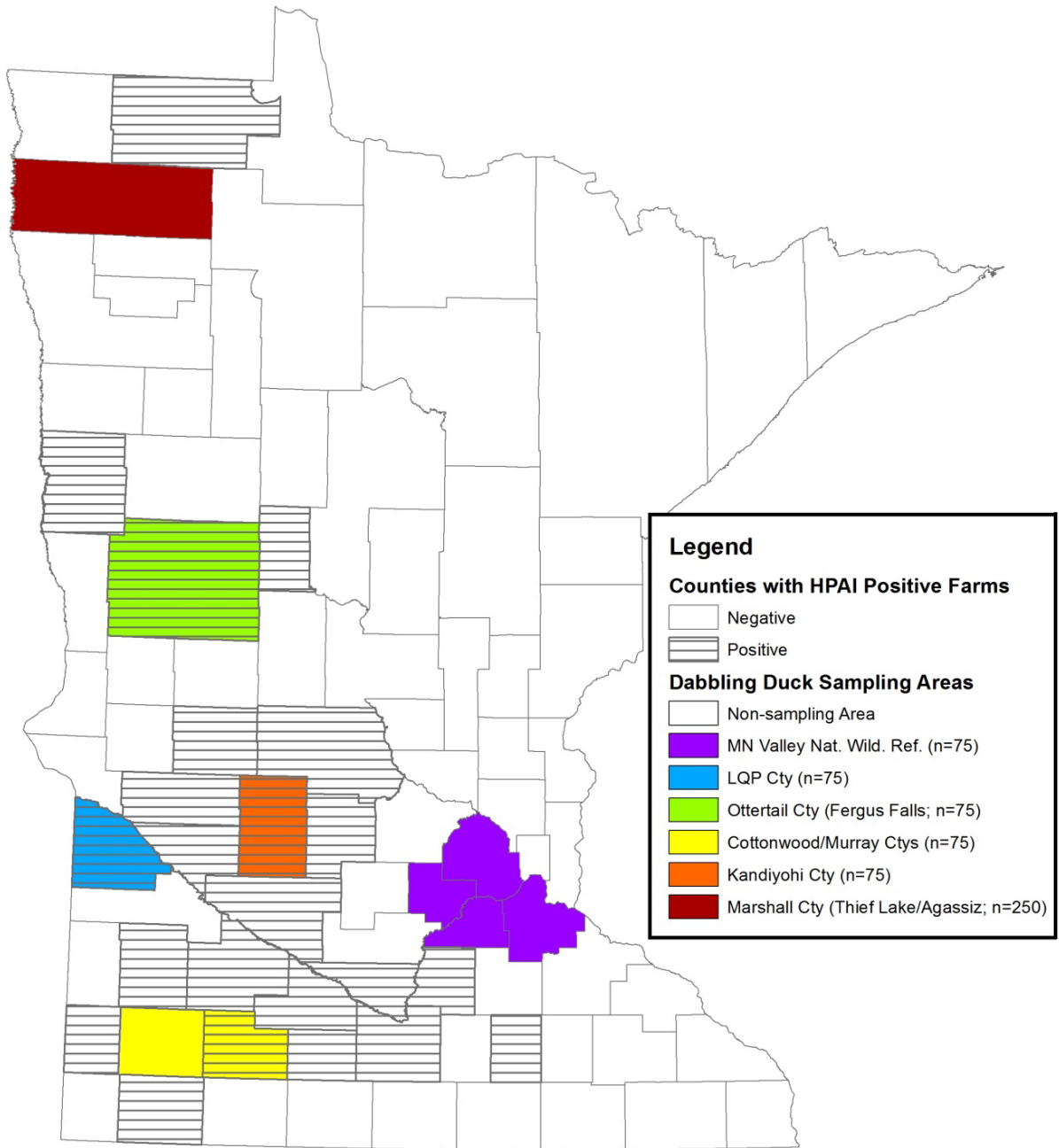


Figure 9. The distribution of USDA targeted MN watersheds for avian influenza sampling in summer, fall, and winter 2015. The three sample sizes noted after watersheds in the legend are the quotas requested by USDA for summer, fall, and winter sampling, respectively.

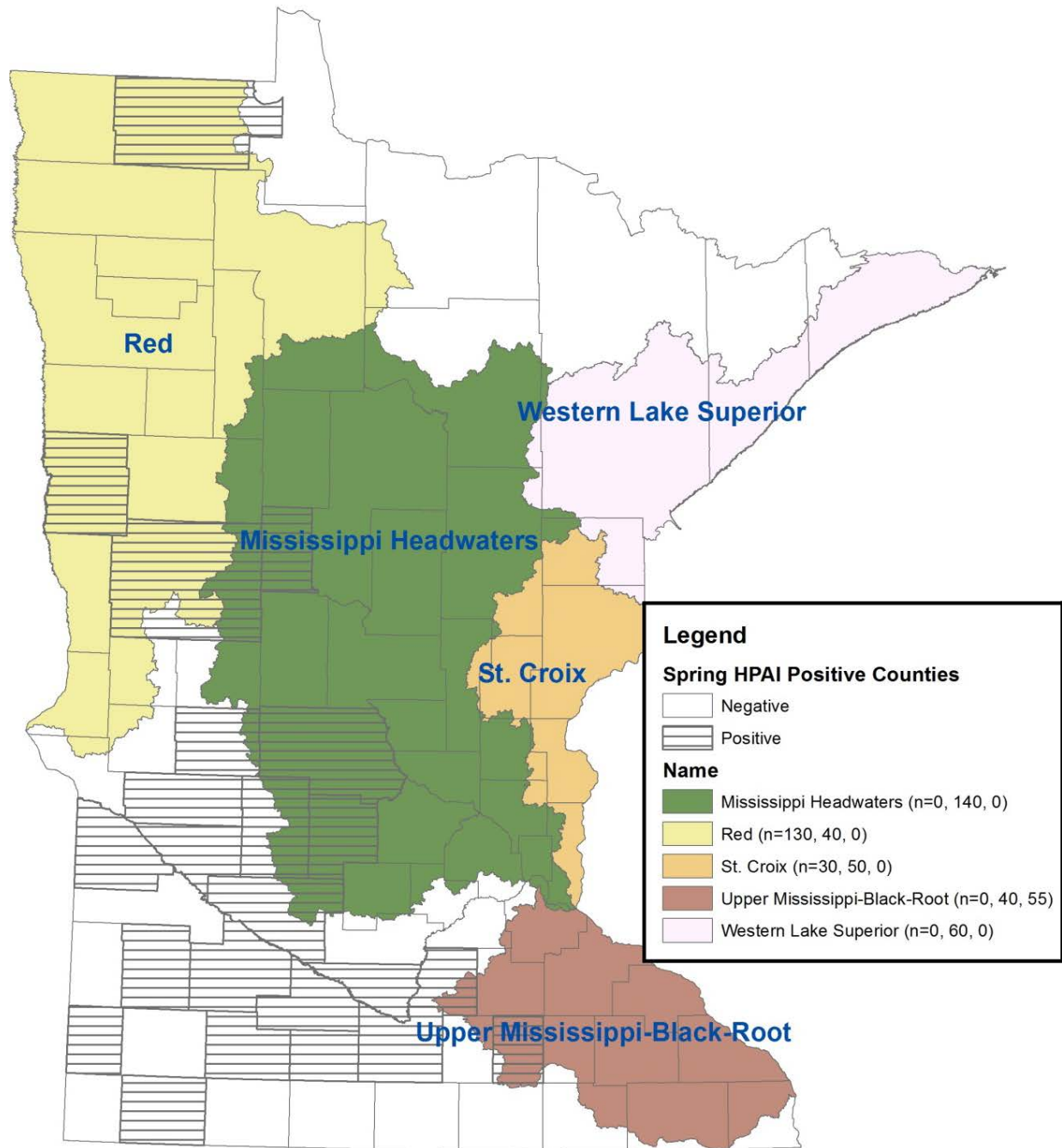


Figure 10. The distribution of MNDNR targeted counties and sampling locations for planned Minnesota fall duck hunter-harvest sampling for avian influenza. Approximately 400 tracheal and cloacal swab samples will be collected in each of the two study area types.

