NAHLN Farm Bill Showcase

Abstracts from 2019 NAHLN Farm Bill Funded Projects

USDA

**United States
Department of Agriculture**

NAHLN Farm Bill Showcase
Presentations from 2019 NAHLN Farm Bill Funded Projects

Wednesday, November 17, 2021
12:30pm – 3:00pm CT

Moderators: Beth Harris and Christie Loiacono

NAHLN Farm Bill Showcase
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Thursday, November 18, 2021
8:30am – 11:00am CT

Moderators: Beth Harris and Christie Loiacono

NAHLN Farm Bill Showcase
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The following projects were presented at the AAVLD/USAHA Joint Committee on NAHLN on October 12, 2021

2019 NAHLN Farm Bill - Capacity Support Projects

Enhancing Biological Safety Level-3 Laboratory Capacity for Early Detection and Diagnosis of Foreign Animal Diseases

Shuping Zhang

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Missouri ranks among the nation's leading states for animal agriculture, particularly in the production of beef cows, hogs and pigs, and turkeys. A 2016 study indicated that the aggregated animal production and processing industries supported 154,268 jobs and paid $6.212 billion in labor income. The animal agriculture industry annually pays approximately $698.7 million in state and local taxes, and $1.4 billion in federal taxes. An outbreak of a foreign animal disease (FAD) could potentially threaten Missouri's economic vigor and Missourians' health and wellbeing. Given the geographic location, an outbreak of a foreign animal disease in Missouri may also affect other Midwest states that are important to the United States' economy and world exports of grain and meat. The University of Missouri (MU) Veterinary Medical Diagnostic Laboratory (VMDL) is a NAHLN Level 1 laboratory and Missouri's only veterinary laboratory accredited by the American Association of Laboratory Diagnosticians (AAVLD). The VMDL serves practicing veterinarians and farmers in Missouri and the Midwest region.

The specific objective of this project is to build MU VMDL Biosafety Level 3 (BSL-3) laboratory testing capacity for the detection of NAHLN scope diseases. The approach and plan of action are as follows: 1) adding new equipment in BSL-3 laboratory, 2) conducting regular exercises in BSL-3 laboratory, and 3) increasing the number of VMDL personnel with full access to BSL-3 laboratory.

Completion of this project has increased the daily testing capacity in BSL-3 laboratory from approximately 500 to 1500 samples, enhanced VMDL's readiness to respond to potential foreign animal disease outbreaks and promoted the recognition of NAHLN by the University of Missouri, Missouri State Legislature, and the public.

Novel Approach to Diagnose and Genetically Characterize High Consequence Pathogens Affecting Animal Health

Christie Mayo[1], Kirsten Reed[3], Izabela Ragan[2], Case Rodgers[3], Natasha Hodges[1], Ted Anderson[1], Ty Sherman[1], Raye Walck[4], Kristy Pabilonia[1], Mark Stenglein[3]

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Diagnostic testing plays a critical role in identifying and addressing infectious diseases that threaten animal and human populations. While there have been some advancements in diagnostic test development, there are substantial impediments in the application or integration of novel technologies in veterinary diagnostic labs. Integration of these platforms could improve testing efficiency, reduce the associated costs of diagnostics, and ultimately informatively guide researchers and veterinarians in the effective management of symptomatic animals (e.g.: bovine respiratory disease). Additionally, symptomatic animals can often be infected with multiple pathogens or multiple strains of the same pathogen and it is often too expensive and time consuming to discriminate the etiologic agents of disease. These diagnostic limitations impact deployment of informed control and mitigation strategies by delaying: 1. adequate and time efficient tracing; 2. epidemiology and transmission risk analysis; 3. evolutionary characterization of the pathogen; and 4. recognition of emerging variants. Our work utilizes a combination of approaches to characterize the genetic composition of viral pathogens (whole genome sequencing) that guides the development of sensitive and targeted multiplex bead-based assays. We have successfully used avian influenza sequencing data to design probes for a ThermoFisher QuantiGene assay on the MagPix platform. The success of this assay in discriminating avian influenza viral strains motivated us to further explore development of a multiplex bead-based assay for diagnosing bovine respiratory disease. This work emphasizes the importance of combining diagnostic platforms (e.g., next generation sequencing with bead-based assays) to generate more robust and cost-effective workflows for deeper interrogation of pathogens affecting animal health. Continued collaborative research and exploration of new technologies, including translational applications from other disciplines, is crucial to the accurate and efficient diagnosis of disease, the reduction of risk/losses to agricultural stakeholders, and the advancement of veterinary diagnostics.

Scalable Field PCR Platform for Seneca virus 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

Seneca virus A (SVA) and Food and Mouth Disease Virus (FMDV) continue to pose challenges for swine producers. Although FMDV has been eradicated from the United States since 1929, the disease it causes is clinically indistinguishable from SVA. The increasing prevalence of SVA has resulted in additional foreign animal disease (FAD) investigations, quarantines, and supply chain delays. To address those challenges, we began development of a field-based adaptive PCR platform utilizing L-DNA. To that end, we have designed primers and probes for SVA, FMDV, and an RNAse P control. Using a Ct of 40, these sequences, in conjunction with commercially available reaction mixture components, correctly identified SVA in each of 44 retained field samples. Those same samples had been previously tested by the NAHLN-approved methodology by Tennessee's state lab. Importantly, the adaptive PCR approach required no extraction step and only lagged by an average of 6.3 cycles. By eliminating that labor/equipment intensive step, a critical obstacle to developing a field-based design was cleared. Instrument design is ongoing to produce a lower cost field instrument, and field-testing is expected to begin within the next year.

Interconnected Electronic Reporting and Transmission of Data

Russ Summers

Arkansas Department of Agriculture, Shared Laboratory Services, Veterinary Diagnostic Laboratory, Little Rock, Arkansas

The Arkansas VDL has traditionally operated with shared access to institutional IT support, and recent changes have diverted this support towards state-wide mandates. Replacement of a LIMS without dedicated in-house support is unlikely to deliver sough-after improvements. Therefore, we have adopted a model which calls for increased partnership with the current vendor of our LIMS. Sporadic interruptions in electrical and/or internet service have also diminished the reliability of meeting stakeholder expectations. The funding opportunity announcement identifies NAHLN priorities in IT as (i) upgrades to LIMS currently in use, (ii) electronic messaging, (iii) pre-accessioning, and (iv) barcoding. We have already accomplished objective (iv) in recent months. In partnership with the vendor of our LIMS, we have custom tailored our scope of work and our deliverables to fulfill objectives (i) through (iii).

Deliverables completed include (i) a web-based FAD accessioning screen, (ii) Numerous custom screens, (iii) NAHLN resulting screens, and (iv) accessioning and resulting screens for aquaculture, an entirely new service area. Deliverables in progress include electronic requisitioning (pre-accessioning) screens.

An unexpected deliverable arising during this project is a multi-state collaboration among Arkansas, Montana, Illinois, and South Dakota to develop upgrades to their VADDS LIMS.

Benefits to NAHLN include a more robust messaging capability, enhanced FAD responsiveness, and a more resilient approach to LIMS in general through cloud hosting and mobile computing.

Laboratory Detection Preparedness for Emerging Diseases in Aquatic Species

Eckstrand C, Torrevillas B, Wolking R, Bradway D, Williams L, Pessier A, Baszler T, Snekvik K

Washington Animal Disease Diagnostic Laboratory, Washington State University,
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Project Objectives: The aquaculture industry has grown tremendously to meet globally increasing seafood production demands. With the continuing rise of high-density production systems in ecologically or geographically novel conditions, in combination with the associated farming practice-related stressors that may prevent adequate disease control in farmed fish populations, emergence of viral fish diseases is inevitable. Well characterized approaches and methods for detection and surveillance of emerging infectious diseases in aquaculture are vital for the implementation of rapid detection, response, and recovery measures to protect vital economic interests and food security. We propose a methodology for detection of aquatic viral pathogens in fish in order to increase capabilities, capacity, and readiness for NAHLN to respond to emerging aquatic infectious diseases.

Objective 1:
Validation of integrated step-wise methodology for diagnosis of aquatic disease using known viral pathogen (sequenced characterized infectious salmon anemia virus isolate)

Objective 2:
Validation of integrated step-wise methodology for diagnosis of emerging aquatic disease using unknown viral pathogens

Deliverables completed: Objective 1 has been completed except for the final summary report, which is currently being prepared in manuscript form. At this time, we have demonstrated laboratory competency in all of the integrated methodologies proposed for characterization of a known pathogen (ISAV for objective 1). These include documented competencies in histopathology descriptions of ISAV lesions, virus isolation/culture, visualization of virions by electron microscopy from virus culture, detection, and de novo assembly of the ISAV genome using WGS technologies (GridIon and MiSeq), and in-situ hybridization. In objective 2 we set out to demonstrate competency in using the methodology outlined in objective 1 on unknown pathogens in fish. We have completed over half of outlined milestones in that we have detected the presence of two uncharacterized viruses (a novel orthoreovirus and an astrovirus).

Deliverables yet to be completed: The remaining deliverables to be completed include demonstrating the ability (or not) to culture the first unknown pathogen (orthoreovirus) and performing electron microscopy to further characterize the structure; and describing the histopathology, electron microscopy and in situ hybridization for the second unknown pathogen (astrovirus). A full summary report of these two discoveries is also pending/in progress.

How the project benefits NAHLN: This project directly benefits NAHLN in that it has significantly increased laboratory capacity preparedness to detect and characterize novel pathogens in aquatic species. WADDL is uniquely suited for the detection of aquatic pathogens as it has a well-established aquatic diagnostics section interfacing with the large fisheries of the Pacific Northwest. This project has allowed for the acquisition of new technologies and personnel, competency training for those new modalities, developed a pipeline for pathogen discovery at WADDL, and allowed for demonstrating competency for detection of known and unknown pathogens. Most importantly we've demonstrated the utility of a diagnostic discovery pathway that can be shared with other NAHLN laboratories to enhance the network's capabilities.

Development and Validation of a Universal Real-Time RT-PCR Assay to Distinguish Between Virulent APMV-1 and APMV-1 of Low-Virulence and Development of Primers-Probes Bank for Rapid Distribution to Field Laboratories in Response to a Virulent APMV-1 Outbreak in the United States

Kiril Dimitrov, David Suarez, Pam Ferro, Martin Ficken, Gabriel Senties-Cue, Megan Schroeder, Alesia Reinisch

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

Newcastle disease (ND), caused by virulent strains of Avian paramyxovirus 1 (APMV-1), is a severe and often fatal infection in naïve chickens and is a threat to the poultry industry worldwide. Occurrence of virulent APMV-1 in commercial poultry results in trade restrictions. ND is considered a foreign animal disease for the U.S., however, outbreaks in poultry occasionally occur, including a recent outbreak in California. The disease has significant economic and social impact. Rapid and specific detection of virulent APMV-1 and the differentiation from non-virulent viruses in suspected flocks is of critical importance for effective control to limit losses and costs involved with outbreak containment.

The current NAHLN APMV-1 test for detecting virulent strains was developed in response to the 2002-2003 California ND outbreak and has highest sensitivity with genotype V viruses. However, APMV-1 are genetically diverse, and are constantly evolving. New variants are circulating in Asia, the Middle East, Africa, Europe, and Central and South America and pose a constant threat to the U.S. poultry industry. The continuous evolution of these viruses presents diagnostic challenges as target-oriented assays might fail to detect emerging variants. Testing APMV-1 isolates from different genotypes with the currently validated test has resulted in several published and unpublished reports of either lower sensitivity or false negative results.

In this project, we created a databank of all publicly available class I and class II APMV-1 sequences. We sequenced 15 previously uncharacterized APMV-1 isolates. These data are utilized to perform a comprehensive in silico analyses of the fusion gene and identify common sequence motifs for each APMV-1 sub/genotype. Results from these analyses are being used to develop an updated real-time RT-PCR assay, targeted around the fusion cleavage site, for sensitive and specific detection of virulent APMV-1 (including the ones affecting pigeons and cormorants). These new assays will be evaluated at the bench for specificity and sensitivity using viruses of all genotypes currently circulating worldwide. A wide spectrum of clinical samples will be used to validate the new assay in five different laboratories.

For high quality control of possible inhibition and extraction failure, we utilized an exogenous internal positive control. An up-to-date sequence databank for NVSL and NAHLN use was created. To facilitate a rapid response in case of an outbreak of ND in the U.S. caused by an emerging strain, a primers/probes bank will be developed (for the NDV targets and XIPC target). The new primers and probes will be aliquoted into working stocks and stored with sufficient volume to test at least 25,000 clinical samples. This relatively inexpensive diagnostic banking program will save valuable time in responding to an outbreak by providing ready-to-use reagents. The outcome of this project will enhance the preparedness of NAHLN laboratories in identifying virulent APMV-1 and responding to the NAHLN-scope emerging Newcastle disease.

Building Capacity to Rapidly Detect Agents of Emerging Infectious Disease

Hon Ip

USGS National Wildlife Health Center, Madison Wisconsin

This project was envisioned as a pilot project to develop experience with two emerging bench top next generation sequencing (NGS) platforms, MinION and Ilumina iSeq 200, with the aim of contributing to the creation of robust procedures for their use. Other goals include identification of best practices for using these platforms to identify viral and bacterial pathogens, to develop bioinformatic analysis tools, and to create training packages for interested laboratories. Initiation of this project experienced delays due to institutional bureaucracy and other disruptions caused by the coronavirus pandemic. Currently, we have the MinION installed and running as either a dongle attached to a laptop computer or standalone as the MK1C instrument. We have demonstrated the ability to characterize the complete genome of SARS-C0V-2 using multiple iterations of the ARTIC procedures and analysis of NGS data is done using bioinformatic pipelines written for a commercial software package. Efforts to recruit the postdoctoral fellow who had been envisaged to implement the laboratory and software development components of this project are presently underway.

Cornell AHDC/NYSVDL Emergency Preparedness Capacity

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College of Veterinary Medicine, Cornell University

Objectives of the project were enhanced flexibility and readiness for detection and differentiation of emerging disease and for response to a high impact animal disease outbreak that would require high volume testing (and receiving).

The grant provided an Illumina iSeq for expansion of sequencing capacity, and a QuantStudio 5 thermocycler to start evaluation and integration for this platform as NAHLN was approving new protocols (currently in use for NAHLN testing under an approved "Request to Deviate from a NAHLN Document").

The AHDC, furthermore, using laboratory fee revenues, proceeded with remodeling two spaces, with Farm Bill funds supplementing equipment: (1) Remodeling of the virology laboratory BSL-2/BSL-3 Flex space into full BSL-3 laboratory space, in order to move NAHLN operations from two previously reserved and AHDC controlled BSL-3 rooms in the College of Veterinary Medicine AHDC based BSL-3 Research 8-room suite, alleviating college research concerns that research operations would be affected by high volume FAD outbreak testing. Remodeling was completed May 2021. The Farm Bill grant contributed a NuAire Reverse Flow Hoods 4 for specimen receiving in the ante room. The KingFisher Flex obtained through the contract was not yet deployed in the remodeled space as it was deployed for community SARS-CoV-2 testing. (2) Combination and remodeling of receiving and adjacent laboratory space to dedicate biosecure receiving space for outbreak response. A first phase, creating access from receiving to the former laboratory space has been completed, with a second, full remodeling phase, planned for Summer 2022. Farm Bill funding contributed equipment for enhanced biosecurity in the remodeled space.

Sequence-Based Real-Time Identification of NAHLN Scope Viral Diseases and Emerging Viral Diseases Using Long Read Approaches

Allison Neujahr, Bruce Brodersen, Dustin Loy, Duan Loy, and Samodha Fernando

University of Nebraska Veterinary Diagnostic Center, Lincoln, Nebraska

Project objectives:

1) Develop a diagnostic tool to rapidly and accurately identify NAHLN scope viral diseases and emerging viral diseases in livestock.

2) Validate sequence-based diagnostic tool developed for accuracy of detecting NAHLN scope viral diseases and emerging viral diseases in relevant matrices.

Deliverables completed:

We have developed a novel molecular diagnostic method that enables sequencing of large genomic regions coupled with genome-based comparisons to rapidly identify known and unknown viral pathogens from different matrices. This flexible approach can be used to identify unknown DNA or RNA viruses from multiple tissue matrices and can be extended to any livestock species or humans. This approach can also be used for bacterial pathogen identification.

We have combined Oxford Nanopore long read sequencing with a robust custom protocol for viral enrichment and developed a custom bioinformatic pipeline to identify viral pathogens rapidly and accurately. This approach relies on the fast-sequencing speed and the longer DNA fragments sequenced through the MinION□ (approximately, 30 kb strands at a speed of 70 bp/second) compared to the current high throughput methods using the Illumina platform. We believe, this approach can be successfully used to identify emerging or novel viral diseases in livestock species rapidly to help early intervention and reduce spread of diseases.

Additionally, we have tested our method using different tissue matrices with mixtures of DNA and RNA viruses and show that we can identify viruses with high confidence. This has allowed the creation of bioinformatically subsampled datasets to identify the minimum number of reads needed to accurately detect the presence and the identity of a viral pathogen.

Deliverables yet to be completed:

The only experiments left to be completed is titration of viral particles to identify the detection limit of the virus in a tissue matrix.

How the project benefits NAHLN:

This project will benefit NAHLN as it has developed a novel diagnostic test platform that can be universally used to detect viral and bacterial pathogens from tissue matrices using long read sequencing methods rabidly without any prior knowledge of the pathogen of interest. As such this method can be used to identify unknown pathogens.

Enhancing the National Animal Health Laboratory Network (NAHLN) Diagnostic Capability and Emerging Disease preparedness through Next-Generation Sequencing

Diego G. Diel

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College of Veterinary Medicine, Cornell University

Early and rapid pathogen detection and identification is the most critical step for adequate and effective control of disease outbreaks. The emerging picornavirus Seneca virus A (SVA) is a foot-and-mouth-disease (FMD)-like vesicular disease virus affecting swine herds in the United States and since 2014 an increase in SVA outbreaks has been observed in swine worldwide. Thus, the objectives of this project are to develop targeted and random NGS protocols for sequencing SVA using MinION platform and also to develop streamlined bioinformatics pipelines in order to provide means for rapid and simultaneous detection, identification, and genetic characterization of SVA and other swine pathogens using the Base2bio online platform.

The targeted whole genome sequencing (WGS) is based on a multiplex PCR amplification targeting 5 fragments of the SVA genome of approximately 1500bp each, with 100bp overlap between amplicons. The targeted WGS protocol was validated on 192 clinical samples from California, collected between 2018 and 2020, that had CT values for RT-qPCR ranging from 10.25 to 38.82. Remarkably, 88.5% of all samples (141 out of 192) resulted in genome coverages >97% with a read depth of at least 20X. The average hands-on time was estimated in 7 hours. An alternative protocol targets the 3D protein with a single pair of primers. Forty-eight clinical samples were sequenced using this protocol and consensus sequences were obtained for all the samples. The total turnaround time for the single gene sequencing, from RNA to load the library is estimated in 5 hours, including the one-step RT-PCR cycling time.

In order to simultaneously detect and characterize multiple pathogens associated with infectious diseases of swine, including vesicular diseases, a random sequencing protocol using nuclease treatment on samples before nucleic acid extraction and sequence-independent single-primer amplification (SISPA) was optimized and evaluated directly in clinical samples. Sequencing data were analyzed with swine specific platforms developed by our group and results show promising perspectives for the detection of co-circulating viruses. Multiple swine viruses were detected, including porcine bocavirus, porcine parvovirus, porcine torovirus, porcine epidemic diarrhea virus. Most, importantly the approach allowed retrieval of complete or near-complete SVA genomic sequences from those clinical samples.

Altogether, our results show that cost-effective MinION sequencing protocols in conjunction with streamlined bioinformatic pipelines provide great potential to enhance our capacity to detect and diagnose as well as improve our understanding of the evolution and disease dynamics of SVA and other swine important swine viruses.

Enhanced surveillance and outbreak surge capacity testing for high consequence, NAHLN-scope animal diseases

Eman Anis and Lisa Murphy

Pennsylvania Animal Diagnostic System-New Bolton Center, University of Pennsylvania

Early detection of and rapid response to emerging animal diseases are critical for safeguarding animal agriculture and public health. Effective responses can prevent or limit negative impacts on animal and human health, food security, and the economy. As part of the National Animal Health Laboratory Network (NAHLN), the Pennsylvania Animal Diagnostic Laboratory System at the University of Pennsylvania's School of Veterinary Medicine (PADLS New Bolton Center) supports timely and accurate detection, rapid response, and appropriate recovery from high-consequence animal diseases. PADLS New Bolton Center has highly-trained and proficiency tested professionals utilizing PCR to analyze animal specimens for the presence of avian influenza (AI), exotic Newcastle disease (END), classical swine fever (CSF), and African swine fever (ASF).

The goal of this proposal was to maintain and enhance surveillance and outbreak testing capacity of PADLS New Bolton Center for high consequence, NAHLN-scope animal diseases. This was accomplished by the purchase of additional laboratory equipment including a KingFisher Flex system, PCR workstations, QuantStudio 5 real-time PCR system, and three freezers. The additional equipment increased our laboratory's PCR testing capacity. In October 2020- October 2021, our laboratory tested approximately 5,500 samples for AI, which is approximately 1000 more samples tested compared with last year. Also, with the purchase of the new freezers our laboratory was able to accommodate additional storage of diagnostic reagents required to perform surveillance and outbreak disease testing. In conclusion, the purchase of these equipment and the PCR workstations enhanced our laboratory surveillance capacity and emergency preparedness and response capabilities in the event of an adverse animal health event or emerging infectious disease.

Preparedness for High Throughput Scaling

Amy Swinford[1] and Brian McCluskey[2]

[1]Texas Veterinary Medical Diagnostic Laboratory, Texas A&M University, College Station, Texas

[2]Trace First Inc.

The objectives of this project were to improve rapid accessioning capabilities through the design of barcode-led workflows to support high throughput testing. All deliverables as outlined in the approved project proposal have been completed. Our laboratory information management system, CoreOne for Labs, now supports customizable barcode label definitions and design capabilities. There is little doubt that barcoded labels for both accessions and specimens in laboratory workflows significantly enhance speed, accuracy, and efficiency in a diagnostic laboratory. Having more control over label design and layout, particularly in the face of a rapid scale-up of testing, benefits the NAHLN by supporting high-throughput testing. Accordingly, we created a custom label generator in our LIMS to support current and future workflows.

Enhancing Antimicrobial Resistance Surveillance via Veterinary Diagnostic Laboratories through XML results messaging

Amy Swinford[1] and Brian McCluskey[2]

[1]Texas Veterinary Medical Diagnostic Laboratory, Texas A&M University, College Station, Texas

[2]Trace First Inc.

The objectives of this project included enhancing our laboratory's ability to support the NAHLN program office's pilot to implement Antimicrobial Resistance Surveillance in the United States through development of an XML message schema; to upgrade the integration of the Sensititre broth microdilution platform with our current laboratory information system (CoreOne for Labs); and to make the XML standard developed publicly available so that other labs in the AMR surveillance program might send data by the XML schema. The integration with the Sensititre instrument has been completed allowing result output from the machine to flow directly to CoreOne for Labs. The XML message schema has been developed and was reviewed by the NAHLN program office with full documentation of the schema shared with the NAHLN program office. The internal mapping to accept the AMR message was completed by the USDA and a test message from the LIMS using the XML schema sent and received by the USDA test endpoint. We are currently working on incorporating an XML message generator in our CoreOne for Labs LIMS that will enable automatic generation and sending of the AMR data in XML format required to support the surveillance effort. Benefits to the NAHLN from this project include increasing the efficiency and potential frequency of reporting of quantitative susceptibility data. This improvement will initially occur from our laboratory but with the standard being made available publicly, all laboratories participating in the pilot can use the standard and improve the efficiency of reporting. More general benefits are to the USDA, FDA, and animal industry partners in informing them of the status of antimicrobial resistance in pathogens important to veterinarians and public health partners.

Strengthening and Enhancement of Kansas State Veterinary Diagnostic Laboratory (KSVDL) Capacity for FAD Emergency Preparedness and Response

Jamie Retallick[1], Lance Noll[1], Bryan Kraus[2]

[1]Kansas State University Veterinary Diagnostic Laboratory, Manhattan, Kansas
[2]Kansas State University, College of Veterinary Medicine, Manhattan, Kansas

Project objectives:

1. Build testing capacity in KSVDL existing BSL3 facility for the purpose of improving detection of the NAHLN scope diseases or emerging diseases.

2. Expand the use of barcodes at KSVDL to track samples involving NAHLN scope programs.

3. Build capacity of KSVDL for surveillance and detection of foreign animal and emerging diseases by supporting laboratory equipment purchases that directly enhance laboratory response capacity to address NAHLN scope disease and emerging disease testing.

Deliverables completed:

Objectives 1: A PCR workstation and -20°C upright freezer were purchased and installed in the Biosecurity Research Institute (BRI), the BSL3 facility that will serve as the testing site for a potential level-3 FAD outbreak.

Objective 2: The prototype for barcoding and sample tracking was complete. All hardware (monitors, computers, kiosks) has been purchased, received, and configured. The LIMS, Vetview, has been upgraded to include sample tracking capability. Processes have been developed for tracking samples. Dashboards have been created for the labs to view sample location, test times and lab messages. Data Innovation drivers have been tested and integration flows with the LIMS has been developed. In addition, a pilot mobile application has been developed for test submission. The mobile application will allow for submitting samples electronically and using the sample tracking functionality.

Objective 3: An additional Applied Biosystems ABI7500 Fast Real-Time PCR System was purchased for the Molecular Diagnostics Laboratory (MDL). The recommended calibration and in-house validation were completed, via parallel testing of select NAHLN assays, to ensure optimum machine performance. An additional KingFisher™ Flex Purification System was also purchased and installed in MDL. Extraction efficiency of the new unit was validated via parallel sample extraction with a previously validated unit of the same make/model.

Deliverables remaining to be completed:

Objective 2: Computers and kiosks will be placed by the end of the calendar year. Implementing changes to operating procedures needs to be complete.

How the project benefits NAHLN:

Objective 1: Addition of a PCR workstation provides equipment that is prerequisite for proper adherence to NAHLN testing steps and reduces the risk of sample contamination during PCR set-up.

An additional freezer increases the amount of NAHLN testing reagents on hand, which bolsters our capacity for immediate FAD response. For example, although standing orders are in place for ASFV reagents, this freezer has allowed for onsite storage of ~3000 reactions worth of testing materials for immediate response.

Objective 2: The additional equipment will increase sample throughput & decreases turn-around-time for NAHLN testing in BSL2 space. We estimate a minimum increase of 202,000 samples tested annually with one additional PCR machine. By replacing the aging extraction unit, the new unit serves to increase reliability of our NAHLN testing infrastructure. Furthermore, this additional NAHLN-approved equipment is now available for transfer into our BSL3 testing facility, in event of an FAD outbreak.

Objective 3: Full implementation of this technology in KSVDL NAHLN testing sections will provide real-time monitoring of all sample stages from receipt on through results reporting, subsequently providing a detailed audit trail. Once implemented, this system can be shared with other NAHLN members to enhance the overall NAHLN network.

Deep Learning Computational Algorithms for Disease Diagnosis by Genome Sequencing

Akhilesh Ramachandran, Sathyanarayanan Aakur, Arunkumar Bagavathi, Fernando Vicosa Bauermann, Sai Narayanan

Oklahoma Animal Disease Diagnostic Laboratory, Oklahoma State University, Stillwater, Oklahoma

Our project goal was to explore the use of a metagenome-based machine learning framework as an innovative platform for disease diagnostic applications. Even though PCR protocols are commonly being used as a reliable diagnostic method for many NAHLN-scope diseases, its capabilities are limited in terms of multiplexing potential and detection of novel pathogens. There are several advantages to using metagenome based diagnostic applications including its pathogen-agnostic nature and theoretical unlimited multiplexing potential.

The main objective of the project was to develop an algorithm that would help in the screening of metagenome data for the presence of pathogen derived nucleic acid sequences. Metagenome sequencing was performed using the MinION platform (Oxford Nanopore). For preliminary algorithm development and testing, metagenome data was generated from archived diagnostic specimens (bovine lung) known to be positive or negative for *Mannehimia haemolytica.* Sensitivity evaluation of the framework and additional development using broader metagenome data generated from archived diagnostic specimens (oral fluids) known to be positive or negative for swine influenza is underway.

Conventional machine learning approaches to metagenome-based diagnosis were initially developed using simulated metagenome data and evaluated using traditional features extracted from k-mer frequencies. On evaluating this baseline algorithm using simulated data, we found a classification accuracy of 47.29% for *M. haemolytica* (vs host genome) and limited multiplexing capability. By analyzing extracted structural features from De Bruijn graphs, the accuracy improved to 73.49% on simulated metagenome data but offered limited transfer to clinical metagenome data. To address this limitation, we employed the use of adversarial co-training and multi-task learning to improve the performance of the graph-based algorithms on clinical metagenome data. The performance improved to 80.03% when both real and simulated data were used for training, but only worked well on binary classification problems such as single pathogen identification. For multiple pathogen identification settings, a very low precision (10.2%) was obtained when considering the six bacterial pathogens associated with bovine respiratory disease complex (*M. haemolytica, P. multocida, T. pyogenes, M. bovis, H. somni, B. trehalosi*). For further improvement of performance, sequence-based features were considered in addition to the graph-based structural features. Using current advances in deep learning such as transformers and attention-based reasoning models two frameworks were developed, called MG-NET and Metagenome2Vec, and they obtained precisions of 61.5% and 63.1%, respectively.

Further studies are ongoing to determine the sensitivity of the two deep learning frameworks on diagnostic samples spiked with varying concentrations of targeted pathogens (*M. haemolytica* and Swine Influenza Virus). Initial results indicate that Metagenome2Vec can differentiate between sequences belonging to unknown pathogens such as viruses that were not trained explicitly on. We aim to improve this multiplexing capability further to build a machine learning-based diagnostic protocol that can be used as a general platform for the screening of emerging and NAHLN scope diseases.

Enhancing the National Animal Health Laboratory Network (NAHLN) Diagnostic Capability Through Electronic Reporting and Transmission of Data

Binu Velayudhan

Athens Veterinary Diagnostic Laboratory, University of Georgia, Athens, Georgia

Project objective: The overarching goal of this proposal was to expand the capacity of VetView NAHLN laboratories to rapidly generate accurate data, collate summary data, and present/export it in a manner easily used for disease epidemiological tracking and rapid reporting of disease data to state and federal authorities. The specific objectives of this project were: 1. To facilitate the upgrades needed by NAHLN laboratories using the VetView LIMS to meet NAHLN requirements for electronic messaging, 2. To expand the ability of VetView LIMS to improve sample management, and 3. Training and Development of Laboratory Personnel.

Deliverables completed / Deliverables yet to be completed: Deliverables completed as part of this project included: Design work was completed for the VetView Application Programming Interface (API) redesign that gain the ability for individual laboratory IT teams to create custom mobile apps and receiving forms, completed development activities for API interface enhancements for the creation and acceptance of an external request, Implemented custom worksheet using new API in a production surveillance site testing environment, requirements and design were completed in preparation for sample tracking development, Initiated development of specimen tracking and location management within the VetView, completed development in the online Portal within VetView for ease of submission and expedition of data entry for case accessioning, and completed development for internal specimen tracking within the laboratory workstations and sections.

Deliverables yet to be competed include meeting with contributors and key personnel to discuss implementation of testing strategy, and training of laboratory personnel to optimally utilize the enhancements.

How the project benefits NAHLN: All NAHLN laboratories use laboratory information management systems (LIMS) to manage their caseload. This process generates a large amount of data that remains stored locally in the LIMS. For LIMS data to be useful and practical for USDA purposes, mechanisms need to be established for straightforward transmission of data to Federal databases. Within the laboratory, the process of data acquisition prior to sample testing can be cumbersome and time-consuming. This in turn, limits a laboratory's testing throughput and thus its capacity to respond to a disease outbreak. The specific objectives of this project were aimed at enhancing VetView LIMS to address these challenges. VetView is a web-based LIMS created and owned by the College of Veterinary Medicine at the University of Georgia. It is currently used by 8 NAHLN laboratories and 6 veterinary teaching hospitals. Successful completion of these objectives and implementation of the LIMS enhancements contribute toward building NAHLN laboratory capacity including online submission, customizable receiving forms, result and worksheet interface, specimen tracking, and electronic messaging of data to State and Federal agencies.

One Health Wyoming: Enhancing Interlaboratory Coordination for Emerging Diseases

William Laegreid

Wyoming State Veterinary Laboratory, Laramie, Wyoming

Emerging infectious diseases, defined as "infections that have newly appeared in a population or have existed but are rapidly increasing in incidence or geographic range," are unexpected events often caused by new pathogens or known pathogens in unexpected host species or places. As a result, emerging diseases are often not recognized in a timely manner, greatly increasing their extent and the cost and complexity of controlling the disease. Contributing to the delay in recognition of a disease emergence event is the structure of our diagnostic services by species – human, domestic animal and wildlife diagnostics typically are handled by separate laboratories. Since introductions of known pathogens across species are an important source of emerging diseases and, in the diagnostic laboratory are essentially equivalent to novel pathogens, the siloing of diagnostic effort is counterproductive to detection of disease emergence. Fortunately, advances in next generation sequencing (NGS) technology are helping to overcome some the issues in detection of disease emergence, allowing for the detection of nucleic acid from any type of pathogen in a generally unbiased manner.   NGS also provides initial information about specific characteristics of the pathogen and its relationship to similar pathogens. This kind of information will help to shape initial efforts at control of the emergence event and is useful for epidemiologic investigations of source and routes of transmission of the pathogen. The output of NGS, nucleic acid sequence, has the advantage of being readily communicated, thus facilitating exchange of pathogen information among labs and other entities as well.

Collaborative participants in this project included the three primary diagnostic centers in the state: the Wyoming State Veterinary Laboratory, Wyoming Game and Fish Wildlife Health Laboratory and the Wyoming Public Health Laboratory, encompassing domestic animal, wildlife, and human diagnostics.

The objective of this project is to stablish a common next generation sequencing (NGS) platform for detection and characterization of emerging pathogens in humans, domestic animals, and wildlife in Wyoming. This project primarily addressed one of the stated NAHLN funding priorities in this program:

Test Procedures, reference materials and equipment. *Evaluate, validate, and implement new sample types, equipment, and technologies for the purpose of improving detection of foreign animal and emerging diseases.*

The following outcomes resulted from this project:

1. Established common NGS technologies between WSVL, WGFD and WPHL. WSVL and WGFD now have in house sequencing capability similar to that in the WPHL, Illumina iSeq 100 for short reads and Oxford Nanopore Minion MK1C for long read data.

2. As we began this project, the onset of COVID-19 provided a perfect disease emergence event requiring interdepartmental collaboration and communication. WSVL worked successfully with WPHL to harmonize assembly pipelines for long read and short read data for SARS CoV-2.
3. Sequence data resulting from this project has provided insight into etiologies and epidemiology of some emerging diseases in Wyoming, including rabbit hemorrhagic disease type 2, canine mycoplasma pneumonia and SARS CoV-2.

In addition to the specific disease related outcomes, this project has greatly enhanced working relationships between WPHL, WGFD and WSVL, which will undoubtedly prove worthwhile in future disease emergence events.

United front to develop harmonized NGS training and procedures to increase the capabilities and capacity of NAHLN laboratories in response to antimicrobial resistance (AMR)

Susan Sanchez

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Project Objectives:

1- Improve capacity through laboratory space organization and equipment acquisition/deployment.

2- Development and training of harmonized SOPs for bacterial DNA extraction, library preparation, and sequencing using the same Illumina platforms across all the laboratories.

The challenge we plan to tackle with this proposal is to develop a model to enhance WGS and NGS capabilities across all NAHLN laboratories from sample/organism to bioinformatics analysis. The specific objective of this application is to pilot a training plan in seven laboratories. This objective will be attained by purchasing standardized equipment across the laboratories, organizing the physical space most appropriate for the purpose, and followed by hiring personnel just for this application, standardizing laboratory procedures, and selecting a bioinformatic downstream demand for WGS that will agree with those used by other federal agencies such as CDC, FDA, etc. If we are successful in our goals, this training model and SOPs could be deployed to other laboratories, and WGS/NGS becomes a NAHLN standard testing capability. We will leverage the different levels of capacity of each of the laboratories involved to achieve successful completion.

Deliverables completed:

1- Equipment such as ISeq and QiAxcel was purchased.
2- Established appropriate workflow and separate areas for the different steps of the process of WGS/NGS in each of the participating laboratories.
3- Equipment will be installed, and personnel trained in the use of the equipment by the vendors.
4- Through a series of conference calls (Zooms) worked on the creation and final development of SOPs. Choose the appropriate training organisms.
5- Laboratories and personnel will perform library preps on four organisms (two Gram positives and two Gram-negatives), following SOPs, to determine the quality of the run.
6- We will explore modes of remote training methodologies. Created a video and presented a mini-symposium to all AAVLD members that were interested (2020).

Deliverable not completed:

1- Travel of personnel to training laboratories will be organized and carried out. The training will be at the level of" train the trainer."

COVID 19 Deliverables added:

1- Include bioinformatic analysis in our deliverables. We are comparing multiple platforms with the "common-line" gold standard.
2- Reduction in the cost of library preparation by reducing expensive critical reagents. Validate the use of x1, x0.5, and x0.25 of the reagents.
3- Publication of all protocols and bioinformatic analysis in detail in a magazine for all laboratories across the NAHLN to use.

How the project benefits NAHLN:

AMR monitoring and the ability of laboratories to be proficient in NGS analysis (including WGS) is critical for the advancement of the NAHLN laboratories. Like in early 2000, PCR became the new and exciting diagnostic technology around which NAHLN was created to prepare for FAD testing and surge capacity if needed. Many laboratories then had never performed a PCR, yet lone have the expertise to do so. The new NAHLN provided funds and training, SOPs, and PT testing to selected laboratories, which promptly used their new knowledge and equipment to develop a test for endemic diseases. This way, not only being readily trained, as if you do not perform a test regularly, you are not prepared on the occasion of an outbreak for a FAD but also serving their constituents and the overall mission of USDA. WGS and NGS are the new frontier technologies today. Our team believes that the successful completion of this grant will demonstrate that laboratories can repeat history, adopt NGS technologies quickly, and develop new test applications. This success will serve NAHLN's future technology addition to the capacity to address NAHLN scope diseases or emerging diseases, including AMR. WGS proficiency and bioinformatics a gateway to FAD and other disease detection in clinical samples.

Summary

Although the project has not been completed, our findings seem to indicate that it is possible to use WGS in a small laboratory with limited funds and be able to analyze the data for some tasks such as bacterial ID, MLST, Serotyping, and the determination of AMR without the assistance of a bioinformatician.

Development of an Interactive Spatial Agrometrics Tool for the Calculation of Livestock (Cattle, Swine and Poultry) Populations in the United States at the County and Parish Level

Akhilesh Ramachandran, Hongbo Yu, Amy Hagerman, Derrell Peel, Emily Cooper

Oklahoma Animal Disease Diagnostic Laboratory, Oklahoma State University, Stillwater, Oklahoma

Project objective: Develop an interactive map of the US that will help in visualizing different animal populations.

Deliverables completed / Deliverables yet to be completed:

- Partially completed: Web based maps for Livestock population estimation.

- Pending: Update and review of data uploads to the mapping platform.

How the project benefits NAHLN:

- The interactive, web-based agrometrics tool will help in the calculation of targeted animal populations relative to their proximity to a point of interest (e.g., NAHLN member laboratory or index case in a disease outbreak).

- Easily integrate data across species and query the data on a multi-state basis.

- Provide regional data that assists NAHLN in test capacity and capability management, determine regional surge capacity, resource allocation, and potentially help identify geographic regions with unmet diagnostic needs.

- The strategic planning and allocation of veterinary stockpile resources.

- Future applications of the tool are envisioned to include real-time mapping of animal movements, predictive modelling of FAD spread, and logistical support for animal health responders.

NAHLN ORDER Message Proof of Concept Commercial Implementation

Michael Martin

Clemson Veterinary Diagnostic Center, Columbia, South Carolina

Project Objective: To demonstrate feasibility of implementing an ORDER message receiving function in a commercial LIMS and make this function available for some NAHLN labs

Project Objective: Discover and correct any missing features in the ORDER message profile and XML schema

Project Deliverables Complete: Basic functionality is complete. Minor edits have been made to the schema including two additional data elements.

Project Deliverables Underway: Workflow for messages that arrive with incomplete code or party mappings is being enhanced. Message inspection function is being modified to display a more user-friendly rendering rather than raw XML. Unmapped additional data ("Epi data") included in order will be returned in result messages for the generated accession.

Benefit to NAHLN: When the ORDER messaging is widely used, data quality will improve, and accession data-entry will be less of a rate-limiting step in the laboratory. Having some LIMS providing the receiver function will—we hope—encourage wider adoption of ORDER message transmission in Epi software and additional LIMS to implement receiving.

Enhancement of Chronic Wasting Disease and Scrapie Diagnostic Testing Capabilities

Kristy Pabilonia, Juan Munoz-Gutierrez

Colorado State University Veterinary Diagnostic Laboratory, Colorado State University, Fort Collins, CO

Background

The Colorado State University Veterinary Diagnostic Laboratory (CSU VDL) in Fort Collins, CO participates in the NAHLN program as a Level 1 laboratory. The CSU VDL is very active in transmissible spongiform encephalopathy (TSE) testing and tests samples submitted from states across the country. The CSU VDL is approved to test for bovine spongiform encephalopathy (BSE), chronic wasting disease (CWD) and scrapie. The table below depicts testing numbers for the last four calendar years.

	Calendar Year			
	2020	**2019**	**2018**	**2017**
Scrapie IHC	8053	9649	9803	9456
CWD IHC	1614	2417	3562	2487
CWD ELISA	38218	63331	39542	43765
BSE ELISA	4891	3723	4471	3791

Purchase and Implementation

The CSU VDL has utilized the Leica Bond Max instrument for CWD and scrapie IHC testing. While this instrument generally fits the needs of the laboratory, the performance of the instrument could be improved upon. The NAHLN has recently approved the Ventana Discovery Ultra instrument for CWD and scrapie IHC testing.

The Ventana Discovery Ultra instrument was purchased and installed in 2020 and a validation study was completed in 2021. This validation consisted of a side-by-side comparison of the Leica Bond Max and the Ventana Discovery Ultra using sheep (scrapie) and cervid (CWD) samples. These included 20 CWD positive cases, 20 CWD negative cases, and 10 scrapie negative cases. The results of the Ventana IHC correlated 100% with the results of the Leica IHC testing. The overall counterstaining of tissues was more intense with the Leica instrument as compared to the Ventana platform but the slides stained with the Ventana platform had significantly less background/non-specific staining as compared to those stained with the Leica platform. This difference improves the quality of our slides and enhances identification of prion-specific immunoreactivity.

The CSU VDL passed the 2021 NAHLN CWD and scrapie IHC proficiency test using the new Ventana platform.

<u>Benefit to the NAHLN</u>

The addition of the Ventana instrument at the CSU VDL provided an additional platform for CWD and scrapie IHC testing to allow for diversification of testing, along with expanded testing capacity. The Ventana Discovery Ultra provides our pathologists with improved IHC slides to interpret. This allows us to provide the best possible results to our clients and ensures that we are detecting all cases of CWD and scrapie from our sample submissions. The instrument also provides increased testing capacity and decreased testing turnaround time for our clients. With the new Ventana instrument, the CSU VDL has maintained the highest quality diagnostic services for CWD and scrapie IHC testing.

Enhancement of Chronic Wasting Disease Testing Capacity

Y. Reddy Bommineni

Bronson Animal Disease Diagnostic Laboratory, Florida Department of Agriculture and Consumer Services, Kissimmee, Florida

A second BOND-MAX Automated Immunohistochemistry (IHC) Stainer was purchased and installed in August of 2020 along with all necessary supplies and reagents. Prior to this purchase, the Bronson Animal Disease Diagnostic Laboratory (BADDL) had only one IHC stainer for Chronic Wasting Disease (CWD) testing. The capacity for the machine was limited to staining 30 slides per run. Due to the time-consuming nature of the CWD IHC staining protocol, an average of 40 slides could be stained per day with approximately 200 per week. During the height of hunting season, 200-300 samples are submitted every two weeks. It was further indicated that many samples were in storage waiting to be submitted to ensure BADDL was not overwhelmed. In consequence, testing on some samples may have been delayed weeks to months because of limited testing capacity. The purchase of the second stainer effectively doubled the capacity of BADDL to test for CWD, an emerging disease of interest to NAHLN and with economic significance to deer producers across the state of Florida. It also decreased the time between sample collection and testing in order to respond earlier to potential disease.

Chronic Wasting Disease (CWD) is a progressive neurological disorder of deer, elk, and moose. It is a prion disease related to Scrapie and Bovine Spongiform Encephalopathy. Florida has over 500 captive Cervidae farms in addition to wild white-tailed deer (four subspecies) and key deer (endangered). White-tailed deer are the most economically important game species in the State of Florida. According to the 2014 census, the white-tailed deer population was 700,000 in the State of Florida. To date, Florida has not had a confirmed positive case of Chronic Wasting Disease; however, every year the disease continues to inch closer to our border.

This project would address essential surveillance needs and safeguard economic interests by doubling the CWD testing capacity of BADDL. In addition, BADDL was selected to be one of the first laboratories to set up CWD messaging. Thus, the outlined project would also aid in CWD electronic reporting and data transmission across NAHLN laboratories accredited to conduct CWD testing.

Enhancing Testing Capacity and Diagnostic Capabilities Using High Throughput Polymerase Chain Reaction Assay

James Trybus

Rollins Diagnostic Laboratory, North Carolina Veterinary Diagnostic Laboratory System, Raleigh, North Carolina

To assist us in enhance our laboratory testing capabilities, Integra Assist plus Pipetting system and Quant studio 5 384 qPCR System were purchased and programmed to use in AIV-matrix testing workflow. As the first step, Integra Assist plus Pipetting system, using Voyager series of pipettes, was custom programmed to meet Quant studio 5-384 qPCR platform (QS384) assay set up needs. AIV matrix PCR assay conditions, including reaction volumes, were standardized on QS384 platforms utilizing NAHLN IAV controls as the reaction template. To meet QS384 well aliquoting needs, Asist plus pipetting system using Voyager series of pipettes program was customized using serial 10-fold and/or 2-fold dilutions of NAHLN assay positive controls. Final reaction volume for QS384 was optimized to 20ul. Four independent runs were set up using each dilution and each dilution was run in triplicate reactions. Data from all 4 independent runs were utilized to optimize the assay conditions and to determine the limit of detection (LOD)on QS-384 platform. Assay performance was compared by simultaneous testing on ABI-7500, using NAHLN approved procedures. Based on the data from 4 independent runs using 10-fold and 2-fold serial dilutions of NAHLN positive amplification controls, AIV Matrix PCR assay LOD for QS384 platform was determined as Ct:35. Detection of control dilutions with a Ct>35 was not consistent among all replicates. Following the standardization of the assay conditions and LOD determination, a total of 90 previously confirmed AIV positive samples (originated from wild birds (oral + cloacal swabs), turkey (tracheal swabs) samples with swine lineage influenza (H1N1, H1N2, and H3N2), and tracheal swab samples from H7N3 low path avian influenza outbreak of 2020) and 11 NAHLN IAV PT samples (from 2021 PT panels) and 90 previously confirmed negative samples were run simultaneously on both QS384 and ABI7500 platforms and data were compared. Assay repeatability and reproducibility was assessed using 30 positive sample and 10 negative samples. For samples with Ct 35 or lower, data obtained QS384 platform showed good agreement with that of ABI 7500. However, for samples with Ct >35, results were inconsistent among the replicates. This observation is consistent with LOD determined in this study.

In conclusion, as part of this NAHLN-Farm bill project, we have standardized assay conditions, including reaction volumes for AIV matrix assay on Thermofisher Quant Studio 384 platform and determined LOD (Ct:35). While Quant studio 5-96 well platform is currently approved for NAHLN testing, it offers similar capacity (maximum of 92 samples per run, excluding the controls) as ABI7500, another approved qPCR platform for NAHLN testing. Whereas QS384 platform allows testing of 384 samples per each run, with a NLHN approved deviation for its use, this will significantly enhance our diagnostic capabilities in the event of an influenza outbreak and surge testing.

Enhancing laboratory preparedness for NAHLN scope diseases

Brett Webb

North Dakota State University Veterinary Diagnostic Laboratory, Fargo, North Dakota

The 2019 Farm Bill funding enabled the North Dakota State University-Veterinary Diagnostic Laboratory (NDSU-VDL) to purchase a KingFisher Flex 96-well magnetic particle processor, an Applied Biosystems 7500, a Qiagen TissueLyzer, along with a master mix hood and a full set of pipettes. The equipment and supplies purchased with this grant fully outfitted the biosafety level 3 laboratory, which will allow the NDSU-VDL to work more safely when conducting high-throughput NAHLN-scope disease testing and provides critical containment for high consequence pathogens. This equipment enabled the laboratory to double its testing capacity to over 1,000 tests per day in the event of an outbreak. The results of the project are well aligned with NAHLN priorities to enhance the nation's capacity to respond to foreign animal disease outbreaks.

Capacity Building in Wisconsin – Enabling Compliance at the National Wildlife Health Center and Surge Support for the Wisconsin Veterinary Diagnostic Laboratory

Jonathan Sleeman and Hon Ip

USGS National Wildlife Health Center, Madison, Wisconsin

Description of Purchases: Two (2) Applied Biosystems QuantStudio 5 real-time PCR thermal cyclers and associated laptop computers were purchased with funds from the 2019 Farm Bill grant.

Abstract: This project has enabled the U.S. Geological Survey National Wildlife Health Center (NWHC) to replace two aging 96-well real-time thermal cyclers which are no longer supported by their manufacturers for two modern instruments (i.e., two Applied Biosystem QuantStudio 5 units) that are in full compliance with NAHLN requirements. Both QuantStudio 5 instruments were successfully order through USGS Contracting, delivered, and validated; and they are both currently in service. Having the additional instruments increased out laboratory's testing capacity by 50%. The NWHC is co-located in the same city with the Wisconsin Veterinary Diagnostic Laboratory, which is a NAHLN Core Laboratory. Having additional platforms that are in common between the two laboratories means that we can support each other during surge testing demands. Increased laboratory testing capacity will also contribute to the NAHLN mission as the added capabilities will enable USGS to conduct continental-scale monitoring of wildlife for NAHLN program diseases.

Needs met: The two new instruments replaced two aging real-time thermal cycler systems that were no longer supported by their manufacturers. Procuring the QuantStudio 5 units has enabled us to seamlessly implement NAHLN protocols for ASF, CSF, FMD, AI, and ND testing without the need for Requests for Deviations. Procuring these new units has resulted in a 50% increase in our laboratory's testing capacity and will enable the U.S. Geological Survey National Wildlife Health Center (NWHC) to support large-scale surveillance testing similar to our previous levels of participation in surveillance for highly pathogenic avian influenza surveillance in wild birds in 2005-2009 and 2014-2018. Thus, our laboratory is now well-positioned with instrumentation to support ASF, RHDV2, and coronavirus surge testing should the need arise.

Benefits to NAHLN: The NWHC is in a unique position as we are located in the same city as a second NAHLN laboratory, the Wisconsin Veterinary Diagnostic Laboratory (WVDL). Having additional and identical real-time PCR platforms at NWHC will enable us to support surge capacity at WVDL should the need arise. Both institutions will have staff pre-qualified or who can be quickly proficiency tested for various assays, including the looming potential need for ASF testing. We have offered WVDL to train our technicians ahead of time so that they can physically assist testing at WVDL for a potential ASF surge. Alternatively, WVDL technicians can be quickly trained in NWHC's laboratory practices and perform the needed testing at NWHC. Finally, WVDL samples can be directly delivered to NWHC for testing and results reported back to WVDL for their reporting channels. In summary, the 2019 Farm Bill greatly expanded the testing capacity at NWHC and provided additional surge capacity for the WVDL.

Acknowledgments

The success of the inaugural year of the NAHLN Farm Bill process would not have been possible without the hard work, dedication, and creativity of many people. The funded projects detailed in these proceedings will help to increase the capabilities, capacity, and readiness of the nation's animal health laboratory network to quickly and effectively respond to animal disease outbreaks. Our partnership and collaborations as a network are invaluable!

A special thank you to all who submitted proposals and presented their data and conclusions for this year's symposium.

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