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rotavirus escapes from the intestinal tract. In this study, we report the experimental infection of nine infant-juvenile cynomolgus monkeys (*Macaca fascicularis*) using a human rotavirus A (RV-A Wa) produced in cell culture. The aim was to assess the suitability of the cynomolgus as a model of rotavirus infection and diarrhea. Six animals were inoculated orally with RV-A Wa by catheter, and three animals were administered orally with saline solution (control group). Clinical and corporal temperatures were monitored every day. The blood was collected in 0, 1st, 3rd, 7th and 10th days post infection (dpi) for measurement of total white blood cells, hematocrit and electrolytes levels. Faeces were collected daily from the 0 to the 10th dpi. Both samples were tested to the rotavirus presence by RT-PCR and qPCR. The study was approved in Ethics Commission for the Use of Animals - CEUA/Fiocruz (LW-35/11). The monkeys inoculated with rotavirus had the subclinical infection form. Every biochemistry and hematological parameters had low alterations comparing animals inoculated with control group animals, but any statistical significance was observed in these parameters, and majority animals had no signals, except one animal, which had occurrence of diarrhea for three days. Nevertheless, the infection occurred in all inoculated animals, the RNA rotavirus was detected in faeces and serum. This monkey model can be used in future to evaluate the efficacy of immunoglobulin Y immunotherapy in rotaviruses disease.

IV495 - CAMELID NANOBODIES, AN ALTERNATIVE TO DIAGNOSIS HANTAVIRUS INFECTION

Pereira, S.S., Fernandes, C.F., Prado, N.D.R., Morais, M.S.S., Luiz, M.B., Moreira, L.S., Mazzarotto, G.A.C.A., Strottmann, D.M., Soares, A.M., Santos, C.N.D., Stabeli, R.G.

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Hantaviruses that belong to the Bunyaviridae family can cause Hantavirus pulmonary syndrome (HPS) in the American continent. The infection in human occurs through inhalation of aerosolized excreta from chronically infected rodents and the association of the disease with

different rodent reservoirs in several geographic areas suggests the development of region-specific antigens. HPS is characterized by fever and vascular leakage, resulting in noncardiogenic pulmonary edema followed by shock. With a case-fatality rate about 50%, a rapid and accurate diagnosis during the early course of the disease is essential to reducing the high mortality rate associated with hantavirus infection. Camelids produce, in addition to conventional antibodies, IgG composed exclusively of heavy chains, in which the antigen binding site is formed only by the single domain, called VHH or nanobody. This work proposes the use of camelid nanobodies against *Araucaria* hantavirus recombinant nucleoprotein (rNH) of a Brazilian hantavirus to develop alternative methods to diagnosis and confirm hantavirus infection. To generate VHHs, the phage display technology was employed. VHH domains were isolated by RT-PCR using cDNA obtained after RNA extraction from peripheral lymphocytes of an immunized Lama glama. Amplicons were cloned into PHEN1 phagemid vector and TG1 *E. coli* strain to construct a VHH immune library with a titer of $2,3 \times 10^{18}$ cfu/mL. Subsequently, VHH domains were displayed fused to M13K07 phage coat protein III and the selection steps performed on immobilized rNH protein. After two round of selection, 69 individual clones recognized specifically rNH protein by ELISA. The positive clones were sequenced, analyzed and the 11 sequences that showed different profiles deposited into GenBank. One of the selected VHHs was purified by Ni-NTA affinity chromatography and recognized specifically the rNH by ELISA, western blotting and surface plasmon resonance. These findings support the idea that selected VHHs could be an alternative tool to diagnosis hantavirus infections. FINANCIAL SUPPORT: CNPQ

PIV7 - DETECTION OF FOUR VIRUSES IN APPLES AND PEARS BY REAL TIME RT-PCR USING 5'-HYDROLYSIS PROBES

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Apple latent viruses such as Apple stem pitting virus, Apple stem grooving virus, Apple chlorotic leaf spot virus and Apple mosaic virus are commonly found in apples and pears. They are main targets of virus elimination procedures from elite and pre-basic material that usually require evaluation of health by processing a large number of samples. Real time RT-PCR offers substantial advantages over conventional RT-PCR for plant virus diagnosis such as immediate availability of results which obviates laborious gel analysis, reduced sample manipulation that reduces amplicon contamination and high sample processing capacity. The objective of this

work was to design primers and probes for a real time RT-PCR protocol for detection of ASPV, ACLSV, ASGV and ApMV. Specific probes labeled with FAM/TAMRA and primers were designed by searching for highly conserved nucleotide fragments in the respective coat protein genes of the four viruses using software CLC Sequence Viewer 6, and used to detect the viruses in tissues of apples and pears. Total RNA was extracted from apple and pear bark scrapings and adsorbed on to silicium dioxide. The StepOnePlus Real Time PCR System was used for thermocycling. Results were analysed graphically using proprietary StepOne Software v2.2.2. Related to the previously known viral status based on RT-PCR and/or biological indexing of the analyzed apple samples, 89.2% (25/28), 96.4% (27/28), 100% (28/28) and 88% (22/25) of infections by ASGV, ASPV, ACLSV and ApMV, respectively were confirmed. In pears, recognition of known pre-existing ASPV infections by primers and probe was 100%. Viral infections were confirmed in a selection of the main commercial cvs. of apples and pears. These results demonstrate the sensitivity and reliability of the designed primers and probes for detection of these pathogens. Real Time RT-PCR using labeled probes represents a valuable tool to increase feasibility of processing large numbers of samples and it is therefore well adapted for control of sanitary quality such as required by healthy plant propagation material certification programs. Financial support: CNPq Proc. Nr. 479609/2011-0

PIV277 - STUDY OF THE STATE OF VIRAL INFECTION IN APIARIES IN THE AREA OF THE PAMPA GAUCHO.

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In recent years, we have seen a sharp and worrying decline of the global bee populations, a phenomenon known as Colony Collapse Disorder (CCD), that is seriously threatening beekeeping and crops that depend on bees for pollination. Among the reasons cited for this decline as suspects, are the viruses. Because the swarms are densely populated and have a high rate of contact between the colony members, relating each other for communication and feeding, bee colonies provide great opportunities for viral transmission. The virus can affect all developmental bee stages, including eggs, brood and adults, and drastically reducing honey production and pollination. Among the family of viruses that affect the bees is the Iflaviridae family, with no scientific records in the hives of *Apis mellifera* in the state of Rio Grande do Sul. This study aims to identify the viruses of this family that are present in beehives of different state cities. Adult

workers of *Apis mellifera* and dead pups were collected from six hives of two apiaries. These individuals were processed at molecular biology laboratory of the Federal University of Pampa, Sao Gabriel campus, where we performed extraction of total RNA, cDNA synthesis and PCR with specific primers for viral detection, as well as a multispecies primer that detects three Iflavirus types (Deformed Wing Virus, Kacugo Viruses and Varroa Destructor Virus). Positive results were obtained for the presence of Varroa destructor virus (VDV-1) with a specific primer for this one, as well as viral amplification in different samples using the multispecific primer, suggesting the presence of other viruses. This is the first record of VDV-1 in South America hives. These results allow a better understanding of the problems that affect or may affect the region apiaries, as well as provides subsidies for new viral detections in *Apis mellifera*. Financial support: CNPq

PIV328 - INFECTION OF TOMATO PLANTS BY THE BEGOMOVIRUS TOMATO CHLOROTIC MOTTLE VIRUS (TOCMOV) INCREASES THE EXPRESSION OF UBIQUITINATION PATHWAY GENES

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Ubiquitination is a post-translational modification that controls the degradation of protein in eukaryotes. The substrate targeted by ubiquitin molecules are degraded by the 26S proteasome complex. The ubiquitination pathway involves an enzymatic cascade that tags the substrate by the attachment of ubiquitin molecules with participation of E1 ubiquitin activating enzyme, an E2 ubiquitin conjugation enzyme and an E3 ubiquitin ligase, that confers specificity to the substrate. Several plant viruses show ability to disturb the ubiquitination pathway by inducing, inhibiting or modifying enzymes, mainly E3 ligases. The aim of the present work is to study expression of genes involved in the ubiquitination pathway during the tomato-begomovirus interaction. An mRNA-Seq from cDNAs libraries of inoculated and non-inoculated tomato near isogenic lines Santa Clara (susceptible) and LAM 157 (resistant) was performed and seven genes of the ubiquitination pathway were identified: one E3 ubiquitin-protein ligase, three F-box proteins, two RING finger proteins and one Ubiquitin-conjugating enzyme E2-like protein. These genes showed significant up-regulation (log₂ fold change > 2.0) when plants were inoculated with ToCMoV (Tomato chlorotic