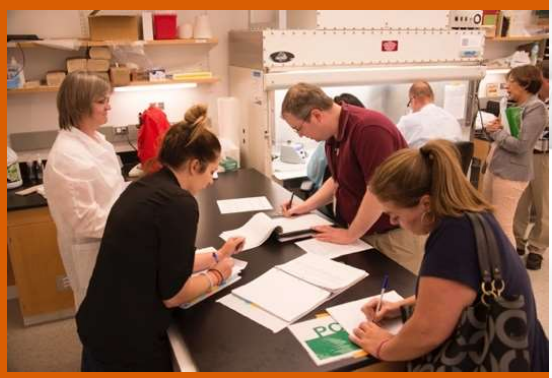

NAHLN Farm Bill Showcase

20 years

2002 - 2022

National Animal Health Laboratory Network

Abstracts from 2020 NAHLN Farm Bill Funded Projects

NAHLN Farm Bill Showcase

Presentations from 2020 NAHLN Farm Bill Funded Projects

Tuesday, November 8, 2022
8:30 AM – 11:15 AM CT

Moderators: Beth Harris & Kelli Almes

NAHLN Farm Bill Showcase

Presentations from 2020 NAHLN Farm Bill Funded Projects

Wednesday, November 9, 2022
12:30 PM – 3:15 PM CT

Moderators: Christina Loiacono & Kelli Almes

NAHLN Farm Bill Showcase

2020 NAHLN Farm Bill Funded Projects

2020 NAHLN Farm Bill - Capacity Support

Novel Loop-Mediated Isothermal Amplification (LAMP) Systems As On-site Diagnostic Tools for Classical Swine Fever and African Swine Fever Viruses

Carol W. Maddox

University of Illinois, Urbana, Illinois

Nucleic acid amplification has proven to be both a sensitive and specific means of detecting pathogens in both animals and humans. Taqman™ real-time PCR revolutionized the ability of well-equipped veterinary diagnostic laboratories to provide reliable results within hours. However, these and smaller labs, particularly field sites in developing countries, would benefit from new LAMP detection which requires much less expensive equipment and easier training. Devastating international infections such as classic swine fever and African swine fever are of great concern to the U.S. as foreign animal diseases that are emerging in the Caribbean/South America and China. Nations near these outbreaks can also benefit by heightened surveillance possible with isothermal amplification and colorimetric detection offered by LAMP. This aim of this proposal is to design ASFV and CSFV primers for LAMP detection and to validate the assay for U.S. and world-wide use.

Objective 1. Develop and validate assays based on loop mediated isothermal amplification (LAMP) systems for the specific and sensitive detection of ASFV. We are ~95% completed with the ASFV LAMP assay and have validated it virus and virus infected tissues with our Co-Investigator at IRTA, Barcelona, Spain, Dr. L. Ganges. We are just completing a manuscript and registration with the Office of Technology Management

Objective 2. Develop and validate assays based on loop mediated isothermal amplification systems for the specific and sensitive detection of CSFV. We are ~35% completed with development and testing of the CSFV 5'UTR LAMP assay (only validated with plasmid containing clone 5'UTR of CSFV and spiked tissues.) Dr. Ganges also hopes to develop type specific detection primers for the major CSFV groups.

The strength of our project is a LAMP assay that assay is rapid, requires very little sophisticated or expensive equipment or technology and thus is suitable for on-site testing. Field collection and processing of samples for surveillance or outbreaks can save tremendous amounts of time and reduce costs and producer losses when responding to the threat of ASF and CSF to US swine herds. All phases of the test can be completed with a beaker of water, hot plate, and thermometer within minutes with exceptional specificity and sensitivity, similar to that of USDA and OIE qPCR and qRT-PCR assays, respectively.

Point-of-Care Detection of ASFV, CSFV and FMDV Using Integrated Nano-Technologies Platform

Jianfa Bai

Kansas State Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, Kansas

African swine fever (ASF), classic swine fever (CSF) and foot and mouth disease (FMD) are three major foreign animal diseases and are listed as select agents by USDA. Nucleic acid detections are generally more sensitive (Eberling et al. 2011; Zsak et al. 2005; Callahan et al. 2002), yet are mostly carried out in laboratory settings and use specialized and sophisticated machines, i.e., KingFisher-96 for extraction, and real-time PCR for detection. Point-of-care detection assays using Integrated Nano-Technologies (INT) platform are under development.

Primers and probes from recently published real-time PCR assays for the detection of foot and mouth disease virus (Wang, Das, et al. 2020), and African swine fever virus (Wang, Xu, et al. 2020) are used in this study. For CSFV, sequences of 5'-untranslated region (5'UTR) were collected and analyzed for primer and probe designs. All primers and probes were synthesized and modified based on INT requirements. Flanking regions encompassing each target were synthesized and cloned to serve as templates for initial analytical evaluations of the assays. Synthesized primers were first evaluated with real-time PCR using SYBR-Green reagent. After verifying each pairs of primers were amplifying the targeted plasmids, they were subjected to testing in the INT system. Preliminary data indicated that all three FAD pathogen templates were tested positive, indicating the system is working. Our next step is to run serial dilutions of the templates on the chips to determine assays' detection limit for each detection assay; at the same time, we will test each assay with some common swine pathogen positive samples to make sure the three assays do not detect other pathogens. Once we have generated satifactory data using cloned plasmids, we will evaluate the assays with real viruses or positive samples in collaboration with FADDL, NVSL.

Animal Virome Panel for Optimization and Validation of Next Generation Sequencing for Endemic, Emerging and Foreign Animal Disease Viruses

Sunil Mor

Veterinary Diagnostic Laboratory, Department of Veterinary Population Medicine University of Minnesota, St. Paul, Minnesota

Project objective

To develop animal virome panel for optimization and validation of next generation sequencing protocols for early detection and surveillance of emerging, zoonotic and foreign animal diseases.

Objective 1- Development and validation of animal virome panel for next generation sequencing.

Objective 2- Inter-laboratory comparison of next generation sequencing using animal virome panel

Objective 3- Standardized guidelines for successful implementation and evaluation of NGS technologies.

Deliverables completed/ yet to be completed

We developed two animal virome panels of selected RNA (n=11) and DNA (n=7) viruses, as a reference material for NGS method optimization and validation from nucleic acid extraction to library preparation. We quantified and pooled RNA and DNA viruses based on TCID50/mL at high and low virus titers and determined their Ct values with PCR. We spiked clinical samples with these pooled viruses and optimized the virus enriched NGS library preparation methodology and bioinformatics pipeline. We detected all viruses in the reference standard successfully.

How the project benefits NAHLN

We believe these two quantified RNA and DNA viral panels will be very helpful for the NAHLN laboratories to use as reference materials for optimization and validation of their NGS assays for viral disease investigation and whole genome sequencing.

VirSlayer -A Quick and Easy to Use Bioinformatics Pipeline for Detection of Endemic, Emerging and Foreign Animal Disease Viruses

Tom Kono[1], Vikash Singh[2], Sunil Mor[2]

[1]Department Minnesota Supercomputing Institute, University of Minnesota, St. Paul, Minnesota
[2]Veterinary Diagnostic Laboratory, Department of Veterinary Population Medicine, University of Minnesota, St. Paul, Minnesota

Project objective:
To develop a simple and easy to use bioinformatics pipeline, which can share with other Veterinary Diagnostics Laboratories and be of use to non-bioinformaticians.

Deliverables completed:

- Finalized design of the pipeline
- Identified testing datasets for development
- Finished scripts for the following steps:
- Read quality summarization
- Read trimming (adapter contamination and low-quality bases)
- Removal of host and human contaminants
- Assembly of short reads into contigs
- Taxonomic classification of contigs
- Wrote accessory/companion scripts for downloading and configuring required reference databases for host depletion, human depletion, and taxonomic assignment

Deliverables yet to be completed:
The next steps for the project are as follows:

1. Finish scripts for classification of sequence as viral or non-viral
2. Finish scripts for annotation of open reading frames
3. Finish pipeline summary report
4. Build container image
5. Write documentation
6. Organize an online or hand-on-training workshop for VDLs

The pipeline scripts are being maintained in a GitHub repository. They will be made publicly available upon completion of the project.

How the project benefits NAHLN:
Installation and training of this robust bioinformatics pipeline will benefit NAHLN laboratories by enhancing their capacity for rapid NGS data analysis and results reporting without bioinformatics expertise. Pipeline will be shared with proper training and SOPs. We will help to download the pipeline on the server or system of interested laboratories.

Agent of Disease Point-Of-Care Genomics (AOD-POCgen): A Simplified Purpose-Built Platform and Improved Diagnostic Tool for Testing of Foreign Animal Diseases

Thomas Hadlock[1], Jacob Neice[1], Chang Lu[1], Steven Hart[2], Albert Rovira[3], Sunil Mor[3]

[1]Department of Chemical Engineering, Virginia Tech, Blacksburg, Virginia; [2]Department of Laboratory Medicine and Pathology, Mayo College of Medicine, Rochester, Minnesota; [3]Veterinary Diagnostic Laboratory, Department of Veterinary Population Medicine, University of Minnesota, St. Paul, Minnesota

Project objectives:
To develop a prototype device that can be used in the field without the need for special expertise in molecular biology.
Objective 1 - Development and experimental validation of AOD-POCgen system.
Objective 2- Optimization of AOD-POCgen for detection of foot and mouth disease virus from field samples.

Deliverables completed or yet to be completed:
Library preparation
This study evaluated a semi-automated, field deployable system for the rapid handling of swine samples including viral RNA extraction, library preparation, and sequencing using the Oxford Nanopore MinION device. The endemic Seneca virus A (SVA) was used to model FMDV. Loop mediated isothermal amplification (LAMP) was utilized to increase starting quantity of RNA recovered from nucleic acid extraction protocols in order to meet minimum requirements of tagmentation based library preparation. A multiplex LAMP primer set (six total primer sets) was designed to evenly amplify three distinct regions covering 16% of the 7300bp genome without needing a thermocycler.

Rapid tagmentation-based libraries prepared with multiplex LAMP system demonstrated a barcoding success rate of 92.9%, and successful alignment rate of 69.2%. Our device achieves LAMP production rates of 331 times starting material and generates libraries of up to 119ng from 1.1ng extracted SVA RNA spiked into TE buffer. Our LAMP sequencing system allowed for detection of SNPs within the primer bounds at up to 98%. We are currently evaluating the efficacy of this device on vesicular fluid samples extracted from SVA-positive pigs. We will also expand the use of this device to simultaneously process multiple samples to reduce reagent cost and library production time.

Hardware
The Bioinformatics team at Mayo Clinic (led by Dr. Hart) was responsible for development and testing of the "edge" computing portion of this project. We selected a Jetson Xavier AGX as the edge device since it contained an 8-core 64-bit CPU and a 512-core Volta GPU with Tensor

Cores to account for the rapid processing necessary for nanopore reads. We then extended the device to have a 1TB SSD as the size of the raw nanopore files is more than the standard 32Gb storage, and also added a 7 ½-inch touchscreen display.

Software Installs

There were three potential informatics solutions that had the capability to process the raw reads in real time SquiggleNet, ReadFish, and UNCALLED. First, Docker containers were created to ensure that all dependencies for each tool could be accounted for and installed. To simplify this process, the Docker files were developed and tested on laptops before attempting to deploy onto the Jetson AGX. Once the foundational package requirements were identified, and the Docker files validated, the next stage was deploying the Docker files onto the Jetson Xavier. Unexpectedly, several errors in installation were found resulting from both conflicts from the base operating system and the ARM-based CPU architecture of the Jetson. In the end, both ReadFish and UNCALLED had an unresolvable dependency (namely the implementation of bwa-aligner). A potential workaround for this has also been identified, but not yet validated.

Data Analysis

With SquiggleNet currently the only viable approach, we then sought to assess the performance of the device-software combination. To provide an unbiased assessment of performance (for later comparison with other tools), we simulated 10,000 reads in "squiggle-space" from the Seneca Virus A genome using DeepSimulator and monitored the compute speed and memory consumption from SquiggleNet. DeepSimulator generates an expected squiggle distribution, which is used by the SquiggleNet AI model to classify whether a squiggle belongs to an expected distribution of reads. Processing required 4GB of memory and completed within 242 seconds. Of the 10K reads, only 52% were correctly assigned to the Seneca A Virus.

From a technical standpoint, the memory and computational speed yielded promising metrics. From a quality standpoint however, much remains to be desired. One would have expected 100% of the reads would be attributed to the Seneca Virus. The poor performance is likely related to simulation data not accurately reflecting the real-life distribution of reads from the nanopore sequencing. Future experiments will use PCR-derived sequence data from the samples, and to work around the bwa-aligner conflict to get additional tooling options.

How the project benefits NAHLN:

The project will enhance the capacity of NAHLN for rapid detection of emerging and foreign animal diseases. This will be helpful in detection and classification of FMDV and other vesicular viruses.

Development and Validation of Real-Time Quaking-Induced Conversion (RT-QuIC) As Confirmatory Testing for Chronic Wasting Disease CWD

Alejandro Banda

Mississippi Veterinary Research and Diagnostic Laboratory, Pearl, Mississippi

PROJECT OBJECTIVES:

Objective 1. Establishment and evaluation of RT-QuIC as a confirmatory diagnostic methodology to detect CWD in biological samples of CWD afflicted deer such as lymph nodes.

Objective 2. Application of RT-QuIC protocols for antemortem CWD diagnosis using saliva, or oropharyngeal fluids

Objective 3. Application of RT-QuIC protocols for use on environmental, soil and branches.

DELIVERABLES COMPLETED:

Objective 1. Establishment and evaluation of RT-QuIC as a confirmatory diagnostic methodology to detect CWD in biological samples of CWD afflicted deer such as lymph nodes.

1. Collection and ELISA testing of deer retropharyngeal lymph node samples.
2. Equipment acquisition for RT-QuIC
3. RT-QuIC troubleshooting and optimization for deer lymph node samples
4. RT-QuIC validation lymph node samples

Objective 2. Application of RT-QuIC protocols for antemortem CWD diagnosis using feces, and saliva

1. Collection saliva and feces specimens
2. Preparation of feces samples spiked with purified CWD prion proteins
3. Establishment of purification and concentration procedures for CWD prion proteins from deer feces
4. RT-QuIC troubleshooting and optimization for feces
5. RT-QuIC validation with feces

Objective 3. Application of RT-QuIC protocols for use on environmental samples soil and branches.

1. Collection of soil and branch specimens
2. Preparation of feed and soil spiked with purified CWD prion positive
3. Establishment of purification and concentration procedures for CWD prion proteins from branch and soil specimens
4. RT-QuIC troubleshooting for branch and soil specimens
5. RT-QuIC validation for branch and soil samples

DELIVERABLES YET TO BE COMPLETED:

Objective 1. Establishment and evaluation of RT-QuIC as a confirmatory diagnostic methodology to detect CWD in biological samples of CWD afflicted deer such as lymph nodes.

1. Development of truncated recombinant prion proteins (seeding substrates)

Objective 2. Application of RT-QuIC protocols for antemortem CWD diagnosis using feces, and saliva

1. Preparation of saliva samples spiked with purified CWD prion proteins
2. Establishment of purification and concentration procedures for CWD prion proteins from saliva
3. Prion protein purification from saliva/feces and ELISA testing
4. RT-QuIC troubleshooting and optimization for deer saliva
5. RT-QuIC validation for saliva samples

Objective 3. Application of RT-QuIC protocols for use on environmental samples soil and branches.

1. Prion protein purification from feed/soil and ELISA testing

HOW THE PROJECT BENEFITS NAHLN:

Current CWD testing protocol requires submission of samples reacting by ELISA testing to be confirmed by IHC, a methodology that involves a degree of subjectivity according to the histopathologist experience. Furthermore, previous studies have demonstrated the lower sensitivity by IHC in comparison with ELISA, especially in samples with lower concentration of CWD prions. This can be a disadvantage with samples yielding borderline ELISA results, which may be diagnosed as negative by IHC. The unanswered question is if these negative results are due to a real true negative sample or because of the lower IHC sensitivity. Furthermore, some laboratories that do not have capabilities for IHC must submit the samples for confirmation to a reference lab and it can take from weeks to months to obtain results depending on reference laboratory. Recent development of highly sensitive testing for prion diseases, known as real-time quaking-induced conversion, RT-QuIC, holds promise for testing tissues from dead animals, clinical fluids or excretions from live animals and environmental samples. This methodology has been successfully applied to the diagnosis of other prion related diseases of humans and animals. RT-QuIC has be used in CWD research, but additional work needs to be done to investigate the usefulness of this technique as a diagnostic tool in clinical CWD settings. Therefore, the benefit of this project includes the development of RT-QuIC methodologies for confirmatory diagnosis of CWD using deer lymph nodes, that could be applied to other prion-related of domestic animals, for the premortem diagnosis using feces or saliva and also the determination of environmental contamination by prions using soil and branch specimens.

Kansas State Veterinary Diagnostic Laboratory (KSVDL) African Swine Fever Outbreak Collaborative Exercises Project

Kelli Almes[1] and Jamie Retallick

Kansas State Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, Kansas
[1]Current affiliation, National Animal Health Laboratory Network, NVSL, Ames, Iowa

The Kansas State Veterinary Diagnostic Laboratory (KSVDL) plays a key role in disease surveillance and response in the state of Kansas. As a Tier 1 member of the National Animal Health Laboratory Network (NAHLN), KSVDL supports early detection of foreign animal diseases (FAD) in the state, ongoing surge testing during an outbreak event, and eventual surveillance testing to facilitate business continuity as guided by federal and state animal health officials. In the event of a foreign animal disease outbreak, the number of tests performed by KSVDL is expected to sharply increase, one of the driving forces behind the funded 2019 NAHLN Farm Bill Project "Strengthening and enhancement of KSVDL capacity for FAD Emergency preparedness and response." To exercise this increased capacity the laboratory, in collaboration with university, state, and federal partners, as well as swine veterinarians within and around the state, conducted multiple exercises including a tabletop exercise (TTX) and functional exercise (FX) focusing on laboratory centric ASF outbreak response activities.

Throughout the course of this project a planning team including representatives from KSVDL, the Kansas Department of Agriculture (KDA), Biosecurity Research Institute at Kansas State (BRI), and the National Agricultural Biosecurity Center (NABC) worked to plan, write, and refine a TTX with three modules, each focusing on a slightly different scenario or aspect of ASF outbreak response. The TTX was held in May 2021 with approximately 70 participants, observers, policy makers, and stakeholders. A survey was completed, and those results were used to help guide the planning process for the FX activities, which was conducted in January 2022.

The FX included mock samples (swab pools) which were accessioned, transported to BSL3 laboratory space, and processed for testing. An electronic submission process was also created within the LIMS test environment to allow electronic submission of client information and uploading of spreadsheets for large numbers of samples while utilizing barcodes for sample identification. This exercise provided a vital opportunity for outbreak planning for the KSVDL Molecular Services section including assessing levels of reagents and supplies that would be needed in an outbreak, creating a work schedule to allow for appropriate transition time between shifts, and allowing staff to get hands-on experience in the BSL3 environment.

This project resulted in numerous benefits to KSVDL, helping to ready them for future outbreaks and prepare to face surge capacity sample numbers. It also yielded deliverables that have been shared with the entire NAHLN and utilized by the NAHLN Exercises and Drills Working Group. The ASF specific TTX was previously presented to the NAHLN EDWG during a core monthly meeting and was used to create a generic FAD TTX template that is housed on the NAHLN Portal. This template was also utilized to facilitate a workshop in conjunction with the

NAHLN/AAVLD Laboratory Emergency Management Committee meeting at the 2022 AAVLD/USAHA conference aimed at helping participants customize the template in order to take it back to their home laboratory and actually conduct the exercise.

Development of Scalable Field PCR Platform for Senecavirus A and Foot and Mouth Disease

Adam Travis[1] and Rick Haselton[2]

[1] Kord Animal Health Diagnostic Laboratory, Tennessee Department of Agriculture, Nashville, Tennessee

[2] Biomedical Engineering, Vanderbilt University, Nashville, Tennessee

Senecavirus A (SVA) and Foot and Mouth Disease Virus (FMDV) continue to pose challenges for swine producers, diagnosticians, and regulatory officials. To address the increasing incidence of foreign animal disease (FAD) investigations caused by SVA, a field-based adaptive PCR platform utilizing L-DNA has been developed. Primers and probes were designed for SVA and FMDV, and RNAse P was selected as an internal control. Using a Ct of 40, these sequences, in conjunction with commercially available reaction mixture components, correctly identified SVA in each of 44 retained FAD samples previously tested at the Kord Animal Health Diagnostic Lab (KAHDL). The adaptive PCR approach required no extraction step and only lagged by an average of 2.2 cycles compared to results from the NAHLN-approved assay. By eliminating that labor/equipment intensive step, a critical obstacle to developing a field-based design was cleared. Additional modifications to a lab-based model include simpler programming interface and chip components, and air-dried reagents have been incorporated into the assay to accommodate less-controlled field conditions. Phased validation of the device is ongoing. Interlaboratory comparisons have produced concurring results, and use of the device by non-developers has assisted with the refining of standardized work instructions.

The next stage of validation, expected to conclude in 2023, will involve a collaborative effort among the developers, KAHDL, state/federal regulatory partners, and a swine producer. Pending completion of the field tests, subsequent FMDV validation work may be proposed at NBAF, the new National Bio and Agro-Defense Facility.

Validation of Real-Time PCR (qPCR) Assays for the OIE Listed Reportable and Emerging Crustacean Disease Pathogens

Lijuan Zhou and Reddy Bommineni

Bronson Animal Disease Diagnostic Laboratory (BADDL), Kissimmee, Florida

Florida has over 1,000 certified aquaculture farms and ~75% of aquaculture products are for human consumption. A major threat to crustacean aquaculture is diseases that can result in significant production losses. The main purpose of this project is to optimize and validate the in-house qPCR or RT-qPCR assays for one emerging virus, decapod iridescent virus 1(DIV1), and seven OIE reportable pathogens, including white spot syndrome virus (WSSV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), Taura syndrome virus (TSV), yellow head virus genotype 1 (YHV1), infectious myonecrosis virus (IMNV), *Hepatobacter penaei*, a proteobacteria that cause necrotizing hepatopancreatitis (NHP), and acute hepatopancreatic necrosis disease (AHPND/EMS), which is caused by a toxin encoded by a gene of a plasmid in *Vibrio parahaemolyticus*. The primers and probes for the 8 pathogens qPCR or RT-qPCR assays are all available in the OIE manual.

Deliverables completed:

1. Pathogens and source of primers and probes for the qPCR assay:

Disease name	Pathogen	Reference	Referred by
Acute hepatopancreatic necrosis disease (AHPND)	*Vibrio parahaemolyticus*	Han et. al., 2015	OIE
(Necrotising hepatopancreatitis (NHP)	*Hepatobacter penaei*	Aranguren et. al., 2010	OIE
Infectious hypodermal and haematopoietic necrosis virus (IHHNV)	IHHNV	Tang et. al., 2001	OIE
Infectious myonecrosis virus (IMNV)	IMNV	Andrade et. al., 2007	OIE
Taura syndrome virus (TSV)	TSV	Tang et. al., 2004	OIE
White spot syndrome virus (WSSV)	WSSV	Durand et. al., 2002	OIE
Yellow head virus genotype 1 (YHV)	YHV	Aranguren et. al., 2012	OIE
Decapos iridescent virus1 (DIV1)/ Shrimp hemocyte iridescent Virus (SHIV)	DIV1/SHIV	Qiu et. al., 2018, 2020	OIE/University of Arizona

2. Singleplex qPCR using AgPath-ID One-Step RT-PCR kit for the eight pathogens was optimized and standardized for different parameters such as buffer, annealing temperature, and other various reaction conditions on QuantStudio 5 and ABI 7500 Real-time PCR

System. Triplicates of the finalized conditions were run successfully with repeatability for 8 targeted pathogens.

2.1. **Assessment of analytical sensitivity** using synthetic crustacean PAC (gBlock DNA fragment from IDT), or plasmid DNA for 8 targeted pathogens:

a. The limit of Detection (LOD) was up to 1:10^9 dilution (about 1-10 copies/µL) for each target.
b. PCR efficiency between 98-99 for each target pathogen.
c. The addition of exogenous internal control XIPC for DNA targets and Escherichia coli bacteriophage MS2 for RNA targets were not affecting the LOD.
d. Two assays targeting two genes in DIV1/SHIV genome were evaluated, LOD and PCR efficiency are the same between the two assays.

2.2. **Diagnostic sensitivity**

a. Shrimp tissue infected with 7 OIE reportable diseases pathogens was purchased from the Aquaculture pathology laboratory, University of Arizona.
    i. 7 pathogens were successfully detected in each tissue sample infected with 7 different pathogens.
    ii. Extracted DNA/RNA from infected tissue was serially diluted with the addition of exogenous DNA or RNA internal controls (same concentration in each dilution)
    iii. The limit of detection was up to 1:106 dilution for each target.
    iv. The addition of XIPC or MS2 internal control was not affecting the LOD.
b. Assessment of d diagnostic sensitivity using spiked tissue samples due to the difficulties to obtain the naturally infected clinical samples.
    i. Uninfected shrimps from a local food store were dissected after 70% Ethanol treatment for at least 24 hours. The head and abdomen tissue were harvested and homogenized from 10 individual shrimps.
    ii. Serial diluted synthetic crustacean PAC (gBlock DNA) was spiked in the uninfected tissue homogenate with XIPC as an internal control. LOD for the 5 DNA targeted diseases is up to 1:10^9 dilution, which is the same as the direct PCR.
    iii. Singleplex qPCR can be used to test the tissue samples pooled up to 10 shrimps.

3. Multiplex qPCR using Path-ID Multiplex One-Step RT-PCR kit and TaqPath 1-Step Multiplex One-Master Mix kit:

a. Triplex qPCR assays for DNA-targeted pathogens AHPND/SHIV/XIPC, and IHHNV/WSSV/XIPC with Path-ID 1-Step RT-PCR kit were successful by direct PCR using serial diluted synthetic DNA, or after DNA spiking in the uninfected tissue.
b. Triplex PCR for DNA-targeted pathogens NHP/IHHNV/WSSV with TaqPath 1-Step RT-PCR kits was successful using serial diluted synthetic DNA as templates.
c. Fourplex PCR for RNA virus IMNV/TSV/YHV/MS2 was performed with TaqPath 1-Step RT-PCR kits using serial diluted virus RNA from infected tissue as templates. TSV and YHV working well. Multiplex PCR for IMNV will be optimized in the next quarter.

Deliverables yet to be completed:

Diagnostic sensitivity assessment using uninfected shrimp tissue with synthetic RNA for the 3 RNA virus.

1. Optimize the multiplex qPCR procedures for the DNA/RNA target pathogens.
2. Complete the SOP for singleplex PCR and multiplex PCR.

Benefit to NAHLN:

The validated crustacean qPCR protocols will share with the NAHLN and USDA.

Development and Validation of Point of Care (POC) Assays for ASF and CSF for Rapid Outbreak Response

Roman Pogranichniy

Kansas State Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, Kansas

Infectious diseases and especially transboundary animal diseases (TADs) pose a constant threat to the US livestock industry. African Swine Fever (ASF) and Classical Swine Fever (CSF) are important TADs affecting swine herd in Asia, Africa and some European countries. Because of heavy trade and movement of goods, animal products, and people between these regions and the US, we are vulnerable to the introduction of these TADs. If an introduction of ASF and CSF were to occur in the US, the swine industry would need reliable point-of-care (POC) molecular, antigen and serological diagnostic assays for rapid, sensitive and specific detection of these important diseases of swine.

To address these needs, we propose to develop and validate antigen and serological POC diagnostic tests for the detection of ASF and CSF. Our team has already developed and validated molecular and antigen detection POC assays for ASF using a collection of experimentally and naturally infected samples at KSU and ARC-Onderstepoort, South Africa. We have also developed a molecular POC test for CSF that is awaiting further validation. Therefore, the first objective of the proposed work includes the development of a POC ASF serological test, and the development of antigen- and antibody-based POC tests for CSF. The reagents, i.e. recombinant proteins and monoclonal antibodies, for these assays will be produced at Kansas State University (KSU) in Dr. Richt's laboratory, using established protocols. These reagents will be made available to BioStone Animal Health LLC to develop the respective lateral flow assays for the detection of ASF and CSF viral antigens and/or antibodies. The second objective of the proposed work will be to evaluate the respective POC assays using samples from experimentally infected animals which were generated and archived at KSU.

Subsequently, evaluation of the POC tests with field samples will be performed by our collaborators in countries where ASF and CSF is endemic using GPS coordinates for sample identification; this will provide USDA Veterinary Services officials better knowledge of animal location for future decision making. This is also an important capability for testing samples from wild animals in the field.

Validation of ASR1 Peptoid-ELISA for the Detection of Chronic Wasting Disease Prion in Samples with Low Prion Burden

Shuping Zhang

University of Missouri Veterinary Medical Diagnostic Laboratory, Columbia, Missouri

Chronic wasting disease (CWD) is a prion disease of captive and free-ranging cervids, including mule deer, white-tailed deer, elk, reindeer, sika, and moose. To control CWD in farmed/captive cervids, the United States Department of Agriculture (USDA) has established a national CWD Herd Certification Program, and many states have implemented surveillance programs, including Missouri.

Currently, laboratory diagnosis of CWD relies primarily on immunohistochemistry (IHC) and enzyme-linked immunosorbent assay (ELISA). Although ELISA is very convenient and sensitive but gives ambiguous results that cannot be confirmed by IHC. Recent research has shown that the aggregated specific reagent 1 (ASR1) peptoids, a group of short peptides developed based on the sequences of PrP19–30 (KKRPKPGG) and PrP100–111 (KPSKPKTNMK) can selectively bind to PrP^{Sc}. In this project, we evaluated ASR1 as an enrichment reagent to improve ELISA sensitivity and specificity. We tested retropharyngeal lymph node (RPLN) homogenates from CWD positive deer (ELISA+/IHC+, n=30), CWD suspect deer (ELISA+/IHC-, n=30), and CWD negative deer (ELISA-, n=30), as well as oral swabs from CWD positive deer (ELISA+/IHC+, n=16) and negative deer (ELISA-, n=30). Our results showed that 30/30 RPLN homogenates from positive deer were positive by ASR1-ELISA, 30/30 RPLN homogenates from suspect deer and 30/30 RPLN homogenates from negative deer were negative. Furthermore, 5/16 oral swabs from CWD positive deer were positive by ASR1-ELISA and 30/30 oral swabs from CWD negative deer were negative by ASR1-ELISA. In conclusion, ASR1 enrichment has no impact on the sensitivity of RPLN ELISA and oral swab ELISA but improves the specificity of RPLN ELISA. The suitability of ASR1 as a CWD prion enrichment reagent for other specimen types needs further evaluation.

Development of Rapid and Reliable Next-Generation Sequencing and Bioinformatics Procedures for Identification, Pathotype Prediction, and Subtyping of iInfluenza A Viruses and Optimization for Use with Clinical Samples

Kiril Dimitrov

Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, Texas

Project objectives:

I. to develop a rapid, sensitive and specific targeted nanopore NGS protocol for influenza A viruses;
II. to optimize the developed protocol for increased specificity and sensitivity using host depletion and RNA capture approaches;
III. to validate the targeted nanopore NGS protocol for influenza A viruses with clinical samples.

Deliverables completed:

- established panel of representative contemporary viruses and viruses similar to those posing a threat to the U.S. poultry industry
- complete genomes of previously uncharacterized or partially characterized IAV
- training of additional personnel in participating laboratories on NGS
  designed of universal barcoded primers for nanopore sequencing of IAV
- training of additional personnel in participating laboratories on primer design and nanopore MinION sequencing
- drafted working protocol with detailed recipes and methods
  established host nucleic acid depletion procedure for treating NA from influenza A viruses. This procedure will also have application in other NGS methods, including random sequencing
- validation data using clinical and experimental samples
- improved the IAV detection capabilities in the participating laboratories
- validation data using experimental samples
- improved the IAV detection capabilities in the participating laboratories
- validation data using FTA cards
- improved the IAV detection capabilities in the participating laboratories
- established SOP and bioinformatics pipeline/procedure optimized bioinformatics pipeline for use on a single personal computer
- validation data using clinical samples
- improved the IAV detection capabilities in the participating laboratories and NAHLN
- improved preparedness and capacity of NVSL and NAHLN laboratories to rapidly respond to an avian influenza outbreak

Deliverables yet to be completed:

- decrease of protocol duration for nanopore sequencing of IAV
- training of additional personnel in participating laboratories on primer design and nanopore MinION sequencing
- determination of optimal parameters and reagent concentrations
  developed simplified bioinformatics pipeline (single PC)
- training of additional personnel in using bioinformatics tools, MinION raw data analysis, and genome assembly
- established RNA capture procedure for treating NA from influenza A viruses. This procedure will also have application in other NGS methods, including random sequencing
- improved IAV detection capabilities in the participating laboratories
- established limit of detection of the developed protocols
- established sensitive protocol for targeted nanopore NGS of IAV
- established statistical measures of the performance of the selected protocol
- improved the IAV detection capabilities in the participating laboratories
- identification of sensitivity, specificity and predictive values of the developed protocol in comparison to the currently validated NAHLN real-time RT-PCR IAV M-gene assay.

How the project benefits NAHLN:

Successful completion of the proposed work and development of test method and bioinformatics procedures will facilitate a rapid response to potential avian influenza outbreaks with the added benefit of pathotyping and characterizing the virus/es causing the outbreak. Furthermore, once established these new diagnostics tools and procedures can easily be adapted for use with many other NAHLN scope viruses, which will enhance the preparedness in NAHLN laboratories and their states for responding to an adverse animal health event.

Validation of KingFisher Duo Prime for Low-Throughput Nucleic Acid Extraction Platform to Enhance Capacity for Rapid NAHLN Scope Disease Testing

Ailam Lim

Wisconsin Veterinary Diagnostic Laboratory, Madison, Wisconsin

Project objective:
Validation of KingFisher Duo Prime as a low-throughput nucleic acid extraction platform to provide NALHN laboratories a more time- and cost-efficient mechanism to extract low numbers of samples in the deep well format. In contrast to the currently approved 96-well system, this smaller format with fast preparation and loading of only a single plate will save plastics and labor to decrease strain on the supply chain and scarce NAHLN laboratories' resources. This low-throughput method will also improve testing turnaround time, which is critical for frequent FAD investigations such as those of the Senecavirus A virus (SVA) Pilot program or now known as the SVA/FMD Surveillance Plan.

Deliverables completed:
Analytical sensitivity for ASF, CSF, FMD, and SVA
Diagnostic sensitivity and specificity for SVA

Deliverables yet to be completed:
Analytical precision for ASF, CSF, FMD, and SVA

How the project benefits NAHLN:
Finding second vendors/platforms for NAHLN assays is a priority for this network. Currently, only the high throughput platform is approved for use in testing African swine fever virus, Classical swine fever virus, Foot-and-mouth disease virus and SVA. An alternative low throughput platform with fast preparation and loading of only a single plate will save resources in supply and labor, as well as improve testing turnaround time for FAD investigations.

Validation of a high-throughput method for the detection of African Swine Fever virus

Nagaraja Thirumalapura and Deepanker Tewari

Pennsylvania Veterinary Laboratory, Harrisburg, Pennsylvania

African Swine Fever (ASF) is a devastating viral disease of swine that threatens global pork production and food security. ASF is a notifiable disease to the World Organization for Animal Health (OIE). The disease has spread to several countries outside Africa including countries in Europe, the Russian Federation, China, Mongolia and Vietnam. African swine fever virus (ASFV) is a double-stranded DNA virus belonging to the *Asfarviridae* family. The virus can transmit through contact, ingestion or by tick belonging to the genus *Ornithodoros.* The virus in its wild suid hosts in Africa causes mild clinical disease and can lead to persist long-term infection. However, most strains of the ASFV cause severe hemorrhagic disease with high mortality approaching 100% in domestic pigs and wild boars. Currently, there are no effective vaccines or treatments available for ASF. Therefore, the disease control measures are limited to culling or quarantine of affected animals in farms.

Currently, a NAHLN approved real-time PCR is used in the United States for ASF surveillance in domestic pigs. The assay targets a gene encoding a highly conserved ASFV structural protein gene, p72 in the viral genome. In present form, the protocol allows extraction of viral DNA from blood and other specimens using either Qiagen DNeasy Blood and Tissue Mini Kit (low throughput) or MagMAX Pathogen RNA/DNA kit on magnetic particle processor (high throughput). The latter option allows extraction of up to 96 samples in a single run. However, subsequent analysis of the extracted DNA using PCR requires manual dispensing of master mix and extracted DNA to 96-well PCR plates potentially limiting number of samples that can be tested with a quick turnaround time.

The objective of the proposed research was to validate an automated liquid handling system for high-throughput detection of ASF virus using the NAHLN approved PCR primers and probe.

With the NAHLN funding support, the laboratory has acquired a KingFisher Apex System, an automated magnetic particle processor for high-throughput extraction of nucleic acid. The instrument is equipped with 2 UV lamps for decontamination and prevent any cross-contamination. The laboratory has upgraded the computer system from EasyCon to MultiCon for an existing epMotion 5070 workstation, a high-throughput liquid handling system for transfer of reagents and samples. The laboratory has 4 staff members who have passed the NVSL ASF proficiency test and one staff member is trained on using the epMotion 5070 workstation. The laboratory is currently working on validating the protocols for high-throughput detection of ASF using proficiency test samples, which would involve: (1) Extraction of viral DNA from samples using the MagMAX Pathogen RNA/DNA kit as described in the NVSL protocol (SOP-DS-

0071), (2) Transfer of PCR master mix into 96-well PCR plates using VIAFLO ASSIST pipetting robot and electronic multichannel pipette and (3) Transfer of extracted nucleic acid into 96-well PCR plates using the epMotion 5070 workstation.

The proposed work is expected to be completed by February 2023.

Providing Laboratory Information Management System (LIMS) Efficiencies for Foreign Animal Disease (FAD) Outbreaks Within Multiple USDA National Animal Health Laboratory Network (NAHLN) laboratories

Angela Pillatzki and John Greseth

South Dakota State University, Brookings, South Dakota

This project is a collaborative effort between NAHLN laboratories at the University of Illinois, Montana Department of Livestock and South Dakota State University working with the software provider Advanced Technology Corporation (ATC) to enhance the capabilities of LIMS to improve responses to foreign animal disease outbreaks and better serve stakeholder needs. The main objectives of the project and related summaries follow:

Target 1 – Electronic requisition screens on WebSuite for FAD
- Developed an electronic requisition platform within the VADDS system
- Mandated multiple fields to facilitate messaging (in progress)
- Developed auto-populate function for consistency and accuracy

Target 2 – Custom accession screen in SANDI for NAHLN submissions (FAD) including refinements
- Developed a custom accession screen to facilitate improved data entry

Target 3 – Improved functionality of the VADDS NAHLN dashboard
- Created separate areas for cases that need to be messaged and those that have already been messaged to facilitate identification of case status
- Updated messaging portal with upload status and download capability
- Cases are sortable by multiple parameters (e.g. disease, species, etc.)
- Simplified HL7 report to foster easier identification of messaging failures
- Implemented auto-refresh so results are more realtime

Target 4 – Result screen/electronic worksheet framework and screens in SANDI form NAHLN testing with documentation
- Built new resulting screens
- New work flow for creating and storing lab worksheets with the goal of becoming paper light
- Updated the instrument interfaces (Molecular Diagnostics and Serology) for improved data transfer to LIMS

Target 5 – Business intelligence and analytics enhancement
- Built new data queries
- Built new financial queries
- Built new tool for user to do custom queries
- New financial dashboard
- New results dashboard
- New case dashbaord

Enhancing Emergency Preparedness at Three NAHLN Laboratories Through Professional BSL-3 Training

Kristy Farmer

Thompson Bishop Spark's State Diagnostic Laboratory, Auburn, Alabama

Project Summary:

Laboratories having access to a BSL-3 lab is of high importance to NAHLN. It is given a weight of 7 (out of 10) in the annual Laboratory Assessment Matrix. BSL-3 laboratories have special engineering and design feature used to increase biosafety and biocontainment, and laboratories have standard operating procedures for work in their BSL-3 labs. But most employees have not been professionally trained to fully understand BSL-3 operations. This could lead to a gap in performing risk assessments and ultimately to a biosafety or biocontainment failure. The University of California, Irvine School of Medicine (UCI-SoM) BSL-3 training program with a focus on a core concept of risk assessment and management will travel on-site to conduct a week-long intensive training on BSL-3 laboratory use for 12 people. This will allow Thompson Bishop Sparks State Diagnostic Laboratory (TBSSDL, Auburn, AL), Mississippi Veterinary Research & Diagnostic Laboratory System (MVRDLS, Pearl, MS), and Bronson Animal Disease Diagnostic Laboratory (BADDL, Kissimmee, FL) to have 4 staff members certified to conduct effective risk assessments of their facilities and safety practices, significantly enhancing the emergency preparedness at these labs in the event of an FAD or high pathogenicity event and activation of the NAHLN network. Additionally, TBSSDL will purchase a high-capacity autoclave for the BSL-3 suite to enhance emergency preparedness by increasing biocontainment.

Deliverables completed:

The University of California, Irvine School of Medicine (UCI-SoM) BSL-3 training program performed a weeklong training at TBSSDL April 18-22, 2022 for 12 people from the 3 NAHLN member laboratories. Training focused on risk assessment, biocontainment, decontamination procedures, donning and doffing appropriate PPE, and emergency preparedness. The training was held in TBSSDL's classroom and in the lab's BSL-3 laboratory with practical demonstrations and exercises.

In progress:

During the training UCI-SoM trainers consulted with TBSSDL on the best features for a new, larger autoclave for the BSL-3 suite. The lab also consulted with the current autoclave vendor and is currently int eh prosses of procuring a PO to purchase a movable, top-loading autoclave.

An Automated Antibiotic Sensitivity Testing System for Electronic Data Management and Sharing the Data with NAHLN

Kristy Farmer

Thompson Bishop Spark's State Diagnostic Laboratory, Auburn, Alabama

Thompson Bishop Spark's State Diagnostic Laboratory (TBSSDL) in Alabama, a level 1 laboratory in the United States Department of Agriculture National Animal Health Laboratory Network (USDA NAHLN), aims to share its antibiotic sensitivity testing (AST) data with NAHLN's antimicrobial resistance (AMR) pilot project. A common AST platform that is used by the participant laboratories to obtain the data for this NAHLN project is a commercially available automated system, Sensititre™. However, until recently, we used to perform Kirby Bauer disk diffusion method for our AST procedure in the Bacteriology Laboratory at TBSSDL. Therefore, in this project, we requested a grant for a fully automated AST technology, Sensititre™ ARIS HiQ AST System with Sensititre™ SWIN™ Software, and associated supplies. To accomplish the goal of this project, the lab was awarded $198,900.00 through 2020 Farm Bill grant. A summary of project activities and its potential benefits to the NAHLN are presented below.

Within the first 2 quarters of the project, a Sensititre™ ARIS HiQ AST System with associated software and supplies were purchased from the Thermo Fisher Scientific (TFS) through Alabama Department of Finance.After receiving the equipment, four laboratory personnel were trained on the equipment by representatives from TFS. Subsequently, the equipment was tested through internal and external verification approaches. In an in-house verification, isolates from 25 clinical cases were tested side by side using the Sensititre™ ARIS HiQ AST System and Kirby-Bauer disk diffusion method. Eight isolates which were tested by the Sensititre™ at TBSSDL were also sent to the National Veterinary Services Laboratories (NVSL) for external verification. The equipment was successfully verified with approximately 98% agreement in both cases. After the verification, the laboratory has been using this Sensititre™ ARIS HiQ AST System for AST on bacterial isolates from clinical and necropsy specimens since October of 2021.

Addition of the Sensititre™ System to the Bacteriology Section has substantially improved AST in TBSSDL. In the past, with the Kirby Bauer disk diffusion method, results were limited by available interpretation criteria from Clinical and Laboratory Standards Institute (CLSI). Adding the minimal inhibitory concentration (MIC) method through Sensititre™ has increased the number of available antimicrobial drugs to be tested; additionally, the MIC method has allowed the lab to test for more patient-organism combinations. This automated platform has also enhanced our capacities to electronically manage AST data. The equipment has enhanced computer software to interpret susceptibility breakpoints. After reading, all results are saved, which can be used to analyze test results for atypical patterns and unusual resistance phenotypes.

Finally, the availability of the Sensititre™ System has let the TBSSDL to participate in the NAHLN AMR Pilot Project. Since January of 2022 the laboratory has been sharing its AST data with the NAHLN. We anticipate, the AST data from TBSSDL will be an excellent addition to the NAHLN's project.

Enhance Capacity for Faster Identification of Bacterial Foreign Animal Diseases at the Thompson Bishop Sparks State Diagnostic Laboratory

Kristy Farmer

Thompson Bishop Spark's State Diagnostic Laboratory, Auburn, Alabama

Thompson Bishop Sparks State Diagnostic Laboratory (TBSSDL) in Alabama is a level 1 laboratory in the National Animal Health Laboratory Network (NAHLN). The lab is committed to assisting in the Foreign Animal Disease (FAD) response strategies of the United States Department of Agriculture Animal Health and Plant Inspection Services (USDA APHIS) by providing rapid and accurate diagnostic and surveillance supports for animal diseases in Alabama. However, until recently, the Bacteriology laboratory at TBSSDL was mostly depended on a series of traditional biochemical methods to identify bacterial and fungal pathogens. With these conventional methods, 3-4 days and sometimes even longer time is required for full identification of a bacterial pathogen. Therefore, in this project, we requested grant for a Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) to enhance our capacity for rapid and accurate identification of bacterial and fungal animal diseases, including exotic and emerging diseases. To accomplish the goal of this project, the lab was awarded $227,025.00 through 2020 Farm Bill grant. A summary of project activities and its potential benefits to the NAHLN are presented below.

Within the first 3 quarters of the project, a MALDI Biotyper® Sirius GP System was purchased from the Bruker, Germany, through Alabama State Purchase Department. After receiving the equipment, 5 lab personnel were trained on the equipment by Bruker representatives. Subsequently, the equipment was tested through internal and external verification approaches. In the in-house verification, initially the equipment was tested to identify 21 ATCC bacterial organisms and 7 ATCC yeasts. Subsequently, 50 clinical cases were tested side by sides using the MALDI Biotyper® Sirius GP System, OmniLog ID System, and RapID system. As an external verification, six isolates identified with the MALDI Biotyper at TBSSDL were sent to the National Veterinary Services Laboratories (NVSL) for further verification. In all cases, the equipment was successfully verified with an approximately 98% agreements. After a successful verification, the lab has been using the MALDI Biotyper® as the primary tool for bacterial and yeast identification since September of 2021.

Addition of the MALDI Biotyper® Sirius GP System to the Bacteriology Section has allowed the TBSSDL to accelerate its bacterial identification process from 3 to 4 days to 1 to 2 days. Moreover, MALDI Biotyper® contains spectral data of about 7000 reference strains in the database. We have also included special libraries for animal bacterial pathogens of bioterrorism importance and *Mycobacteria* on our system. Thus, this technology would allow us to identify significant animal bacterial pathogens that could be missed with conventional biochemical procedures. As such, we anticipate that this rapid diagnostic platform will let the TBSSDL to play better roles in assisting the USDA APHIS in its FAD response strategies. Moreover, in the past, sometimes conventional methods could not provide enough information to identify an isolate conclusively. As such, we had to ship all questionable isolates to the National Veterinary Service Laboratory (NVSL) at Ames in Iowa for identification. Thus, with the availability of this platform,

we also anticipate that we would likely send fewer routine cultures to NVSL, which would shorten the turnaround time to our clients and reduce the testing burden of NVSL.

Overall, with the availability of the MALDI Biotyper® Sirius GP System, the TBSSDL is currently well-equipped for providing rapid and accurate diagnostic services for bacterial and fungal animal diseases in Alabama and to play better roles in the FAD response strategies of the USDA APHIS.

Arizona Veterinary Diagnostic Laboratory NAHLN Enhancement (Priority 2) 2020 Cooperative Agreement Project Proposal, Farm Bill Section 12101

Sara Plevel

Arizona Veterinary Diagnostic Laboratory, Tucson, Arizona

The Arizona Veterinary Diagnostic Laboratory (AzVDL) purchased a Kingfisher Plus and a QuantStudio 5 to increase capacity. The new Kingfisher was very helpful as the AzVDL took on significantly more HPAI surveillance than we had done in the past.

A Kingfisher unit was purchased. The unit had a slightly larger footprint than the counters in the area it was to be utilized and a C Leg table was purchased as well to house it. New pipettes were ordered as well as a Smart Check unit from Rainin be able to keep track of in-house calibration checks of pipettes to maintain ideal operation.

Biosafety was upgraded with new PAPR units and a portable shower out that can be utilized to improve workflow capacity during outbreak situations.

New computers and monitors were purchased to upgrade QA, IT and molecular workstations.

Enhancing Swine Diagnostics for the Detection and Differentiation of ASF and CSF from Other Economically Significant Septicemic Diseases: Development of Novel Multiplex High Throughput Taqman qPCR Panels and Point-of-Care Isothermal Diagnostic Platforms

Hemant Naikare

Tifton Veterinary Diagnostic & Investigational Laboratory, University of Georgia, Tifton, Georgia

PROJECT OBJECTIVES:

1) develop multiplex Taqman qPCR panels for the detection and differentiation of African Swine Fever (ASF) and Classical Swine Fever (CSF) from other high consequential economically significant septicemic diseases affecting swine

2) develop Isothermal Helicase Dependent Amplification (HDA) assays for potential point-of-care testing for ASF and CSF pathogens in resource limited settings.

The current NAHLN standard operating procedures approved for use at the NAHLN laboratories for PCR based detection of ASF and CSF are uniplex (single target) assays. Till date, simultaneous detection of ASF and CSF with internal extraction control in the same reaction is not available for the NAHLN laboratories. Development of high-throughput multiplex Taqman PCR panels for these swine pathogens will enhance the testing capacity as 2X more samples could be tested. This would result in a reduction in the turn-around-time of disease detection by screening multiple septicemia causing pathogens in the same PCR reaction. This will also result in cost savings due to reduction in consumption of reagents and consumables compared to singleplex testing. By in-house validation of proposed novel assays using core reagents from atleast two different reputable manufacturers on multiple PCR platforms, we will be able to offer multiple options of validated testing methods and reduce the burden on the supply chain of reagents and consumables for conducting the tests. Scarcity of reagents has been a huge bottleneck for molecular testing and is evident in the current COVID-19 pandemic crisis.

We propose to enhance ASF and CSF diagnoses in suspect cases and also rule in/ rule out other septicemic disease conditions affecting swine operations such as Porcine Reproductive and Respiratory Syndrome (PRRS), Erysipelas, Salmonellosis, Aujesky's disease (Pseudorabies), Pasteurellosis, Streptococcosis and Listeriosis. This project aims to result in the following deliverables: 1) development of a core multiplex Taqman qPCR panel (Panel I: ASF, CSF, PRRS, Internal control); 2) development of two additional sets of swine septicemic panel: Panel II-Erysipelas, Salmonellosis, Pasteurellosis, Internal control; 3) Panel III- Aujesky's disease (PRV), Streptococcosis, Listeriosis, Internal control). Development of these three panels will offer diagnostic solutions for multiple septicemic swine diseases.

We also propose to develop a novel isothermal HDA assay for ASF and CSF (singleplex and multiplex) with a potential to serve as a point-of-care testing option. Isothermal amplification utilizes the action of a thermostable helicase enzyme, rather than heat to separate the nucleic acids and it will allow DNA replication to occur at a single uniform temperature

(~64°C) with a simple heat block that could be deployable in resource limited settings without the need for specialized equipment or complex software for data interpretation. We anticipate to deliver a proof of concept for HDA assay and subsequently incorporate the process in systems such as “Amplivue” or “Solana” from Quidel Corporation that has commercially developed these assays using HDA or use lateral flow assay strips. Overall, these novel molecular diagnostic tools for ASF, CSF and other septicemic diseases will address a crucial unmet need of the swine industry in the US and worldwide.

HOW THE PROJECT BENEFITS NAHLN:

Improved capacity and capability to conduct high throughput testing at the NAHLN laboratories for multiple swine septicemic diseases and development of point of care testing options that could be useful in resource limited setting to enable prompt and rapid NAHLN emergency response in a disease outbreak situation.

Establishing Messaging Through Newly Acquired Orchard Sequoia LIMS

Craig Bowen

Indiana Animal Disease Diagnostic Laboratory, West Lafayette, Indiana

Project objective:
The ADDL has been using Orchard Sequoia LIMS for COVID-19 PCR testing for humans, and had intended to expand its use to all of our animal testing. It has been determined that this product will not meet the needs for our animal diagnostics. We received NAHLN approval to apply this grant funding to support training of IT staff in support of improved NAHLN messaging.

Deliverables completed / Deliverables yet to be completed:

- IT staff have been assigned to each training course.
- All identified individuals are registered and currently taking the HL7 course
- All identified individuals are registered for the SNOMED Foundation course
- One of 4 identified individuals has completed both the SNOMED Foundation and Developer course
- One individual has registered for the SNOMED Implementation course
- Additional HL7 training is being explored to further increase the knowledge of our IT staff.

How the project benefits NAHLN:
While the intended benefit of the initial project was to build the interface through the Orchard Sequoia LIMS for messaging results to USDA, it became apparent that this software product was not going to meet the demands of the ADDL. With approval by the NAHLN, we have switched gears and are pursing internal improvements to our homegrown LIMS. With the Farm Bill funding, we are providing training in HL7, SNOMED, and other messaging technologies to multiple individuals in our information technology department. The benefits have already started to show as a new system on the ADDL side has been built and utilized to message results during the HPAI event. As messaging proficiency tests come along, our IT staff is modifying this new system to assess and switch all of our messaging to NAHLN through this new system. Additional benefits include a more resilient messaging system, added ability to resend messages, and sending messages in real-time rather than once a day. An indirect benefit (but also very important) is that the ADDL now has more than one person knowledgeable with the messaging process to maintain continuity of business when IT staff are sick or leave. The ADDL looks forward to utilizing the increased knowledge of our IT staff with improvements and expansion of our messaging capabilities.

Enhancing Diagnostic and Surveillance Capabilities for Early Detection of Emerging and Foreign Animal Diseases Using High Throughput Sequencing

Sunil Mor

University of Minnesota Veterinary Diagnostic Laboratory, St. Paul, Minnesota

Project objective:
The objective of proposed project is implementation of automated library preparation and sequencing tools for developing rapid, low-cost and highly scalable assays for early detection of emerging and FAD viruses.

Deliverables completed:
The equipment for library automation and Oxford Nanopore GridION for sequencing have been installed at MVDL for same day high throughput sequencing. The protocols are being optimized for regular use.

How the project benefits NAHLN:
This project enhances the NGS capacity of MVDL. The protocols developed for the use of this equipment will benefit NAHLN laboratories.

Increasing NAHLN ASF Response Capacity in Minnesota

Albert Rovira

University of Minnesota Veterinary Diagnostic Laboratory, St. Paul, Minnesota

African swine fever (ASF) is one of the most important threats to US agriculture. As described in the USDA ASF Response Plan (The Red Book), an introduction of ASF in the US would result in a comprehensive surveillance plan to identify additional infected premises, establish control zones, authorize animal movement and determine freedom from disease. While the details of such plan have not been decided and would likely vary depending on the characteristics of the outbreak, an ASF outbreak in a state with high swine farm density like Minnesota is projected to result in the need to test thousands of samples during a period of several months. While the Minnesota veterinary diagnostic laboratory (VDL) has the capability of testing for ASF by PCR, the process is not very efficient, and the capacity to test large numbers of samples for a prolonged period of time would be very limited. Therefore, there is an urgent need to improve the testing capacity for ASF in the state of Minnesota.

After evaluating the whole ASF PCR process at the VDL, we have identified two main areas of improvement that are described in this proposal: template addition and amplification steps. To improve the efficiency of the template addition step we propose to purchase a liquid handler that can combine the samples from four 96-well extraction plates into one 384-well PCR plate. To improve the efficiency of the amplification step, we propose to purchase a thermal cycler that can process 384 samples at a time. The combined improvements in these two areas will result in a 2.7x increase in the efficiency of the ASF PCR process. Currently, the VDL is able to test 279 samples (3 batches of 93 samples) in a 8-hour shift, without markedly affecting the regular testing volumes for diagnosis and surveillance of other diseases. After implementation of the changes described in this proposal, the VDL will be able to complete 756 samples (2 batches of 378 samples) in a 8-hour shift. This proposal aligns with priority 2 of the NAHLN 2020 Farm Bill request for proposals: enhancing emergency preparedness.

The overall goal of this project is to improve the capacity of the Minnesota VDL to respond to an ASF outbreak by improving the efficiency of the ASF PCR test. Two areas of improvement have been identified:

Objective 1: To increase the efficiency of the template addition step of the ASF PCR test

Objective 2: To increase the efficiency of the amplification step of the ASF PCR test

Potential Problems and Anticipated Solutions

- Adapting current protocols to new equipment can sometimes result in unexpected problems that can usually be solved by working with the equipment manufacturer. This is why one of the criteria when selecting a new piece of equipment is the availability and quality of the technical service.
- Access to positive and negative field samples for the detection of ASF can be challenging. A small set of samples will be requested from USDA/NAHLN. In addition, PRRSV positive and negative samples will be used as a surrogate to evaluate the new equipment with large batches of samples.

NAHLN Messaging Audit Check

Jim Trybus

North Carolina Department of Agriculture and Consumer Services Veterinary Division, Raleigh, NC

Project objective:
NAHLN HL7 messaging provides laboratories an electronic, automated, and standard method for transmitting test results, animal, and farm information to the USDA to aid in animal disease response. USALIMS is a laboratory information management system used in a number of state diagnostic laboratories (17 during the time of this project) that is utilized to generate accessions, reports, and HL7 messages for NAHLN tests. USALIMS provides an HL7 message generator that allows laboratories to automatically generate and send HL7 messages from their LIMS to the USDA. There is no interaction or verification from the end users prior to message being sent. Also, laboratories do not receive warnings that their data may be incomplete during data entry, and currently have no mechanism for proactively verifying that the HL7 message information is complete and will transmit successfully to the USDA.

This project's objective was to change the end user process, so that they can perform data verification prior to results being sent to the USDA. This allows labs to easily verify that HL7 required fields have been filled in and ensure more complete data is being sent to the USDA. This process would be added to USALIMS software so that each lab can perform this message data check more efficiently and reduce the number of errors going into the messaging pipeline.

Deliverables completed / Deliverables yet to be completed:
Creation of an Audit Check Process (computer programming code graphical user interface) for USALIMS

How the project benefits NAHLN:
HL7 messaging provides an electronic, automated, standard method for transmitting test results, animal, and farm information to the USDA to aid in animal disease response. 17 state diagnostic laboratories utilize USALIMS for disease reporting. This project enhances the efficiency of USALIMS users, through a software enhancement, to verify messaging data is complete prior to transmitting to NAHLN and the USDA. The increased efficiency and reduced errors in the messaging process will get the disease testing data to the USDA and NAHLN in a timely manner and reduce the need for trouble shooting of faulty messages of all parties.

Enhancing BSL-3 Capabilities of Ohio Animal Disease Diagnostic Laboratory

Dominika Jurkovic

Ohio Department of Agriculture, Animal Disease Diagnostic Laboratory, Reynoldsburg, OH

Project Objectives:

1. Enhance storage sample capacity for FAD sample handing in the Ohio ADDL BSL-3 Facility.
2. Increase FAD testing capacity in the Ohio ADDL BSL-3 Facility.
3. Increase number of BSL-3 analysts with appropriate training and experience.

Completed Deliverables:

Objective 1: Two laboratory refrigerators with keyed door locks were purchased and installed in 2021. One refrigerator is dedicated for sample receiving and the other refrigerator is for the storage of samples post testing. The new units also increase redundancy if a refrigerator in the BSL-3 laboratory is placed out of service for maintenance or repair.
Objective 2: Two QuantStudio 5 PCR platforms and two King Fisher Flex Nucleic Acid Purification Systems were purchased and installed in 2021. Both are being utilized during the current HPAI outbreak.
Objective 3: Personnel completed BSL-3 Laboratory Training at UCI National BSL-3 Training Laboratory in 2022.

Pending Deliverables:

Objective 3: Training of additional BSL-3 staff is pending. This training will be completed after new PAPRs are received to be part of an updated in-house training program and refresher courses.

Benefits to NALHN:

This project increases the testing capacity of the Ohio ADDL which aligns with NAHLN's priority to ensure effective emergency preparedness and capacity to response to foreign animal disease outbreaks by increasing sample storage capacity, testing capabilities, and the number of trained BSL-3 testing staff.

Sample Processing Efficiency, Accuracy and Integration with CoreOne LIMs

Kurt Williams, Mark Ackermann, Donna Mulrooney, Susie Strangfield, Susan Tornquist

Oregon Veterinary Diagnostic Laboratory, Carlson College of Veterinary Medicine, Oregon State University, Corvallis, Oregon.

Project objectives:
Streamline workflow to increase throughput and capacity, adding to emergency preparedness capabilities. This will be accomplished by incorporating barcode scanning into several specimen handling processes throughout the laboratory during implementation of a new LIM system.

Deliverables completed:
The OVDL has upgraded existing computer workstations, replacing outdated hardware with new, more powerful computers and newly integrated devices including handheld barcode scanners, desktop scanners, label printers, iPads, and integrated lab equipment middleware to process accessions without reducing throughput as was happening with our outdated hardware. In addition to increasing throughput, the new computer hardware allowed our IT Team to upgrade security measures across the OVDL to provide increased protection against unforeseen cybersecurity events.

How the project benefits the NAHLN:
A direct benefit is provided to the NAHLN by significantly increasing OVDL laboratory capacity preparedness and the ability to provide surge testing during a disease outbreak. The OVDL has provided surge testing during multiple avian influenza outbreaks and high throughput testing of animal and human samples during the COVID-19 pandemic. Further streamlining sample handling processes allows a continued increase in capacity and high throughput testing, positioning the OVDL as an immediate resource within the NAHLN network, enhancing the network's capabilities for providing a rapid response during disease outbreak events in the Pacific Northwest and nationally.

To Increase Real-Time PCR Testing Capacity of Foreign Animal Diseases at the Clemson Veterinary Diagnostic Center

Radhika Kakani

Clemson Veterinary Diagnostic Center, Columbia, SC

Objectives: To increase testing capacity with

a. Installation of a biosafety cabinet at the sample receiving area will shorten the sample processing time by eliminating the need to use a biosafety cabinet in another section of the laboratory.
b. Purchase of QuantStudio 5 real-time PCR system to increase laboratory testing capacities by nearly 100 samples per 8-hour shift.

Deliverables completed:

a. A biosafety cabinet was purchased and installed in the sample receiving area in June 2021. Lab staff are using it to process samples in the receiving section.
b. Real-time PCR system, QuantStudio 5, was purchased and put into service in June 2021. Lab technicians participated in the inter-lab comparison of ASF proficiency testing in 2021. Validation of the QS 5 was completed to run ASF and CSF tests. Additionally, method verification has been completed to detect other animal pathogens.
c. A -20^{o}C freezer was purchased and put into service in October 2021. We are using it to store critical reagents, and supplies required for PCR testing (not in the original proposal. Obtained a deviation when we are left with more funds after receiving trade-in discounts for QS 5).

How the project benefits NAHLN:

The Clemson Veterinary Diagnostic Center (CVDC) is approved currently to test for high-consequence pathogens such as Avian Influenza, Avian Paramyxovirus, Swine Influenza, African Swine Fever Virus, Foot and Mouth Disease Virus, and Classical Swine Fever Virus. The addition of a biosafety cabinet in our sample receiving area proved to shorten sample processing time, resulting in a quick turnaround of results. This valuable addition not only reduced the sample processing time but also protects employees from potential pathogen exposure.

The QuantStudio 5 real-time PCR system was validated by NAHLN for the detection of Classical Swine Fever and Foot and Mouth Disease. It is anticipated that this real-time PCR system will be validated for the detection of other NAHLN-scope diseases in near future. The real-time PCR system enhanced the CVDC emergency preparedness by increasing testing capacity for the purpose of improving surveillance, and detection of foreign animal and emerging pathogens, which directly enhances the CVDC response capacity and in turn NAHLN capacity to address specific NAHLN scope disease testing. Further, the PCR system also enabled the lab to detect multiple pathogens simultaneously.

Enhancing Electronic Reporting and Data Transmission

Tanya LeRoith

VA Tech Animal Laboratory Services, Blacksburg, VA

Project Objective:
To integrate molecular diagnostic equipment into the LIMS, develop a user-friendly client webstation for sample submission and result delivery, and to update the LIMS for NAHLN reporting and messaging requirements.

Deliverables completed:
Molecular diagnostics equipment is fully integrated into the LIMS which allows for faster result reporting and eliminates errors in manual reporting. LIMS update is complete.

Deliverables to be completed:
Webstation development is still on-going by the vendor. NAHLN messaging is under development.

How the project benefits NAHLN:
ViTALS uses Orchard Harvest Software as its laboratory information management system. The vendor primarily supports public health and human diagnostic laboratories, with complete laboratory system integration and electronic lab reported to Public Health Information Network Messaging Systems and the CDC, USAMRIID, or NMRC. Orchard's advanced decision-support rules and laboratory analytics were attractive to veterinary diagnostic laboratories, and the configuration abilities are extremely useful for customizing individual laboratory needs.

This project meets the needs of the NAHLN by establishes rules and systems for other NAHLN labs that may invest in Orchard Software.

Enhancing Chronic Wasting Disease Testing Capability

Tanya LeRoith

VA Tech Animal Laboratory Services, Blacksburg, VA

Project Objective:
To increase CWD testing capacity in Virginia by standing up CWD ELISA and IHC testing

Deliverables completed:
Set-up of ELISA testing has been completed to the point of successfully passing the NAHLN proficiency test and being approved for CWD testing. Immunohistochemistry processes have been completed.

Deliverables to be completed:
IHC troubleshooting of methods to be able to successfully pass the proficiency test. Configuration of the LIMS for reporting to the Virginia Department of Wildlife Resources (VDWR).

How the project benefits NAHLN:
No testing capacity for CWD was available in either of Virginia's NAHLN laboratories. The Virginia Department of Wildlife Resources (VDWR) has a testing requirement of approximately 3500 samples during hunting season (October through January), and approximately 2500 samples in early February. Rapid turnaround time is imperative and can be delayed due to staffing shortages or other priorities in other laboratories. Increasing testing capacity in Virginia allows for a shorter turnaround time especially when timely results are critical and removes testing pressure from other laboratories.

Moorefield Animal Health Diagnostic Laboratory-Enhancement of NAHLN Emergency Preparedness

Erika D. Alt

Moorefield Animal Health Diagnostic Laboratory, Moorefield, WV

Project Objectives:
The objectives of the project included 1) Maintain disease surveillance testing capacity for high consequence, NAHLN-scope animal diseases. 2) Enhance outbreak testing capacity for high consequence, NAHLN-scope animal diseases. 3) Improve emergency preparedness and response capabilities in the event of an adverse animal health event or emerging infectious disease through the implementation of new equipment and technologies.

Deliverables Completed:
KingFisher Flex was purchased and incorporated into the laboratory in October 2021.

How the project benefits NAHLN:
This project aligns with NAHLN's mission by directly supporting animal health diagnostic testing for early detection, rapid response, and appropriate recovery from high-consequence animal diseases. In addition, the purchased equipment strengthens our laboratory's response capacity and capability to an animal disease outbreak by preparing our laboratory to manage a sudden influx of samples during an animal disease event.