SWINE HEALTH INFORMATION CENTER - FINAL RESEARCH GRANT REPORT

Project Title: Development and validation of a single-tube, triplex RT-PCR assay for differential detection of highly virulent Chinese strains of pseudorabies virus
Project identification number: 16-250-SHIC
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Date Report Submitted: 2018-07-18

Industry Summary:

Pseudorabies virus (PRV) causes pseudorabies or Aujeszky's disease in livestock and wild mammals; however pigs are the main host and reservoir for this virus. It causes deadly disease in newborn piglets, respiratory problems in growing and fattening pigs, and reproductive problems in pregnant sows. Like other herpesviruses, PRV establishes a lifelong infection in the nervous system followed by subsequent shedding of infectious virus. Pseudorabies has spread throughout the world, but Canada, Greenland, and Australia are considered free of this disease. In 2004, PRV was eliminated from the US commercial swine herds, but the virus remains in some localized feral swine populations. China is considered the largest pork producer in the world. The earliest documented PRV outbreak in China was in 1947. Since 1990s, more than 80% of pigs in China have been vaccinated and the clinical disease was well controlled. In late 2011 however, a newly PRV virus (variant) which cause severe disease surfaced in PRV vaccinated pig herds in Northern China. Since then, this virus has spread across China causing severe economic losses.

Early detection of PRV is essential to contain spread and prevent economic losses. There are a number of highly sensitive laboratory assays available for detection of PRV in general, however none of them can differentiate pigs infected with the highly pathogenic PRV strains from those infected with the classical PRV strains. The ability of the assay to differentiate PRV variant, classical, and vaccine strains enable veterinary authorities to provide a quick and appropriate response if an outbreak occurs. The newly discovered PRV variants from China and the PRV from other countries (classical viruses) show some minor differences in their genetic material. In this study we have successfully developed a single, highly-sensitive, highly-specific laboratory assay that can detect and differentiate highly infectious PRV variants from the classical strains simultaneously. The assay has a built-in internal control to ensure good assay performance, and it can be completed within three hours from the time the sample is received in the laboratory. The newly developed assay was able to detect as low as one infectious PRV particle in a sample. The assay did not detect any other similar herpesviruses or swine viruses encountered in modern swine farms. The assay shows high consistency between replicates, and also when performed on different days, on different instruments by different laboratory technicians. The clinical sensitivity and specificity of the new assay was validated using clinical samples (nasal and oral swabs, whole blood, serum, tissues) collected from pigs prior to and after infection with either classical (Bristol) or variant (JS 2012 and HeN1) PRV strains. The specificity of the assay was further

confirmed by testing over 300 clinical samples (serum, tissues, and swabs) collected from Canadian and US national herds. The assay was validated at the Kansas State University Veterinary Diagnostic Laboratory and at the National Veterinary Services Laboratory in Iowa. In conclusion, in this study we have successfully developed and validated a highly sensitive and specific laboratory diagnostic assay for rapid detection and differentiation of newly emerged highly lethal PRV variant strains discovered in China from the classical PRV strains and commercially available PRV DIVA vaccine viruses. This assay will be made available to the National Veterinary Service Laboratories (NVSL) and other State and University Veterinary Diagnostic Laboratories in the USA, and Canadian Animal Health Laboratories (CAHLN) for routine diagnostics and epidemiological studies.

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Keywords: Pseudorabies, virulent, variant, detection, Real-time, PCR, assay, DIVA