**2018 Project Abstract**
For the Period Ending June 30, 2018

**PROJECT TITLE:** Avian Influenza distribution, evolution, and impacts on Ring-billed and Herring gulls in Minnesota
**PROJECT MANAGER:** Marie Culhane
**AFFILIATION:** University of Minnesota
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**FUNDING SOURCE:** Environment and Natural Resources Trust Fund
**LEGAL CITATION:** M.L. 2015, Chp. 76, Sec. 2, Subd. 10 - Emerging Issues Account
M.L. 2017, Chapter 96, Section 2, Subdivision 18

**APPROPRIATION AMOUNT: $213,443**

## Overall Project Outcomes and Results

Questions remain surrounding the origin of the virus that caused the 2015 H5N2 highly pathogenic avian influenza outbreak in Minnesota poultry. Since gulls are frequently sighted near poultry farms, we conducted a project from October 2016 to September 2017 to test Ring-billed gulls for avian influenza virus to determine the role gulls played in the outbreak. We also evaluated the effects on overall gull health due to avian influenza virus infections.

We visited three breeding gull colonies (in Big Stone [Marsh Lake's Hermit and Big Islands], Cass [Leech Lake's Little Pelican Island], and St. Louis [St. Louis River's Interstate Island] counties) and six landfills (in Kanabec, Dakota, Kandiyohi, Cottonwood, Blue Earth, and Rice counties). We caught, measured, sampled, tagged and released 1,346 gulls from which we collected swabs, blood, age, weight, and keel and head-to-beak length. For resighting identification, each gull received metal and alpha-numeric colored leg bands.

Highly pathogenic viruses were not detected in the gulls, but two H5 low pathogenicity viruses were detected, which is interesting in that these may mutate into highly pathogenic strains under certain conditions in various avian species. Out of all gulls tested, 26% were positive for avian influenza.

We calculated body condition index using weight and body measurements for every gull as an indicator of gull health. For every body condition score unit increase, the odds of being avian influenza positive decreased 16%. We found significant seasonal and age dynamics in virus prevalence, with juvenile gulls during fall migration having the highest apparent prevalence (68.36%).

The high avian influenza prevalence within gulls, particularly in young gulls, warrants further targeted surveillance efforts of gulls and other related species. Genetic analyses of the avian influenza viruses found in this project's gulls appear distinct from the 2015 highly pathogenic H5N2 avian influenza viruses. Our results suggest gulls are not part of the poultry transmission cycle of avian influenza and gulls of good body condition are less likely to be avian influenza infected.

## Project Results Use and Dissemination

Results from this project have been used to secure funding for an additional avian influenza virus surveillance project, funded by the Minnesota Agricultural Experiment Station, intended to track the possible dissemination of avian influenza viruses by Ring-billed gulls throughout Minnesota.

Presentations were made via teleconference and informational sheets were distributed to promote voluntary participation in the study to the Minnesota Turkey Growers, the Minnesota Chicken and Egg Producers, the Minnesota Audubon Society, and the Minnesota Ornithologists Union.

Presentations were made including project overviews and preliminary results at the following venues:

- a scientific symposium during the North Central Avian Disease Conference meeting on March 13, 2017 in St. Paul, MN
- the Fisheries, Wildlife, and Conservation Biology Club on the University of Minnesota campus on April 4, 2017
- the Conservation Sciences Department Brown Bag Lunch Seminar on April 18, 2017;
- the International Avian Influenza Symposium in Brighton, UK in April 2018 including two abstracts:
  ·"Comparison of oral and cloacal swabs for the detection of influenza in gulls"
  ·"Avian Influenza virus surveillance in ring-billed and herring gulls in Minnesota."

The peer-reviewed manuscript (1), "Avian Influenza Prevalence and Viral Shedding Routes in Minnesota Ring-billed Gulls (*Larus delawarensis*)." Manuscript # v1848-041718-RegR, authors Todd Froberg, Francesca Cuthbert, Chris Jennelle, Carol Cardona, and Marie Culhane, was accepted on 20 July 2018 for publication in the Avian Diseases Journal Special Issue, Proceedings of the 10th International Symposium on Avian Influenza.

Todd Froberg presented this work as his Master's Thesis Defense on December 21, 2017 in a seminar to the University of Minnesota Fisheries, Wildlife, and Conservation Biology Department titled, "Seasonal Dynamics of Avian Influenza Viruses in Ring-billed Gulls in Minnesota." In attendance were numerous students and faculty from the University of Minnesota College of Veterinary Medicine, and colleagues from the Department of Natural Resources and United States Fish and Wildlife Service. This work was submitted as a thesis (2) to the faculty of the University of Minnesota by Todd Froberg in partial fulfillment of the requirements for the degree of Master of Science under the advisement of Francesca Cuthbert. Mr. Todd Froberg is now an employee of the Minnesota Department of Natural Resources. A manuscript will be submitted to the Journal of Wildlife Diseases under the working title, "Use of a Netlauncher to Capture Non-breeding Gulls at Landfills in Minnesota." The genetic sequence data is in the public domain for knowledge sharing in the influenza research database.

## Summary of Separately Submitted Documents:

(1) Todd Froberg, Francesca Cuthbert, Chris Jennelle, Carol Cardona, and Marie Culhane. "Avian Influenza Prevalence and Viral Shedding Routes in Minnesota Ring-billed Gulls (Larus delawarensis)." Manuscript # v1848-041718-RegR. Accepted on 20 July 2018 for publication in Avian Diseases Special Issue, Proceedings of the 10th International Symposium on Avian Influenza. DOI: 10.1637/11848-041718-Reg.1 (PDF), 21 pages.

(2) Froberg, Todd (2018). Seasonal Dynamics of Avian Influenza Viruses in Ring-billed Gulls (*Larus delawarensis*) in Minnesota. University of Minnesota M.S. thesis. June 2018. Major: Conservation Biology. Advisor: Francesca Cuthbert. (PDF); xi, 68 pages.

# Environment and Natural Resources Trust Fund (ENRTF)
# Emerging Issues Account M.L. 2016 Work Plan

**Date of Report:** December 21, 2018

**Date of Next Status Update Report:** December 21, 2018 is the FINAL REPORT

**Date of Work Plan Approval:** July 12, 2016

**Project Completion Date:** June 30, 2018

**Does this submission include an amendment request?** NO

**PROJECT TITLE: Avian Influenza distribution, evolution, and impacts on ring-billed and herring gulls in Minnesota**

**Project Manager:** Marie Culhane

**Organization:** University of Minnesota

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**Location:** Two gull breeding colonies, 3 wildlife refuge areas, and 3 private farm fields in Minnesota – all to be determined. Possible breeding colony sites include Gull and Pelican Islands, tribally owned islands in Leech Lake, and Interstate Island in Duluth, MN.

| **Total ENRTF Project Budget:** | | |
|---|---|---|
| | **ENRTF Appropriation:** | **$213,443** |
| | **Amount Spent:** | **$213,407** |
| | **Balance:** | **$36** |

**Legal Citation:** M.L. 2015, Chp. 76, Sec. 2, Subd. 10 - Emerging Issues Account
M.L. 2017, Chapter 96, Section 2, Subdivision 18

**Appropriation Language:**

$1,000,000 the first year is from the trust fund to an emerging issues account authorized in Minnesota Statutes, section 116P.08, subdivision 4, paragraph (d)

Carryforward (a) The availability of the appropriations for the following projects are extended to June 30, 2018: (3) Laws 2015, chapter 76, section 2, subdivision 10, Emerging Issues Account.

**I. PROJECT TITLE:** Avian Influenza distribution, evolution, and impacts on ring-billed and herring gulls in Minnesota

**II. PROJECT STATEMENT:**

We propose to examine the impacts of avian influenza virus infections in Minnesota's ring-billed (*Larus delawarensis*) and herring gulls (*Larus argentatus*) to determine if they have suffered from or played a potential role in the deadly highly pathogenic avian influenza H5 outbreak in Minnesota in 2015. The introduction of highly pathogenic avian influenza H5 to Minnesota was the most devastating animal disease ever to reach this state in recent memory. These Eurasian H5 viruses (clade 2.3.4.4) found in North America in 2015 are the only highly pathogenic avian influenza viruses known to circulate in wild birds (likely through migratory routes). Although reservoir species like dabbling ducks can survive infections (depending on highly pathogenic avian influenza strain) and potentially distribute virus, there is a major gap in our surveillance and understanding of avian influenza dynamics in birds such as gulls. The capacity of these viruses to mutate and change is an eminent threat for susceptible wild birds and domestic poultry. Of critical concern are information gaps preventing us from understanding the origins of last year's outbreak or what lingering consequences there are on our wild bird populations. It is disconcerting that the origins of the outbreak remain undetermined, leaving us with several questions to be answered.

Therefore, our goals of this proposed project are to determine:
1) if gulls played a role in the past outbreak.
2) the presence or distribution of avian influenza in gulls and the evolutionary potential of avian influenza viruses in Minnesota's gulls.
3) possible ways to predict or prevent a massive China-like outbreak in Minnesota's wild birds.
4) if there are any negative effects of avian influenza infection in the migratory or breeding gulls in Minnesota.

Given that highly pathogenic avian influenza H5 has only been confirmed in a single Cooper's Hawk (*Accipiter cooperii*) and has not been detected in wild ducks in Minnesota, our overall goal of the project is to perform avian influenza surveillance testing on gulls in Minnesota. Gulls are a known host for avian influenza viruses, can migrate long distances and occur in relatively large numbers in Minnesota and Midwestern U.S., yet are under-represented, compared to ducks, in surveillance efforts in Minnesota. Poultry and grain farmers frequently report large flocks of gulls on farms and in fields and have questioned the role these birds may play in distributing avian influenza on the landscape. Furthermore, gulls are commonly infected with avian influenza viruses and are species that, when infected, facilitate avian influenza virus change. This makes them obvious species to study to determine if they played a role in bringing the highly pathogenic avian influenza H5 virus to Minnesota or, now that the Minnesota landscape has been seeded with the highly pathogenic avian influenza H5 virus, if the gulls are capable of moving the virus around the state and infecting other wild birds. These scenarios are entirely plausible and merit investigation because gulls have long migrations, they can move avian influenza viruses internationally, and the 2015 highly pathogenic avian influenza H5 outbreak that devastated MN birds was the result of a virus that had changed and that contained portions of viruses from Europe and Asia. Therefore, gulls could have been a contributing factor to this outbreak. Nonetheless, gulls remain underrepresented in current Minnesota avian influenza surveillance strategies as they are non-game species and access to gulls is not as easy as for hunter-killed ducks.

The outcomes of this comprehensive avian influenza surveillance of ring-billed and herring gulls in Minnesota are:
1) track virus evolution in these gulls through whole genome sequencing of the viruses detected,
2) define the role of one or both gull species in the avian influenza outbreak in Minnesota, and
3) help us better understand the potential negative effects of the highly pathogenic avian influenza H5 virus outbreak on the many inhabitants - wild, domestic, avian, and human - of Minnesota's shared ecosystem.

This project will include banding and avian influenza testing of gulls in breeding colonies, gulls on farm fields, and gulls in wildlife areas during the fall and spring migrations. The information generated will be novel and help determine the role gulls may have had, if any, in the spread of avian influenza in Minnesota and the impacts the avian influenza infection may have had on them. If banded gulls are available for recapture, these studies could help us discover the possible negative effects, such as delayed migration or weight loss, that avian influenza infection may have on the gulls studied. Furthermore, our studies may reveal risk factors or threats of infection to two threatened or endangered species in the Great Lakes area - the Caspian Tern (*Hydroprogne caspia)* and Common Tern (*Sterna hirundo*). Our results have the potential to inform the decisions made by natural resource managers and guide future surveillance activities for avian influenza and other wild bird diseases in Minnesota.

**III. OVERALL PROJECT STATUS UPDATES:**

**Project Status as of January 10, 2017:**
Approval of the research plan was sought from the University of Minnesota Institute for Animal Care and Use Committee (IACUC) in September 2016 and granted. Graduate student Todd Froberg was hired and enrolled in graduate school. Wildlife technician Elizabeth Rasmussen was hired. Undergraduate student helper Gavin Aguilar was hired. Todd and Elizabeth completed necessary IACUC training to catch, test, and release gulls.

Drs. Francesca Cuthbert, Timothy White, and Marie Culhane trained Todd on gull identification, sex determination, sample collection, weight collection, and body measurements in August 2016. Todd Froberg received permits to capture, band, test and release birds from the US Fish and Wildlife Service and the Minnesota Department of Natural Resources.

USFWS wildlife biologists, Dr. Tom Cooper and Dave Fronzak, trained Todd and Beth in September 2016 on proper and safe use of the net launcher to safely and efficiently trap and sample gulls. Dr. Chris Jennelle from the Minnesota Department of Natural Resources reviewed protocol on proper blood sampling procedures.

Todd, Marie, and Elizabeth promoted voluntary participation in the study from the Minnesota Turkey Growers Association, the Minnesota Chicken and Egg Producers, the Minnesota Audubon Society, and the Minnesota Ornithologists' Union at meetings in September, October, November and December 2016.

In fall 2016, 6 field and farm locations in 4 counties (Wright, Kandiyohi, Stearns, and Carver) of Minnesota were evaluated as suitable sampling and trapping sites, of which 2 sites were successful trapping locations from which samples were obtained. A total of 107 birds were captured, banded, tested, and released in Fall 2016 during 1 field site visit and 3 farm visits. There were 13 ring-billed gulls from Carver County, MN (the field site) and 91 ring-billed gulls and 3 herring gulls from Kandiyohi County, MN (the farm site). Additionally, 25 Franklin's gulls were caught and sampled in collaboration with the USDA Wildlife Services team at a DDG ethanol plant in Fairmont MN on August 11, 2016. A single cloacal swab ID TF110416003 collected on 11-4-2016 from a ring-billed gull with band ID 0994-08032 at Kandiyohi County tested positive for influenza A virus by real-time reverse transcriptase polymerase chain reaction (rRT-PCR) test at the University of Minnesota MidCentral Research and Outreach Center Laboratory (MCROC Lab). rRT-PCR tests for H5 and H7 avian influenza virus were negative at MCROC Lab. Whole genome sequencing of the swab ID TF110416003 CL is pending for influenza A virus genes at the University of Minnesota College of Veterinary Medicine. All 106 other gulls were negative for avian influenza virus by rRT-PCR on both cloacal swabs and oropharyngeal swabs at MCROC Lab. Blood samples were collected from all 107 birds and serology tests for avian influenza A virus antibodies are pending at MCROC Lab.

All gulls were in good health at the time of capture and release. There were no capture-associated mortalities in the Fall 2016 sampling season. Gulls were observed feeding in all 6 of the suitable trapping locations identified in the 4 counties above. Gulls were captured while feeding and loafing in Carver and Kandiyohi counties. Gulls were observed to co-mingle with multiple *Anatidae species* (Mallards, Northern Shovelers, Buffleheads, Goldeneyes, Northern Pintails, Gadwalls, and Canada Geese) at the trapping site in Stearns County. At the Carver County site, Gulls were observed co-mingling with *Anatidae species* (Mallards and Canada Geese). At the Kandiyohi County trapping site, gulls were observed co-mingling with *Cathartidae species* (Turkey Vulture) and *Sturnidae species* (European Starlings) and were in close proximity with domestic turkeys (e.g. they were roosting on the roofs of the barns). In Wright County, gulls were observed co-mingling with *Anatidae species* (Mallard, Wood Duck, and Gadwall). Ring-billed gulls were observed to co-mingle with Mallards, Buffleheads,

Pied-billed Grebes, Double-crested Cormorants, American Coots, Canada Geese, and Trumpeter Swans at Lake Harriet (Hennepin County) and Lake Phalen (Ramsey County).

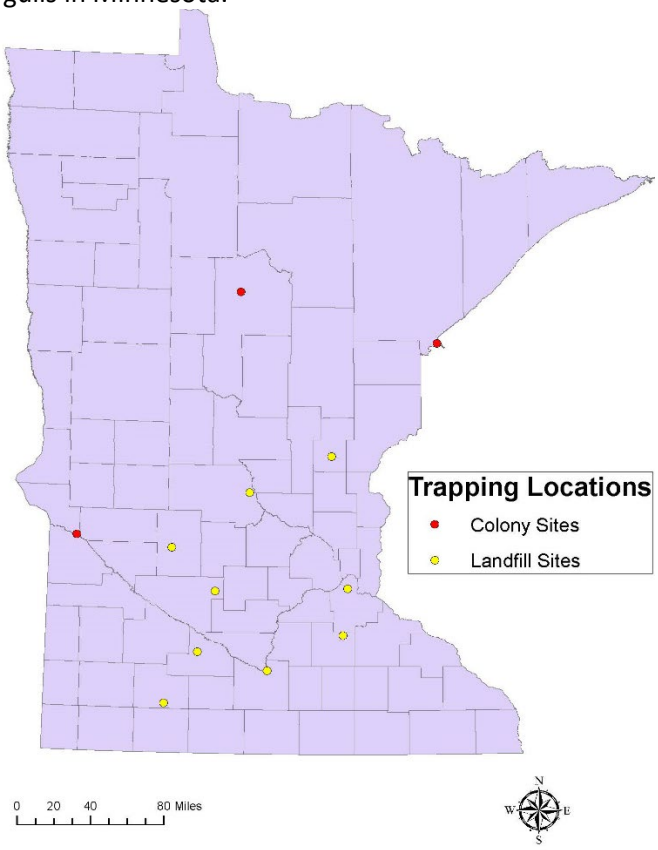
**Amendment Request 02/03/2017:**
The work plan needs to be amended as follows: The ENTRF Budget for Activity 1 is $103,372, not $109,276 as was listed in the rev6-30-2016 work plan and not $85,517 as was listed in the rev6-30-2016 budget. The budget spreadsheet incorrectly listed personnel budget for Activity 1 as $29,460. The correct personnel budget for Activity 1 is $47,315 (which is 53% of the total personnel budget which is and has always been correctly listed as $89,273 total for personnel). There has been $0 spent on Activity 1. The balance should be $103,372 and this is correctly listed on both the amended work plan rev 2-3-2017 and amended budget rev 2-3-2017.
The ENTRF Budget for Activity 2 is $110,071, not $104,167 as was listed in the rev6-30-2016 work plan and not $83,766 as was listed in the rev6-30-2016 budget. The budget spreadsheet incorrectly listed personnel budget for Activity 2 as $19,738. The correct personnel budget for Activity 2 is $41,958 (which is 47% of the total personnel budget; the total has always been correctly listed as $89,273 for total personnel). Furthermore, the budget incorrectly listed Professional/Technical Services Contract as $2,012. The correct budget for Professional/Technical Services Contract is $6097. There has been $16,514 spent on Activity 2. The balance should be $93,557 and this is correctly listed on both the amended work plan rev 2-3-2017 and amended budget rev 2-3-2017.
**Amendment Approved by LCCMR 02/08/17**

**Amendment Request 03/01/17:**
Please extend the availability of funds to the project, e.g., please allow a no-cost extension, to June 30, 2018.
**Amendment Approved by LCCMR: May 30, 2017**

**Project Status as of July 2017:**
In January 2017, the Minnesota Turkey Growers and the Minnesota Board of Animal Health provided GPS location information of poultry farms (chicken and turkey) within Minnesota under a data transfer and confidentiality agreement. Todd, Francesca, and Chris used the geographic locations of the farms and a land-use cover type map to identify high and low-density poultry areas, heavy agricultural areas, wetlands, MSW landfill facility locations and breeding gull colonies to determine ideal trapping locations. It was determined from poultry farm GPS locations that four high-density poultry sites and three lower-density poultry sites for trapping locations during migration periods would help identify spatial prevalence of AI in gulls in relation to poultry farms. The four high-density areas are Kandiyohi, Cottonwood, Blue Earth, and Rice counties. The three lower-density areas are Dakota, Kanabec, and Sherburne counties. In addition to migration trapping sites, three colony sites were chosen based on past reports of gull numbers and their respective locations in the state spatially. The three colony sites include Little Pelican Island on Leech Lake in Cass County, Interstate Island on the St. Louis River in St. Louis County, and lastly, Hermit Island on Marsh Lake in Swift County.

In February 2017, the UMN and DNR collaborators met as a group to discuss the results of the mapping exercises and to determine suitable sites for Spring migration sampling and breeding colony sampling. To meet our goals of testing the required number of birds, the decision to sample sanitary landfills was made as these areas are frequented by numerous gulls at predictable times. Given the very brief stopover period of gulls at wildlife management areas (WMAs) during spring migration (e.g., one day) and the associated difficulty in capturing significant numbers of birds for sampling at these locations, we elected to employ strategies implied in the study design to include sanitary landfill locations across the state (Figure 1). These locations have generally maintained sizable numbers of gulls (>100) that are available to be captured and sampled. Furthermore, gulls are not obligate landfill visitors and turnover of individuals is significant, which supports the assumption that these birds are representative of the greater population of gulls in MN. Given our limited sampling time frame, this additional sampling strategy allows us to ensure our sample quotas are achievable.

Figure 1. Trapping locations for gulls in Minnesota.




In March 2017, we recruited Minnesota poultry producers for the project at the Annual Meeting of the Minnesota Turkey Growers held in St. Paul, MN on March 13 and 14. The recruitment resulted in 4 farmers, two in Stearns County, 1 in Wright, and 1 in Sibley, showing keen interest in the project. Despite regular contact and visits to these farms, trapping gulls on actual farm property proved elusive for spring migration. However, all farmers agreed to continue to cooperate through late summer and fall. Each farmer also mentioned that gulls were more frequently on farm properties in August and September rather than March or April. On June 21, 2017, project collaborator Dr. Carol Cardona presented a research update of our project to the Summer Meeting of the Minnesota Turkey Growers in Brainerd, MN. The project was well received and an additional 3 poultry farmers expressed interest in having our team collect bird samples on their farms during early Fall migration. Mr. Steve Olson of the Minnesota Turkey Growers will be in contact and receive updates from the newly recruited poultry farmers.

During the Spring migration, a total of 150 gulls were captured using a net launcher, banded, and sampled. Morbidity and mortality associated with the trapping was very low, with only 2 ring-billed gulls humanely euthanized by cervical dislocation during the spring migration period due to a broken wing. Injuries were caused during capture in the net or from handling during the pre-processing period. Gulls were observed co-mingling with a diversity other species at different trapping locations as follows:

- Blue Earth County - *Accipitridae* (Red-tailed Hawk and Bald Eagle), *Cathartidae* (Turkey Vulture), *Corvidae* (American Crow).
- Cottonwood County - *Anatidae* (Blue-winged Teal, Northern Shoveler, Mallard, Canada Goose, and Bufflehead) and *Sturnidae* (European Starling).

- Dakota County - *Accipitridae* (Bald Eagle), *Anatidae* (Canada Goose, Mallard), *Cathartidae* (Turkey Vulture), *Corvidae* (American Crow), and *Sturnidae* (European Starling).
- Kanabec County - *Accipitridae* (Bald Eagle, Red-tailed Hawk), *Cathartidae* (Turkey Vulture), *Columbidae* (Rock Pigeon), *Icteridae* (Red-winged Blackbird), *Passeridae* (House Sparrow).
- Kandiyohi County - *Anatidae* (Canada Goose), *Cathartidae* (Turkey Vulture), and *Sturnidae* (European Starling).
- Rice County - *Accipitridae* (Bald Eagle)*, Cathartidae* (Turkey Vulture), *Corvidae* (American Crow), and *Sturnidae* (European Starling).
- Sherburne County - *Accipitridae* (Bald Eagle)*, Cathartidae* (Turkey Vulture), *Corvidae* (American Crow), and *Sturnidae* (European Starling).

Due to the warm, wet spring, trapping gulls in large numbers on farm fields was not possible. Most farmers got into the fields later than expected due to wet fields. In addition, with the early thaw and warm weather, gulls arrived early and began to nest earlier than in typical years. This combination of factors led to a majority of gulls completing migrations to colonies before most farmers were working in the fields, leaving little opportunity to trap gulls in association with field work. Nevertheless, the spring trapping work was fruitful.

As spring migration came to a close, the project work focused on the breeding colony testing. Working closely with our collaborators at the Minnesota Department of Natural Resources and the Leech Lake Division of Resource Management, we sampled at three gull breeding colonies in Minnesota - Hermit Island on Marsh Lake in Big Stone County, Little Pelican Island on Leech Lake in Cass County, and Interstate Island on the St. Louis River in St. Louis County. Three visits were made to Marsh Lake, 6 visits to Interstate Island, and 3 to Leech Lake. A total of 470 birds were captured, banded, measured, sampled, and released in May and June 2017. In addition, 475 environmental swabs were collected from the nesting areas. The breeding colony sampling is on-going throughout the summer and will conclude in July or August 2017.

From May 2017 through June 2017, the Minnesota Department of Natural Resources provided unanticipated in-kind funding to the project for sampling supplies and serological testing of blood samples. This briefly available funding string allowed baseline serological testing of 565 samples, and allows LCCMR project dollars to go further by sampling additional birds. Additionally in May and June 2017, the Minnesota Poultry Testing Laboratory quickly obtained and validated for routine use on wild birds in their laboratory the IDEXX AI MultiS-Screen Ab Test ® and delivered timely results on all sera obtained from the birds sampled in the project.

The Institutional Biosafety Committee (IBC) granted approval on June 2, 2017 under Protocol ID: 1508-32918H. Please note that the IACUC approval granted approval in 2016 under Protocol # 1609-34152A.

**Project Status as of December 31, 2017:**

From July 2017 to December 31, 2017, 883 additional gulls were caught, sampled, and released at 3 breeding colonies and 6 landfills. We captured and sampled a total of 1346 ring-billed gulls during 2016-2017, including 257 juveniles, 295 hatch-year, 727 adults and 67 unknown age birds. In addition, we collected 715 environmental feces samples. All environmental fecal samples were collected at the breeding colonies during the summer sample collection period. Since H5 and H7 highly-pathogenic avian influenza virus was not detected in any of our samples by rRT-PCR, we ran a model to predict apparent prevalence when "0" positives are found. Considering the sample size we collected, we determined that there was a 95% probability that overall H5 and H7 highly-pathogenic avian influenza virus sample prevalence was between 0.0-0.22%.

Our results across colonial sites and seasons varied, but a consistent finding was juvenile and hatch year birds had higher avian influenza virus prevalence. Furthermore, swabs from the oropharynx and cloaca showed a significant difference in avian influenza virus prevalence. Oropharyngeal swab testing yielded true avian influenza virus prevalence rate estimates of 15.41% avian influenza virus prevalence compared to just 2.50% prevalence of avian influenza virus when only cloacal swab results were analyzed. More birds (26.06%) were avian influenza virus positive for any combination of

cloacal or oropharyngeal or both compared to 8.14% of all gulls that were positive for both oral and cloacal samples. These results indicate, as other studies have shown, that birds of the Charadriiformes more commonly shed virus via the oropharyngeal route which has likely implications regarding transmission to other species and surveillance strategies. It is important to consider that avian influenza virus was detected in both oropharyngeal and cloacal specimens of 104 birds and occasionally in only cloacal (33 birds), making it essential to swab both oropharyngeal and cloacal for avian influenza virus surveillance efforts in gulls.

During our study, only two H5 avian influenza viruses were detected by subtype specific rRT-PCR and neither has been confirmed as HP avian influenza virus by gene sequencing. It will be important to further analyze all swabs that tested positive for avian influenza virus with Ct values <30 and subject them to whole genome sequencing (WGS) to further characterize the viruses detected similar to work described by others. WGS is being attempted through collaborations with scientists at both the University of Minnesota and, at no additional charge, the J. Craig Venter Institute in La Jolla, California. The whole genome sequences will provide insight into the virus genes harbored by gulls and analyses of the genes will be attempted to elucidate the direction of virus movement between wild birds and domesticated poultry. Phylogenetic relationships will be inferred separately for each influenza gene sequence alignment using the maximum likelihood methods available. Sequences will be examined to determine if they share a common ancestry with the domestic HPAI H5 viruses of 2014-2015 or from other years and locations. The implications for not finding H5 avian influenza virus by rRT-PCR could mean that gulls do not harbor or circulate H5 avian influenza viruses or that timing and geographic location is imperative of detection for H5 Avian influenza virus during outbreaks. Gulls are an underrepresented species within avian influenza virus surveillance efforts and warrant additional research, as Charadriiformes species constitute a large source of the avian influenza virus isolates. Ring-billed gulls are ideal candidates for further research as they share habitat with a diverse group of species commonly associated with avian influenza viruses. Some of these species, including blue-winged teal (*Anas crecca*) and northern pintail (*Anas acuta*), make long seasonal migrations. Northern pintails in particular have been linked to intercontinental migrations, potentially exposing gulls or other species in North America to Eurasian influenza strains. Our colony location prevalence rates did positively correlate with higher avian influenza virus prevalence and greater avian species diversity.

As of December 31, 2017, we have not detected H5 highly pathogenic avian influenza virus and these preliminary results suggest that gulls likely did not have a direct role in transmission to poultry facilities. Their role in avian influenza virus movements overall is unclear. Fall is a high-risk time period for avian influenza virus in Minnesota thus targeted seasonal surveillance is important and should be designed for optimum age, season, and spatial heterogeneity. Research funds could be more effectively extended in future studies if both oral and cloacal swabs are collected from birds and then those swabs are combined into one tube. Gull movements, community behavior ecology and bird diversity in colonies is likely very important, but is currently not known and offer opportunities for future research. Body condition index (BCI) also needs to be further explored to determine the negative effects that avian influenza virus may have on gulls. Our preliminary results indicate body condition index was correlated with influenza A virus status in hatch year birds. That is, for every unit increase in BCI score, there is a 16% decrease in avian influenza virus positive prevalence. Low BCI in the birds could have potential carry-over effects on young birds (delayed migration) or avian influenza virus susceptible bird species (raptors).

Work to perform and analyze Whole Genome Sequencing as part of both Activities 1 and 2 is still underway and may require additional funds in order to complete. If so, an amendment request will be submitted.

Our study has some limitations in time, sample size, and geographic space. Nevertheless, in the last 6 months of the work we will strive to obtain valuable variables to further evaluate the ecology and evolution of avian influenza viruses within ring-billed gulls in Minnesota. The differences in prevalence rates on colonies may indicate the importance of geographic isolation of colonies and that migration routes for ring-billed gulls may play an important role for avian influenza virus exposure within ring-billed gulls, especially when compared to migrations other common avian influenza virus harboring species. Our group has proposed future research of avian influenza virus surveillance for ring-billed gulls to other funding sources. In these new proposals, we build off this work supported and completed here but we hope to focus surveillance efforts on hatch-year or juvenile birds in late summer and fall, a larger geographic scale, and a larger sample size.

**Amendment Request (02/16/2018):** We would like funds to be shifted between budget activities in order to complete the still pending Whole Genome Sequencing for both Activities 1 and 2. In particular, we would like to shift $8,104 from Travel

in Minnesota to Equipment/Tools/Supplies. The Travel In Minnesota funds were not spent down because our team had far fewer than budgeted overnight stays. However, Whole Genome Sequencing has proved difficult and requires additional testing, such as simple, direct Sanger sequencing of the hemagglutinin and neuraminidase genes, which are proving to be necessary first steps prior to proceeding to the more complicated Whole Genome Sequencing. **Amendment Approved by LCCMR 3/12/2018**

## Project Status as of December 21, 2018:

One of our project goals was to determine if avian influenza viruses of Minnesota gulls are genetically diverse and likely to reassort. The genetic makeup, genetic evolution and direction of virus movement between wild birds and domestic poultry is not fully known but can be assessed through whole genome sequencing of the avian influenza virus found in gulls in our current and proposed surveillance. Analyses of the genetic sequences obtained in our surveillance efforts will determine the genetic composition of influenza viruses in gulls and those that may have potential poultry area contact, as inferred by satellite and camera monitoring. We hypothesize that, through whole genome sequencing of influenza A viruses, the gull viruses will be reassortants containing internal gene segments of different geographical and/or avian host origin, such as Minnesota turkey-origin 2015 highly pathogenic avian influenza H5N2.

To achieve this, we used the following approach and methodology. Gulls were baited into walk-in traps. All trapped birds were banded and released. The birds were also sampled with swabs (cloacal and oral); blood was collected for influenza antibody tests. Swabs were tested for avian influenza virus with rRT-PCR following standard methods (Spackman 2008) in the Midwest Central Research and Outreach Center laboratory in Willmar, MN. All positives with PCR cycle threshold values <30 (strong positives) were subjected to whole genome sequence (WGS) analysis by influenza researchers at the J. Craig Venter Institute (JCVI). The JCVI is a world leader in genomic and bioinformatics research fueled by a team-centered, multidisciplinary approach to large research initiatives. JCVI has a long track-record of creative and interdisciplinary approaches to genomics and bioinformatics. Numbers of strong positives for WGS were 38 cloacal swabs and 29 oral swabs.

The JCVI successfully sequenced influenza A virus genes from 49 of the 67 strong positive samples and the complete genome was sequenced from 45 samples. The genetic sequence information was successfully submitted to GenBank. GenBank ® is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences (*Nucleic Acids Research*, 2013 Jan;41(D1):D36-42). GenBank is part of the International Nucleotide Sequence Database Collaboration. The sequences are also easily retrievable and available for analysis at the Influenza Research Database (IRD). The IRD, accessible at http://www.fludb.org, is a free, open, publicly-accessible resource funded by the U.S. National Institute of Allergy and Infectious Diseases through the Bioinformatics Resource Centers program. IRD provides a comprehensive, integrated database and analysis resource for influenza sequence, surveillance, and research data, including user-friendly interfaces for data retrieval, visualization and comparative genomics analysis, together with personal login-protected 'workbench' spaces for saving data sets and analysis results. IRD integrates genomic, proteomic, immune epitope, and surveillance data from a variety of sources, including public databases, computational algorithms, external research groups, and the scientific literature.

A table of all the influenza A virus strains identified as a result of this project and the associated accession numbers for each segment of each strain is provided at the end of this report as Table S1. This project contributed 45 complete genomes to the influenza database, increasing the sequencing information available for Minnesota's wild birds by 12%.

Analyses completed on these sequences to date suggest that the influenza A viruses detected in Minnesota ring-billed gulls in 2017 were not genetic precursors to, nor were they genetic descendants of, the highly pathogenic H5N2 influenza A viruses that infected Minnesota's domestic poultry in 2015. Rather they are distinct North American gull-lineage viruses of H13NX subtype with occasional evidence of reassortment with gull-lineage viruses from Asia and South America. In addition, the Minnesota gull influenza A viruses have only moderate genetic diversity among geographic locations and minimal genetic diversity within geographic locations.

| | Count of Subtype by Location | | | | | |
|---|---|---|---|---|---|---|
| **Location** | **H?N6** | **H13N2** | **H13N6** | **H13N8** | **mixed** | **Grand Total** |
| Mora Landfill | | | 1 | | | 1 |
| Kandiyohi County Landfill | | 4 | | 1 | | 5 |
| Ponderosa Sanitary Landfill | 1 | 2 | 3 | | 1 | 7 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Marsh Lake Big Island | 2 | | 8 | | | 10 |
| Little Pelican Island Leech Lake | | | | 26 | | 26 |
| **Grand Total** | **3** | **6** | **12** | **27** | **1** | **49** |

In addition to the sequencing data analyses, a manuscript describing the influenza A virus detection rates by specimen type for the gulls was accepted for publication in a special edition of Avian Diseases. The full manuscript is attached as supplement 1 to this report. Finally, this work was noteworthy in that it fulfilled the requirements for a Master's thesis in Conservation Biology for Mr. Todd Froberg. His thesis is attached as a supplement 2 to this report.

**Overall Project Outcomes and Results:**

In summary, this project provided novel and important information regarding the avian influenza prevalence and viral shedding routes in Minnesota ring-billed gulls and the genetic characteristics of the influenza A viruses detected. We addressed a gap in influenza A virus surveillance by sampling 1346 ring-billed gulls (*Larus delawarensis*) during Spring and Fall migrations and at three breeding sites in 2017 across Minnesota. Results indicated noticeable age-cohort dynamics in AI virus prevalence within ring-billed gulls in Minnesota. Immunologically naïve juveniles represented the cohort with the highest prevalence rate (57.8%). Regardless of age, more gulls had AI virus detected in oropharyngeal than in cloacal swabs.

Our results varied among colony sites and seasons, but a consistent finding was that juvenile and hatch year birds had higher avian influenza virus prevalence than adults. Furthermore, swabs from the oropharynx and cloaca demonstrated a significant difference in avian influenza virus prevalence. Oropharyngeal swab testing yielded true avian influenza virus prevalence estimates of 23.55%, versus 10.64% for cloacal swab testing. These results suggest, as other studies have shown, that gulls more commonly shed avian influenza virus via the oropharyngeal route which may facilitate transmission to other species and have implications for surveillance strategies. Although our results indicate that gulls shed virus predominately through the oropharyngeal cavity it is important to consider the apparent prevalence bias of sampling only the oral cavity. If only oral cavities were sampled, our estimates of sample prevalence would have been negatively biased by 2.5% considering all sampled birds together. Using this approach, avian influenza virus detection would have been missed in 34 birds. This negative bias would have been highest at 6.4% if we conditioned the analysis on local birds, resulting in 86 missed detections. The negative bias would have been negligible for adults at <1% yet would still result in 12 missed detections. This example supports the practice of swabbing both oropharyngeal and cloacal cavities for avian influenza virus surveillance efforts in gulls. If funding is limited, then oropharyngeal and cloacal swabs should be taken and pooled into one tube.

During our study, we identified only two H5 avian influenza viruses that were detected by subtype specific rRT-PCR and neither was confirmed as highly pathogenic avian influenza virus by gene sequencing. We further analyzed all swabs that tested positive for avian influenza virus with Ct values <30 and subjected them to whole genome sequencing to further characterize the viruses detected and found H13N6, H13N8, and H13N2 viruses. Analyses of these genes showed that there was apparently no virus movement between wild gulls and domestic poultry in Minnesota in the time period studied.

**Table of Overall Summary of Results by Specimen Type and Age.** Avian influenza virus rRT-PCR positive Cycle Threshold (Ct) value results for ring-billed gulls sampled in Minnesota during 2017, by birds of known age and shedding route. Using set theory, OP is the set of all possible oropharyngeal positive results, CL is the set of all possible cloacal positive results, OP-CL is the set of oropharyngeal positive results that do not also include cloacal positive results, CL-OP is the set of cloacal positive results that do not also include oropharyngeal positive results, and OP∩CL is the set of oropharyngeal positive results that are also associated with cloacal positive results.

| Age | Shedding Route | Positive/Total [A] | Ct Average[B] | Ct Range |
|---|---|---|---|---|
| All | OP | 301/1345 | 33.72 | 26.13–39.38 |
| | OP-CL | 197/1346 | 33.72 | 26.13–39.38 |
| | CL | 136/1346 | 32.01 | 15.94–38.97 |
| | CL-OP | 32/1346 | 32.69 | 22.71–38.97 |
| | OP∩CL | 104/1346 | 32.16 | 15.94–39.23 |
| Local | OP | 84/293 | 31.94[CD] | 27.13–37.98 |
| | OP-CL | 38/296 | 32.35 | 27.13–37.98 |
| | CL | 64/294 | 30.93[D] | 18.67–38.97 |
| | CL-OP | 18/296 | 33.00 | 24.55–38.97 |
| | OP∩CL | 46/296 | 30.86 | 18.67–37.00 |
| Juvenile | OP | 133/257 | 34.47[A] | 26.13–39.38 |
| | OP-CL | 88/257 | 34.99 | 27.39–39.38 |
| | CL | 53/257 | 32.61[BC] | 15.94–38.55 |
| | CL-OP | 8/257 | 32.61 | 29.81–37.63 |
| | OP∩CL | 45/257 | 33.29 | 15.94–39.23 |
| Adult | OP | 83/728 | 34.33[A] | 28.89–37.79 |
| | OP-CL | 70/727 | 34.66 | 30.44–37.79 |
| | CL | 19/728 | 33.94[AB] | 21.35–37.65 |
| | CL-OP | 6/727 | 35.60 | 31.16–37.63 |
| | OP∩CL | 13/727 | 32.85 | 21.35–37.65 |

[A]Total number collected is different for CL and oropharyngeal OP swabs, because we failed to collect an OP swab from one local bird and thus had only a CL swab to test.

[B]Average Ct values of positives by age group that do not share a capital letter are significantly different with a *P* value of <0.05 when grouping information using Bonferroni method of multiple comparisons and 95% CI.

The high AI virus prevalence within ring-billed gulls, particularly in immunologically naïve birds, warrants further targeted surveillance efforts of ring-billed gulls and other closely related species. Sequence analyses completed on the viral genes identified, suggest that our data group separately from highly pathogenic H5NX avian influenza viruses that devastated Minnesota poultry in 2015 which is interesting and suggests that gulls are not part of the poultry transmission cycle (see Figure 2 as an example). Additional analyses will be conducted and we look forward to further research using the data generated from this successfully completed project.

**Amendment 2-15-2019 Overall Project Outcomes and Results:**

To summarize, this project achieved its original goals. Specifically, each of these goals (below in italics) was met in the following ways (below in bold):

*1) if gulls played a role in the past outbreak.*

**A) No, gulls did not play a role in the outbreak since the outbreak virus, H5N2, was not detected in our gulls. Additionally, there was no unequivocal evidence that the viruses detected in our study gulls possessed viral genes that were descendants or ancestors of the outbreak virus.**

*2) the presence or distribution of avian influenza in gulls and the evolutionary potential of avian influenza viruses in Minnesota's gulls.*

**B) Avian influenza in gulls was determined to be widely distributed throughout our 9 study areas, with the exception of the Cottonwood county landfill in Windom, MN, the only location where we collected a sufficient number of specimens yet there were no avian influenza viruses detected in either visit to this location. Of note, Waconia county also had no avian influenza viruses detected but the number of birds tested during our only visit was very small (n=13).**

*3) possible ways to predict or prevent a massive China-like outbreak in Minnesota's wild birds.*

**C) We successfully developed a way to effectively capture, swab, test, and release a large population of gulls throughout the state of Minnesota. Should the need arise to sample gulls in the future in response to an outbreak, we have developed the methods to do so through this study. We have also been able to share our refined gull sampling methods with avian influenza researchers throughout the globe, including researchers in China and Russia, two countries where highly pathogenic avian influenza viruses can emerge.**

*4) if there are any negative effects of avian influenza infection in the migratory or breeding gulls in Minnesota.*

**D) Both the migratory and breeding gulls in Minnesota were overall healthy populations in that they were abundant and successfully raising chicks. We did, however, detect a statistically significant lower body weight in gulls that were avian influenza virus positive, suggesting that avian influenza virus infections cause some level of illness but not death. Specifically, we calculated body condition index using weight and body measurements for every gull as an indicator of gull health. For every body condition score unit increase, the odds of being avian influenza positive decreased 16%. We found significant seasonal and age dynamics in virus prevalence, with juvenile gulls during fall migration having the highest apparent prevalence (68.36%).**

We met the stated outcomes of this project. In particular, our achievements for each outcome are as follows:

*1) track virus evolution in these gulls through whole genome sequencing of the viruses detected,*

**We successfully sequenced 45 complete genomes of avian influenza viruses out of 67 attempts. Table S1, at the end of this report, contains all the influenza A virus strains identified because of this project and the associated accession numbers for each segment of each strain. We increased the genetic sequencing information available for Minnesota's wild birds by 12%. Through our analysis of these sequences, we were able to track the avian influenza virus evolution in Minnesota's gulls to two distinct origins. In particular, we found that the H13 genes from the avian influenza viruses detected in the Marsh Lake breeding colony are from viruses that have likely evolutionary origins similar to those of gull-lineage viruses in the Atlantic flyway. In contrast, the Leech Lake gull breeding colony has avian influenza viruses that have a likely evolutionary origin similar to that of gull-lineage viruses in the Pacific flyway. That Minnesota can serve as a location that receives avian influenza viruses with origins as diverse as the Pacific and Atlantic flyways is interesting as it implies**

**that avian influenza viruses from all continents could travel through gulls to Minnesota under favorable conditions. We are particularly eager to validate these findings and have shared the avian influenza virus genetic information in the public Influenza Research Database. As more avian influenza virus researchers share their data, more concrete connections and evolutionary relationships between viruses can be established.**

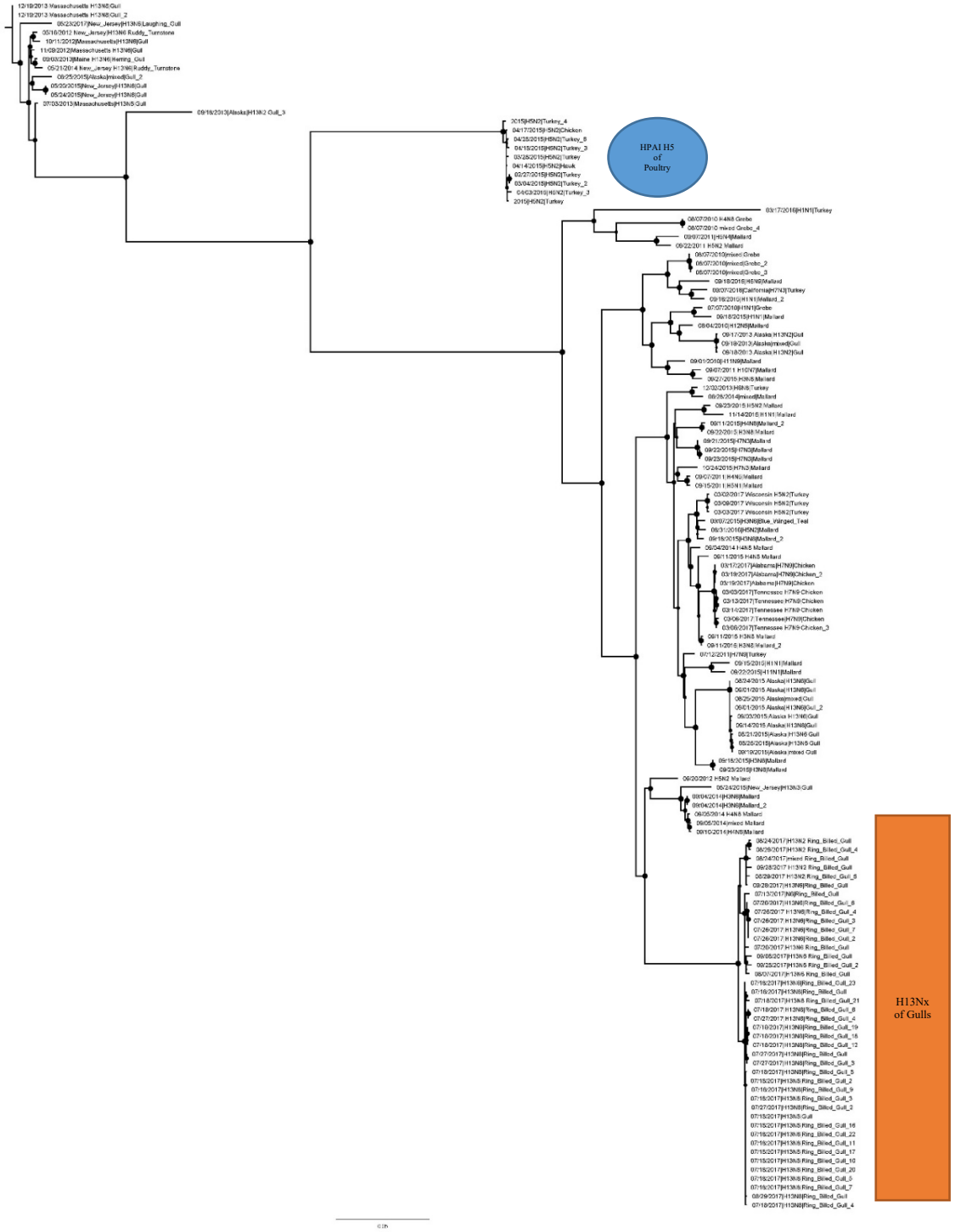
*2) define the role of one or both gull species in the avian influenza outbreak in Minnesota, and*

**Our analyses of the gull avian influenza virus gene sequences indicate the ring-billed gulls did not contribute to nor were they recipients of the highly pathogenic H5 avian influenza viruses that caused the outbreak in Minnesota. The ring-billed gulls did not have H5 viruses detected nor did any of the other genes of the influenza viruses share high similarity to the outbreak strains.**

*3) help us better understand the potential negative effects of the highly pathogenic avian influenza H5 virus outbreak on the many inhabitants - wild, domestic, avian, and human - of Minnesota's shared ecosystem.*

***Fortunately, the gulls in our study were all healthy, abundant, raising chicks, and migrating successfully. While we did detect avian influenza viruses in lower weight gulls, nevertheless, these gulls were showing no other negative effects from the infection. Moreover, gulls in Minnesota are successfully migrating to wintering ground as is apparent from the re-sighting data in locations are far as Florida. Additionally, several of the gulls in our study returned to Minnesota to the breeding colonies and were re-sighted and actively invested in their nests.***

**Figure 2.** Maximum likelihood tree from influenza A virus gene segment 1, polymerase basic 2 (PB2). All sequences without a state code came from Minnesota. Node shape size (black circles) indicates support for the node placement. Also included in the tree are all PB2s from Minnesota avians available in GenBank from birds sampled between 2010-2017 and PB2s from all H13NX from the same time across United States (mostly from gulls). All identical sequences and partial sequenced 1500 base pairs in length were removed. The cluster of H5 highly pathogenic avian influenza viruses of poultry are represented by the blue circle. The gulls from our study are represented by the orange rectangle.



**IV. PROJECT ACTIVITIES AND OUTCOMES:**

**ACTIVITY 1:** Testing of Gulls in Breeding Colonies for Avian Influenza

**Description:** Gulls are underrepresented in Minnesota surveillance efforts despite frequent reports of gulls on poultry farms. Additionally, gulls are the most frequently detected AI positive wild birds in global surveillance for avian influenza. Our teams will visit two gull breeding colonies in Minnesota. Each colony will be visited once weekly for 4 weeks. At each visit, we will collect 100 fecal samples from the environment and catch 50 gulls by snare or box trap. The gulls will be banded with a uniquely numbered aluminum leg band plus a combination of colored plastic bands to facilitate future identification and recapture. Additionally, an oral swab, cloacal swab, and blood sample will be collected from each gull. All swabs will be tested for avian influenza by a rapid polymerase chain reaction test. Any avian influenza positives will be further characterized by completing whole genome sequencing of the avian influenza found. The blood samples will be tested for antibodies to avian influenza to determine any previous exposure and susceptibility to subsequent exposure. Studying gulls in the breeding colonies allows us to obtain numerous samples from a resident population over time, thus increasing our chances of detecting waves of influenza infection, definitively identifying and observing gulls, and assessing both adult, hatch year, and juvenile birds.

**Summary Budget Information for Activity 1:**

**ENRTF Budget:** $103,372
**Amount Spent:** $103,372
**Balance:** $0

| Outcomes of Activity 1 | Completion Date |
|---|---|
| Visit gull colonies; capture, and band gulls; collect samples to test for avian influenza and observe the population of the colony | May 2017 |
| Test Swab samples and Blood samples for avian influenza for avian influenza to determine influenza infection status in the colony of gulls | June and July 2017 |
| Perform Whole Genome Sequencing on avian influenza positive samples to characterize the viruses and compare their genetic structure to those of the H5 avian influenza outbreak of 2015 | August 2017 |
| Analyze Whole Genome Sequencing results to determine directionality of virus movement, virus origins, and introduction of new virus genes into Minnesota | September 2017 |

**Activity 1 Project Status as of January 2017:**

Not yet started. This activity will begin in May 2017 at two colonies in Minnesota – Gull Island in Leech Lake and Interstate Island Wildlife Management Area in Lake Superior. Permits have been approved by the state of Minnesota Department of Natural Resources to trap, sample, and band gulls on Interstate Island. The Leech Lake Division of Resource Management gave permission to work on Gull Island.

**Activity 1 Project Status as of July 2017:**

In May and June 2017, 3 breeding colonies were visited and trapping resulted in capture of 470 birds. Trapping began at Hermit Island on Marsh Lake in Big Stone County on May 10, 2017 and continued until May 24, 2017 when most nests had hatched. We estimated approximately 1,000 ring-billed gull nests, 10 American White Pelican nests, and 200 Double-crested Cormorant nests on Hermit Island. A total of 72 gulls (71 ring-billed gulls, 1 Franklin's gull) were caught on Hermit Island using walk-in-nest traps. Gulls were observed co-mingling with various other species on Marsh Lake including; *Anatidae* species (Canada Goose), *Ardeidae* (Black-crowned Night-Heron, Great Blue Heron, Great Egret), *Laridae* (Common Tern, Caspian Tern), *Pelecanidae* (American White Pelican), *Phalacrocoracidae* (Double-crested Cormorant), and *Scolopacidae* (Ruddy Turnstone, Marbled Godwit).

Trapping began at Interstate Island on May 13, 2017 and concluded with near-complete hatching on June 7, 2017. We estimated approximately 13,000 nesting pairs of ring-billed gulls, 15 nesting pairs of herring gulls, 150 nesting pairs of Common Terns, and 5 nesting pairs of Canada Geese on Interstate Island. A total of 279 ring-billed gulls were caught on Intestate Island using a net launcher and hand nets. There was no direct chick or adult mortality associated with trapping events on Interstate Island. Gulls were seen co-mingling with multiple species on Interstate Island including; *Anatidae* (Canada Goose, Mallard, Ring-necked Duck, Common

Merganser, and Lesser Scaup), *Laridae* (Common Tern), *Phalacrocoracidae* (Double-crested Cormorant), and *Scolopacidae* (Ruddy Turnstone).

Trapping began on Little Pelican Island May 31, 2017 and is currently still in progress with about 80% of eggs hatched to date. A total of 119 birds have been caught at Little Pelican Island on Leech Lake using walk-in-nest traps. A diversity of other species nest on Little Pelican Island including Caspian Terns, Double-crested Cormorants, and American White Pelicans. There has been no official estimate of nesting numbers to date. Gulls have been seen co-mingling with; *Anatidae* (Canada Goose, Mallard, Bufflehead, Common Goldeneye, and Lesser Scaup), *Icteridae* (Red-winged Blackbird), *Laridae* (Common Tern, Caspian Tern), *Phalacrocoracidae* (Double-crested Cormorant), *Pelecanidae* (American White Pelican), *Scolopacidae* (Ruddy Turnstone, Sanderling, Least Sandpiper) and *Charadriidae* (Black-bellied Plover).

The influenza A virus PCR test results on the gulls in the breeding colonies are in the table below. All positive influenza A virus test results were obtained from oropharyngeal swabs. All influenza A virus PCR positive oropharyngeal swabs were tested for H5 and H7 influenza A virus by PCR and were negative. Whole genome sequencing of each of the positive swabs is pending at the University of Minnesota. Interestingly, all of the cloacal swabs collected from gulls in breeding colonies have been negative to date.

| | | **INFLUENZA A VIRUS PCR RESULTS** | | | |
|---|---|---|---|---|---|
| **County** | **Trapping Date** | **POSITIVE** | **NEGATIVE** | **Pending** | **Total** |
| ***Big Stone (****Hermit Island on Marsh Lake )****TOTAL*** | | ***1*** | ***71*** | | ***72*** |
| | 5/10/2017 | 0 | 19 | | 19 |
| | 5/23/2017 | 0 | 8 | | 8 |
| | 5/24/2017 | 1 | 44 | | 45 |
| ***Cass*** *Little Pelican Island on Leech Lake)* ***TOTAL*** | | ***3*** | ***116*** | | ***119*** |
| | 5/31/2017 | 0 | 36 | | 36 |
| | 6/1/2017 | 3 | 38 | | 41 |
| | 6/9/2017 | 0 | 42 | | 42 |
| ***St. Louis (****Interstate Island)* ***TOTAL*** | | ***0*** | ***219*** | ***60*** | ***279*** |
| | 5/13/2017 | 0 | 57 | | 57 |
| | 5/25/2017 | 0 | 50 | | 50 |
| | 5/26/2017 | 0 | 68 | | 68 |
| | 5/27/2017 | 0 | 44 | 10 | 54 |
| | 6/6/2017 | 0 | | 31 | 31 |
| | 6/7/2017 | 0 | | 19 | 19 |
| **Grand Total** | | **4** | **406** | **60** | **470** |

The influenza A virus antibody test results from the IDEXX ELISA assays on the sera from the gulls in the breeding colonies are in the table below.

| County / Trapping Date | Influenza A virus Serum Antibody test results | | | |
|---|---|---|---|---|
| | POSITIVE | NEGATIVE | Quantity Not Sufficient | Total Tested |
| ***Big Stone (****Hermit Island on Marsh Lake )****TOTAL*** | ***19*** | | | ***19*** |
| 5/10/2017 | 19 | | | 19 |
| ***Cass*** *Little Pelican Island on Leech Lake)* ***TOTAL*** | ***98*** | ***21*** | | ***119*** |
| 5/31/2017 | 28 | 8 | | 36 |
| 6/1/2017 | 37 | 4 | | 41 |
| 6/9/2017 | 33 | 9 | | 42 |
| ***St. Louis (****Interstate Island)* ***TOTAL*** | ***176*** | ***97*** | ***6*** | ***279*** |
| 5/13/2017 | 38 | 19 | | 57 |
| 5/25/2017 | 27 | 20 | 3 | 50 |
| 5/26/2017 | 38 | 29 | 1 | 68 |
| 5/27/2017 | 33 | 19 | 2 | 54 |
| 6/6/2017 | 25 | 6 | | 31 |
| 6/7/2017 | 15 | 4 | | 19 |
| **Grand Total** | **293** | **118** | **6** | **417** |

Overall, for the breeding colony testing as of July 1, 2017, 417 adult birds have been trapped and sampled. Although not all PCR tests for avian influenza A virus are completed yet, there have been 4 positive gulls; 3 from Leech Lake and 1 from Marsh Lake and all positive PCR test results were obtained from oropharyngeal swabs. Whole genome sequencing of each of the positive swabs is pending at the University of Minnesota. All the environmental swab testing for influenza A virus by PCR is pending. The percentage of birds with serum antibodies for avian influenza virus is high (>50%).

**Activity 1 Project Status as of December 31, 2017:**

Activity 1 work was completed during the summer at three gull breeding colony locations in Minnesota (Interstate Island, Hermit and Big Island, Little Pelican Island). For data analysis, the summer time-period was split into two time periods (Summer 1, Summer 2) to target two age cohorts (adults, hatch year) across the 3 colony locations (Table 1).

***Summer 1***

Summer 1 sample collection period began on 13 May 2017 and ended on 9 June 2017. During this time, we captured and sampled 469 adult ring-billed gulls and the influenza A virus prevalence rate was 0.9% (.25-2.28%). In addition, we collected 465 environmental fecal samples across the 3 colony locations during Summer 1 period resulting in 11 influenza A virus positive samples (2.36%).One environmental fecal sample from Big Stone County was positive for North American lineage H5 influenza A virus by rRT-PCR (Table 2). At Interstate Island in St. Louis County, we captured 279 adult ring-billed gulls, of which no gulls tested positive for influenza A virus. Therefore, we can say with 95% confidence that influenza A virus prevalence levels within the Interstate Island adult population were between 0.0-1.11%. Furthermore, we collected 290 environmental fecal samples at Interstate Island in St. Louis County, with one positive sample resulting in a 0.344% environmental prevalence rate of influenza A virus. On Hermit Island in Big Stone County, we captured and sampled 71 adult ring-billed gulls, with an influenza A virus prevalence rate of 1.48% (0.37-8.0%). Additionally, we collected 49 environmental fecal samples of which 8 samples were influenza A virus positive by rRT-PCR (16.33%), with one sample the aforementioned North American Lineage H5 influenza A virus positive by rRT-PCR. On Little Pelican Island in Cass County, we captured and sampled 119 adult ring billed gulls, yielding a prevalence rate of 3.54% (0.97-8.82%), and we collected 126 environmental fecal samples, resulting in 2 influenza A virus positive samples (1.59%).

***Summer 2***

Summer 2 sampling period started on 13 July 2017 and ended 27 July 2017. During this period, we trapped on 8 occasions at 3 colony locations, capturing and sampling 294 hatch year ring-billed gulls, detecting a total influenza A virus prevalence of 36.16% (30.46-42.20%). Additionally, we collected 250 environmental fecal samples from the 3 colonies, detecting 9 positives (3.6%) (Table 3). At Interstate Island in St. Louis County, we captured and sampled 75 hatch year ring-billed gulls with an influenza A virus prevalence rate of 7.02% (2.32-15.66%). The100 environmental fecal samples had zero positives (0.0%). On Big Island in Big Stone County, we captured and sampled 134 hatch year ring-billed gulls, detecting an influenza A virus prevalence rate of 29.07% (21.31-37.89%) and collected 100 environmental fecal samples of which seven were influenza A virus positive (7.0%). On Little Pelican Island in Cass County, we captured and sampled 85 hatch year ring-billed gulls. The influenza A virus prevalence rate was 73.07% (61.55-83.11%). We collected 50 environmental fecal samples with2 positive samples (4.0%).

Work to perform and analyze Whole Genome Sequencing (Activity 1 Outcomes 3 &4) is still underway.

**Table 1.** Influenza A virus true prevalence rates for each colony location during each summer period. Summer 1 sample collection period began on 13 May 2017 and ended on 9 June 2017. Summer 2 sampling period started on 13 July 2017 and ended 27 July 2017.

| *Season* (County) | *Age*[1] | *N* | *Estimate*[2] | *95% LCI*[3] | *95% UCI*[4] |
|---|---|---|---|---|---|
| *Summer 1 (Total)* | AD | 469 | 0.0090 | 0.0025 | 0.0228 |
| Big Stone county | | 71 | 0.0148 | 0.0037 | 0.0800 |
| Cass county | | 119 | 0.0354 | 0.0097 | 0.0882 |
| St. Louis county | * | 279 | -- | 0.0000 | 0.0111 |

| *Season (*County*)* | *Age*[1] | *N* | *Estimate*[2] | *95% LCI*[3] | *95% UCI*[4] |
|---|---|---|---|---|---|
| *Summer 2 (Total)* | HY | 294 | 0.3616 | 0.3046 | 0.4220 |
| Big Stone county | | 134 | 0.2907 | 0.2131 | 0.3789 |
| Cass county | | 85 | 0.7307 | 0.6155 | 0.8311 |
| St. Louis county | | 75 | 0.0702 | 0.0232 | 0.1566 |

*There were zero positives detected at St. Louis County during Summer 1, therefore with our estimates we can determine with 95% confidence that prevalence levels were between 0.0-1.11% in adults.

[1]Age is expressed by AD (adults) or HY (hatch year).

[2]Estimate is the true prevalence rate.

[3]95% Lower Confidence Interval for true prevalence.

[4]95% Upper Confidence Interval for true prevalence.

**Table 2:** Environmental fecal samples taken from each colony location during Summer 1 sampling period.

| Season (*County*) | N | # Positive | % Positive |
|---|---|---|---|
| Summer 1 (Total) | 465 | 11 | 0.0237 |
| *Big Stone County* | 49 | 8* | 0.1633 |
| *Cass County* | 126 | 2 | 0.0159 |
| *St. Louis County* | 290 | 1 | 0.0034 |

*One positive environmental fecal sample from Big Stone County contained low pathogenicity avian influenza virus H5.

**Table 3.** Environmental fecal samples taken from each colony location during Summer 2 sampling period.

| Season (*County*) | N | # Positive | % Positive |
|---|---|---|---|
| Summer 2 (Total) | 250 | 9 | 0.0360 |
| *Big Stone County* | 100 | 7 | 0.0700 |
| *Cass County* | 50 | 2 | 0.0400 |
| *St. Louis County* | 100 | 0 | 0.0000 |

**Activity 1 Project Status as of December 21, 2018:**

Whole Genome Sequencing (Activity 1 Outcomes 3 &4) has been completed on the strong positives. Only Marsh Lake and Leech Lake breeding colonies had influenza A virus positive samples and none were H5 Highly Pathogenic Avian Influenza. The subtypes of influenza A virus detected in the breeding colonies were H13N6 at Marsh Lake and H13N8 at Leech Lake.

| | ***Count of Each Subtype*** | | | |
|---|---|---|---|---|
| ***Breeding Colony Location*** | **H?N6** | **H13N6** | **H13N8** | **Grand Total** |
| *Marsh Lake Big Island* | 2 | 8 | | 10 |
| *Little Pelican Island Leech Lake* | | | 26 | 26 |
| *Grand Total* | **2** | **8** | **26** | **36** |

The H13 genes from the avian influenza viruses detected in the two colonies are genetically distinct from each other with Marsh Lake H13s having greater than 15% nucleotide difference from the Leech Lake H13s. The H13s from Leech Lake are of North American gull-lineage viruses from the Pacific flyway. Interestingly, the H13s from Marsh Lake are of North American gull-lineage viruses from the Atlantic flyway. The strength of these HA gene phylogenetic relationships requires further research. Despite having unique H13 genes, there is little genetic diversity in the internal genes of the influenza A viruses detected in the gulls of the breeding colonies, with all genes having greater than 98% similarity to each other. Furthermore, the N8 genes also share greater than 99% nucleotide identity with each other. Similarly, the N6 genes are highly similar, sharing greater than 98% nucleotide identity.

**Activity 1 Overall Project Outcomes and Results:**

Breeding colony sample collection occurred in two phases – Summer 1 and Summer 2. Summer 1 sample collection period began on 13 May and ended on 9 June. During this time, we captured and sampled a total of 469 adult RBGUs and avian influenza virus prevalence was 0.9% (0.25-2.28%). In addition, we collected 465 environmental fecal samples across the 3 colony locations during Summer 1 period resulting in 11 avian influenza virus positive samples (2.36%). One environmental fecal sample from Big Stone County was positive for North American Lineage H5 avian influenza virus by rRT-PCR (Table 1.3). At Interstate Island, St. Louis County, we captured 279 adult RBGUs; no gulls tested positive for avian influenza virus. Therefore, we can say with 95% confidence that avian influenza virus prevalence within the Interstate Island adult RBGU population was between 0.0-1.11%. Furthermore, we collected 290 environmental fecal samples at Interstate Island, but found only 1 positive sample which resulted in a 0.344% prevalence rate of avian influenza virus. On Hermit Island, we captured and sampled 71 adult RBGUs, and estimated avian influenza virus prevalence as 1.48% (0.37-8.0%). Additionally, we collected 49 environmental fecal samples; 8 samples were avian influenza virus positive by rRT-PCR (16.33%), and one sample, the aforementioned North American Lineage H5 was avian influenza virus positive by rRT-PCR. On Little Pelican Island, Cass County, we captured and sampled 119 adult RBGUs, yielding prevalence of 3.54% (0.97-8.82%); the 126 environmental fecal samples contained 2 avian influenza virus positive samples (1.59%).

Summer 2 sampling period started on 13 July and ended 27 July. During this time period, we trapped 8 days at 3 colony locations, capturing and sampling 294 local RBGUs. These samples yielded a total avian influenza virus prevalence of 36.16% (30.46-42.20%). Additionally, we collected 250 environmental fecal samples from the 3 colonies, detecting 9 positives (3.6%) (Table 1.4). At Interstate Island, we captured and sampled 75 local RBGUs and estimated avian influenza virus prevalence rate of 7.02% (2.32-15.66%). The 100 environmental fecal samples had 0 positives (0.0%). On Big Island, we captured and sampled 134 local RBGUs, detecting an avian influenza virus prevalence rate of 29.07% (21.31-37.89%) and collected 100 environmental fecal samples of which 7 were avian influenza virus positive (7.0%). On Little Pelican Island, we captured and sampled 85 local RBGUs and estimated avian influenza virus prevalence as 73.07% (61.55-83.11%). We collected 50 environmental fecal samples with 2 positive samples (4.0%).

**Final Report Summary:**

Breeding colony site avian influenza virus prevalence varied greatly with rate estimates of 29.07%, 73.07%, and 7.02% respectively for Big Stone, Cass, and St. Louis county colonies. Why estimates varied to this extent at our research sites is

not fully understood but anecdotal observations of avian species diversity at each colony site appeared to correspond with higher prevalence of avian influenza virus. At the colony with the lowest prevalence rate, Interstate Island, we recorded 11 different avian species present over our study period and the site had the lowest diversity of species (4) nesting at the colony compared to our other sites. For example, on Little Pelican Island where we document the highest avian influenza virus prevalence, we recorded 15 different avian species present at some point during our visits; this location also had the highest species diversity of species (6) nesting on the island. Finally, we observed 13 different avian species on Big and Hermit islands in Big Stone and this location had 3 different species nesting on the island, adjacent to a nearby site with breeding great blue herons (*Ardea herodias*) and great egrets (*Ardea alba*). It appears that life history characteristics and local bird community dynamics play a large role in avian influenza virus exposure and prevalence.

**Activity 2**: Testing of Gulls during Spring and Fall Migration for avian influenza ($110,071)

In the late summer, gulls leave their breeding colonies and intermingle with other migratory birds during the fall migration, creating a situation for exchange of avian influenza viruses among species. Migratory gulls also return to Minnesota in the spring during which time they again mix with other wild birds and exchange avian influenza viruses. We will live-capture gulls via netting techniques weekly for three consecutive weeks during spring and fall migration through Minnesota wildlife areas and on farm fields near poultry farms. This effort will result in a total of 36 gull netting occurrences (18 in the spring and 18 in the fall). During each netting occurrence, our goal will be at least 20 birds captured at each site, with oral swabs, cloacal swabs and blood samples collected from each bird. All swabs will be tested for avian influenza by a rapid polymerase chain reaction test. Any avian influenza positives will be further characterized by completing whole genome sequencing of the avian influenza found. The blood samples will be tested for antibodies to avian influenza to determine any previous exposure and susceptibility to subsequent exposure. Studying gulls during the migration allows us to obtain numerous samples from a migratory population over time, thus increasing our chances of mixed influenza infections as the birds co-mingle with other wild birds and potentially have interactions with domestic birds. We can also definitively identifying and observe gulls, their timing of migration, their presence on or near domestic bird areas, and assessing both adult and juvenile birds.

**Summary Budget Information for Activity 2:**

**ENRTF Budget:** **$** 110,071
**Amount Spent:** **$** 110,035
**Balance:** **$** 36

| **Outcomes of Activity 2** | **Completion Date** |
|---|---|
| **Visit areas with fall migrating gulls, capture, band, and sample gulls for avian influenza** | Aug/Sep/Oct 2016 |
| Test Swab samples for avian influenza to determine infection status after close and prolonged contact in the breeding colonies; Test Blood samples for avian influenza to determine immune status after waves of infection in the breeding colonies. | Sep/Oct /Nov 2016 |
| Perform Whole Genome Sequencing on avian influenza positive samples to characterize viruses and identify mixed virus infections possibly obtained from exposure to other wild and domestic birds. | Nov/Dec 2016 |
| Analyze Whole Genome Sequencing results to further determine directionality of virus movement between species. | January 2017 |
| **Visit areas with spring migrating gulls; capture, band, and sample gulls for avian influenza** | Feb/Mar 2017 |
| Test Swab samples for avian influenza to determine infection status after migrating long distances and returning to Minnesota; Test Blood samples for avian influenza to determine any exposure to avian influenza after wintering in distant locations. | Mar/Apr/May 2017 |
| Perform Whole Genome Sequencing on avian influenza positive samples to characterize the virus and determine the genetic structure | May/June 2017 |
| Analyze Whole Genome Sequencing results to further determine if mixed infections are present, if infection with highly pathogenic viruses occurred, and if new virus genes are being introduced to Minnesota by gulls. | June 2017 |
| | |
| ***Visit areas with fall migrating gulls, capture, band, and sample gulls for avian influenza*** *AS NEEDED based on ACTIVITY 1 results, timing, interest, and available funds* | *Aug/Sep/Oct 2017* |
| *Test Swab samples for avian influenza* | *Sep/Oct 2017* |

| | |
|---|---|
| *Test Blood samples for avian influenza* | *Oct/Nov 2017* |
| *Perform Whole Genome Sequencing on avian influenza positive samples* | *Nov/Dec 2017* |
| *Analyze Whole Genome Sequencing results* | *January 2018* |

**Activity 2 Project Status as of January 10, 2017:**

A total of 107 birds were captured, banded, sampled, and released in Fall 2016 during 1 field site visit and 3 farm visits. There were 13 ring-billed gulls from Carver County, MN (the field site) and 91 ring-billed gulls and 3 herring gulls from Kandiyohi County, MN (the farm site). Cloacal swabs and oropharyngeal swabs and blood were collected from all birds. All swabs were tested for influenza A virus resulting in 214 total tests completed on swabs as of January 10, 2017. A single cloacal swab ID TF110416003 collected on 11-4-2016 from ring-billed gull with band ID 0994-08032 at Kandiyohi County tested positive for avian influenza virus using an influenza A virus real-time reverse transcriptase polymerase chain reaction (rRT-PCR) test at the University of Minnesota MidCentral Research and Outreach Center Laboratory (MCROC Lab). rRT-PCR tests for H5 and H7 avian influenza virus were negative at MCROC Lab. Finding a low pathogenic avian influenza such as this was expected. In fact, we expected to find as high as 15% of the gulls positive for avian influenza, consistent with studies in gulls in Europe over the last 5 years. Instead, we found only one. Our finding <1% of our Minnesota gulls positive is more consistent with North American studies of gulls which have yielded 5.3% avian influenza positives over the last 5 years of surveillance. The influenza from that gull is definitely NOT H5 or H7 (the two subtypes that can be highly pathogenic) because those tests were negative. It is likely H2 or H13, common gull avian influenza subtypes of North American and Europe, and successful whole genome sequencing will tell us exactly what type of influenza it is and whether it has other genes or parts of virus that were found in the turkeys during 2015. Whole genome sequencing of the swab ID TF110416003 CL is pending for influenza A virus genes at the University of Minnesota College of Veterinary Medicine. All 106 other gulls were negative for avian influenza virus by rRT-PCR on both cloacal swabs and oropharyngeal swabs at MCROC Lab. Blood samples were collected from all 107 birds and serology tests avian influenza A virus antibodies are pending at MCROC Lab.

**Activity 2 Project Status as of July 2017:**

The single positive sample from the Fall 2016 migration, swab ID TF110416003 CL from a ring-billed gull in Kandiyohi County, was subjected to whole genome sequencing between February 2017 and April 2017. Gene sequences were obtained for only four of the eight genes in the influenza A virus genome – hemagglutinin, nuclear export protein, nucleocapsid protein and matrix. All genes obtained share 99% nucleotide similarity to that of A/northern pintail/Ohio/15OS5861/2015 H5N2, a low-pathogenic H5N2 virus isolated from a North American duck in Ohio in 2015. This H5 is NOT related to the Highly Pathogenic Avian Influenza H5N2 viruses from the 2014-2015 outbreak in poultry in the United States, but rather a low-pathogenic H5 found in wild ducks in the North and Central America. Indeed, TF110416003 CL shares <80% hemagglutinin gene nucleotide similarity to A/turkey/Minnesota/7172_1/2015 H5N2, one of the Highly Pathogenic Avian Influenza H5N2 viruses isolated from domestic turkeys in Minnesota during the 2015 outbreak. Additional attempts to obtain the remaining gene sequences (neuraminidase, polymerase basic protein 1, polymerase basic protein 2 and polymerase acidic protein genes) from swab ID TF110416003 CL are still pending.

On March 30, 2017 spring migration trapping began in a high-density poultry area of Cottonwood County where 12 ring-billed gulls were caught. In April 2017, successful trappings continued at various locations with varying numbers of birds caught at each location. In the high-density poultry areas, we caught 56 ring-billed gulls at Kandiyohi County, and 14 ring-billed gulls at Blue Earth County. In lower density poultry areas, we caught 29 ring-billed gulls in Dakota County, 2 herring gulls and 37 ring-billed gulls in Kanabec County to conclude the spring migration period on May 5, 2017. The spring migration was much shorter than anticipated, possibly due to the warm weather that allowed the gulls to start nesting earlier than normal. One hundred fifty (150) gulls were caught in the spring migration of 2017 and the following results were obtained:

- Blue Earth County –

- Collection date 4-7-2017
  - 14 ring-billed gulls tested, 1 gull of 14 positive for avian influenza virus by PCR tests of individual oropharyngeal swab, all 14 gulls negative for avian influenza virus by PCR tests of individual cloacal swabs.
  - Serum successfully collected from 13 of 14 birds and sera positive for antibodies to avian influenza virus suggesting a population of gulls with mixed immunity and likely recent exposure to avian influenza virus.

- Cottonwood County –
  - Collection date 3-30-2017,
    - 12 ring-billed gulls tested, all 12 negative for avian influenza virus by PCR tests of individual oropharyngeal swabs and cloacal swabs.
    - Serum successfully collected from 5 of 12 birds. All 5 sera positive for antibodies to avian influenza viruses, suggesting previous exposure to avian influenza virus and a detectable immune response.
- Dakota County –
  - Collection date 4-3-2017
    - 16 ring-billed gulls tested, and 10 birds positive for avian influenza by PCR (8 birds POSITIVE for avian influenza by PCR tests of the oropharyngeal swabs, 1 bird POSITIVE for avian influenza by PCR of the cloacal swab, and 1 bird POSITIVE on both the oropharyngeal swab and cloacal swab).
    - Sera successfully collected from 13 of the 16 birds and 6 sera (46%) positive for antibodies to avian influenza viruses and 7 negative for antibodies to avian influenza viruses, suggesting a population of gulls with mixed immunity and likely recent exposure to avian influenza virus.
  - Collection date 4-13-2017
    - 13 ring-billed gulls tested, all 13 negative for avian influenza virus by PCR tests of individual oropharyngeal swabs and cloacal swabs.
    - Sera successfully collected from 8 of the 13 birds with 5 sera (62%) positive for antibodies to avian influenza viruses and 3 negative for antibodies to avian influenza viruses, suggesting a population of gulls with mixed immunity and prior exposure to avian influenza virus.
- Kanabec County –
  - Collection date 5-5-2017
    - 40 gulls tested (2 herring and 38 ring-billed) all 40 negative for avian influenza virus by PCR tests of individual oropharyngeal swabs; 20 negative for avian influenza virus PCR tests of individual cloacal swabs; and 20 pending test results for avian influenza virus PCR tests of individual cloacal swabs.
    - Sera successfully collected from 39 of 40 birds with 17 sera positive for antibodies to avian influenza viruses and 22 negative for antibodies to avian influenza viruses, suggesting a population of gulls with mixed immunity.
- Kandiyohi County –
  - Collection date 4-1-2017
    - 46 ring-billed gulls tested all 46 negative for avian influenza virus by PCR tests of individual oropharyngeal swabs and cloacal swabs.
    - Sera successfully collected from 45 of 46 birds with 27 sera positive for antibodies to avian influenza viruses and 18 negative for antibodies to avian influenza viruses, suggesting a population of gulls with mixed immunity.
  - Collection date 4-10-2017
    - 10 ring-billed gulls tested all 10 negative for avian influenza virus by PCR tests of individual oropharyngeal swabs and cloacal swabs.

        - Sera successfully collected from all 10 birds with 7 sera positive for antibodies to avian influenza viruses and 3 negative for antibodies to avian influenza viruses, suggesting a population of gulls with mixed immunity
- Rice County –
    - Visit date 4-19-2017
        - No gulls were observed at the landfill site on this date and none were trapped or tested.
- Sherburne County –
    - Visit date 4-28-2017
        - Many gulls were observed; however, gulls did not congregate in large groups in the netting area. No gulls were trapped or tested.
- Overall, during spring migration 150 gulls were sampled from 5 counties. From each gull, an oropharyngeal swab and cloacal swab was collected and a total of 300 swabs from gulls were tested for avian influenza virus by PCR. A total of 11 birds (7.3%) were positive for influenza A virus by PCR with 9 of the 11 birds positive for influenza A virus by PCR on only the oropharyngeal swab. Only 1 of the 11 birds was positive for influenza A virus by PCR on both the oropharyngeal swab **and** cloacal swab, and a single bird positive for influenza A virus by PCR on only the cloacal swab. All influenza A virus PCR positive swabs were tested for H5 and H7 influenza A virus by PCR and were negative. Whole genome sequencing of each of the positive swabs is pending at the University of Minnesota.
- Sera were successfully collected from 133 of the 150 gulls. The Minnesota Poultry Testing Laboratory conducted Influenza A Virus antibody tests of the sera using the IDEXX AI MultiS-Screen Ab Test ®. Of those 133 gull sera tested, 74 (55.6%) were positive and 59 were negative for serum antibodies to avian influenza virus by the IDEXX ELISA serological test. Further testing of the 74 positive sera, or a represent subset therein as a cost-saving measure, for H5 subtype specific antibodies is pending.

- Summary Table of Results of the PCR and Serology Tests for Activity 1 Spring Migration

| | **Blue Earth** High-density poultry area | **Cottonwood** High-density poultry area | **Dakota** Low-density poultry area | **Kanabec** Low-density poultry area | **Kandiyohi** High-density poultry area | **Total** |
|---|---|---|---|---|---|---|
| **Number of Birds Sampled** | 14 | 12 | 29 | 39 | 56 | 150 |
| Number of influenza A virus PCR positive cloacal swabs**/**total cloacal swabs collected (% positive) | 0/14 (0%) | 0/12 (0%) | 2/29 (6.9%) | 0/20 *[21 pending]* (0%) | 0/56 (0%) | 2/150 (1.3%) |
| Number of influenza A virus PCR positive oropharyngeal swabs**/**total oropharyngeal swabs collected (% positive) | 1/14 (7.1%) | 0/12 (0%) | 9/29 (31%) | 0/39 (0%) | 0/56 (0%) | 10/150 (6.7%) |
| Number of birds positive for influenza A virus by PCR on either oropharyngeal or cloacal swab**/**total birds sampled (% positive) | 1/14 (7.1%) | 0/12 (0%) | 10/29 (34%) | 0/39 *[partial testing on 21 birds)* (0%) | 0/56 (0%) | 11/150 (7.3%) |
| Number of bird sera positive for antibodies to influenza A virus by IDEXX ELISA serological test**/**total sera tested (% positive) | 7/13 (53.8%) | 5/5 (100%) | 11/21 (52.4%) | 17/39 (43.6%) | 34/55 (61.2%) | 74/133 (55.6%) |

**Activity 2 Project Status as of December 31, 2017:**

Spring 2017 tests for avian influenza virus RT-PCR tests on the gulls were completed in August 2017 and the results are summarized here. During Spring 2017 we captured and sampled 148 ring-billed gulls from 5 counties during the time period of 30 March 2017 to 5 May 2017. The avian influenza virus prevalence rate in Spring 2017 was 7.82% (3.97-13.59%) among all gulls sampled (n=148). Adults (n=108) had a 7.80% (3.24-14.82%) prevalence rate and the prevalence rate for juveniles (n=40) was 7.89% (1.66%-21.46%) prevalence. (Table 4).

**Table 4. avian influenza virus true prevalence rates for all age groups trapped from 5 counties during spring 2017 migration period (30 March 2017 to 5 May 2017).**

| *Season* (County) | Age[1] | N | Estimate[2] | 95% LCI[3] | 95% UCI[4] |
|---|---|---|---|---|---|
| *Spring* (multiple counties) | ALL | 148 | 0.0782 | 0.0397 | 0.1359 |
| | JUV | 40 | 0.0789 | 0.0166 | 0.2146 |
| | AD | 108 | 0.0780 | 0.0342 | 0.1482 |

[1]Age is expressed by ALL (all birds captured) JUV (juveniles) or AD (adults)

[2]Estimate is the true prevalence rate.

[3]95% Lower Confidence Interval for true prevalence.

[4]95% Upper Confidence Interval for true prevalence.

**Fall 2017**

Fall 2017 (Fall 2) efforts resulted in 327 captured and sampled ring-billed gulls, including 130 adults and 197 juveniles from 6 counties during the period of 2 August 2017 to 28 September 2017. The total avian influenza virus prevalence rate in Fall 2017 (Fall 2) was 68.36% (62.64-73.79%) for all gulls sampled (n=327). Adults (n=130) had a prevalence rate of 61.54% (52.10-70.56%) and juveniles (n=197) had a prevalence rate of 72.67% (52.10-70.56) (Table 5).

**Table 5. avian influenza virus true prevalence rates for all age groups trapped from 6 counties during Fall 2017 (Fall 2) migration period (2 August 2017 to 28 September 2017).**

| *Season (*County*)* | *Age*[1] | *N* | *Estimate*[2] | *95% LCI*[3] | *95% UCI*[4] |
|---|---|---|---|---|---|
| Fall 2 (multiple counties) | ALL | 327 | 0.6836 | 0.6264 | 0.7379 |
| | JUV | 197 | 0.7267 | 0.6534 | 0.7938 |
| | AD | 130 | 0.6154 | 0.5210 | 0.7056 |

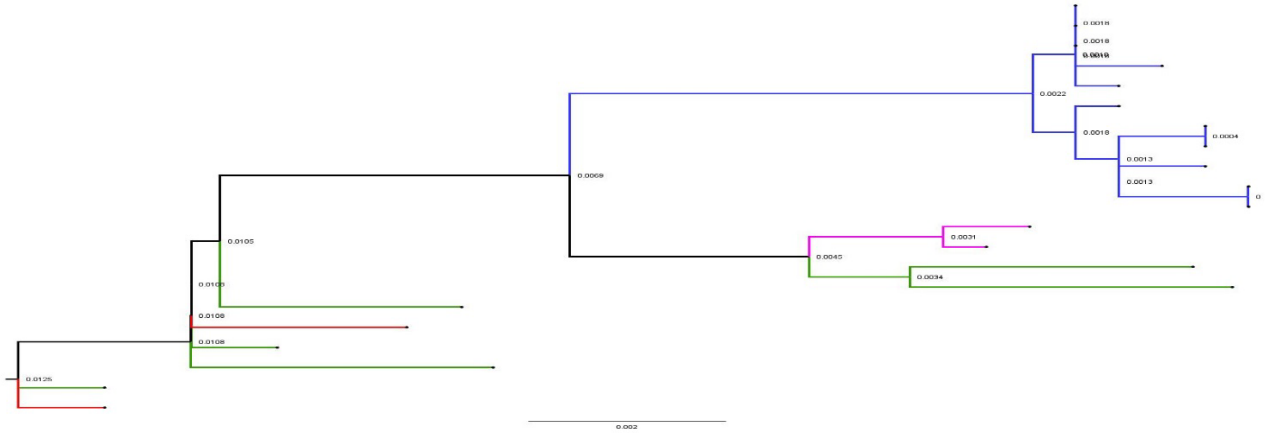
[1]Age is expressed by ALL (all birds captured) JUV (juveniles) or AD (adults)
[2]Estimate is the true prevalence rate.
[3]95% Lower Confidence Interval for true prevalence.
[4]95% Upper Confidence Interval for true prevalence.
Work to perform and analyze Whole Genome Sequencing (Activity 2 final two Outcomes) is still underway.

## Activity 2 Project Status as of December 21, 2018:

Work to perform and analyze Whole Genome Sequencing (Activity 2 final two Outcomes) is completed. H13N6 avian influenza viruses were identified at Mora and Ponderosa landfills, H13N2s at Kandiyohi and Ponderosa landfills, and H13N8 at only the Kandiyohi county landfill. Ponderosa landfill had the most subtypes of avian influenza virus detected and there was evidence of mixed infections in gulls collected here. Analyses of all genes suggest mixing of viruses may occur at landfills and much more in-depth analyses are warranted.

| | Count of Subtype | | | | | |
|---|---|---|---|---|---|---|
| **Location** | **H?N6** | **H13N2** | **H13N6** | **H13N8** | **mixed** | **Grand Total** |
| Mora Landfill | | | 1 | | | 1 |
| Kandiyohi County Landfill | | 4 | | 1 | | 5 |
| Ponderosa Sanitary Landfill | 1 | 2 | 3 | | 1 | 7 |
| **Grand Total** | **1** | **6** | **4** | **1** | **1** | **13** |

**Figure 3**. A simplified maximum likelihood tree of the PB2 genes of influenza A viruses detected during our study to demonstrate their relationships to each other. Kandiyohi county sequences are red, Ponderosa landfill sequences are green, Marsh Lake breeding colony sequences and Leech Lake breeding colony sequences are blue. The phylogenetic relationships inferred by this tree suggest there is mixing of viruses at Kandiyohi and Ponderosa landfills from Marsh Lake.



**Activity 2 Overall Project Outcomes and Results:**

Six landfills throughout Minnesota were chosen as sampling sites during migration periods. These six sites were selected based on their proximity to poultry facilities. Three landfills sites were located within counties with a high density of poultry facilities (Blue Earth, Cottonwood, and Kandiyohi Counties) and three landfills were located in counties with a low density of poultry facilities (Dakota, Kanabec and Rice Counties). In this study, we focused on ring-billed gulls (RBGUs) because this species is very opportunistic in diet and foraging behavior and is common in Minnesota's poultry producing counties during spring and fall migration periods, often associating with human activities and coming in close contact with poultry facilities. The migration season was considered as either Spring (March-May) or Fall (August -November). The avian influenza virus testing results for the migration periods are summarized in the tables below.

We found significant seasonal, spatial, and age-cohort dynamics in avian influenza virus apparent prevalence within ring-billed gulls in Minnesota. The highest apparent prevalence estimate was 68.36% for juvenile gulls during the fall migration period. Each season immunologically naïve juveniles consistently had the highest prevalence.

We captured 666 gulls during 22 trapping attempts in the fall trapping season, including 338 adults (51%) and 328 juveniles (49%). Species composition during the fall trapping season included 420 ring-billed gulls, 243 Franklin's gulls, and 3 herring gulls. During the 22 trapping attempts, we were successful (1 or more birds caught) on 18 attempts for a fall capture success rate of 82%. Our mean catch rate during the fall was 30.27 birds, comprising of a mean catch rate of 15.43 adults and 14.83 juveniles per trapping attempt. Two Franklin's gulls and three ring-billed gulls (< 1% mortality rate) were killed from the net blast; 12 additional gulls received minor injuries, including lacerations or broken feathers (2% injury rate).

We captured 150 gulls, ring-billed exclusively, on 13 trapping attempts during the spring season, including 108 adults (72%) and 42 juveniles (28%). During spring trapping season, we made 13 trapping attempts; 7 were successful with 1 or more gulls captured. Spring capture success rate was 54% and the mean catch rate was 11.54 birds, comprising a mean catch rate of 8.31 adults and 3.23 juveniles per trapping attempt. Two ring-billed gulls (1.3% mortality rate) died during trapping and three ring-bills received minor injuries, including lacerations or broken feathers (2% injury rate).

During the combined spring and fall 2017 trapping seasons we trapped on 35 occasions, capturing a total of 816 gulls, including 446 adults (55%) and 370 juveniles (45%). Species composition of the gulls was: 570 ring-bills, 243 Franklin's gulls and 3 herring gulls (Table 2.3). During the 35 trapping attempts, 25 resulted in a successful attempt for a combined mean capture success rate of 71%. Over the spring and fall trapping seasons of 2017 we had a mean catch rate of 23.31 birds, resulting in 12.82 adults and 10.49 juveniles captured per trapping attempt. Seven birds (2 Franklin's, 5 ring-billed gulls) were killed that were directly hit by the net or the projectile net weights (< 1% mortality rate). Fifteen additional birds sustained minor injuries including lacerations or broken feathers after struggling in the net or while awaiting processing in the poultry crates (2% injury rate). An antibiotic cream was applied to any minor injuries having exposed flesh. All birds with minor injuries were released and demonstrated normal flight.

**Avian influenza virus true prevalence rates for all age groups trapped from 5 counties during the spring 2017 migration period (30 March 2017 to 5 May 2017).**

| *Season* (County) | Age[1] | N | Estimate[2] | 95% LCI[3] | 95% UCI[4] |
|---|---|---|---|---|---|
| *Spring* (multiple counties) | ALL | 148 | 0.0782 | 0.0397 | 0.1359 |
| | JUV | 40 | 0.0789 | 0.0166 | 0.2146 |
| | AD | 108 | 0.0780 | 0.0342 | 0.1482 |

[1]Age is expressed by ALL (all birds captured) JUV (juveniles) or AD (adults)

[2]Estimate is the true prevalence rate.

[3]95% Lower Confidence Interval for true prevalence.

[4]95% Upper Confidence Interval for true prevalence.

**Avian influenza virus true prevalence rates for all age groups trapped from 6 counties during Fall 2017 (Fall 2) migration period (2 August 2017 to 28 September 2017)**.

| *Season (*County*)* | *Age*[1] | *N* | *Estimate*[2] | *95% LCI*[3] | *95% UCI*[4] |
|---|---|---|---|---|---|
| Fall 2 (multiple counties) | ALL | 327 | 0.6836 | 0.6264 | 0.7379 |
| | JUV | 197 | 0.7267 | 0.6534 | 0.7938 |
| | AD | 130 | 0.6154 | 0.5210 | 0.7056 |

[1]Age is expressed by ALL (all birds captured) JUV (juveniles) or AD (adults)
[2]Estimate is the true prevalence rate.
[3]95% Lower Confidence Interval for true prevalence.
[4]95% Upper Confidence Interval for true prevalence.

## Final Report Summary:

Our results showing a 68% peak avian influenza virus infection during late summer to fall (August-November) coincide with results of European studies and as expected, prevalence was highest (72.67%) in juveniles during the Fall. Susceptibility to disease could explain the high virus prevalence in RBGUs in the Fall as individuals potentially are exposed to other virus strains via intra- and interspecific interactions with other birds after they disperse from the colony site. Thus, we suggest that investigators concentrate on fall as a primary risk period for avian influenza virus transmission and evolution. Our surveillance on gulls in Minnesota suggest that fall should still be considered the most likely time period for avian influenza viruses to be introduced into poultry facilities.

Our research demonstrated that using a Coda Netlauncher at landfills is a safe and effective method to capture gulls. The most common species caught was the ring-billed gull. High capture rates for this species were possible because ring-bills are the most abundant gull species in the state, they are common as spring and fall migrants in the trapping study area and they often feed at landfills. Based on our success, trapping at landfills is a very useful strategy for conducting future research on gulls and possibly other co-occurring avian species.

Our study resulted in a capture success rate of 71%, a mean catch rate of 23.31 birds per trap attempt, injury rate of 2% and a mortality rate of <1%. Using the Coda Netlauncher, we also attempted to trap gulls in agricultural fields, ball fields and sewage treatment plants but had very limited success. For example, we attempted capture nine times away from landfills with a low capture success (11%). Also, gulls appeared extremely wary in these more natural settings, causing our catch rate (13 individuals) to be considerably lower than in the landfills. We were able to increase capture success only after birds were pre-baited and acclimated to sites with a dummy netlauncher. Attempting to capture non-breeding gulls away from a landfill or other predictable food source, requires significant investment in time and funds when compared to trapping landfill sites. None of our trapping attempts on the landfills required pre-baiting or use of a dummy netlauncher, and we were able to return to trap multiple times at the same sites without birds becoming aware and avoiding the netlauncher.

Although our overall capture success rate was 71%, success would have been higher if we targeted multiple species and not only ring-billed gulls. For example, when Franklin's gulls were the single species in the trap area, we passed opportunities to deploy the net. Furthermore, during multiple attempts, ring-billed gulls were in the net area, but we did not shoot as we waited for additional gulls to enter the trap area. Also, we often did not deploy the net when we perceived unsafe conditions for gulls (i.e. when too many gulls were in the net area and we predicted injury might occur from entanglement or prolonged holding time during processing). Finally, we chose not to attempt a capture on multiple occasions when gulls were in flight over the trap area to avoid hitting birds in the air with the net or net launcher projectiles.

Another factor affecting capture and success rate was season of trapping. For example, the number of days gulls were present at landfills was shorter in the spring than fall and capture success and mean catch rates were lower in spring (54%; 11.5 birds) than fall (82%; 32.3 birds). As a result, we trapped for a shorter period of time in the spring (13 attempts), compared to the fall (22 attempts). During fall, birds appeared easier to bait and capture likely due to their energetic needs during this migratory time period. For most fall migrants is not as urgent, and birds are not pressed by the need to begin their breeding effort as early as possible. Moreover, our results indicate we caught a larger proportion of juveniles during fall (45%) versus spring season (28%), which is likely due unsurprisingly to the greater abundance of juveniles available for capture following the breeding season. We also had a higher mean catch rate per trapping attempt in the fall of 15.43 adults and 14.83 juveniles, compared with 8.31 adults and 3.23 juveniles in Spring. Again, this is likely a result of the greater

abundance of juveniles available for capture during the fall and the fact that many juveniles at this time have not been exposed to the stress of capture attempts by humans.

**V. DISSEMINATION:**
**Description:** Presentations to stakeholders at scientific meetings, symposia, conferences

**Status as of January 10, 2017:** Presentations were made via teleconference and informational sheets were distributed to promote voluntary participation in the study from the Minnesota Turkey Growers, the Minnesota Chicken and Egg Producers, the Minnesota Audubon Society, and the Minnesota Ornithologists Union

**Status as of July 2017:**
Presentations were made including project overviews and preliminary results at a scientific symposium during the North Central Avian Disease Conference meeting on March 13, 2017 in St. Paul, MN. Todd presented to the Fisheries, Wildlife, and Conservation Biology Club on the University of Minnesota campus on April 4, 2017 and to the Conservation Sciences Department Brown Bag Lunch Seminar on April 18, 2017.

**Status as of December 31, 2017:**
Todd Froberg presented this work as his Master's Thesis Defense on December 21, 2017 in a seminar to the University of Minnesota Fisheries, Wildlife, and Conservation Biology Department titled, "Seasonal Dynamics of Avian Influenza Viruses in Ring-billed Gulls in Minnesota." In attendance were numerous students and faculty from the University of Minnesota College of Veterinary Medicine, and colleagues from the Department of Natural Resources and United States Fish and Wildlife Service. This work will be part of his Master's thesis consisting of two publications, "Seasonal Dynamics of Avian Influenza Viruses in Ring-billed Gulls in Minnesota" and "An Effective Method for Capturing Gulls on Landfills." Journal publication options include submission to the Journal of Wildlife Diseases *and Animals* — Open Access Animal Science and Animal Welfare Journal, among others.

This work will be presented at the International Avian Influenza Symposium in Brighton, UK in April 2018 in two abstracts, "Comparison of oral and cloacal swabs for the detection of influenza in gulls" and "Avian Influenza virus surveillance in ring-billed and herring gulls in Minnesota." Both can eventually be submitted as brief Research Notes manuscripts to be published in the proceedings book in the last issue of the journal Avian Diseases in 2018. All manuscripts will go through a peer-review process by members of other avian influenza experts.

**Status as of December 21, 2018:**
This work was submitted as a thesis to the faculty of the University of Minnesota by Todd Froberg in partial fulfillment of the requirements for the degree of Master of Science under the advisement of Francesca Cuthbert. Mr. Todd Froberg is now an employee of the Minnesota Department of Natural Resources. A manuscript will be submitted to the Journal of Wildlife Diseases under the working title, "Use of a Netlauncher to Capture Non-breeding Gulls at Landfills in Minnesota." The genetic sequence data is in the public domain for knowledge sharing in the influenza research database. The results from this work were instrumental in the team gaining new funding from the Minnesota Agricultural Experimental Station to study the role gull species play in the disease ecology of avian influenza in Minnesota, with Culhane as the principal investigator.

**Overall Project Outcomes and Results:**
We performed a study to determine if gulls played a role in bringing the highly pathogenic H5 avian influenza virus to Minnesota or if the gulls are capable of moving the virus around the state and infecting other birds. Therefore, we embarked on a year-round project that included banding and avian influenza virus testing of gulls in breeding colonies and in both rural and urban areas during the fall and spring migrations. We began in September 2016 when gulls left their breeding colonies and intermingled with other migratory birds during the fall migration, creating a situation for exchange of avian influenza virus among species. When the migratory gulls returned to Minnesota in the spring, they again mixed with other wild birds and had the opportunity to exchange avian influenza virus. We live-captured and banded gulls via netting techniques weekly during spring and fall migration in both rural and urban areas and intensively sampled adults, juveniles, hatch-year birds and

the environment of the breeding colonies. This effort resulted in more than 500 gulls banded and sampled during spring and fall migration, over 750 gulls banded and sampled in the breeding colonies, and 300 environmental samples of the breeding colonies. All birds had both oral and cloacal swabs individually tested for avian influenza virus by a polymerase chain reaction tests. Gulls in breeding colonies experienced waves of avian influenza virus infections and at the peak, 58% of the birds tested were avian influenza virus positive by PCR (July 26, 2017 at Marsh Lake). These avian influenza virus positives were further characterized by completing whole genome sequencing and results suggest that gulls do not play a role in virus dissemination to and from poultry. Rather, gull avian influenza viruses appear unique to gulls but can be comprised of genes from avian influenza viruses of gulls from multiple continents and flyways.

Not only were we able to detect waves of influenza infection, but we also definitively identified the gulls, and assessed adult, hatch year, and juvenile birds. Overall, approximately 11% of all samples collected are avian influenza virus positive to date and need to be further characterized by completing avian influenza virus whole genome sequences. The information generated is novel and will help determine the role gulls may have had in the spread of avian influenza virus in Minnesota. We want to capitalize on the success of our recently completed fieldwork and research, which ended in October 2017, to further explore the data and pursue other important avenues of AI research. We found pronounced AI prevalence fluctuations during our yearlong surveillance and we now want to explore the ecological drivers of these waves of avian influenza virus infection and how they affect viral genetic diversity. Our research demonstrated that this method works well for capturing large numbers of gulls for research. This technique also has potential for capturing other species at landfills. For example, during our study, we incidentally caught American crows (*Corvus brachyrhynchos*) and rock pigeons (*Columba livia*). We also had opportunities to trap European starlings (*Sturnus vulgaris*), house sparrows (*Passer domesticus*), Brewer's blackbirds (*Euphagus cyanocephalus*) and Canada geese (*Branta canadensis*) that were all in the capture area. Additionally, we commonly observed bald eagles (*Haliaeetus leucocephalus*), turkey vultures (*Cathartes aura*) and eastern wild turkeys (*Meleagris gallopavo silvestris*) that were near our trapping site and we believe these individuals could be lured to the trap site with more attractive species-specific bait.

The use of a netlauncher as a trapping technique is very appealing and easy to use without regulatory restrictions that accompany use of cannon or rocket net explosives or charges. Although the netlauncher system is more expensive for original set up, the overall cost of the launcher is acceptable, when compared to its increase in sample size per time expended over walk-in-nest traps, funnel traps, or other wire/mesh based single bird traps. Netlaunchers can be loaded and unloaded from a vehicle, carried, set up, fired, and disassembled by a single investigator. There are some disadvantages to consider when using this method on landfills including entanglement with uncovered waste and limited mobility once set up. Despite losing seven gulls as a result of our capture efforts (or 0.8% capture myopathy), the relative success of using a coda netlauncher outweighs the costs, and should be considered as a capture technique for both abundant, endangered, or at-risk bird populations.

## Final Report Summary:

Birds within the orders Charadriiformes (shorebirds; gulls) and Anseriformes (waterfowl) are reservoir hosts for low pathogenicity avian influenza viruses, but their role in the transmission dynamics of highly pathogenic avian influenza viruses is unclear. The novel reassortant highly pathogenic avian influenza virus H5N2 that emerged in Minnesota in 2015 was devastating to the poultry industry resulting in massive financial losses and destruction of millions of domestic poultry. To date, waterfowl have been the predominant focal species for most avian influenza virus surveillance and epidemiological studies, yet gulls, in particular, have been shown to harbor reassortant avian influenza viruses of both North American and Eurasian lineages and are underrepresented in North American surveillance efforts. To address this gap in surveillance, 1346 ring-billed gulls (*Larus delawarensis)* were sampled during spring and fall migrations and at three breeding sites in 2017 across Minnesota. We found significant seasonal, spatial, and age-cohort dynamics in avian influenza virus apparent prevalence within ring-billed gulls in Minnesota. The highest apparent prevalence estimate was 68.36% for juvenile gulls during the fall migration period. Each season immunologically naïve juveniles consistently had the highest prevalence. Spatial heterogeneity was detected at nesting colony sites; St. Louis County exhibited low prevalence estimates in both adults (0.0%) and juveniles (7.02%), whereas Cass County exhibited highest prevalence in adults 3.54% and juveniles (73.07%). No highly pathogenic avian influenza viruses were detected in sampling efforts, but the high prevalence of low pathogenicity avian influenza viruses within ring-billed gulls, particularly in immunologically naïve birds and all age-cohorts in fall 2017 warrant further targeted surveillance efforts of ring-billed gulls and other closely related species. Identification

of the prevalence of low pathogenicity avian influenza viruses H5 and H7 viruses is the highest priority for future research as these variants have the greatest potential for mutating into highly pathogenic forms in poultry.

We used a modified -74.32 m2 net propelled by a Coda Netlauncher to demonstrate a safe an effective method to capture gulls on landfills in Minnesota. We captured 816 gulls during spring and fall 2017, including three species common in Minnesota: ring-billed gull (*Larus delawarensis*), herring gull (*Larus argentatus*), and Franklin's gull (*Leucophaeus pipixcan*). During 35 trapping events, our capture success rate was 71% and the mean bird catch rate was 23.31 birds per trapping event. Additionally, bird mortalities and injuries were minor, with a < 1% mortality rate and 2% minor injury rate. The netlauncher is easy to use and can be set by only one investigator in 10-15 minutes; no firing or handling of dangerous explosives or charges common to cannon and rocket nets is required. Results from our study demonstrate that the Coda Netlauncher is a safe and effective tool for capturing non-breeding gulls in an anthropogenic interface.

## VI. PROJECT BUDGET SUMMARY:

### A. ENRTF Budget Overview:

| Budget Category | $ Amount | Overview Explanation |
|---|---|---|
| Personnel: | $ 89,273 | Marie Culhane, principal investigator, project design and oversight, publication assistance and analysis of results, 5% FTE for one year ($9,440); Todd Knutson, collaborator, whole genome sequencing and bioinformatics, 2% FTE for one year($2,942). [Note: Both Culhane and Knutson are contract faculty and staff, respectively, with annually renewable appointments at the U of MN and required by contract to seek out funding certain percentages of their salaries]. Todd Froberg, graduate student in wildlife ecology and research assistant, 50% FTE for one year ($44,522); TBN, wildlife data technician, to participate in field studies, collect samples from wildlife, data management, 75% FTE for one year ($32,369). |
| Professional/Technical/Service Contracts: | $9,100 | Undergraduate student workers, for data entry and data collection, $13.00/hour for 700 hours over one year |
| Equipment/Tools/Supplies: | $87,375 | |
| | | Binoculars for bird observation — $100<br>Waterproof notebooks for recording bird observations — $20<br>Gloves and other personal protective equipment for field sampling — $427<br>Sample preservation of collected samples — $34<br>Mailers for samples — $100<br>3040 avian influenza polymerase chain reaction tests of bird and environmental fecal samples for detection of avian influenza at U of MN MidCentral Research and Outreach Center Laboratory at $22.86 per test — $36,576<br>120 Whole Genome Sequence of AI viruses detected at U of MN College of Veterinary Medicine @$85.70 each and direct Sanger Sequencing of the Hemagglutinin and Neuraminidase genes @ $67.53 each — $14,245<br>880 wild bird blood sample serology tests at U of MN MidCentral Research and Outreach Center Laboratory @$6.00 each — $3,140 |
| Travel Expenses in MN: | $19,591 | weekly car rental at $228 per week for 10 weeks over the time of the project; mileage charges of $0.17 per mile X approx. 6300 miles per University rate |
| **TOTAL ENRTF BUDGET:** | | **$213,443** |

**Explanation of Use of Classified Staff:** Not Applicable N/A

**Explanation of Capital Expenditures Greater Than $5,000:** Not Applicable N/A

**Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation:** 1.32

**Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation:** 0.34

**B. Other Funds:**

| Source of Funds | $ Amount Proposed | $ Amount Spent | Use of Other Funds |
|---|---|---|---|
| **Non-state (in-kind support from Federal and Tribal)** | $15,000 | $0 | In-kind Services To Be Applied To Project During Project Period: Salary of White is covered by USDA ($2,500), Salary of Mortensen is covered by LL DRM (2% FTE), Salary and benefits of Cooper are covered by USFWS ($10,000 ) |
| | | | |
| **State (in-kind support from U of MN)** | $5,000 | | In-kind Services To Be Applied To Project During Project Period: Salaries of Cardona (1%FTE) and Cuthbert (1%FTE) are covered by the U of MN |
| **State (in-kind support from U of MN)** *The U of MN does not charge the State of Minnesota its typical overhead rate of the total modified direct costs (aka indirect costs). The U of MN indirect cost rate increases to 53% effective 7/01/2017, so for the project period of 01/01/2017 - 06/30/2018, we have 181 days at 52% and 365 days at 53%.* | $101,118 | | The University of Minnesota does not charge the State of Minnesota its typical overhead rate of the total modified direct costs (equipment, capital expenditures, charges for patient care, rental costs, tuition remission, scholarships and fellowships, participant support costs and the portion of each subaward in excess of $25,000 are excluded). These in-kind funds will provide general office and laboratory support during the project. |
| **State (in-kind support from DNR)** | $5,000 | $0 | In-kind Services To Be Applied To Project During Project Period: Salary of Jennelle is covered by MN DNR (6% FTE) |
| **TOTAL OTHER FUNDS:** | **$126,118** | **$0** | |

**VII. PROJECT STRATEGY:**

**A. Project Partners:**

**Carol Cardona, DVM, PhD, DACPV,** collaborator, is Professor and Pomeroy Chair of Avian Health at the University of Minnesota, in the Department of Veterinary and Biomedical Sciences. She is an internationally recognized expert in the fields of avian diseases and avian influenza infections in poultry. Dr. Cardona is also the laboratory director of the University of Minnesota's Mid-Central Research and Outreach Center (MCROC) where the majority of the testing will occur.

**Tom Cooper,** collaborator, Chief of the R3 Migratory Bird Program, U.S. Fish and Wildlife Service, will provide expertise and equipment for gull netting and handling.

**Marie Culhane, DVM, PhD** (receiving funds of $9,440 for wages and benefits), principal investigator, is an infectious disease expert with a DVM and a PhD from the
University of Minnesota. Dr. Culhane is a member of the joint OIE/FAO influenza working group (OFFLU) and has been actively involved in swine, human, and avian influenza global research and surveillance for 9 years. She designed the project plan and will assist with test result interpretation and selecting gene sequence analyses.

**Francesca Cuthbert, PhD,** collaborator, is a Distinguished Teaching Professor in the Department of Fisheries, Wildlife and Conservation Biology at the U of MN. Dr. Cuthbert has a wealth of experience with both species of gulls in the state (including rocket netting ring-billed gulls) and her lab has records on gull nesting colonies in MN to guide the colony surveillance efforts. She has conducted research on waterbirds for more than 30 years.

**Todd Froberg,** (receiving funds of $44,522 for wages and benefits), Graduate Student, U of MN, is pursuing a master's degree in Conservation Biology. He recently worked as an intern with the DNR and will perform the gull studies under the advisement of Drs. Cuthbert, Jennelle, and Culhane.

**Chris Jennelle, PhD,** collaborator, is a Research Scientist with the Wildlife Health Program at the Minnesota

Department of Natural Resources. He has a strong background in the design, data collection, and analysis of surveillance data for wildlife diseases including avian influenza, chronic wasting disease, and *Mycoplasma gallisepticum*. His research interests also include quantitative modeling, parameter estimation, and prediction of wildlife disease dynamics.

**Todd Knutson, PhD**(receiving funds of $2,942 for wages and benefits), collaborator, is a post-doctoral associate at the U of MN in the Department of Veterinary Population Medicine. Dr. Knutson will use his expertise in the analysis and whole genome sequences of viruses to reveal the evolutionary changes in the virus and direction of transmission of virus genes found in wild birds and their introduction into domestic poultry.

**Steve Mortensen**, collaborator, Fish, Wildlife & Plants Director, Leech Lake Band of Ojibwe Division of Resource Management, will provide his expertise regarding the gull breeding colonies in the Leech Lake area and assist with sampling.

**Steve Olson,** collaborator, Executive Director of the MN Turkey Growers Assn, MN Turkey Research & Promotion Council, Chicken & Egg Assn of MN, Midwest Poultry Federation, will assist with coordination and outreach to the poultry producers of Minnesota to allow us access to fields where gulls will be captured and released.

**Tim White,** collaborator, USDA APHIS Wildlife Services, will use his expertise as a Wildlife Disease Biologist to assist in opportunistic sampling, occasional field sampling, and educating the citizen scientists and other team members in wild bird handling and sampling.

### B. Project Impact and Long-term Strategy:

This project will provide valuable information to help maximize understanding of the role of gulls in introducing avian influenza infections into Minnesota and the potential negative impact of spillback of infection to gulls. This project will focus on gull breeding colonies in Minnesota, where waves of influenza infection have been described to occur in other countries. Research will also extend out to the farmlands and wetlands of Minnesota, where mixing with other migratory birds and domestic poultry may occur. Results will inform future international wildlife avian influenza surveillance plans and the Minnesota highly pathogenic avian influenza response plan , identify the potential risk to endangered terns that share breeding colonies with these gulls, and formulate predictive risk models of avian influenza. The whole genome sequencing of the viruses found in the gulls will be subjected to detailed evolutionary and genetic analyses to determine directionality of virus movement (e.g., poultry to gulls or gulls to poultry; Asia to North America or North America to Asia) and the most likely common ancestor strain in gulls and other birds.

With the many collaborators here, we are able to reach across sectors and disciplines to address this highly pathogenic avian influenza problem head-on. We have the capacity to determine when and where to attach real time, satellite monitoring devices on gulls to investigate gull movement dynamics and for avian influenza virus spread in Minnesota. Tracking is an expensive endeavor, but after a year of comprehensive AI surveillance, future efforts could be greatly refined to the higher risk gull species and the landscape for surveillance narrowed to a specific colony or wildlife area in the state. The results generated from this study can lead to longer-term studies that are capable of providing objective data that more definitively assesses the cumulative effects of avian influenza infections on gulls and terns in Minnesota. Knowledge of the level of avian influenza burden in gulls in Minnesota is necessary to understand avian influenza epidemiology in other wild birds, to evaluate the effect of avian influenza infection on the health of wild birds at the level of the individual and of the population, to understand the co-evolution of avian influenza and its wild bird hosts, and to understand the selective pressures on wild birds. Finally, through collaborative and data-sharing efforts at the University of Minnesota and state agencies such as the Minnesota Department of Natural Resources, this project will be part of a larger effort to understand and better implement surveillance efforts for diseases such as avian influenza and Newcastle disease that have potential tremendous impact on Minnesota's shared ecosystem inhabitants, both wild and domestic birds.

### C. Funding History:

| Funding Source and Use of Funds | Funding Timeframe | $ Amount |
|---|---|---|
| *USDA APHIS Outbreak studies on HPAI (Cardona) to develop environmental testing (water) for AI by PCR and refine whole genome sequences pipeline | September 2015 to September 2016 | $ 299,058 |
| *RARF, Surveillance for High-consequence Poultry Diseases in Wild Bird Reservoirs: Influenza and Newcastle Disease (Redig) | July 2015 to June 2016 | $140,509 |

| | | |
|---|---|---|
| *AES GAR for WRC response, (Willette) Incorporating Captive Managed Avian Collections into Minnesota's Avian Influenza Response Planning | August 2015 to August 2016 | $14,976 |

* Not to be considered matching or in-kind funding (as defined by UMN policy)

**VIII. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS:** Non-applicable N/A

**IX. VISUAL COMPONENT or MAP(S):** Non-applicable N/A

**X. RESEARCH ADDENDUM:**

The research plan provided below is an abbreviated version of the forthcoming research addendum. A more comprehensive and detailed research addendum is currently undergoing peer-review via a process involving U of M research faculty (invited reviewers: Dr. Julia Ponder and Dr. Meggan Craft) and non-U of M subject matter experts (invited reviewers: Dr. Hon Ip and Dr. Jeff Hall, United States Geological Services National Wildlife Health Center).

***Avian Influenza distribution, evolution, and impacts on ring-billed and herring gulls in Minnesota.***

Gulls, shorebirds, and wading birds are members of the large group of birds in the order Charidiiformes. The Charidiiformes have yielded the most AI positive samples in global surveillance strategies. In the Mississippi flyway, and particularly in Minnesota, this order of birds, particularly gulls, has been under represented in surveillance efforts. Frequently, poultry farmers and grain farmers report large flocks of gulls on farms and in fields and have questioned the role that gulls may play in the spread of AI from wildlife to domestic poultry. To address this gap in knowledge, we propose a collaborative surveillance approach with the U of MN, DNR, U.S. Fish and Wildlife Service, and USDA-WS that will include banding and AI testing of ring-billed and herring gulls in breeding colonies and netting of gulls on farm fields or in wildlife areas during the fall and spring migrations. The information generated via the gull studies will be novel and help determine the role, if any, that gulls may have in the spread of AI in Minnesota.

Catching, Banding, and Sampling Strategy for Activities 1 and 2: In the late summer (August), gulls leave their breeding colonies and intermingle with other migratory birds during the fall migration (September, October), creating a situation for exchange of AI viruses among species. Migratory gulls also return to Minnesota in the spring (March, April) during which time they again mix with other wild birds and exchange AI viruses. We will live-capture gulls via netting techniques weekly for three consecutive weeks during spring and fall migration (Activity 2) through Minnesota wildlife areas and on farm fields near poultry farms. This effort will result in a total of 36 gull netting occurrences (18 in the spring and 18 in the fall). During each netting occurrence, our goal will be at least 20 birds captured at each site, with OP swabs, CL swabs and blood samples collected from each bird. 40 samples will be collected (an OP and CL swab from each of 20 birds caught each visit) at each occurrence for a total of 1,440 bird samples x $22.86 per PCR test) = $32,918. When gulls are caught during migration, we will collect blood and extract sera for antibody testing to determine any previous exposure and susceptibility to subsequent exposure. Antibody positive sera will be further evaluated for subtype-specific influenza antibodies by hemagglutination inhibition (HI) testing. The processing and testing of the sera will be approximately $6/sample. For the anticipated 720 sera collected from birds during migration, we have budgeted $4,320. For the estimated 15% positive PCR results, we have budgeted for whole genome sequences on the estimated 216 positives at a total of $18,511.20.

The gulls return to their breeding colonies by May each year and spend the summer in the breeding colonies. As part of Activity 1, two breeding colonies will be chosen. Each breeding colony will be visited once weekly for 4 weeks. At each visit, we will collect 100 fecal samples from the environment and catch 50 gulls by snare or box trap. The gulls will be banded with a uniquely numbered aluminum leg band plus a combination of colored plastic bands to facilitate future identification and recapture. Additionally, an oral (OP) swab, cloacal (CL) swab, and blood sample will be collected from each gull. All swabs (n=800 fecal/environmental samples and 800 bird swabs) will be tested for AI by a rapid PCR test. Any AI positives will be further characterized by completing whole genome sequencing of the AI found. For the anticipated 1600 PCR tests, we have budgeted $36,570. For the estimated 15% positive PCR results, we have budgeted for whole genome sequences on the estimated 240 positives at a total of $20,568.

Serology testing**.** When gulls are caught, we will collect blood and extract sera for antibody testing to determine any previous exposure and susceptibility to subsequent exposure. Antibody positive sera will be further evaluated for subtype-specific influenza antibodies by hemagglutination inhibition (HI) testing. The processing and testing of the sera will be approximately $6/sample.

AI testing by PCR and whole genome sequences of PCR positives.
Swabs and tubes filled with brain heart infusion broth will be provided for sampling birds ($1 each). Each swab will be tested by RT-PCR conducted at the U of MN Mid Central Research and Outreach Center laboratory (MCROC), directed by Dr. Carol Cardona, at a cost of $21.86 each. The total cost, therefore, of a PCR test is $22.86.

Each AI positive sample we detect will be subjected to whole genome sequencing (WGS) at a cost of $85.70 per sample in the laboratory of Dr. Douglas Marthaler, for a total of $17483 for WGS. We anticipate detecting AI positives in these birds by PCR testing in approximately 10-20% of the samples. Each AI PCR positive sample will be subjected to WGS at a cost of $85.70 per sample.

Whole Genome Sequence Analysis
WGS will provide us with insight into the virus genes harbored by the wild bird population in Minnesota and analyses of the genes will elucidate the direction of virus movement between wild birds and domestic poultry.
The WGS results will be analyzed by a molecular virologist, Dr. Douglas Marthaler, to determine the genetic composition of influenza viruses in gulls on or near poultry farms. The evolution of the virus genes in gulls will be elucidated and we will determine the flow of influenza virus genes between the domestic poultry population and wild birds in Minnesota.

Supplies:
Blood tubes and collections swabs are $1.00 each. A large supply has been provided by the USDA at no charge. Personal protective equipment ($1427), notebooks ($50), mailers ($490), binoculars ($250), sample preservation ($100) require a total budget of $2317.00.

**XI. REPORTING REQUIREMENTS:**

**Periodic work plan status update reports will be submitted no later than January 1, 2017 and July 1, 2017 and December 2017. A final report and associated products will be submitted between June 30 and August 15, 2018.**

**The final report was submitted on December 21, 2018.**

Table S1. Strain Names and GenBank Accession numbers for gene sequences obtained from this study.

| Organism Name | Subtype | Segment 1 PB2 | Segment 2 PB1 | Segment 3 PA | Segment 4 HA | Segment 5 NP | Segment 6 NA | Segment 7 MP | Segment 8 NS |
|---|---|---|---|---|---|---|---|---|---|
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI0939/2017) | H13N6 | MH764167 | MH764166 | MH764165 | MH764160 | MH764163 | MH764162 | MH764161 | MH764164 |
| Influenza A virus (A/ring-billed gull/Minnesota/OPMNAI0939/2017) | H13N6 | MH763906 | MH763905 | MH763904 | MH763899 | MH763902 | MH763901 | MH763900 | MH763903 |
| Influenza A virus (A/ring-billed gull/Minnesota/OPMNAI0946/2017) | H?N6 | MH763945, MH763946 | MH763944 | MH763943 | -N/A- | MH763941 | MH763940 | MH763939 | MH763942 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI0693/2017) | H?N6 | MH763913 | MH763912 | MH763911 | -N/A- | MH763909 | MH763908 | MH763907 | MH763910 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI0790/2017) | H13N8 | MH764103 | MH764102 | MH764101 | MH764096 | MH764099 | MH764098 | MH764097 | MH764100 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI0798/2017) | H13N8 | MH764111 | MH764110 | MH764109 | MH764104 | MH764107 | MH764106 | MH764105 | MH764108 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI0806/2017) | H13N8 | MH764119 | MH764118 | MH764117 | MH764112 | MH764115 | MH764114 | MH764113 | MH764116 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI0808/2017) | H13N8 | MH764127 | MH764126 | MH764125 | MH764120 | MH764123 | MH764122 | MH764121 | MH764124 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI0809/2017) | H13N8 | MH763980 | MH763979 | MH763978 | MH763973 | MH763976 | MH763975 | MH763974 | MH763977 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI0811/2017) | H13N8 | MH764033 | MH764032 | MH764031 | MH764026 | MH764029 | MH764028 | MH764027 | MH764030 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI0814/2017) | H13N8 | MH764135 | MH764134 | MH764133 | MH764128 | MH764131 | MH764130 | MH764129 | MH764132 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI0815/2017) | H13N8 | MH763988 | MH763987 | MH763986 | MH763981 | MH763984 | MH763983 | MH763982 | MH763985 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI0818/2017) | H13N8 | MH764143 | MH764142 | MH764141 | MH764136 | MH764139 | MH764138 | MH764137 | MH764140 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI0821/2017) | H13N8 | MH764017 | MH764016 | MH764015 | MH764010 | MH764013 | MH764012 | MH764011 | MH764014 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI0827/2017) | H13N8 | MH764151 | MH764150 | MH764149 | MH764144 | MH764147 | MH764146 | MH764145 | MH764148 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI0829/2017) | H13N8 | MH764009 | MH764008 | MH764007 | MH764002 | MH764005 | MH764004 | MH764003 | MH764006 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI0833/2017) | H13N8 | MH764159 | MH764158 | MH764157 | MH764152 | MH764155 | MH764154 | MH764153 | MH764156 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI0891/2017) | H13N6 | MH764001 | MH764000 | MH763999 | MH763994 | MH763997 | MH763996 | MH763995 | MH763998 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI0926/2017) | H13N6 | MH763921 | MH763920 | MH763919 | MH763914 | MH763917 | MH763916 | MH763915 | MH763918 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI0947/2017) | H13N6 | MH764175 | MH764174 | MH764173 | MH764168 | MH764171 | MH764170 | MH764169 | MH764172 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI0968/2017) | H13N8 | MH764191 | MH764190 | MH764189 | MH764184 | MH764187 | MH764186 | MH764185 | MH764188 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI1197/2017) | mixed | MH764212 | MH764210, MH764211 | MH764208, MH764209 | MH764200 | MH764204, MH764205 | MH764202, MH764203 | MH764201 | MH764206, MH764207 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI1199/2017) | H13N2 | MH764095 | MH764094 | MH764093 | MH764088 | MH764091 | MH764090 | MH764089 | MH764092 |
| Influenza A virus (A/ring-billed gull/Minnesota/OPMNAI0800/2017) | H13N8 | MH764075 | MH764074 | MH764073 | MH764068 | MH764071 | MH764070 | MH764069 | MH764072 |
| Influenza A virus (A/ring-billed gull/Minnesota/OPMNAI0801/2017) | H13N8 | MH763962, MH763963, MH763964 | MH763961 | MH763960 | MH763955 | MH763958 | MH763957 | MH763956 | MH763959 |
| Influenza A virus (A/ring-billed gull/Minnesota/OPMNAI0806/2017) | H13N8 | MH764041 | MH764040 | MH764039 | MH764034 | MH764037 | MH764036 | MH764035 | MH764038 |
| Influenza A virus (A/ring-billed gull/Minnesota/OPMNAI0809/2017) | H13N8 | MH764025 | MH764024 | MH764023 | MH764018 | MH764021 | MH764020 | MH764019 | MH764022 |
| Influenza A virus (A/ring-billed gull/Minnesota/OPMNAI0814/2017) | H13N8 | MH763972 | MH763971 | MH763970 | MH763965 | MH763968 | MH763967 | MH763966 | MH763969 |
| Influenza A virus (A/ring-billed gull/Minnesota/OPMNAI0815/2017) | H13N8 | MH763938 | MH763937 | MH763936 | MH763931 | MH763934 | MH763933 | MH763932 | MH763935 |
| Influenza A virus (A/ring-billed gull/Minnesota/OPMNAI0816/2017) | H13N8 | MH763866 | MH763865 | MH763864 | MH763859 | MH763862 | MH763861 | MH763860 | MH763863 |
| Influenza A virus (A/ring-billed gull/Minnesota/OPMNAI0817/2017) | H13N8 | MH764049 | MH764048 | MH764047 | MH764042 | MH764045 | MH764044 | MH764043 | MH764046 |
| Influenza A virus (A/ring-billed gull/Minnesota/OPMNAI0834/2017) | H13N8 | MH763898 | MH763897 | MH763896 | MH763891 | MH763894 | MH763893 | MH763892 | MH763895 |
| Influenza A virus (A/ring-billed gull/Minnesota/OPMNAI0919/2017) | H13N6 | MH764081 | -N/A- | -N/A- | MH764076 | MH764079 | MH764078 | MH764077 | MH764080 |
| Influenza A virus (A/ring-billed gull/Minnesota/OPMNAI0926/2017) | H13N6 | MH763882 | MH763881 | MH763880 | MH763875 | MH763878 | MH763877 | MH763876 | MH763879 |
| Influenza A virus (A/ring-billed gull/Minnesota/OPMNAI0935/2017) | H13N6 | MH763930 | MH763929 | MH763928 | MH763922, MH763923 | MH763926 | MH763925 | MH763924 | MH763927 |
| Influenza A virus (A/ring-billed gull/Minnesota/OPMNAI0966/2017) | H13N8 | MH764057 | MH764056 | MH764055 | MH764050 | MH764053 | MH764052 | MH764051 | MH764054 |
| Influenza A virus (A/ring-billed gull/Minnesota/OPMNAI1199/2017) | H?N6 | -N/A- | MH764087 | MH764086 | -N/A- | MH764084 | MH764083 | MH764082 | MH764085 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI1283/2017) | H13N2 | MH764220 | MH764219 | MH764218 | MH764213 | MH764216 | MH764215 | MH764214 | MH764217 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI1301/2017) | H13N2 | MH764228 | MH764227 | MH764226 | MH764221 | MH764224 | MH764223 | MH764222 | MH764225 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI1320/2017) | H13N8 | MH763874 | MH763873 | MH763872 | MH763867 | MH763870 | MH763869 | MH763868 | MH763871 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI1420/2017) | H13N6 | MH764236 | MH764235 | MH764234 | MH764229 | MH764232 | MH764231 | MH764230 | MH764233 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI1501/2017) | H13N2 | MH764244 | MH764243 | MH764242 | MH764237 | MH764240 | MH764239 | MH764238 | MH764241 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI1529/2017) | H13N6 | MH764252 | MH764251 | MH764250 | MH764245 | MH764248 | MH764247 | MH764246 | MH764249 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI1539/2017) | H13N6 | MH764260 | MH764259 | MH764258 | MH764253 | MH764256 | MH764255 | MH764254 | MH764257 |
| Influenza A virus (A/ring-billed gull/Minnesota/OPMNAI1286/2017) | H13N2 | MH764065, MH764066, MH764067 | MH764064 | MH764063 | MH764058 | MH764061 | MH764060 | MH764059 | MH764062 |
| Influenza A virus (A/ring-billed gull/Minnesota/OPMNAI1315/2017) | H13N2 | MH763890 | MH763889 | MH763888 | MH763883 | MH763886 | MH763885 | MH763884 | MH763887 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI1025/2017) | H13N6 | MH763954 | MH763953 | MH763952 | MH763947 | MH763950 | MH763949 | MH763948 | MH763951 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI0955/2017) | H13N8 | MH764183 | MH764182 | MH764181 | MH764176 | MH764179 | MH764178 | MH764177 | MH764180 |
| Influenza A virus (A/ring-billed gull/Minnesota/CLMNAI0974/2017) | H13N8 | MH764199 | MH764198 | MH764197 | MH764192 | MH764195 | MH764194 | MH764193 | MH764196 |

**Environment and Natural Resources Trust Fund**

**M.L. 2015 Project Budget**

**Project Title: Avian Influenza distribution, evolution, and impacts on Ring-billed and Herring Gulls in Minnesota**

**Legal Citation: M.L. 2015, Chp. 76, Sec. 2, Subd. 10 - Emerging Issues Account**

**Project Manager: Marie Culhane**

**Organization:** *University of Minnesota*

**M.L. 2015 ENRTF Appropriation: $213,443**

**Project Length and Completion Date: 2 year;** *June 30, 2018*

**Date of Report December 21, 2018**

| ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET | Activity 1 Budget | Amount Spent | Activity 1 Balance | Activity 2 Budget | Amount Spent | Activity 2 Balance | TOTAL BUDGET | TOTAL BALANCE |
|---|---|---|---|---|---|---|---|---|
| **BUDGET ITEM** | | | | | | | | |
| **Personnel (Wages and Benefits)** | **$47,315** | **$47,315** | **$0** | **$41,958** | **$41,958** | **$0** | **$89,273** | **$0** |
| Marie Culhane, principal investigator, project design and oversight, publication assistance and analysis of results, 5% FTE for one year, 75 % salary, 25% benefits = $9,440 | | | | | | | | |
| Todd Knutson, collaborator, whole genome sequencing and bioinformatics, 2% FTE for one year, 75% salary, 25% benefits = $2,942 | | | | | | | | |
| Todd Froberg, graduate student in wildlife ecology and research assistant, 50% FTE for one year, 52% salary, 48% benefits = $44,522 | | | | | | | | |
| TBN, wildlife data technician, to participate in field studies, collect samples from wildife, data management, . 75% FTE for one year, 75% salary, 25% benefits = $32,369 | | | | | | | | |
| **Professional/Technical/Service Contracts** | **$3,003** | **$3,003** | **$0** | **$6,097** | **$6,061** | **$36** | **$9,100** | **$36** |
| Undergraduate student workers, for data entry and data collection, $13.00/hour for 700 hours over one year = $9,100 | | | | | | | | |
| **Equipment/Tools/Supplies** | **$46,596** | **$46,596** | **$0** | **$48,883** | **$48,883** | **$0** | **$95,479** | **$0** |
| Binocular for bird observation (5 @$50 each) = $250 | | | | | | | | |
| Waterproof notebooks for recording bird observations (5@$10 each) = $50.00 | | | | | | | | |
| Gloves and other personal protective equipment for field sampling = $1427 | | | | | | | | |
| Sample preservation of collected samples = $100 | | | | | | | | |
| Mailers for samples (12@$41 each) = $490 | | | | | | | | |

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|---|---|---|---|---|---|---|---|---|
| Polymerase Chain Reaction tests of bird and envirommental fecal samples for detection of Avian Influenza at U of MN Mid Central Research and Outreach Center Laboratory at on 3040@ $22.86 per test = $69494.40 and direct Sanger Sequencing of the Hemagglutinin and Neuraminidase genes @ $67.53 each | | | | | | | | |
| Whole Genome Sequence of AI viruses detected at U of MN CVM on 120 @$85.70 each = $10,284 | | | | | | | | |
| Serology on wild bird blood samples collected at U of MN Mid Central Research and Outreach Center Laboratory on 880@$6.00 each = $5280 | | | | | | | | |
| **Travel expenses in Minnesota** | **$6,458** | **$6,458** | **$0** | **$13,133** | **$13,133** | **$0** | **$19,591** | **$0** |
| Overnight lodging and per diem meals in greater MN is $179 per person X 4 people X 34 nights = $24,344 per U of MN reimbursement rates | | | | | | | | |
| weekly car rental at $228 per week for 10 weeks = $2,280 | | | | | | | | |
| mileage charges of $0.17 per mile X 6300 miles = $1,071 per U of MN rates | | | | | | | | |
| **COLUMN TOTAL** | **$103,372** | **$103,372** | **$0** | **$110,071** | **$110,035** | **$36** | **$213,443** | **$36** |