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remain of unknown etiology. In 2008, a2-month-old mixed-breed unvaccinated puppy was presented at a private animal hospital in the city of Rio de Janeiro, Brazil with a history of vomiting, depression and watery diarrhea. At the time of hospitalization, fecal sample was collected and stored at -20ºC prior to virologic examination. Nucleic acid was purified from 10% (w/v) fecal suspensions in Tris-Ca++ using a combination of phenol/ chloroform/ isoamyl-alcohol and silica/guanidin thyocianate and genomic RNA was purified according to published TRIzol® extraction protocols. The diagnostic panel included CPV, canine coronavirus (CCoV), canine calicivirus (CCaV) and rotavirus (RV-A) using either conventional PCR or transcriptase reverse (RT)-PCR. The fecal sample tested negative for CPV and RV, but positive to CCoV and CCaV. The PCR products obtained were purified and then subjected to direct sequencing using BigDye Terminator Cycle chemistry. Nucleotide and amino acid similarity with Genbank database was assessed using BLAST tool. According to phylogenetic analysis carried out within RdRp region, the CCaV strain detected in this study presented 98% of aa identity with other canine and feline calicivirus strains deposited in the GenBank. The phylogenetic analysis of the fragment of the gene encoding for transmembrane protein M of CCoV strains also allowed the characterization of this strains as CCoV-I genotype.These results pointed out the role of CCoV and CCaV in a clinical diahrrea case and may suggest a possible role of RNA virus as agents of enteritis either alone or in mixed in the ethiology of acute gastroenteritis in puppies.

VV396 - PHYLOGENETIC ANALYSIS OF RNA-POLYMERASE BLOCK III OF RABIES VIRUS ISOLATED FROM THE MAIN RABIES RESERVOIRS IN BRAZIL

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The RNA polymerase L of rabies virus (RABV) contains clusters of amino acids (aa) conserved in blocks along of the protein. The clusters are named with roman numbers I to VI; between these six aa blocks has highly conserved domains. The block III, aim of this research, has a catalitic domain, between the 530 and 1177 aa with four motifs (A, B, C and D) that is the more conserved region of protein. Conserved areas in the virus genome, as RABV, could be used in evolution studies. In this study were analyzed 10 RABV strains isolated from the main rabies reservoirs inBrazil, bats (n=5) and canids (n=5). Viral RNA was reverse transcribed and amplified by polymerase chain reaction (RT-PCR) with primers targeting the L gene and the partial nucleotide sequence of the L gene was determined. After the sequences edition was determined the genetic diversity (intragroup variability) and genetic divergence (variability between lineages studied and the others species of lissaviruses). The nucleotide comparative analysis shown variation but the putative proteins sequences shown was conserved. The intragroup diversity between RABV strains was 1.7%. When the RABV sequences studied were compared with other lissaviruses sequences, available in GeneBank, the same mutation standard was identified but with more variation. The higher divergence was identified between the RABV studied and the West Caucasian Bat Virus (10%). This is the first report of RABV RNA-polimerase in Braziland the association between epidemiological and genetic information contributes to greater understanding of the biology of rabies nature, allowing the taking of appropriate controls measures.

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VV397 - DETECTION OF SAPOVIRUS IN FECAL SPECIMENS COLLECTED OF PIGLETS IN SWINE FARMS FROM AMAZON REGION, BRAZIL

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The caliciviruses (CVs) – genera Norovirus (NoVs) and Sapovirus (SaVs) belong to the Caliciviridae family and are related to gastroenteritis in humans and animals. Several strains isolated from pigs had genetic similarities with those observed in humans, suggesting a possible zoonotic transmission of these viruses. The aim of this study was the detection and molecular characterization of CVs in piglets from five swine farms located in the Pará State, Brazil. The stool samples were collected from individual pigs during the phase of nursing (less than 28 days of age) and post weaning (29 to 60 days of age) from January/2008 to February/2009. At the moment of suspensions preparation, the specimens were classified as watery/pasty and normal stools that should correspond to diarrheic and asymptomatic animals, respectively. A total of 130 specimens (69 nursing and 61 post weaning pigs) were collected and tested by reverse transcription-polymerase chain reaction (RT-PCR) using the primers 289/290, for detection of CVs. The positives samples were sequenced and analyzed by BioEdit software (v. 7.0.5.3) and the sequences compared with others registered in GenBank. A positivity of 12.3% (16/130) was observed, being all characterized as SaVs, 14 as belong to genogroup GIII-B and one as GVII. One was not classified. NoVs was not detected in these samples. The frequency of SaVs was higher in nursing (20.3%-14/69) than in post weaning pigs (3.7%-2/61). SaVs was detected in two of the

five (40%) studied farms. About the positive cases, 56.2% (9/16) were classified as diarrheic. The results obtained in this study (12.3%) were similar to the ones described in pigs from Belgium (11.9%) and higher than other realized in Brazil (10%). This study was pioneer in Amazon Region, and demonstrated for the first time the circulation of SaVs in swine farms from Pará State. It also was important considering the lack of information about these agents in Brazil.

HVV398 - GENETIC IMMUNIZATION FOR PAPILOMAVIRUSES CONTROL: CONSTRUCTION OF A VACCINAL VECTOR BASED ON THE GENE L2 Bovine Papillomavirus TYPE 1

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The cattle industry is one of the main highlights of the Brazilian agribusiness on the international stage. However, some diseases have caused considerable damage including bovine papillomatosis which is an infectious tumorous disease related to bovine papillomavirus (BPV) and characterized by the formation of tumors in tissues of the skin and mucosa. Currently we know 11 different types of bovine papillomavirus, among which the BPV types 1, 2 and 4 are oncogenic. So far there is no vaccine or treatment for the papilomaviroses control. Different studies have reported that the L2 protein may be a candidate for the development of prophylactic vaccine strategies against BPV. On this paper, our objective was to construct a vector vaccine from pCINeo plasmid (Promega ®) and the gene of BPV1 L2, evaluating in vitro L2 antigen expression in mammalian cells. The L2 gene was amplified by PCR from the complete genome of BPV1, using specific oligonucleotides containing an AU1 epitope in the forward primer and subsequently cloned into pGEM-T Easy vector (Promega®) and subcloned in expression vector pCIneo, generating the construct pCIL2B1. The construct, pCIL2B1, obtained was confirmed by PCR and sequencing. 293 cells were transfected with pCIL2B1 and the capacity of this construction to express the L2 gene and produce their protein in mammalian cells was confirmed by RT-PCR and western blot (using antibody against the epitope AU1). The results confirmed the L2 gene expression in mammalian cells and the consequent production of the protein L2 BPV-1.

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VV399 - SEROPREVALENCE OF BOVINE HERPESVIRUS 1 AND 5 IN SAO PAULO STATE, BRAZIL

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Studies in Brazil have demonstrated high rates of seroconversion to bovine herpesvirus in cows. The BoHV-1 is considered one of the most important viral agents, manifested by Infectious Bovine Rhinotracheitis (IBR) and Infectious Pustular Vulvovaginitis/ Balanoposthitis (IPV/ IBP), and also associated with conjunctivitis and abortion. The BoHV-5 is the causal agent of herpetic encephalitis. Both are transmitted by nasal exudates, respiratory droplets, genital secretions, semen, fetal fluids and tissues. The detection of antibodies against BoHV-1 and 5 in serum is an important goal to demonstrate the presence of the virus in the herd. Thus, this study aimed to determine the infections prevalence of BoHV-1 and 5 in cattle of Sao Paulo State through a seroepidemiological survey conducted by qualitative analysis of the samples, using the virus-neutralization, a method recommended by the World Organization for Animal Health (OIE). We analyzed 6,902 serum samples from cows aged 24 months or above, no history of vaccination against herpesvirus, from 1,073 properties located around the State, collected from October to December 2001. It was also evaluated serological cross-reactions between antibodies produced against BoHV-1 from those against BoHV-5. Of the total analyzed, 71.53% (4,937/6,902) were reactive for herpesvirus. Among the reactive samples, 92.30% (4,557/4,937) had cross-reaction to BoHV-1 and BoHV-5, 5.51% (272/4,937) reacted only against the BoHV-1 and 1.46% (72/4,937) was reactive only against the BoHV-5. A total of 27,86% (1,923/6,902) of samples did not react to any of the serotypes. In relation to BoHV-1, 70.36% (4.856/6.902) were reactive, 28.93% (1.997/6.902) were negative and 0.71% (49/6.902) contaminated or toxic. Concerning BoHV-5, 67.20% (4.638/6.902) were reactive, 32.01% (2.209/6.902) were negative and 0.80% (55/6.902) could not be assessed due to contamination or toxicity. These results demonstrate the high percentage of serological cross-reactivity between BoHV-1 and 5 and the high rates of reactive animals confirms the significant spread of these viruses in cattle in the Sao Paulo State, Brazil.

VV400 - MOLECULAR DETECTION OF BOVINE VIRAL DIARRHEA VIRUS IN BOVINE FOLLICULAR FLUIDS AND SEMEN

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