An indirect Enzyme-Linked Immunosorbent Assay for Detecting Antibody Response in Pigs Infected by Emerging Porcine Seneca Valley Virus

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INTRODUCTION

Seneca Valley virus (SVV), is a single-stranded non-enveloped RNA virus. SVV belongs to the genus Senecavirus, family Picornaviridae. Important members in the family also include poliovirus, rhinovirus, hepatitis A virus, foot-and-mouth disease virus (FMDV) and swine vesicular disease virus (SVDV). Historically, the association of SVV with swine vesicular disease was speculative, since the virus had also been isolated from pigs without clinical symptoms, and experimentally inoculating pigs with SVV isolates were unable to reproduce the disease. Recently, multiple studies from Brazil, Canada, China and the US provided evidence that SVV is a potential causative agent of idiopathic vesicular disease (IVD) in pigs (Leme et al., 2015; Singh et al., 2012; Vannucci et al., 2015; Wu et al., 2016; Zhang et al., 2015). In some of those pigs tested as SVV positive, clinical signs of anorexia, lethargy, lameness, and vesicular lesions were observed. Gross lesions could be found on the oral mucosa, snout, nares, distal limbs, especially around the coronary bands (Singh et al., 2012). In addition, our previous study confirmed that SVV is the causative agent of IVD by experimentally infecting pigs with SVV recovered from a full-length cDNA clone. The clinical presentations of SVV resemble those caused by other economically more devastating transboundary pathogens that caused vesicular disease, including vesicular exanthema of swine virus (VESV), FMDV (**Figure 1**), and SVDV, which may lead to foreign animal disease investigations. Due to the clinical resemblance of SVV to the more pathogenic FMDV and SVDV, a serological test is required for diagnosis and differentiation. In addition, early identification of the cause of the lesions will help decrease the spread of the pathogenic SVV.

SVV infection

FMDV infection

Figure 1. Surface lesions observed on SVV infected piglets (left panel) and FMDV infected piglets (right panel). The left panel was adapted from Chen Z., et al., 2016, and the right panel was adapted from the Texas A&M College of Veterinary Medicine. (http://www.cvm.tamu.edu/fadr/Disease.aspx?DID=2700)

OBJECTIVES

1. To produce recombinant SVV VP2 protein as an antigen for use in diagnostic assay development
2. To develop an SVV VP2-based indirect ELISA

ACKNOWLEDGEMENTS

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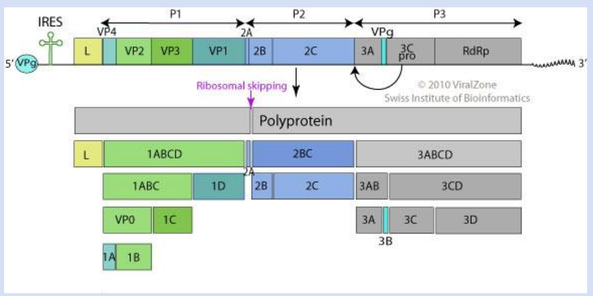


Figure 2. Schematic diagram of the full-length SVV genome and viral protein expression strategies (http://viralzone.expasy.org/697).

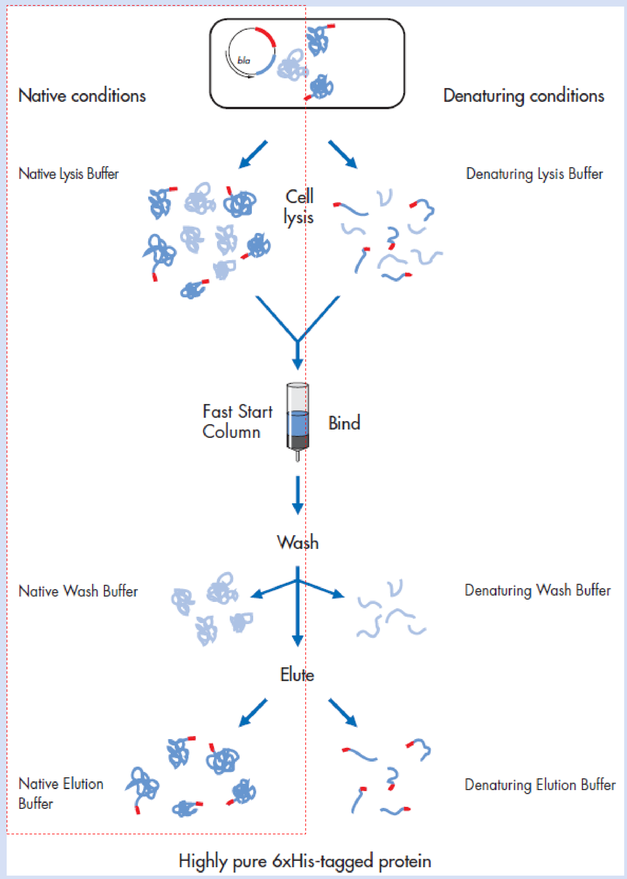


Figure 4. The schematic of purification strategy for 6His-tagged VP2 fusion protein using Ni-NTA beads from QIAGEN. Briefly, after induction with 0.1 mM IPTG overnight, bacterial cells were harvested and lysed with native lysis buffer supplemented with lysozyme followed by sonication. The soluble expressed VP2 fusion protein in native buffer was bind to Ni-NTA beads through 6His-tag. The nonspecific binding proteins were washed away with native wash buffers containing low concentrations (20, 50, 80 mM) of imidazole. Finally, the VP2 fusion protein was eluted with native elution buffer containing 250 mM imidazole.

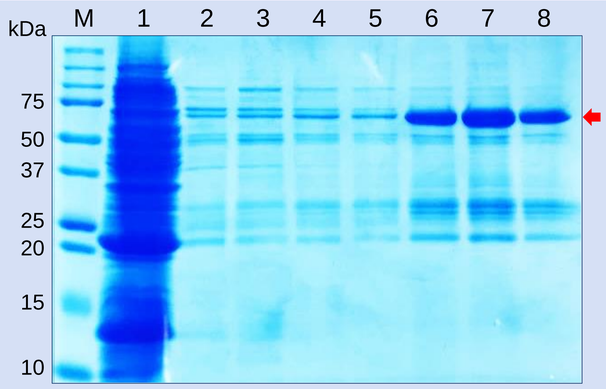
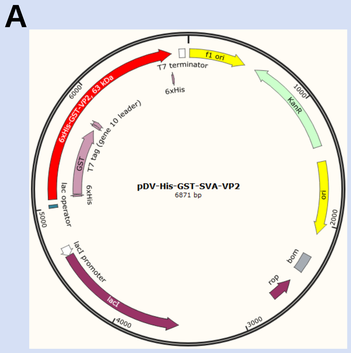


Figure 5. Detection of purified VP2 fusion protein by SDS-PAGE electrophoresis followed with coomassie blue staining. M: Protein maker; 1: flow through; 2~5: wash buffer; 6~8: elution buffer (250 mM imidazole).

A



B

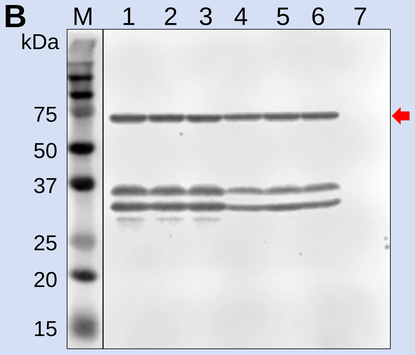
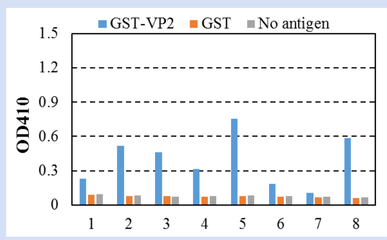
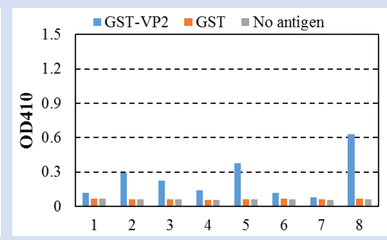


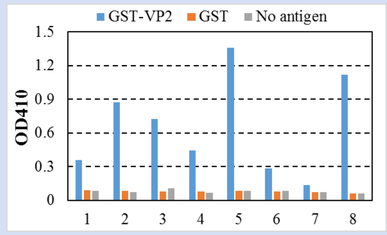
Figure 3. Expression of SVV VP2 protein as a fusion protein (6His-GST-VP2-6His) in *E coli*. A) The map of expression plasmid which expresses 6His-tag and GST fused VP2 protein (Generated with Snapgene software). B) Detection of VP2 fusion protein by western blot analysis using mAb against 6His-tag. Lane 1~3: protein expression was induced at RT with 0.1, 0.5, or 1.0 mM IPTG overnight, lane 4~6: protein expression was induced at 37 ^{o}C with 0.1, 0.5, or 1.0 mM IPTG for 4 hours, lane 7: no induction.



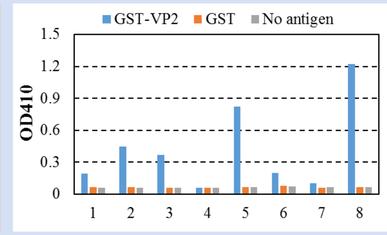
Coating antigen: 100 ng/well
Serum dilution: 1/20



Coating antigen: 100 ng/well
Serum dilution: 1/50



Coating antigen: 200 ng/well
Serum dilution: 1/20



Coating antigen: 200 ng/well
Serum dilution: 1/50

Figure 6. Indirect ELISA detecting swine IgG response to SVV in serum samples collected from experimentally infected piglets. 1~5: serum samples collected from SVV infected piglets at 14 dpi or 21 dpi; 6 and 7: serum samples collected from SVV negative piglets; 8: mouse mAb against His-tag.

RESULTS AND DISCUSSION

- The recombinant SVV VP2 protein was expressed in *E coli*. and purified as a soluble protein. The specificity of the protein was confirmed by Western blot (Figure 3B) and the purity of the protein was determined by SDS-PAGE (Figure 5);
- The purified SVV VP2 protein was used as an antigen for indirect ELISA assay development. Specific IgG response was detected from piglets infected by SVV at 14 and 21 days post infection (Figure 6).
- Further validation of this ELISA is needed, including the determination of test cutoff value, diagnostic sensitivity and diagnostic specificity, as well as the comparison to that of FMDV specific ELISAs.
- Outcomes of this study provide additional tools to aid in SVV and FMDV epidemiological surveillance and outbreak investigation.