Autores cristine cerva [1], Fabiana Quoos Mayer [1], Márcia Regina Loiko [2], Ana Paula Muterle Varela [2], Samuel Paulo Cibulski [2], Thais Fumaco Teixeira [2], Diane Alves Lima [2], Paulo Michel Roehe [2]

Instituição [1] SEAPI - Secretaria da Agricultura Pecuária e Irrigação (Av. Getúlio Vargas, 1384 - Menino Deus, Porto Alegre - RS, 90150-004), [2] UFRGS - Universidade Federal do Rio Grande do Sul (AVENIDA SARMENTO LEITE, 500, PORTO ALEGRE – RS, BRASIL - CEP 90035-190)

Resumo

Porcine circovirus associated disease (PCVAD) is one of the causes of negative economic impact on pig farming systems described worldwide. Losses include expenditures with treatment, increased mortality rates, and decreased productivity. One of the most relevant manifestations of PCVAD is the post-weaning multisystemic wasting syndrome (PMWS). The main pathogen present in PMWS is porcine circovirus type 2 (PCV2). However, observational and experimental studies have shown that other agents may be involved in the pathogenesis and clinical manifestation. High-throughput sequencing combined with metagenomics analyses make it possible to identify the total microbiota in a given sample, regardless of microorganism culture. In order to contribute to the knowledge of the viruses involved in PMWS, the present study carried out the high-throughput sequencing of swine sera and subsequent analysis of the resulting metagenome. Sixteen serum samples collected on a farm in Rio Grande do Sul, from 80 and 100 days old pigs with clinical signs of PMWS, were examined. Data revealed that in addition to PCV2 sequences, porcine parvovirus type 1 through 6 (PPV1 to 6) were recovered from samples of pigs affected by PMWS. The PPV1 capsid protein sequence identified in this study presented one of the mutations found only in the pathogenic strains of this virus, suggesting PPV1 involvement in the disease. The conserved motifs found on parvovirus capsid proteins have catalytic properties and are related to virus entry into host cells inducing viral infectivity. In this study, PPV2, 3, 5 and 6 were shown to have these conserved domains, indicating that these viruses may be involved in the development of PMWS. The occurrences of PCV2 and PPV1 to 5 have already been described in pigs with PMWS, so this study reinforces previous results. PPV6 was recently described in China, Europe and the United States, and the studies did not correlate the virus to any specific disease. The present study is the first report of PPV6 in pigs presenting PMWS signals. However, further studies are necessary to be able to attribute the relationship between PPV6 in the development of SMDS.

Palavras-chaves: High-throughput sequencing, metavirome, PCVAD, PCV2, PMWS

NOVEL H1N2 SWINE INFLUENZA VIRUS REASSORTANT STRAIN DERIVED FROM THE PANDEMIC H1N1/2009 VIRUS

Autores Rejane Schaefer [1], Danielle Gava [1], Vanessa Haach [2], Janice Reis Ciacci Zanella [1], Maurício Egidio Cantão [1]

Instituição [1] EMBRAPA SUÍNOS E AVES - Empresa Brasileira de Pesquisa Agropecuária (BR153, Km110, Vila Tamanduá, Concórdia, SC), [2] ICBS/UFRGS - Universidade Federal do Rio Grande do Sul (Rua Sarmento Leite, 500 Porto Alegre, RS)

Resumo

Respiratory infections in swine caused by influenza A virus (IAV) represent a burden for the swine industry and a threat for public health due to the emergence of viruses with pandemic potential. IAV subtypes H1N1, H1N2 and H3N2 are commonly found in Brazilian swine herds associated with mild to severe acute respiratory disease cases, as the only pathogen, or in association with other viral or bacterial agents. Recently, an increase in the viral genetic diversity in swine has been detected after reassortment events between pandemic H1N1 influenza (H1N1/2009) virus and human origin H1N2 and H3N2 viruses. Herein, we describe the whole-genome sequencing of three H1N2 IAVs (28/15-1, 28/15-2 and 65/15-2) isolated from pigs in 2015 in farms located in the states of Santa Catarina and Paraná. Nasal swab and lung samples collected from pigs with respiratory clinical signs tested positive for IAV by reverse-transcription PCR targeting the matrix gene. Virus isolation was performed in SPF chicken eggs and the isolated viruses were subtyped by RT-PCR as H1N2. Virus RNA was extracted

from allantoic fluids of infected eggs and the eight gene segments were amplified by RT-PCR using PathAmp FluA reagents. DNA libraries were prepared and submitted for sequencing using Ion Torrent system. Influenza genomes were assembled using Newbler V 2.9 with high coverage (180x). Nucleotide alignments of the hemagglutinin (HA) and neuraminidase (NA) gene segments were generated for related human and swine IAVs, collected globally and downloaded from the Influenza Virus Resource available in GenBank. The phylogenetic relationships of the datasets were inferred by using the Neighbour Joining method. The phylogenetic analysis showed that all genes of the three viruses, with the exception of the neuraminidase (NA), belonged to the H1N1/2009 cluster. The HA sequence of 28/15-1, 28/15-2 and 65/15-2 influenza viruses shared 98 to 99% of nucleotide identities. The NA segment of H1N2 viruses was closely related to an H3N2 virus that was introduced in swine in Brazil in the late 1990's. Interestingly, H1N2 viruses (28/15-1 and 28/15-2) were isolated from pigs of the same farm in which H1N1/2009 and H3N2 IAVs were also circulating. This study highlights the importance of performing full genome sequencing of influenza virus isolated from swine in order to detect novel reassortant viruses that might represent a threat for humans.

Palavras-chaves: Influenza A virus, Pandemic H1N1/2009 influenza virus, Reassortant, Swine

EVALUATION OF BOVINE VIRAL DIARRHEA VIRUS EXCRETION BY RT-PCR IN SEMEN OF BULLS FROM CATTLE HERDS FROM MATO GROSSO STATE

Autores: Sandro Ribeiro da Costa [1], Raphael Campos Quinteiro [1], Gabriela Molinari Darold [1], Glaucenyra Cecília Pinheiro da Silva [1], Paulo Victor Braga de Almeida Santos [1], Amauri Alcindo Alfieri [2], Michele Lunardi [1]

Instituição [1] UNIC - Universidade de Cuiabá (Rua Manoel José de Arruda, 3100, Jardim Europa, Cuiabá - MT. CEP: 78065-900), [2] UEL - Universidade Estadual de Londrina (Rodovia Celso Garcia Cid, PR 445 KM 380, Londrina - PR. CEP: 86051-990)

Resumo

Pestivirus infections in ruminants are associated with several diseases affecting cattle, with relevant impact on animal production. Bovine viral diarrhea virus (BVDV) belongs to *Pestivirus* genus of the *Flaviviridae* family and has a single-stranded positive-sense RNA genome. In view of the consequences caused by the BVDV, a better understanding of the venereal transmission in the livestock is relevant. Currently, beef industry of Mato Grosso stands out as having the largest cattle herd in Brazil and the main structure for exporting meat production. Therefore knowing the role of bulls as disseminators of the virus through semen is necessary so that adequate strategies of control and prevention can be implemented and directed to the management practices in the state. This study aimed to investigate the excretion of BVDV in semen of bulls from beef cattle of Mato Grosso state. A total of 64 bulls, aging 28 weeks to 10 years old, from non-vaccinated beef herds without a history of reproductive failure, from six municipalities, representing four out of the six macroregions of the state, were evaluated. RNA was purified with TRIzol LS from DEPC-water diluted fresh semens. In order to amplify the 5'UTR region of the BVDV genome, a RT-PCR assay employing M-MLV reverse transcriptase, Platinum *Taq* DNA Polymerase and the primer pair 324/326, was carried out. In this study, the excretion of BVDV through semen was not detected in the 64 beef bulls, evidencing that these animals apparently were not persistently or acutely infected by this viral agent by the time of collection of the semen. Importantly, negative semen samples spiked with BVDV isolated in cell culture, and used as positive controls, yielded amplicons with the expected length in all RT-PCR analyses. The absence of BVDV excretion through semen of evaluated bulls may be explained by the fact that all the animals analyzed belonged to cattle herds with extensive management, characterized by reduced herd turnover and reproduction by natural mating system. Due to previous