one type of CCoV was associated with CPV: CCoV-I/CPV (2), CCoV-IIa/CPV (7), CCoV-IIb/CPV (1) while for five samples two CCoV subtypes (CCoV-I/IIa) were detected. Based on clinical reports, four CCoV-I positive puppies presented mild enteric signs (soft diarrhea), while two CCoV-IIa positive puppies showed more severe clinical signs (vomiting, hemorrhagic diarrhea) and one died. In puppies with mixed infection, the clinic signs varied from mild to severe with five fatal outcome. Dual infection of CCoV (CCoV-I/IIa) along with CPV was detected in three non-survivors puppies. This is the first report of multiple infections caused by CCoV-I/IIa and CPV. These results suggest that dual CCoV infection associated to CPV may affect the severity and outcome of the disease. Financial support: FAPERJ, CNPq, PROPPI-UFF.

VV211 - DETECTION OF CANINE HERPESVIRUS TYPE 1 IN THE BLOOD SAMPLES USING QPCR AND DETERMINATION OF VIRAL LOAD.

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The canine herpesvirus is a contagious infectious agent that is transmitted through direct contact with infected animals. The viruses are eliminated by oronasal and genital tract secretions. It is a virus with a latent feature which maintains its infectivity and dissemination to other animals. This work aims apply qPCR to identify canine herpesvirus type 1 (CHV-1) infection quantifying viral load. For this, it was used qPCR reaction directed to thymidine kinase gene, previously standardized, in dog blood samples. Were tested 138 DNA extracted from dog whole blood samples collected in Botucatu region, São Paulo, Brazil. The viral load quantification was performed comparing with absolute standard curve made using recombinant plasmidial DNA. Nine samples were positive showing single melting curve and viral load from 15,35 to 2,48x105 copies/µl. To check specificity, PCR products were submitted to DNA sequencing showing results with 100% of identity to CHV-1 (acession AF361075). Therefore, this data supports the identification of the agent and specificity of assays used. Thus, we observe that qPCR showed high analytical sensitivity and specificity quantifying viral load at low level. Considering that there are no reports with detection of HCV-1 in blood samples, the qPCR is an important tool for CHV-1 viral load quantification. Financial Support: FAPESP (2011/04795-2).

VV215 - EXPANDED SCREENING FOR SIMIAN FOAMY VIRUS (SFV) IN NEW WORLD PRIMATES IDENTIFIES TWO NOVEL SFV LINEAGES IN TAMARIN AND UAKARI MONKEYS FROM BRAZIL

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While simian foamy viruses (SFV) have been identified in almost all Old World primates and have been shown to cross into humans, little is known about SFV infection in New World primates (NWP) and their ability engaging into zoonotic transmissions. Expanded screening of additional NWP genera and species using both serology and PCR is necessary to better understand the distribution and prevalence of SFVs in NWPs. Blood samples were collected from 99 captive NWPs at the Primatology Center of Rio de Janeiro and the Rio de Janeiro Zoo. Plasma was screened for SFV antibodies by EIA and Western blot assays. Genomic DNA was tested by PCR for polymerase and LTR-gag regions. Phylogenetic inference was used to determine genetic relationships. Total SFV seroprevalence was 37% (37/99). SFV antibody was detected in seven genera. In contrast, a lower prevalence of 17% (17/99) was found using generic PCR methods with sequences detected in seven genera. Phylogenetic analysis inferred co-speciation of SFV and NWPs, including new SFVs in uakaris (SFVuak) and tamarins (SFVtam). SFVuak is the first example of SFV infecting the Pitheciidae family of primates. Our results indicate that serology is more sensitive than PCR for detection of SFV in NWPs, suggesting that studies using only PCR may underestimate prevalence and species distribution. Our finding demonstrates further the broad distribution of SFV in NWPs, including the identification of divergent viruses in uakaris and tamarins. Our results suggest that humans exposed to NWPs are at risk for infection with diverse SFVs. The new SFV sequences identified here will facilitate development of better molecular assays for the detection of these viruses.

VV218 - EVALUATION OF NESTED AND SEMI NESTED PCR IN DETECTION OF EQUINE

INFECTIOUS ANEMIA VIRUS INFECTION IN HORSES OF PANTANAL REGION, BRAZIL

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Equine infectious anemia (EIA) is caused by a retrovirus and its primary mode of transmission comprises transfer of blood from infected animals to healthy ones. In Brazil, EIA notification is mandatory and its confirmation is based in serological tests followed by euthanasia of infected animals. AGID and ELISA are serological techniques recommended because have good accuracy and reliable results for detection of positive animals; however, some cases may display false results. The importance of this disease in the national context, make the development of direct diagnostic techniques very important to confirm disease. Molecular techniques such PCR, including those recommended by OIE, were designed using laboratory-adapted virus strains; thus these methods are not suitable for detection of field isolates of EIA virus, which in turn rises the possibility of false-negative and false-positive results. Dong et al. (2012) proposed a nested PCR for the amplification of EIAV proviral sequences using outer primer pair, EIAVltr-1F/EIAVltr-1R and inner primer pair, EIAVltr-2F/EIAVltr-2R amplifying from the LTR to the tat gene. Based on that, the aim of this study was to evaluate nested PCR technique in samples from Brazil. We tested five combinations of these primers, using 12 DNA samples extracted from positive horses from Pantanal (endemic area) in both serological techniques. The best results were obtained with the semi-nested method using the outer primers EIAVltr-2F and EIAVltr-1R and inner primers EIAVltr-2F and EIAVltr-2R. The technique allow us to detect ten (83,3%) positive samples, while in the nested PCR only six samples were amplified. Two samples were sequenced showing a high variable region of the EIAV proviral DNA. In conclusion semi-nested PCR was better for use in Brazilian samples. Also it is necessary further standardization of molecular techniques for use in conjunction with the herein described. Financial support: FAPESP

VV235 - ROTAVIRUS INFECTIONS AMONG POULTRIES AND WILD BIRDS IN RIO DE JANEIRO STATE

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Rotavirus (RV) infections pose a major concern to the poultry industry due to substantial losses caused by decreasing in productivity. The frequency of RV infections among wild birds however, is poorly understood. RV belongs to the Reoviridae family. The viral particle possesses a nonenveloped capsid surrounding a genome composed of 11 dsRNA segments. The antigenic properties of the 6th segment (VP6), classify RV into 8 species A - H. RV belonging to the species A-C and H infect humans and animals while species H only infect humans. Species D-F infect only in animals, particularly birds. This study evaluated the frequency of RV infections among poultry and wild, migratory or resident, birds in the state of Rio de Janeiro. Thus far, 403 fecal samples were collected from wild birds from 2005 to 2010 and 63 cloacae swabs collected from poultries in 2012. Screening was performed by real time and conventional RT-PCR using primers that amplify the VP6 encoding gene of avian RV species A (RVA) and D (RVD). Among wild birds RVA was detected in 12.1% (49) of the samples; RVD was detected in 4 samples (1%). The RV-positive samples were obtained from birds from the Charadriidae and Scolopacidae families that are considered to be migratory in our country. Among poultries, RVA was detected in 6 (9.5%) samples. The VP6 encoding gene of 4 of those samples was submitted to sequencing. Those samples were also inoculated in MA-104 cells for virus isolation. The results showed that RV infections are common among wild migratory birds - a fact that can impact the ecology of RV infections as such birds could disseminate the virus to long distance regions. Characterization of RV stains detected among migratory birds is of utmost importance for understanding the ecology, distribution and evolution of RV infections. Moreover, the knowledge of the epidemiology RV infections among poultry is of great interest to reduce such infections and therefore improve the production of poultry farms. Financial support: FAPERJ, CNPq.

VV251 - MOLECULAR EPIDEMIOLOGY OF BOVINE VESICULAR DISEASES IN THE SOUTHEAST, NORTHEAST AND MIDWEST OF BRAZIL

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The cattle industry is one of the highlights of Brazilian agribusiness on the world stage. One important factor