Testing for Senecavirus A in Biological Products

Background

Senecavirus A (SVA), also known as Seneca valley virus, is a positive single stranded RNA virus belonging to Picornaviridae family. The virus causes disease in young pigs. The pathological lesions in infected animals are similar to reportable diseases such as foot and mouth disease, vesicular stomatitis, swine vesicular disease, and vesicular exanthema. Disease outbreaks have been reported in Australia, Brazil, New Zealand, China, and Canada. Outbreaks have increased in the United States rapidly over the last few years.

At the 2017 annual meeting of the American Association of Swine Veterinarians (AASV), a biologics company reported SVA contamination in two lots of porcine-derived trypsin. The identification was made by whole genome sequencing and virus isolation. The finding raised concerns with pork industry representatives regarding the possibility of SVA contamination in veterinary biological products, including those already licensed and in commerce. In response, the Center for Veterinary Biologics (CVB) immediately initiated testing vaccine samples available in the CVB repository that were known to be manufactured using porcine-derived ingredients. The repository contains samples of all vaccines on the market that have current expiration dates.

Selection of Biological Product Samples

CVB prioritized the highest risk biologics for testing. Those included biologics used in swine and manufactured using swine serum and swine-derived trypsin. For their initial testing, CVB selected forty-two (42) samples of high risk biologic serials from the repository.

Testing Procedure

CVB included trypsin lots routinely used, including the two trypsin lots identified as contaminated during the AASV meeting. For positive controls, CVB used trypsin and vaccine samples spiked with control plasmids or with SVA. For subsequent testing, CVB incorporated thirty-five (35) CVB repository available serials of inactivated autogenous and mycoplasma bacterins.

CVB screened multiple cell lines. For the biologics sample testing, they chose the fast growing and easily available chicken embryo fibroblast (CEF) cell culture substrate for SVA isolation. They adopted the sensitive Fowler real-time rt-qPCR testing method for amplification and screening of viral RNA. RNA was extracted directly from the biologics samples, serum and trypsin samples, and the infected CEF cell cultures. Independent confirmatory testing was completed at Iowa State University (ISU) laboratory in Ames, Iowa and VS' Foreign Animal Disease Diagnostic Laboratory (FADDL) in Plum Island, New York.

Results and Discussion

The testing results are summarized in the Tables below. CVB testing found all biologics samples screened negative except for two vaccine serials manufactured by one firm that was positive for

SVA nucleic acid. Both of the vaccine serials tested positive in confirmatory testing at the ISU laboratory as well as at FADDL. Of the several trypsin and swine serum samples screened, CVB only found one serum lot positive by rt-qPCR. ISU and FADDL confirmed this result. This serum lot was used during the manufacturing of the two serials that were identified as SVA contaminated in the initial CVB screening. All control samples performed as expected. To date, CVB has not found vaccine serials, serum, or trypsin samples positive for SVA contamination during screening.

CVB further characterized the SVA present in two PCR positive vaccine serials and one PCR positive serum sample found during their initial screening. The quality of both the cDNA necessary for nucleic acid library preparation, and the viral RNA for direct sequencing was compromised because the contaminated serum used in the preparation of the two serials was inactivated by gamma irradiation at 47kGy. Thus, the whole genome sequencing (WGS) method failed to detect SVA in any of these three positive samples. However, PCR amplification and Sanger Sequencing attempts were useful in successfully generating an SVA-specific 1.8kb nucleic acid fragment. This SVA nucleic acid fragment was similar to the SVA-OH2 strain isolated in 2015 and available at GenBank with identification #KU058183.1. **Despite the identification of SVA nucleic acid in these samples, CVB did not detect viable virus in any of the samples tested.**

Table 1. Susceptibility of Various Cell Lines for SVA Infection

Cell Line Type	SVA Titer in Logs $TCID_{50}$	Virus Amplification Identification by	
		CPE*	IFA*
B-ST cells	8.2	+	+
CEF	8.1	+	+
CrFk	7	+	+
DF-1	5.8	+	+
GPC	9.5	+	+
H1299	8.8	+	+
MA-104	6.9	+	+
MDBK-A	7.6	+	+
MDCK	6.6	-	+
McCoy	6.2	-	+
PK-15N	7.5	+	+
PK-13B	6	+	+
VERO	7.3	+	+

*CPE=Cytopathogenic effect; IFA=Immunofluorescent Antibody test

Table 2. Biologics & Animal Origin Ingredients Test Results for SVA Contamination

Sample Type	Serials/Lots/Samples (Total #)	Serials/Lots/Samples Identified Positive by	
		Cell Culture Test (Total #)	PCR Method (Total #)
Modified Live Virus Vaccines	42	0	2
Autogenous Vaccines	7	0	0
Mycoplasma Bacterins	28	0	0
Serum lots	4	0	1
Trypsin lots	6	0	0
Spiked CEF samples	2	2	NA
Spiked PCR Samples	7	NA	7

Conclusions

CVB could not reproduce the results presented at AASV meeting. CVB tested 77 biologics samples with the highest risk of contamination with SVA. CVB found two vaccine serials, which were manufactured by a single firm using the same lot of swine serum, positive for SVA nucleic acid. CVB testing **did not detect viable virus in any of the samples**, and testing supports that the **serum had been adequately irradiated to inactivate the virus**. CVB found none of the screened porcine-derived trypsin samples positive for SVA. **Based on CVB's testing, it is reasonable to conclude that there is no evidence to suggest viable SVA is present as an extraneous agent in any of the vaccines that were evaluated. Further, it is very unlikely that SVA was spread by contaminated vaccines.**

The CVB has implemented SVA contamination screening for all incoming Master Seeds, incoming Master Cells, and materials with animal origin ingredients. The CVB published a draft notice recommending biologics manufacturers implement SVA contamination screening of all seed materials and animal origin ingredients.

Also, the CEF cell culture system is non-permissive to FMD virus amplification. Plans are underway at the CVB to use lamb kidney and BHK21 cells for standardizing a test for viable SVA contamination detection, which will also detect FMD in animal origin ingredients. CVB is working with industry to review all testing of animal origin ingredients regulations and will propose updated guidance in the near future.