

SHIC Final Research Grant Report

I. Project title and number: Development of reagents and serological assays for Seneca Valley Virus. SHIC # SA1600754; NPB project #15-188 SHIC.

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II. Industry Summary

Senecavirus A (SVA), formerly named Seneca Valley Virus (SVV), is a pathogenic RNA virus. In July 2015, severe outbreaks were identified in swine herds and were characterized by vesicular lesions along the hoof, snout and mouth. The disease has been detected in swine, cattle and wild mice and the primary concern of the virus is not only the development of vesicular lesions, but the fact that the disease is clinically indistinguishable from the highly contagious, look-a-like Foot-and Mouth disease virus (FMDV). Currently, the veterinary diagnostic community does not have a commercially available antibody test for herd surveillance or identification of new outbreaks.

The Swine Health Information Center has stated that they would like to have new antibody detection assays developed that provide more rapid, cost-effective methods to assist diagnostic detection in tissues or sera and to differentiate SVA from other diseases of swine. To address these needs, we proposed to utilize our well established methods to fulfill the following objectives towards the design of specific reagents and serological assays for SVA diagnostics.

Overall Objectives - The overall objective of this proposal was to develop and validate diagnostic reagents and tests for Senecavirus A (SVA) antigen and antibody detection. The Specific objectives include:

1. The development of specific expressed protein and antibody reagents for diagnostic assay development and confirmation of virus isolation attempts, including reagents for immunohistochemistry (IHC), fluorescent antibody (FA) staining and development of serological and antigen capture assays.
2. The development and validation of first generation serological assays for detection of antibody responses to SVA. These assays included an indirect ELISA, fluorescent microsphere immunoassay (FMIA) and a fluorescent focus neutralization (FFN) assay.

Our endeavors to produce specific antibody-based reagents (objective #1) began with the purification of SVA virus and the expression of individual virus proteins. First, a South Dakota SVA virus strain was successfully grown to high titers within our laboratory. The virus was purified and used to immunize rabbits for the production of antibodies. In addition, a variety of recombinant SVA proteins were also injected into rabbits; and after a 3 month immunization regimen, the serum was harvested and resulting antibodies were purified and used for early research. These antibodies proved valuable in tests used for the isolation and characterization of additional virus strains (Figure 3).

Tests that detect antibodies (serological tests) are important to help differentiate SVA from other diseases causing similar symptoms. Through the scope of this project, we developed several different serological tests which are able to capture and quantitate antibody found in serum, and identify animals that have been recently exposed to the virus. Specifically, a SVA virus strain isolated in our lab, was used to produce an IFA test designed to detect a general antibody response. It was also used to produce a virus neutralization test to quantify neutralizing antibody responses in infected pigs (Figures 4 & 5). These tests have been optimized and are currently in the validation stages of development. These new tests should allow producers to determine the serostatus of a herd and deduce if animals have been recently infected. These two tests can be used for routine diagnostic testing and are anticipated be available summer 2016 at the SDSU Veterinary Diagnostic Laboratory.

Two other diagnostic assay platforms were developed to evaluate SVA serological responses at different stages of infection. The ELISA and FMIA tests rely on the use of expressed proteins of SVA to capture antibodies that develop after exposure and are routinely

used in veterinary diagnostic laboratories for high throughput herd surveillance. The ELISA and FMIA were validated and compared against the IFA test (Table 2). All three tests were shown to be highly sensitive and in agreement with each other, but additional test development will be necessary to increase the specificity of the ELISA assay.

In brief, Seneca Valley Virus infection can imitate other foreign animal vesicular diseases such as FMD. New serologic tests specific for SVV infection, including an IFA, serum neutralization, direct ELISA and FMIA were developed to allow producers and veterinarians to determine whether pigs have been recently exposed to this virus. An additional blocking ELISA is currently in development to allow for further specificity.