3. UFOP - Universidade Federal de Ouro Preto, Campos Morros do Cruzeiro, s/n - Bauxita, Ouro Preto - MG, 35400-000

The emerging infectious diseases play an important role in public health, and zoonoses have a great part in this scenario. In the context of emerging viruses, it is believed that with environmental degradation, some species of small mammals are now approaching anthropic disturbed areas, increasing contact with both human populations with domestic animals and herds, which could increase the flow of zoonoses between natural and anthropic system. Among the viral agents associated with these zoonoses, the following families should be highlighted: Bunyaviridae, Flaviviridae and Poxviridae. The aim of this study is to develop and validate a qPCR platform to detect potential zoonotic viral agents circulating in rodents distributed in the geographical extension of the state of Minas Gerais by qPCR. This technique was chosen for its high sensitivity and robustness considering the possibility of numerous samples. For positive controls specific sequences were chosen and assembled in gene blocks (gBlocks™), cloned into pGEM-T easy vector (Promega), and used for the validation of the designed primer pairs. Specific primers were designed for all viruses of interest including Apeu virus (APEU), Juquitiba virus (JUQV), Yellow Fever virus (YFV), St. Louis encephalitis virus (SLEV), Vaccinia virus (VACV) and Pseudocowpox virus (PCPV). Standard curves ranging from 1.0ng to 1.0fg were prepared, reactions were performed using the SYBR® Green PCR Master Mix (Applied Biosystems), and the detected efficiency for each primer pair were: 94% (APEU), 108% (JUQV), 103% (YFV), 99% (SLEV), 96% (VACV) and 96% (PCPV). The linearity coefficients (R2) were close to 1. These initial results indicate that the platform can be used for detection of the targeted viruses in field samples, which will be prepared and used as template for our reactions in the near future.

VV145 - MUTATIONS IN THE CAPSID PROTEIN OF PORCINE CIRCOVIRUS TYPE 2 INDICATE A VIRAL IMMUNE EVASION

Gava, D.[1]; Schaefer, R.[1]; Serrão, V.H.B.[2]; Cantão, M.E.[1]; Silva, M.C.[3]; Zanella, J.R.C.[1]

1. Embrapa Swine and Poultry Research Center, Animal Health Laboratory, EMBRAPA

2. Institute of Physics of São Carlos – University of São Paulo-USP

3. Diagnostic Center For Animal Health (CEDISA)

Postweaning multisystemic wasting syndrome, the most common clinical manifestation of porcine circovirus type 2 (PCV2) diseases (PCVD), was first described in 1996 in Canada and in 2000 in Brazil. PCVD has caused economic losses with high mortality rate in Brazilian pig farms until the introduction of PCV2 vaccine in 2008. During December of 2012, a suspect PCVD case was reported in 57-67 days-old pigs in a vaccinated wean-to-finish farm in Southern Brazil. The affected pigs showed coughing, dyspnea, enlargement of inguinal lymph nodes, wasting and diarrhea. The objective of this study was to diagnose and characterize the PCV2 infection in a PCV2 vaccinated pig herd. Lymph node, kidney and lung tissue samples were collected and processed according to conventional methods for histopathology (H&E), real-time PCR and immunohistochemistry (IHC). DNA sequencing was performed by Sanger method. The obtained sequences were analyzed and assembled with Phred/Phrap/Consed softwares. Phylogenetic analyses of the whole genome and the ORF2 gene (capsid protein) were performed by Neighbor-Joining method in MEGA 5.2 software. Using molecular modeling methodology (I-TASSER) a structural model of the capsid protein was obtained. The structural model was validated and the mutated residues were identified. PCV2 infection was demonstrated by H&E and confirmed by IHC. A high viral load was detected by real-time PCR (5.67x1011 DNA copies/uL). Sequence analysis revealed a PCV2-b genotype. The comparison among the ORF2 amino acid sequence with other PCV2 sequences revealed three amino acids substitutions, in domains F57I, N178S and A190T. The results of the structural analysis showed a substantial change on the physical-chemical characteristics of one residue. Moreover, the analysis also indicates a possible disruption in the secondary structure of the capsid protein around the 178 residue which is an important region for antibodies recognition. Therefore, modifications in the viral protein conformation could have caused an inefficient antibody binding that was observed by the lack of vaccine efficacy. This mechanism could explain the recent vaccine failures detected in swine in Brazil. However, additional studies

using in vivo and in vitro models are needed to confirm this hypothesis.

VV155 - PESTIVIRUS CONTAMINATION IN CELL CULTURES

da Silva, M.S.; Mósena, A.C.S.; Weber, M.N.; Silveira, S.; Torikachvili, M.; Dupont, P.M.; Budaszewski, R.F.; Streck, A.F.; Canal, C.W.

UFRGS - Universidade Federal do Rio Grande do Sul, Avenida Paulo Gama, 110 - Farropilhas, Porto Alegre - RS, 90040-060

The genus Pestivirus of the family Flaviviridae compriseS RNA viruses that often cause diseases in ruminants and pigs. Pestiviruses contamination in cell cultures and fetal calf serum (FCS), widely used as a nutrient in cell culture, is frequently reported in the literature. Viral contaminations may negatively affect diagnostic tests, vaccine production and research. In this study, 41 cell lines of different animal origins were analyzed by the reverse transcription-polymerase chain reaction (RT-PCR) targeting the 5′ untranslated region (UTR). Eighteen (43.9%) samples were positive and the amplification products from eight were sequenced. Phylogenetic analyses revealed that five strains are closely related to BVDV-1, two to BVDV-2 and one to 'HoBi'-like viruses. The current findings reinforce the need for a constant pestivirus control in FCS and cell cultures to keep the biological safety and correct diagnostic results. Financial support: CAPES, CNPq, FAPERGS and Propesq/UFRGS.

VV166 - BETACORONAVIRUS LINEAGE 2A DETECTION IN YOUNG ALPACAS (VICUGNA PACOS) FROM FARMS IN ANDEAN REGION OF SOUTHERN PERU

Luna, L.; Gregori, F.

LABMAS/USP - Laboratório de Biologia Molecular Aplicada e Sorologia. Departamento de Medicina Veterinária Preventiva e Saúde Animal da Universidade de São Paulo, Av. Prof. Dr. Orlando Marques de Paiva, 87, São Paulo – SP, 05508-270

Coronavirus is a large family of viruses that may cause a diversity of symptoms (e.g. respiratory, enteric, nervous), infecting many animal and human hosts. Considering the high mortality rate in young alpacas due to occurrence of diarrhea and data regarding the etiology is scarce, the objective of this work was to detect coronavirus in 50 stool samples of young alpacas from 3 farms located at the Andean region of southern Peru by a RT-nested PCR test targeting a fragment of RpRd gene (RNA dependent RNA polymerase) followed by nucleotide sequencing. A total of 11 samples resulted positive and the nucleotide phylogenetic analysis of sequences demonstrates that all were characterized as Betacoronavirus Lineage 2a, a group frequently found in both domestic and wild ruminants. These results confirm coronavirus circulation in the sampled area and may be implicated with the diarrhea in young alpacas.

VV167 - ANALYSIS OF ANTIBODY LIBRARY VARIABILITY FROM CHICKEN IMMUNIZED WITH BOVINE HERPESVIUS TYPE 1

Japolla, G.; Almeida, R.G.; Penido, B.A.F.; Brito, D.E.M.W.; Souza, L.R.G.

UFG - Universidade Federal de Goiás, Av. Esperança, s/n - Setor Itatiaia, Goiânia - GO, 74001-970

The bovine herpesvirus type 1 (BoHV-1) is a member of the family Hesperviridae and is recognized as an important agent of economic loss in cattle. The economic impact caused by the BoHV-1 is expressed, among other features, by embryonic death, abortion, birth of debilitated animals, reduced reproductive efficiency of cows and bulls, poor feed conversion and growth delay in calves. In order to produce and analyze the variability of antibody library, two chickens were immunized with BoHV1 (105.5 DICC/50mL). Then the chickens were euthanized and their spleens were quickly removed for total RNA extraction and cDNA synthesis. The light and heavy chains of antibodies genes were amplified to produce the single chain Fv fragments (scFv). The whole scFv repertoire were cloned into phagemid vectors, expressed as fusion proteins in filamentous bacteriophages and amplified by the E. coli bacteria infection. Plasmidial DNA from 15 clones was extracted and the product was analyzed in agarose gel 2%. The DNA was digested with the restriction enzyme BstNI in order to evaluate the variability of the library through the analysis of the restriction fragments. The results showed that only two of the 15 clones share the same fragment size characterizing the other 13 as unique clones with specificities theoretically distinct. In another study, this variability of antibodies generated by chickens has not been verified, probably due to immunodominânce of some antigen. In this work, the chickens didn't live