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OTHERS

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Identification and genetic comparison of VP1 gene of Seneca Valley Virus in Brazil

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Introduction: Vesicular disease has been described recently in Brazil and was associated to Seneca Valley virus (SVV). The clinical presentations were characterized by vesicular lesions in sows and acute losses of neonatal piglets. SVV has been identified in swine herds from United States, Canada, Australia, Italy, China and New Zealand. Few reports in Brazil characterized the virus circulating in outbreaks in the country and performed the phylogenetic analysis to better understand the epidemiology of the virus. Based on that, the objective of this study was to identify the SVV circulating in Brazil and perform the phylogenetic comparison with other virus from Brazil, US and China.
Materials and Methods: Thirteen samples: vesicular fluid (n=1), lungs (n=5), lymph nodes + spleen (n=7), submitted to our laboratory from clinical vesicular disease had the total RNA extracted using RNeasy™ kits (Qiagen) The RNA (10 µl) was used as the template in a one-step RT-PCR with the primers SVV-1C556F (5'-TCGGTTTACTCCGCTGATGGTTGG-3') and SVV-2A22R (5'-AGGACCAGGATTGGTCTCGATATC-3')destined from SVV VP1 gene and using the following cycling conditions: 30 min at 42 °C, 5 min at 94 °C, and then 35 cycles of 1 min at 94 °C, 1 min at 55 °C and 1.5 min at 72 °C, followed by 5 min at 72 °C and held at 4 °C. PCR products were analyzed by electrophoresis using 1.5% agarose gels, ran at 120 Volts for 40 min and visualized using a gel documentation system. The positive samples had the DNA sequencing performed and compared with other SVV sequence available at online public database. The RNA samples were also tested for vesicular stomatitis.
Results: The vesicular fluid sample was PCR positive for SVV and all samples were PCR negative for vesicular stomatitis. The SVV positive sample shared 99% of identity with the Brazilian SVV strains: SVV/BRA/GO3/2015, SVV/BRA/MG2/2015 and SVV/BRA/MG1/2015 and 98% of identity with strains BRA/UEL-SVV-A1/15 and BRA/UEL-SVV-B2/15. To North American SVV strain USA/IA40380/2015 shared 97% of identity and to USA/SD41901/2015 and USA/IA46008/201 shared 96% of identity. And to a Chinese strain CH-01-2015 was observed 96% of identity.
Conclusion: A considerable sequence identity between our strain and the other Brazilian strains were found. Evidence of the introduction of the SVV and the distribution of this virus in the country still unclear, however, further studies are being made to better understand and identify the distribution of SVV in Brazil. The characterization of this virus could contribute to future control strategies, especially to avoid positive replacement animals.

Disclosure of Interest: None Declared

Keywords: Brazil, SVV, Vesicular disease

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Whole Genome Sequencing on the Identification of Pathogens Involved with High mortality in Piglets

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Introduction: Idiopathic vesicular disease (IVD) is a sporadic condition that affects swine herds worldwide. At the end of 2014, swine herds across Brazil showed outbreaks similar to IVD causing high mortality and the presence of vesicles in piglets, and erosions on the snouts and coronary bands of sows. Samples of infected animals were shipped to Lanagro (Agriculture Ministry Official Laboratory) and tested for vesicular diseases including foot and mouth disease with negative result. Some studies have identified the association of *Seneca valley virus (SSV)* in pigs affected with IVD. Therefore, a metagenomic study in samples from brain, kidney and vesicles fluid and epithelium (VFE) of infected animals was carried out as a diagnostic tool to identify possible pathogens involved with this condition using two approaches.
Materials and Methods: Approximately 0.05 g of tissue samples from brain, kidney and VFE of infected pigs were submitted to RNA and DNA extraction at the Embrapa Swine and Poultry NB3 Lab. Approximately 2ug of RNA was reverse transcripted using high capacity cDNA kit and random primers. DNA and cDNA from individual tissue samples were pooled together from 3 animals for each tissue, and shipped to PathGEN Dx Pte. (Singapore). The first pathogen screening was conducted with the PathGEN® PathChip, which contains 50,000 viruses and 20,000 bacteria, developed by Affymetrix®. Following the identification of the possible infectious agents, the DNA and cDNA samples were enriched by RT-PCR using PathGEN primers, and the sequencing library was created using standard protocol. Raw pair-end reads were generated with the Illumina MiSeq (2x250bp). Following sequencing the *Seqyclean* program was used to clean the data, removing adapters, contaminants, short reads < 70bp, and reads with Phred < 20. All reads were mapped using BWA program against two known Seneca Virus sequences and assembled using Newbler.
Results: Using the PathGEN® chip we identified partial sequences of human rhinovirus (HRV) only in Brain samples. The SVV has a structural similarity up to 40% with HRV which can be responsible for the results. Using the MiSeq technology for sequencing allowed us to build partial fragments of the SVV in two samples (brain and kidney), and the complete sequencing in VFE of infected animals.
Conclusion: SVV sequence was identified and associated with IVD observed in Brazil since 2014. SSV was found in three different tissues: brain, kidney and VFE when sequencing approach was used, and only in the brain using PathChip. The whole SVV sequence assembled in this study shows some variations when compared to the ones published.

Disclosure of Interest: None Declared

Keywords: Metagenomic Sequencing, PathGEN®, Seneca Virus