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

Date of Report: June 30, 2016

Date of Next Status Update Report: January 1, 2017

Date of Work Plan Approval:

Project Completion Date: June 30, 2017 (pending extension to June 30, 2018 during 2017 legislative session)

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:	**$0**
	Balance:	**$213,443**

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

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 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. So, gulls could be a contributing factor of this outbreak. Nonetheless, gulls remain under-represented 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 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 1, 2017:

Project Status as of July 1, 2017:

Project Status as of January 1, 2018:

Project Status as of July 1, 2018:

Overall Project Outcomes and Results:

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 overtime, 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:	**$** 109,276
Amount Spent:	**$** 0
Balance:	**$** 109,276

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 1, 2017:

Activity 1 Project Status as of July 1, 2017:

Activity 1 Project Status as of January 1, 2018:

Activity 1 Project Status as of July 1, 2018:

Activity 1 Overall Project Outcomes and Results:

Final Report Summary:

Activity 2: Testing of Gulls during Spring and Fall Migration for avian influenza ($104,167)
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 overtime, 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:**	**$** 104,167
	Amount Spent:	**$** 0
	Balance:	**$** 104,167

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 1, 2017:

Activity 2 Project Status as of July 1, 2017:

Activity 2 Project Status as of January 1, 2018:

Activity 2 Project Status as of July 1, 2018:

Activity 2 Overall Project Outcomes and Results:

Final Report Summary:

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

Status as of January 1, 2017:

Status as of July 1, 2017:

Status as of January 1, 2018:

Status as of July 1, 2018:

Overall Project Outcomes and Results:

Final Report Summary:

VI. PROJECT BUDGET SUMMARY:
A. ENRTF Budget Overview:

<table>
<tr><th>Budget Category</th><th>$ Amount</th><th>Overview Explanation</th></tr>
<tr><td>Personnel:</td><td>$ 89,273</td><td>Marie Culhane, principal investigator, project design and oversight, publication assistance and analysis of results, 5% FTE for one year ($9,440); Doug Marthaler, collaborator, whole genome sequencing and bioinformatics, 2% FTE for one year($2,942) [Note: Both Culhane and Marthaler are contract faculty 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).</td></tr>
<tr><td>Professional/Technical/Service Contracts:</td><td>$9,100</td><td>Undergraduate student workers, for data entry and data collection, $13.00/hour for 700 hours over one year</td></tr>
<tr><td>Equipment/Tools/Supplies:</td><td>$87,375</td><td></td></tr>
<tr><td></td><td></td><td>
<table>
<tr><td>Binoculars for bird observation</td><td>$100</td></tr>
<tr><td>Waterproof notebooks for recording bird observations</td><td>$20</td></tr>
<tr><td>Gloves and other personal protective equipment for field sampling</td><td>$427</td></tr>
<tr><td>Sample preservation of collected samples</td><td>$34</td></tr>
<tr><td>Mailers for samples</td><td>$100</td></tr>
<tr><td>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</td><td>$36,576</td></tr>
<tr><td>120 Whole Genome Sequence of AI viruses detected at U of MN College of Veterinary Medicine @$85.70 each</td><td>$6,141</td></tr>
<tr><td>880 wild bird blood sample serology tests at U of MN MidCentral Research and Outreach Center Laboratory @$6.00 each</td><td>$3,140</td></tr>
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Travel Expenses in MN:	$27,695	Overnight lodging and per diem meals in greater MN is $179 per person X 4 people X 34 nights per University reimbursement rates; 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, Jenelle, 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.

Doug Marthaler, PhD, (receiving funds of $2,942 for wages and benefits), collaborator, is an assistant professor at the U of MN in the Department of Veterinary Population Medicine. Dr. Marthaler will use his expertise in the analysis and WGS 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 sequencig 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 WGS 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 WGS 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 WGS 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 WGS 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.

WGS 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 of these have 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. A final report and associated products will be submitted between July 1 and August 15, 2017 (or between June 30 and August 15, 2018 pending extension during 2017 legislative session).