

AN EMERGING PORCINE SAPELOVIRUS IN THE UNITED STATES: GENETIC CHARACTERIZATION AND DIAGNOSTIC TOOL DEVELOPMENT

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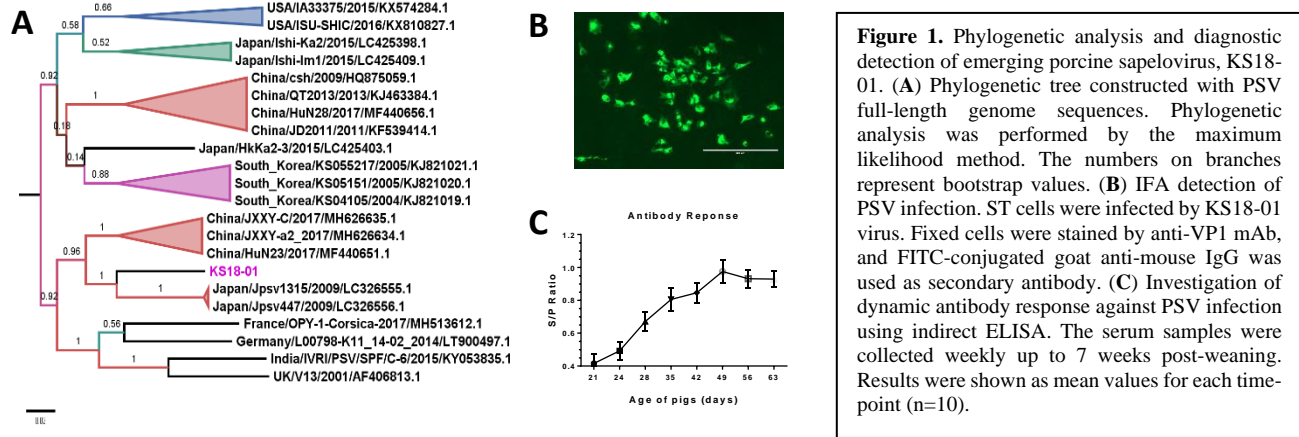
Abstract: An emerging porcine sapelovirus was isolated in a diagnostic specimen from a US swine farm, designated as PSV KS18-01. Full-length genome sequence was obtained through next-generation sequencing. Phylogenetic analysis showed that the virus is more closely related to two Japanese strains but is distantly related to two known US strains. PSV specific diagnostic tools were developed, including the monoclonal antibodies against VP1 and VP2, and a VP1-VP2 antigen-based indirect ELISA. Using this assay, the dynamic response of PSV antibody was investigated in a group of post-weaned pigs that naturally exposed with PSV. The availability of the PSV isolate (KS18-01) and the specific diagnostic reagents and assays provide important tools for PSV control and prevention.

Porcine Sapelovirus (PSV), previously named as porcine enterovirus 8, belongs to the genus *Sapelovirus* in the family *Picornaviridae* (1). PSV is a non-enveloped, positive-sense single-stranded RNA virus. It has a genome of approximately 7.5 kb, with a typical genomic organization of 5' untranslated region -Leader-polyprotein (P1-P2-P3)-3'UTR. The polyprotein is synthesized and proteolytically cleaved into seven non-structural proteins (2A, 2B, 2C, 3A, 3B, 3C, 3D) and four structural proteins (VP4, VP2, VP3, VP1) (2). The PSV capsid is icosahedral arrangement with VP1, VP2, and VP3 on the surface, while VP4 embedded in the internal side of the virion. Host antibodies are mainly directed toward the viral capsid proteins, in which VP1 was reported having the ability to stimulate strong antibody response during the infection (2).

PSV infection is commonly asymptomatic, but clinical disease of respiratory failure, diarrhea, reproductive disorder, and polioencephalomyelitis have been reported in swine farms from many countries (3-5). Neurotropic strains have been reported from China, India, the United Kingdom, and the United States (US). However, little is known about the disease prevalence, morbidity and mortality caused by PSV infection. PSV isolates and diagnostic tools are needed for the studies of PSV pathogenesis and epidemiology.

In this study, we isolated an emerging PSV in a fecal sample from the US swine farm. The virus isolation was performed on ST cells using the method as described previously (6). The new isolate was designated as PSV KS18-01. The full-length genome sequence was obtained through metagenomic sequencing (7). The genome size of KS18-01 was 7,453 bp, encoding a polyprotein of 2,316 amino acids (aa). Compared to other PSV strains, KS18-01 genome has 79.88-87.91% nucleotide (nt) identity. The 5'-untranslated region (5'-UTR) is the most

conserved region of the genome, while the most variable region is the VP1 gene with 72.13-88.8% nt identity (78.2-97.5% aa identity) to other strains. Phylogenetic tree was constructed with all available PSV sequences in GenBank using Maximum-likelihood method (MEGA 7). KS18-01 were grouped together with two Japanese strains, Jpsv1315 and Jpsv447, but is distantly related to two known US strains with 85.5% nucleotide (nt) identity to ISU-SHIC strain, and 85.7% nt identity to USA/IA33375/2015 strain (Fig. 1A). This suggests that KS18-01 emerged as a novel strain in the US.



Next, we developed specific diagnostic reagents and assays for detection of PSV infection. Initially, recombinant proteins of VP1, VP2, and VP1-VP2 were generated by cloning and expression of corresponding genes of KS18-01 isolate using the methods described previously (8). The recombinant VP1-VP2 protein was expressed as a linked protein with GGGGS linker between VP1-VP2. Individual VP1 or VP2 antigen was used to immunize BALB/c mice for monoclonal antibody (mAb) production using the methods as we described previously (9). A total of nine mAbs were generated for VP1 and VP2. This panel of mAbs were tested in IFA using the ST cells infected with KS18-01 virus. All mAbs showed strong reactivity with the virus (Fig. 1B). Specificity of these mAbs were further confirmed in Western blot analysis using the lysate of infected cells. VP1 was specifically detected as a 28.3 kDa protein band, while VP2 was specifically detected as 26.1 kDa protein band.

Subsequently, we developed indirect ELISA for serological detection of pig antibody response against PSV infection. When comparing VP1, VP2 and VP1-VP2 as the coating antigen for ELISA, the linked protein VP1-VP2 showed the highest optical density (OD) reading. Thus, VP1-VP2 protein was used for ELISA validation. A total of 604 serum samples (503 positives and 101 negatives) from experimental animals were used, and their infection status was verified by IFA using KS18-01 infected ST cells. Receiver Operating Characteristic (ROC) analysis (MedCalc) determined the optimal cutoff of 0.6 with maximal diagnostic sensitivity of 99.2% and diagnostic specificity of 97.0%. Using a single lot of internal control serum, PSV ELISA exhibits a within-plate CV of 5.13%, within-run CV of 5.96%, and between-runs CV of 7.73%, indicating this assay is highly repeatable.

The PSV ELISA was further applied to investigate the kinetics of host antibody response in a group of commercial piglets after natural exposure of PSV infection. A total of ten post-weaning piglets were monitored from three-nine weeks old. Blood samples were collected weekly. Results showed that during the first week post-weaning (three-week-old), antibody

response was very weak; after that, an increased pattern of antibody response was observed with the antibody level peaked at 28 days post-weaning (49 days of age) (Fig. 1C). This result correlates with the amount of viral RNA detected in fecal samples.

Clinical signs were monitored daily for this group of pigs. One of the pigs showed neurological signs of circling and abnormal gait for 2 days during the six weeks post-weaning. Rest of the pigs did not exhibit any clinical signs. It is unclear whether the neurological signs were directly related to PSV infection, and whether immune response had protected rest of the pigs from the disease. PSV has been reported to be associated with multiple diseases, including diarrhea, reproductive failure, neurological disorder, but most of the pigs are commonly asymptomatic (10). The pathogenic outcomes resulted from the infection of different PSV strains are unknown. So far, only one Korean study fulfilled the Koch's postulates of PSV caused enteritis and diarrhea in piglets (11). Additional pathogenesis studies are required for in depth characterization of different PSV strains, especially the newly emerging strains. The virus isolate, diagnostic reagents and assays generated in this study will be important tools in aid of future pathogenesis studies as well as development of vaccines and therapeutics against PSV infection.

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