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# Rapid Detection and Characterization of Emerging Foreign Animal Disease Pathogens

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**Abstract**
To best safeguard human and animal health requires early detection and characterization of disease events. This must include effective surveillance for emerging infectious diseases. Both deliberate and natural outbreaks have enormous economic and public health impacts, and can present serious threats to national security. In this project, we developed novel next generation detection technologies to protect the agricultural economy and biosecurity. The first technology is a multiplexed assay to simultaneously detection 10 swine viral and bacterial pathogens. The second one is the Lawrence Livermore Microbial Detection Array (LLMDA) which can detect more than 10,000 microbial species including 4219 viruses, 5367 bacteria, 265 fungi, 117 protozoa and 293 archaea. We analyzed a series of swine clinical samples from past disease events to demonstrate the utility of the assays for faster and cheaper detection of emerging and foreign animal disease pathogens, and their utility as s routine diagnosis and surveillance tool. A second goal of the study is to better understand mechanisms of African swine fever virus (ASFV) infection in pigs to aid the development of countermeasures and diagnostics. There is no vaccine available for ASF. ASF outbreak is on the rise on several European countries. Though ASF is not currently in the U.S., a potential outbreak in the U.S. would be detrimental to the swine industry and the US agricultural economy. We pursued a genome-wide approach to characterize the pig immune responses after ASFV infection. We used RNA sequencing and bioinformatics methods to identify genes and pathways that are affected during ASF infection. We have identified a list of most differentially expressed genes that are in the immune response pathways.

**Background and Research Objectives**
Agriculture accounts for $1.24 trillion, or 12.3 percent, of the Gross Domestic Product in the United States. Any deliberate or natural disruptions resulting from the introduction of new infectious diseases can produce enormous impacts, which can affect domestic consumption, exports, and in the case of zoonotic pathogens, public health. Recent examples include the introduction of influenza H1N1 and porcine epidemic diarrhea virus (PEDV) in swine. In addition, foreign animal diseases, such as African swine fever (ASF) and classical swine fever (CSF), remain constant threats. African swine fever virus (ASFV) is an important threat to U.S. pork production. An outbreak of the disease in the U.S. would result in an immediate suspension of pork exports which account for 30% of production. There are no commercial vaccines currently available. The best assurance for the timely identification of known and unknown threats is to employ techniques that can detect endemic, emerging, and foreign animal diseases using a single test.

Our overarching goal is to enhance the scientific foundation for advanced research in agricultural and animal disease pathogens, and increase our broad engagement with global agricultural pathogen surveillance. We have three main objectives of this project. 1) Develop a novel and comprehensive pathogen detection and surveillance strategy for early detection of known, emerging, and new foreign animal disease outbreaks; 2) Characterize host-ASFV interactions and understand host immune mechanisms for ASFV infection; 3) build a strategic partnership with the National Bio and Agro-Defense Facility at Kansas State.

Additionally, we established a national advisory group with leaders from the Department of Homeland Security, U.S. Department of Agriculture, and the National Pork Board to have quarterly calls to seek their advice on the broad utility of our technologies. We have given a number of presentations at national and international conferences about our research. Some examples are: American Association of Veterinary Laboratory Diagnostics, Global African Swine Fever Research Alliance, Biodefense World Summit. In collaboration with K-State, we have submitted several research proposals to USDA and DHS to study disease mechanisms of foreign animal disease pathogens, and to develop faster and cheaper detection technologies. Lastly, as part of the development of strategic partnership with K-State, we have participating in graduate student and young faculty education and training. We have hosted several graduate students and faculty and trained them on LLNL array technologies and bioinformatics tools to facilitate technology transfer to K-State.

**Scientific Approach and Accomplishments**

Over the course of the study, we have made accomplishments towards three main technical objectives:

1. We developed a swine respiratory disease multiplex panel to rapidly detect 10 viral and bacterial pathogens including African Swine Fever, Classical Swine Fever, Porcine Circovirus Type 2, Suid Herpes Virus 1, Porcine Reproductive and Respiratory Syndrome, Swine Influenza, Actinobacillus pleuropneumoniae, Haemophilus parasuis, Mycoplasma hyopneumoniae, and Streptococcus suis.  The multiplex assay panel utilized Luminex bead based technology which has several benefits. Luminex assays are higher-throughput and more cost-effective than traditional PCR singleplexed assays. Most diagnostic labs have the instrument available and are already familiar with the technology.  Luminex beads are stable up to 2 years at 4 degree Celsius. Another main advantage is that the embedded ASF and CSF beads can be taken out for routine screening of domestic diseases. When there is a CSF or ASF outbreak, or a need for increased ASF or CSF surveillance, the beads can be included into the panel for testing as deemed appropriate. We performed bioinformatics design and testing of various target and near neighbor DNA samples and analyzed the sensitivity of the panel. The assay has been transitioned to K-State for further evaluation with pig clinical samples. Additionally, a veterinary diagnostic company, Biovet (St-Hyacinthe, Québec, Canada) has also shown interest in licensing possibilities and has done some evaluation of the assay panel

2. We evaluated the use of Lawrence Livermore Microbial Detection Array (LLMDA) in detection of pathogens from swine clinical samples, analyzed the array's sensitivity and specificity. We tested clinical samples from a study using an experimental infection model for the analysis of Porcine Circovirus Type 2 (PCV2) and Porcine Reproductive and Respiratory Syndrome (PRRSV), the two most common viral pathogens in the swine industry. LLMDA sensitively detected both PCV2 and PRRSV. Additionally, LLMDA detected several bacterial and viral co-infections in a number of clinical samples, which demonstrated the utility of LLMDA in routine clinical diagnosis and surveillance. We also conducted a follow on study to use LLMDA to assess the association between pig microbiomes and the health outcomes of experimentally infected pigs. The LLMDA array has been transitioned to KSU and is now in use as a detection and diagnostic tool for swine viral and bacterial infections and microbiome studies.

3. We characterized the ASFV infection mechanisms by conducting a comprehensive whole transcriptome analysis to determine the pig genes and pathways that are affected by ASFV. This study was performed in collaboration with the Australian Animal Health Laboratory where the pig infection, viremia and phenotypic characterization was done. We performed RNAseq analysis using next generation sequencing technology, the Illumina NextSeq, and bioinformatics methods to determine the most differentially regulated genes after ASFV infection. Additionally, we also compared the mechanisms of pigs infected with two types of ASFV, a low pathogenic strain and a high pathogenic strain. We identified a list of top most differentially regulated genes that could be used as potential markers for viral infection and diagnostics and to inform future vaccine development.

**Impact on Mission**

A comprehensive suite of novel technology platforms to rapidly detect and characterize foreign animal disease pathogens will help protect the agricultural economy, food supply, public health, and biosecurity of the nation. Our effort in this study aligns with the Laboratory's strategic biosecurity mission to safeguard the nation against emerging biological threats, and enhances our core competency in biodetection and diagnostics. We have made significant progress towards building a long-term strategic partnership with the National Bio and Agro-Defense Facility (NBAF) at Kansas State as well as other federal stakeholders. The partnership between LLNL and K-State is at a multitude of levels including scientific research, education and outreach and future joint funding opportunities through government and industry. Additionally, we have expanded our international collaborations with the Australian Animal Health Laboratory and we have started discussions with several countries in Africa on emerging animal and zoonotic disease diagnosis and surveillance.

**Conclusion**

In conclusion, we have achieved several research goals as demonstrated by publications, presentations, and research proposals. We have transferred our technology to Kansas

State and set a strong foundation for long-term strategic partnership with NBAF. We have submitted several research proposals to DHS, USDA and pork industry. We will continue to pursue additional research opportunities both in the US and with international partners. The LLMDA has recently been commercialized by Affymetrix. The Luminex 10-pathogen assay is being evaluated for potential commercialization.

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