HELIO GELLI PEREIRA AWARD
CHRYSANTHEMUM STUNT VIROID IN BRAZIL: SURVEY, IDENTIFICATION, BIOLOGICAL AND MOLECULAR CHARACTERIZATION AND DETECTION METHODS

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In Brazil, ornamental flowers and plants market moves in the wholesale and retail more than 2 billion 12 dollars, annually, and chrysanthemum stands out as one of the most valuable commercial species. The 13 stunting disease induced by Chrysanthemum stunt viroid (CSVd) has become a serious problem in 14 chrysanthemum production systems worldwide. CSVd incites colour breaking and retards flowering, but in 15 many situations, not induces visible symptoms, facilitating its spread in the field, and passing unnoticed 16 when cross international borders. In Brazil, there are few studies on this pathogen, with a single report of 17 its possible occurrence in chrysanthemum in the State of São Paulo. This work aimed to: (i) carry out a 18 survey, identify and characterize viroids present in chrysanthemum crops in the State of São Paulo; (ii) 19 challenge of chrysanthemum varieties with a CSVd isolate; and (iii) develop diagnostic methods to 20 strengthen quarantine and indexing programs. Our survey showed that the CSVd is widely disseminated in 21 chrysanthemum crops in São Paulo State. All varieties of chrysanthemum evaluated were susceptible, 22 without symptoms. The development of oligonucleotides for conventional RT-PCR and RT-qPCR will 23 allow the use of these techniques for diagnosis with high sensitivity, 100,000 times more sensitive than 24 sPAGE. Dot-blot was sensitive and useful for diagnosis of large number of samples. The complete genome 25 sequencing of a CSVd isolate showed high percentage of nucleotide identity compared with other isolates 26 deposited in databases. This is the first identification and molecular characterization of the CSVd in Brazil.

STUDY OF HUMAN VIRUS IN SURFACE WATER SURFACE: QUANTIFICATION, INTEGRITY, INFECTIVITY AND MOLECULAR CHARACTERIZATION.


Universidade Federal de Santa Catarina, Departamento de Microbiologia, Imunologia e Parasitologia.

This study aimed to quantify human viruses, HAdV, JCPyV, HAV and RVA in surface waters used for human consumption, as well as evaluate the integrity, infectivity and perform a molecular characterization of HAdV present in these matrices. Three sites in the city of Florianópolis-SC were selected: Site 1) Peri Lagoon; Site 2) Source water; Site 3) Public supply water system. Water samples were collected, processed and viral quantification was determined by qPCR. Viral integrity was evaluated by enzymatic assay (DNase I) and infectivity by plaque assay (PA) and integrated cell culture using enzymatic assay and transcribed mRNA (ICC-et-RT-qPCR). The results found that 93% (67/72) of the samples were positive for HAdV, 45.8% (33/72) for RVA, 13.8% (10/72) for JCPyV and 12.5% (9/72) for HAV. The evaluation of HAdV integrity and infectivity of the samples showed that in Peri Lagoon 83% (10/12) were undamaged and 75% (9/12) infectious; Source water 66% (8/12) were undamaged and 58% (7/12) infectious; Public supply water system 58% (7/12) were undamaged and 25% (3/12) infectious. HAdV-2 was the prevalent serotype of the HAdV. When PA and ICC-et-RT-qPCR were compared, ICC-et- RT-qPCR was accurate, efficient, sensitive and rapid.

CHARACTERIZATION OF NOROVIRUS INFECTIONS IN CHILDREN ADMITTED IN A PEDIATRIC HOSPITAL FOR GASTROENTERITIS IN BELÉM, NORTHERN BRAZIL.


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Several viruses have been associated with acute gastroenteritis (AGE), and group A rotavirus (RVA) and norovirus (NoV) are the most prevalent. This study aimed to assess their prevalence among children hospitalised for diarrhoea during a three-year surveillance study. From May 2008-April 2011, overall positivity rates of 21.6% (628/2904) and 35.4% (171/483) were observed for RVA and NoV, respectively. The seasonality observed indicated distinct patterns when both viruses were compared. This finding may explain why hospitalisation for AGE remains constant throughout the year. Continuous AGE monitoring is needed to better assess the patterns of infection.

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An unusual strain of human rotavirus G3P[3] (R2638 strain) was detected from a 1-year-old child patient during the epidemiological survey of rotavirus in the state of São Paulo, Brazil in 2011. The aim of this study was to carry out sequence analyses of the two outer capsid proteins (VP4 and VP7) of the R2638 strain detected in order to obtain further information of the genetic relationships between human and animal rotaviruses. Rotavirus G3P[3] was detected using a commercial immunoenzymatic assay, SDS-PAGE, and genotyped by RT-PCR. The analysis of the genetic relationship between human and animal rotaviruses was carried out by sequencing the VP7 and VP4 genes. The VP7 gene of the R2638 strain displayed the highest nucleotide identity to the canine strains A79-10 (96.6%) and CU-1 (96.2%) isolated in USA. The VP4 sequence showed the highest nucleotide identity to P[3] canine rotavirus strain RV52/96 isolated in Italy at 94.1%. Furthermore, the VP4 genes of P[3] strains could be discriminated into two phylogenetically distinct clusters. The present study reinforces the hypothesis that animal’s rotaviruses might be able to cross the species barriers, and the lack of systematic surveillance of rotavirus infection in small animals hinders the ability to establish firm epidemiologic connections. Moreover, in 2006 rotavirus vaccine was included in the Brazilian Immunization Program, and selective vaccine pressure could increase the circulation of uncommon strains. This is the first report of G3P[3] in over 20-year period of monitoring in Brazil.
BV11 - EVOLUTIONARY HISTORY AND SPATIOTEMPORAL DYNAMICS OF RODENT-BORNE HANTAVIRUS
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Hantavirus (Family Bunyaviridae) are mostly associated to rodents and transmitted to man by inhalation of aerosolized infected excreta of these animals. The human infection by Hantavirus can lead to severe diseases such as hemorrhagic fever with renal syndrome (HFRS) in Asia and Europe, and pulmonary syndrome (HPS) in the Americas. To determine the origin, spreading and evolutionary dynamics of rodent-borne hantavirus, were collected 190 N gene sequences of rodent-borne hantavirus identified from 30 countries over the past 25 years (1985 to 2010). Recombinant sequences and identify identical sequences were not included in the study. Nucleotide sequences were aligned and the spatiotemporal and demographic dynamics of dissemination of rodent-borne hantavirus was reconstructed using the Bayesian Markov Chain Monte Carlo (MCMC) approach using the BEAST 1.7.4 program. It was estimated that the N gene of hantavirus carried by rodents evolved at a rate of 6.8 x 10^-4 (2.5 x 10^-4 - 1 x 10^-3) nucleotide substitutions per site per year and that rodent-borne hantaviruses originated around 2,000 years ago. However, the rodent-borne hantavirus had a large period of slow growth and about 500 years ago started a rapid spread worldwide that coincides with the human traveling between continent. Hantaviruses associated to Murinae and Arvicolinae subfamilies, probably, were originated in Asia 500-700 years ago spreading toward Siberia, Europe, Africa and North America. Hantaviruses associated to Neotominae subfamily, probably, emerged 500-600 years ago in Central America and spread toward North America. Finally, hantaviruses associated to Sigmodontinae occurred in Brazil 400 years ago and were, probably, originated from Neotominae-associated virus from northern South America. These data offer subsidies to understand the time-scale and worldwide dissemination dynamics of rodent-borne hantaviruses. Financial support: FAPESP

BV74 - FOLLOWING THE STEPS OF AN EMERGING VIRUS ON ITS WAY INTO THE CELL BY LASER-SCANNING CONFOCAL FLUORESCENCE MICROSCOPY
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Arboviral infections are a major public health problem worldwide. Of the 210 different species of arboviruses circulating in Brazil, Mayaro virus (MAYV) is the fourth in number of infected individuals, behind only dengue, yellow fever and Oropouche viruses. Although Mayaro fever in humans is even more debilitating than dengue and its urbanization from the Amazon region is imminent, the disease is largely neglected and many details of the replication cycle of the virus are still unclear; including the early events of infection. The aim of this work was to analyze the behavior of MAYV particles during their entry into host cells. To this purpose, MAYV was labeled with the lipophilic fluorescent probe DiD without impairment to viral infectivity and the fluorescent signals were tracked in the host cells by laser-scanning confocal fluorescence microscopy in real time. Our results show that MAYV entry into cells occurs by an endocytic mechanism involving fast internalization of the endocytosed cargo, since fluorescent signals from labeled virus particles could be visualized inside the cell a few seconds after virus binding to receptors on the cell surface. Following DiD fluorescence dequenching at the single particle level, we could capture the moment of the fusion between the viral envelope and the endosomal membrane, that was shown to occur faster (around 3 min post-binding) than for other arboviruses. This work provides unique kinetic insights into the entry of virus particles in living cells. Understanding the dynamics of virus infection may provide important insights to the development of antiviral strategies. Financial support: CAPES, CNPq, FAPERJ, FINEP, INBEB and PRONEX.
Oral Presentation

4. Fundação Oswaldo Cruz, FIOCRUZ, Augusto de Lima, 1715, Belo Horizonte, Minas Gerais

The Dengue virus express non-structural proteins which are involved primarily with the intracellular viral multiplication steps. However, there are several reports that these proteins can interact with cellular proteins and substantially change their functions and, therefore, control the rates of cell multiplication and the secreted-protein expression profile, such as cytokines and chemokines. One of these non-structural proteins are NS1. After your synthesis, NS1 associates to membranes where it remains as the only viral protein in infected cells. In addition, in vivo assays have shown that NS1 can accumulate in the liver of animals and changes some cellular functions. Data obtained by our group, suggest that the expression of NS1 changes the activation profile of MAP kinases MEK/ERK and NF-kb pathway proteins. These signaling pathways, like many others, are activated at level of plasma membrane in response to extracellular stimuli that can be mediated by GPI-anchored proteins that are associated with membrane micro domains named lipid rafts in wich, according to data from literature; NS1 is also associated by a GPI-anchor. To investigate the importance of lipid rafts in triggering of signaling events mediated by NS1 we are analyzing the distribution of caveolin-1 (the main marker of rafts) in cells expressing NS1 or DENV-infected by western blot of membrane fractions extracts. Our preliminary data have shown that the expression of NS1 can promotes changes in the distribution of caveolin-1 in HepG2 cells, suggesting that this protein may be causing changes in cellular signaling pathways. To refine these results we are generating cells silenced for caveolina-1 that will be very useful to assess the involvement of lipid rafts in signaling events mediated by expression of NS1 in liver cells. The identification of role of lipid rafts into signaling events elicited by NS1 can be useful to understand the complex relations between cells and Dengue virus.

Supported by: UFOP, EAPEMIG, CNPq and CAPES.

BV219 - Jatropha curcas extract inhibits HIV-1 inducing internalization of CD4 receptor

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Highly active antiretroviral therapy (HAART) has been used as standard treatment to HIV-1 infection; however, virus resistance contributes to therapeutic failure. Therefore, searching for new class of drugs to reduce HIV-1 infection has been the best alternative to control multi-resistant virus since an effective vaccine against HIV-1 is not available. Here, we screened extracts from Jatropha curcas to evaluate cytotoxicity and antiviral activity against HIV-1 in lymphocytes CD4+ (MT-4 cells). Our results showed that the fraction THS eluted in hexane decreased HIV-1 infection up to 80% in a dose-dependent manner. The maximum inhibition of HIV-1 infection was observed at 260µg/ml of THS. Cell viability experiments were showing no toxicity in these concentrations. Although there was no effect of this compound in the production and release of the virus, THS blocked HIV-1entry mediated by CD4 internalization. Flow cytometry analysis showed that CD4 receptor was downregulated from the plasma membrane in MT-4cells after 10 min of treatment with THS. Confocal microscopy confirmed that this compound promotes CD4 internalization into cellular vesicles. The treatment with PKC inhibitor (GO6983) revert the effects of CD4 internalization suggesting that THS specifically affects HIV-1 entry mediated by CD4 internalization. Flow cytometry analysis showed that CD4 receptor was downregulated from the plasma membrane in MT-4cells after 10 min of treatment with THS. Confocal microscopy confirmed that this compound promotes CD4 internalization into cellular vesicles. The treatment with PKC inhibitor (GO6983) revert the effects of CD4 internalization suggesting that THS specifically affects HIV-1 entry mediated by CD4 internalization. Flow cytometry analysis showed that CD4 receptor was downregulated from the plasma membrane in MT-4cells after 10 min of treatment with THS. 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Confocal microscopy confirmed that this compound promotes CD4 internalization into cellular vesicles. The treatment with PKC inhibitor (GO6983) revert the effects of CD4 internalization suggesting that THS specifically affects HIV-1 entry mediated by CD4 internalization.

BV226 - An antibody dependent Dengue infection enhancement is mediated by homologous anti-envelope IgGs: In vivo and in vitro observations


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The main correlate of protection for dengue virus (DENV) infection is the generation of neutralizing antibodies against the envelope glycoprotein, particularly the domain III (EDIII), which is involved with host cell receptor recognition. The protection correlate is mainly determined in vitro by virus neutralization assays carried out with non- Fc receptor bearing cells. In order to investigate this point, the DENV2 EDIII was obtained as a purified recombinant protein retaining native biological properties. This protein was used as a vaccine antigen, co-administered with or without different adjuvants

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in BALB/c mice. Animals immunized with inactivated DENV or non-immunized mice were studied as controls. The induced antibody responses were studied and immunized mice were challenged with a homotypic DENV2 (JHA1) strain. The antibodies generated before and after infection were tested regarding neutralization activity. Immunized mice developed a Th2 immune response pattern with high levels of IgGs capable of neutralizing the virus in in vitro assays carried out with non-Fc receptor bearing cells. However, under in vivo infection challenges, we found that animals immunized with EDIII developed hematological disturbances, tissue damage and increased tissue viral load earlier than non-immunized mice. As a consequence, immunized mice died earlier than non-immunized mice. In addition, sera from EDIII-immunized mice were shown to induce increased levels of infection in Fc-receptor bearing cells. The present results indicate that a strictly humoral and homologous immune response directed against DENV EIII causes a homotypic ADE by increasing the infection level of Fc bearing cells and accelerating the onset of damage symptoms in vivo. The contribution presented in this work is the first evidence that the process of developing dengue vaccines should be reviewed.

**BV250 - INVESTIGATION OF YELLOW FEVER VIRUS-INDUCED ENDOPLASMIC RETICULUM STRESS**

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The yellow fever is a hemorrhagic disease of great relevance in Africa and South America, where it occurs as small outbreaks. It is caused by the yellow fever virus (YFV), a flavivirus such as the Dengue Virus, both being transmitted by mosquitos. During its life cycle, the YFV uses the endoplasmic reticulum for translation of viral proteins and assembly of new viral particles. The accumulation of misfolded proteins in this organelle triggers the endoplasmic reticulum stress (ERS), which leads to the dissociation of the binding immunoglobulin protein (BiP) from ATF6, PERK e IRE1. Once released, these factors become active and start to mediate the ERS. ATF6 is transported to Golgi, where is cleaved. PERK and IRE1 homodimerize, are phosphorylated and become active. PERK phosphorylates and inactivates eIF2α. IRE1 is a RNAse that alternatively splices XBP1 mRNA, leading to the production of a response for ERS. One of these responses is the overexpression of the nuclear transcription factor CHOP, which regulates the expression of pro and anti-apoptotic genes. In this study, we investigate the ERS induced by the infection of VERO cells by YFV through western-blotting and fluorescence microscopy. We infected VERO cells with YFV, using a multiplicity of infection of 1. We analyzed cell viability by the LIVE/DEAD assay and we observed that by 72 hours post infection, cells undergo cell death. The ERS induction was noticed by CHOP overexpression. Moreover, we observed phosphorylation of eIF2α, ATF6 cleavage and spliced XBP1 18 hours post infection. BiP expression levels remained unaltered. Apoptosis was analyzed by the TUNEL assay and it was observed 72 hours post infection. Our results suggest that the YFV induces ERS in VERO cells through PERK, ATF6 cleavage, XBP1 splicing and CHOP overexpression. Financial Support: Capes, CNPq, FAPERJ, Pronex, INWEB

**EV43 - GIANT VIRUSES ISOLATION FROM DIFFERENT BRAZILIAN HABITATS: URBAN AND NATURAL ENVIRONMENTS**

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The nucleocyttoplasmic DNA large viruses (NCDLV) are a group formed by the largest viruses known. In the last years, an increase in their study was observed, given that some of them have many interesting features that set this group apart from other viruses, Mimiviridae and the Marseilleviridae families belong to NCDLV, and have been isolated from some locations as cooling towers, antartic lakes, oceans, etc. However, despite Acanthamoeba spp. (its natural host) presents a global distribution, there is a lack of information regarding these viruses in some regions of the world. Brazil is a country with many habitats where this study can be performed, representing an open field for giant viruses study. Therefore, the objective of this work was to perform the isolation of giant viruses from three Brazilian regions marked by different levels of pollution and urbanization. We collected about 425 samples of water and soil from three regions differently affected by human activity: the artificial lake of Pampulha in Belo Horizonte (high pollution level), Central Lake in Lagoa Santa (intermediary pollution level) and the Serra do Cipó National Park (low pollution level). These samples were enriched, filtered (membranes of 0.2 μm) and then, the isolation of giant viruses was attempted in amoebae of Acanthamoeba castellani specie, by the observation of cytopastic effect. Other tests were also performed, including real-time PCR (for Mimiviridae and Marseilleviridae), helicase and polymerase genes sequencing optical and electronic microscopy. Four
EV61 - DETECTION OF HUMAN ADENOVIRUSES IN SURFACE WATER AND SEDIMENTS IN SANGRADouro RIVER, SANTA Catarina, Brazil

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Sediments are suggested to play an important role in transmission of enteric viruses in the environment because of the sorption to and desorption from these biosolids which can control the transport of viruses and other waterborne pathogens through the water column. Human Adenovirus (HAdV) is highly prevalent in both sewage and biosolids and it has been included in all three of the U.S. Environmental Protection Agency's contaminant candidate lists, which prioritize currently unregulated drinking water contaminants (EPA, 2012). The Sangradouro River located in Florianopolis, Santa Catarina, Brazil receives the Peri lagoon water during the rainy episodes and also inadequate disposal of wastewater and sewage from neighborhood houses and hostels. In this study, HAdV were quantified either in surface water or sediment samples by real-time PCR (qPCR) along the Sangradouro River. A total of 48 samples were collected in six points with different sediment characteristics during the summer season of 2013. Two liters of surface water samples were concentrated by negatively charged membranes and 20g of sediment samples were concentrated and clarified using glycine buffer followed by polyethylene glycol (PEG) precipitation. The HAdV genome was detected in 17/24 (70.8%) ranging from 105 to 108 genome copies (gc) per liter and 10/24 (41.7%) ranging from and 109 to 1010 gc/L in surface water and sediment samples, respectively. Higher concentrations of gc of HAdV in sediment samples may be due to its organic material composition which plays an important role in the protection of viruses against sunlight inactivation. On the basis of this preliminary study, we conclude that the HAdV can be potentially found in high amounts in sediments due to its stronger affinity to biosolids. This study will continue by searching other enteric viruses in these samples, looking for seasonal contribution of the enteric virus prevalence and also infectivity assays for future risk assessment studies. Financial support: CNPq/TWAS and CNPq Universal 470808/2009-8.

EV233 - ENVIRONMENTAL SURVEILLANCE OF POLIOVIRUSES IN RIO DE JANEIRO IN SUPPORT TO THE ACTIVITIES OF GLOBAL POLIO ERADICATION INITIATIVE.

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Polioomyelitis is an acute infectious disease that occurs following an infection caused by one of three poliovirus serotypes (serotypes 1, 2, and 3). Since the implementation of global polio eradication initiative, the incidence of wild poliovirus transmission has dramatically dropped (> 99%). However, wild polioviruses remain endemic in three countries (Afghanistan, Pakistan and Nigeria) but cases of re-emergency have been reported in previously polio-free countries (ex. Somalia and Kenya). Environmental surveillance of polioviruses has been used as a supplementary tool in monitoring the circulation of wild poliovirus and/or vaccine derived poliovirus (VDPV), even in the absence of acute flaccid paralysis (AFP) cases. This study aimed to isolate and characterize poliovirus and Non-Polio Enteroviruses (NPEV) from wastewater samples collected at one of the stations of sewage treatment (ETE Alegria/ Cedae), located in Rio de Janeiro city. From December 2011 to June 2012 and from September to December 2012, 31 samples were collected and concentrated. Isolation in RD and L20B cell cultures, followed by RT-PCR was able to detect enteroviruses in 27/31 samples (87%). Poliovirus was isolated in 8/27 positive samples (29.6%): Sabin1 = 1 sample, Sabin 2 = 5 samples, Sabin 3 = 2 samples. The remaining isolates were NPEV. All polioviruses were classified as Sabin-like based on the nucleotide sequences of the VP1 gene. VDPVs were not detected. The following NPEV have been identified: 1 Echovirus 3; 11 Echovirus 6, 7 Echovirus 7, 2 Echovirus 20; 4 Coxsackievirus B4 and 2 Coxsackievirus B5. Environmental surveillance has been used successfully in monitoring the circulation of enteroviruses and it is of crucial importance in the final stages of the WHO global polio eradication initiative. Our results show the continuous circulation of Sabin-like poliovirus and non-polio enteroviruses in the analyzed area during the study period.

Financial support: CNPq, FAPEMIG, CAPES, MAPA.
EV409 - ROTAVIRUS DIVERSITY IN TREATED AND UNTREATED SEWAGE WATER FROM SIX DIFFERENT CITIES OF URUGUAY


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Transmission of Group A Rotavirus (RVA) between human populations through surface waters contaminated with sewage water (SW) is a serious health problem worldwide. The aim of this study was to determine and characterize the contamination of SW in different areas of Uruguay by RVA. We analyze the presence of RVA in SW collected samples from six different cities located on the North, west and east region of Uruguay. The SW samples were collected from four cities without sewage treatment plant (STP) placed on the north and west region that discharge directly its SW in the Uruguay River (SW-UR), while the others two cities from the east region have STP with UV treatment system (SW-STP). In all the 6 cities, 42ml of SW were collected, and viral concentration was made by using an ultracentrifugation protocol. In the case of SW-UR, the samples were collected during an entirely year (from 02/11 to 02/12), fortnightly in each city. In the case of SW-STP, the samples were collected bimonthly (from 09/11 to 06/12), and the collection was made at affluent and effluent in each STP. Viral RNA extraction was conducted from the previously concentrated viruses, performed by commercial kit according to manufactures instructions and cDNA was generated using random hexamer primers. Worldwide standardized specific Nested Multiplex PCR protocol directed against outer capsid protein genes VP4 and VP7 were conducted for RVA genotype determination. The RT-PCR analysis of the SW-UR samples (n=126) showed a positivity of 41% (n=51) and in the case of SW-STP (n=20), 40% were positive at affluent and 10% at effluent. The diversity of genotypes detected were as follow: 1) SW-UR: G2, P[6], P[9]; 2) SW-STP: G2, P[8], P[4], VP4 and VP7 consensual fragment of the positive samples are under sequencing process to confirm the genotypes through phylogenetic analysis. These results are the first evidence of the environmental dissemination and diversity of RVA in different regions of Uruguay. Financial support: Polos de Desarrollo Universitario, Universidad de la República (UdelaR). Comisión Sectorial de Investigación Científica (CSIC) UdelaR

EV450 - QUANTITATIVE DETECTION AND RECOVERY OF INFECTIOUS ENTEROVIRUS IN DIFFERENT TREATED SEWAGE SLUDGE MATRICES


Companhia Ambiental do Estado de São Paulo, Cetesb, Av. Prof. Frederico Hermann Jr., 345

Treated sewage sludge is increasingly applied to agricultural land as fertilizer, and as a beneficial alternative to conventional means of disposal. However, the reuse is often restricted due to the presence of toxic metals and pathogens that can lead to the contamination of ground water and food chain. The objectives of this study were to determine the concentration of Enterovirus in treated sewage sludge from different treatment processes and to evaluate the effectiveness of the method concentration by the recovery of poliovirus 1 (PV-1). Treated sewage sludge samples were collected from four wastewater treatment plants (WWTP) in São Paulo, Brazil, with four different sampling events at each plant. Two samples were collected each month from November 2011 to June 2012. The equivalent of 12g of total solids (gTS) of sample was eluted in 3% beef extract. The solids were separated by centrifugation and the viruses in the supernatant were concentrated by organic flocculation. The pellet was resuspended in phosphate buffer and treated with chloroform. For the recovering evaluation the samples were spiked with approximately 400 plaque forming units (PFU) of PV-1. Eluted viruses were enumerated by the single-layer plaque assay using the human rhabdomyosarcoma (RD) cell line. The mean recovery efficiency of the method was 32%, with significant difference (p<0.05) values comparing the four treatment processes. The mean concentration of indigenous Enterovirus and the mean recovery efficiency of the samples, considering each treatment process were the following: dewatering (41.4PFU/gTS, 52.2%), composting (<0.25PFU/gTS, 38.9%), mesophilic anaerobic digestion,FeCl3, and organic polymer (5.4PFU/gTS, 20.6%), mesophilic anaerobic digestion, FeCl3, and lime (<0.25PFU/gTS, 16.2%). The method evaluated is considered simple and presented recovery percentages variable from 11.5% to 85.3%. However it should be taken into account that such performance suffers the influence of the sample matrix.

EV501 - DETECTION OF HUMAN BOCAVIRUS IN RAW WATER SAMPLES OF RIO DOS SINOS WATERSHED, RIO GRANDE DO SUL, BRAZIL

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Human bocavirus (HBoV), a member of the Parvoviridae family, was first described in 2005 when it was isolated from respiratory tract secretions of young children with acute respiratory disease. Although HBoV is frequently associated with respiratory infections in children, it has also been detected in stool samples, leaving unclear its role as a causative agent of gastroenteritis. Since HBoV can be excreted through feces, its presence in water should be considered as a possible source of transmission. Therefore, the aim of this study is to evaluate the presence of HBoV in raw water samples collected from ten drinking water treatment plants localized within the Rio dos Sinos watershed, Rio Grande do Sul, Brazil. 500 ml of each sample was collected and concentrated using an adsorption-elution method, followed by the extraction of the viral DNA. The presence of HBoV genome was detected by conventional PCR using primers designed to align in highly conserved regions of the HBoV genome, targeting the nonstructural protein NP1. The reaction products were submitted to electrophoresis on 2% agarose gel in a TBE buffer, stained with SYBR Safe DNA Gel stain (Invitrogen) and visualized by UV light. So far, 29 water samples were analyzed from January to October 2011 and 5 of them were positive to HBoV genome. Those samples were previously tested for enteroviruses and rotaviruses by conventional PCR and they all resulted negative for those viruses. These preliminary results suggest that HBoV may be included as an additional marker of fecal contamination in water samples and further studies are necessary to evaluate its risks for public health. Financial support: CNPq, Feevale, Capes, FAPERGS.

**HV14 - UNEXPECTED DETECTION OF BOVINE G10 ROTAVIRUS IN A BRAZILIAN CHILD WITH DIARRHEA**

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Rotavirus group A (RVA) G3 genotype has broadest host range. The aims of this study were to carry out Bayesian phyllogenetic analyses using the nucleotide sequences of VP7 gene available in GenBank in order to investigate the evolutionary dynamic between RVA G3 strains originating in humans, wild and domestic animals; quantify the mutation rates; and estimate the most recent common ancestors. For 5 bovines, 3 simians, 2 environmental, 8 canines, 22 equines, 3 felines, 5 rabbits, 5 porcines, 2 caprines, 3 murines, and 199 human G3 strains; the entire or partial VP7 ORF sequences and the year of isolation could be retrieved from GenBank. The Bayesian inference method available in the software BEAST v. 1.6.2 was used in order to analyze the phylogenetic relationship. Based on 257 sequences, the mutation rate was estimated to be 1.7318 x 10^-3 (1.4374-2.075 x 10^-3) nt substitution/site/year. The TMRCA inferred for G3 strain was calculated to be 1786 (1765-1810). It was possible to separate three distinct Lineages of G3 by phylogenetic analysis. All of them contain animals and humans strains; however, Lineage II contains the majority of human G3 strains, and they are associated with urban environments. Phylogeography and temporal analysis, suggested that G3 strain emerged in Asia and scattered through the globe in rural environments. The urban context of RVA G3 circulation was later observed 100-110 years ago, and the data analyzed also suggested that the urbanization process took place in Asia, and posteriorly in Europe and the Americas. The Bayesian Phylogenetic analysis suggests that a transmission between human and animals may be the ancestral characteristic of the G3 strain, and its urbanization is a later phenomenon. The most recent common ancestor of this strain was dated back to 1786; however the emergence of the majority human urban Lineage II could be tracked back to around 1904. This data suggests that the urbanization of the RVA and its fixation on human population may be associated with the industrialization process associated with the change from rural settlements towards a predominantly urban population. Also, urbanized strains are apparently more prevalent than rural strains. The complexity that naturally arises from this changing environment is an ideal situation to the emergence of a new zoonotic virus, as indicated by the recent epidemics of SARS-COV, and H1N1. Financial Support: PPG-CCD-SES/SP; IAL.

**HV86 - THE EFFECT OF HOST IL28B GENOTYPE ON CLINICAL OUTCOMES OF HEPATITIS A AND B**


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Despite advances in therapy and vaccine development, viral hepatitis infections still account for morbidity and mortality worldwide. Genetic diversity of host immune response, such as IL28B SNPs, may contribute to explain the variability in the outcome of hepatitis A and B infection. Host IL28B genotype has significant influence on treatment response to chronic hepatitis patients. Whether IL28B genotype influences directly as an antiviral agent, in non-treated patients, is still
unknown. For these reasons, this study aim to compare the genetic profile of IL28B SNPs to different outcomes of hepatitis A and B viral infection. For this purpose, samples from patients with different hepatitis clinical outcomes, confirmed by serology, were genotyped for IL28B by TaqMan real time PCR. In the total, 144 samples were enrolled into 3 cohorts: 100 acute, 23 chronic and 21 fulminant hepatitis. Concerning acute samples, 86 belongs to hepatitis A and 14 hepatitis B group. Between fulminant samples, 6 belong to hepatitis A, 2 from hepatitis B and 13 non-viral hepatitis patients. Genotyping results of IL28B17, 60 and 75 were confronted with different characteristics factors (hepatitis type, group, clinical outcome, viral/non-viral). Statistic results showed that only clinical outcome were significant associated with IL28B haplotypes and alleles (p<0.05), independently IL28B75G variant allele were more frequent in acute patients (93.5%) than in chronic ones (6.5%). IL28B75AA haplotype and the absent of IL28B17G variant allele were more frequent in chronic patients (60.6%) than in fulminant ones (39.4%). IL28B17TT haplotype and the absent of IL28B17G variant allele were more frequent in acute patients than in chronic (87.8%/12.2%) and fulminant ones (87.8%/12.2% and 71.1%/28.9%, respectively). IL28B60CC haplotype and the absent of IL28B60T variant allele were more frequent in acute patients than in chronic ones, 93.9% and 6.1%, respectively. IL28B75AA haplotype and the absent of IL28B75G variant allele were more frequent in acute patients (93.5%) than in chronic ones (6.5%). With the high resolution molecular typing of clinical groups was verified that there is evidence of the influence of IL28B SNPs with the outcome of hepatitis A and B. This study is the first that shows association between hepatitis A and B outcomes with IL28B in Brazil. Understanding how the host factor influences the immune response to viral infection provides new opportunities to control these diseases and for the development of effective therapeutics, which justifies the study of this locus.

**HV103 - ARARAQUARA VIRAL RNA LOAD IN PATIENTS WITH HANTAVIRUS CARDIOPULMONARY SYNDROME**


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Hantaviruses (Bunyaviridae) are rodent-borne emerging viral with a worldwide distribution and with high lethality in Americas. In the Americas, hantaviruses are known to cause Hantavirus pulmonary syndrome (HPS), this one is an increasing health problem in Brazil, especially in Central Plateau and Southeastern, where circulates the Araraquara virus, which may be the most virulent hantavirus of world. To understand the role that viral load plays in the pathogenesis in patients with HPS, we quantified Araraquara virus S segment viral RNA in blood samples from 20 acutely ill patients, divided into teen samples from patients in acute period, and teen samples obtained from survivors (convalescent phase). To detection and quantitation of Araraquara virus RNA of S segment was used a one-step SYBR Green real-time RT-PCR. From the sera of 20 human HPS patients, the hantavirus genome was amplified in 10 sera by quantitative RT-PCR, including 2 samples that have not been amplified previously by conventional RT-PCR. These 2 samples had low viral loads (3,67x104 and 2,64x104 copies/ml of serum) that were likely below the detection capacity of conventional RT-PCR. The analysis of viral load demonstrated high plasma levels of viral RNA during acute infection phase (2,64x104 and 3,78x106 copies/ml). We observed that high plasmatic viral load of Araraquara virus are inversely correlated with IgG antibody concentration. In 10 survivors who had samples obtained after the acute phase, not was observed detection of viral RNA, however high levels of IgG antibody was observed. These results suggest that patients with high viral loads on admission are more likely to have severe disease.

**HV290 - DETECTION AND GENOTYPING OF NOROVIRUS IN BLOOD AND STOOL SAMPLES OF CHILDREN HOSPITALIZED WITH ACUTE GASTROENTERITIS IN BELÉM, PARÁ, BRAZIL.**


Norovirus (NoV) are currently considered the major cause of acute gastroenteritis (AGE) in children less than 5 years old, causing more than 1 million hospitalizations/year and around 200,000 deaths/year in this age group. The most common symptoms of the infection by NoV are diarrhea, vomiting and fever. However, studies have demonstrated other extra-intestinal symptoms, like disseminated intravascular coagulation, seizures, encephalopathy, and necrotizing enterocolitis, and until now, little is known about its ability to spread outside the gut. The present study, aims to investigate the role of NoVs causing viremia in children hospitalized for AGE, as well as to correlate the presence of NoVs RNA in serum with clinical severity and stool viral load. Paired stool and serum samples were collected from 85 pediatric patients under 6 years hospitalized for AGE from March to September 2012 in Belém, Brazil. Enzyme-linked immunosorbent assay (EIA) and reverse transcription quantitative PCR (RT-qPCR) were used to detect and
HV336 - COMPARATIVE ANALYSIS OF RASSF1A PROMOTER METHYLATION LEVELS BETWEEN HEPATOCELLULAR CARCINOMA (HCC) AND NON-HCC TISSUES FROM BRAZILIAN HEPATITIS C VIRUS CHRONIC CARRIERS.

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Hepatocellular carcinoma (HCC) is one of the most common tumors worldwide. HCC is frequently diagnosed after the development of clinical deterioration at which time survival is measured in months. Hepatitis C virus (HCV) infection is a major cause of chronic hepatitis and a major risk factor for HCC in Brazil. Hypermethylation of promoter regions of tumor suppressor genes has been shown to facilitate the development of human cancers, including HCC. This study was performed to determine whether occurrence of aberrant methylation of RASSF1A gene promoter might be associated with hepatocarcinogenesis in Brazilian HCV chronic carriers. Methylation levels were measured by bisulphite pyrosequencing of DNA extracted from formalin-fixed, paraffin-embedded liver tissues. Twenty-five samples, including 15 HCC, two cirrhotic and eight normal liver tissues were analyzed. In each sample, the percent of methylation was determined for six promoter CpG islands (1 to 6). At each of them, low, intermediate and high levels of methylation were measured for normal liver, cirrhotic and HCC tissues, respectively. Mean percents of methylation were as follows. CpG1: 14.7% (normal tissues), 29.4% (cirrhotic) and 59.2% (HCC); CpG2: 11.3%, 26.4% and 65.4%; CpG3: 12.4%, 31.2% and 72.3%; CpG4: 11.1%, 29.1% and 57.9%; CpG5: 16.6%, 34.9% and 61.3%; CpG6: 16.4%, 35.7% and 60.0%. Understanding epigenetic changes in HCV-associated HCC will help to elucidate the pathogenesis and may lead to the identification of molecular markers for liver cancer prognosis, diagnosis and treatment.
RVA genotypes circulation during the study period has epidemiological implications, particularly with respect to Rotarix effectiveness.

HV455 - ANALYSIS OF THE SPATIAL DISTRIBUTION OF DENGUE IN Aedes Aegypti Mosquitoes in a Neighborhood from São José do Rio Preto (SP)

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Dengue is an important arboviral infection with worldwide distribution and Aedes aegypti mosquitoes are the main vectors of dengue viruses (DENV 1-4) in Brazil. São José do Rio Preto, which is located at the northwestern region of São Paulo State, has been presenting high incidences of the disease every year and the geographic information systems (GIS) can contribute for a better comprehension of dengue distribution and for more effective surveillance measures in cities that present an endemic circulation of the disease. Adult trap information combined with spatial analysis can provide fundamental information of DENV spread and transmission. The aim of our study was to associate viral surveillance in mosquitoes with spatial analysis to identify possible hot spots of DENV transmission. We have placed MosquitoTM traps twice a week in an area that comprises 25 census tracts and 102 blocks from May to October 2012. The specimens were pooled and labeled according to genus/species, gender and collection site. The geocoding was performed with TerraView open software (DPI/INPE). Our analysis was performed with mosquitoes collected from the epidemiological week 10 until the epidemiological week 44. Approximately 340 traps were positive for the presence of Aedes aegypti mosquitoes, among 1,332 traps that were installed in the study area. We were able to detect DENV-1, DENV-2 and DENV-4 in four adult traps of three different census tracts. One of these tracts can be considered a hot spot for dengue transmission because we were able to find an infected adult male, which is an indication of local vertical transmission. In this tract, DENV transmission would occur without the presence of infected human subjects. Our preliminary data indicate that census tracts can present different risks of dengue transmission and control measures should be applied according to viral surveillance in mosquitoes and humans alike.

HV462 - EPSTEIN-BARR VIRUS AND CHRONIC ADENOTONSILLAR HYPERTROPHY.

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Epstein-Barr virus (EBV), the main cause of infectious mononucleosis, is a herpesvirus that infects B lymphocytes of 95% of humans. Chronic adenotonsillar hypertrophy (CAH) is a poorly understood disease and the most frequent indication for tonsillectomy in the world. This study assessed the EBV infection in palatine tonsils, adenoids, nasal secretions and peripheral blood in patients with CAH undergoing tonsillectomy at the University of Sao Paulo Hospital in Ribeirao Preto, Brazil. A total of 180 patients with CAH, without symptoms of acute respiratory infections, who underwent tonsillectomy in 2011-2012 were enrolled. EBV detection and quantification were done by qPCR. EBV genome was detected in 137 (76%) of patients. Overall, 122 (68%) had detectable EBV in palatine tonsil tissue samples, 110 (61%) in adenoids, 76 (42%) in respiratory secretions, 51 (28%) in the peripheral blood and 10 (7.3%) in all four tested sites. The median viral loads of EBV in palatine tonsils and adenoids were respectively 7.65 x 102 and 7.28 x 102. Approximately 55% of the patients had EBV viral loads higher than 105 copies/g in palatine tonsils and adenoids, respectively. The median EBV loads in respiratory secretions and peripheral blood were respectively 7.65 x 102 and 7.28 x 102. There was no relationship of high viral loads in all of the tested samples with the different degrees of tonsillar hypertrophy. The results suggest that EBV infection is not a cause of chronic adenotonsillar hypertrophy and is not related with the grades of palatine tonsils hypertrophy. However, the presence of high viral loads of EBV in the palatine tonsils and adenoids was associated with simultaneous detection of EBV in respiratory secretions and peripheral blood. This suggests that these lymphoid tissues may function as reservoirs of EBV-infected cells, and that children with hypertrophic tonsils are important sources of EBV shedding, which assures the virus transmission to susceptible hosts.


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Human enteroviruses (HEVs) are responsible for a wide spectrum of clinical diseases. HEVs comprise a large genus in the Picornaviridae family, with 113 serotypes delineated into four species (A–D) based mostly on their phylogenetic relationships. Members of the enterovirus B species cause a central nervous system (CNS) infection, like encephalitis, meningoencephalitis and mainly aseptic meningitis. The aim of this study was to establish the occurrence of HEVs in patients with infections of the CNS in São Paulo State, Brazil. This retrospective study was conducted in convenient surveillance clinical specimens (cerebrospinal fluid and/or stool, swab anal, and brain biopsy) collected from 922 patients with infections of the CNS, in a period of 2004 to 2012. We investigated the presence of enterovirus (EVs) in these samples by cell culture to isolate the viral agent. The samples that showed cytopathic effect (CPE) in the cell culture were submitted by Indirect Immunofluorescence (IFA), using monoclonal antibodies (Chemicon, USA), RT-PCR and VP1 partial sequencing to identification of EVs isolated. Enteroviruses were identified in 15.2% (140/922) of all CNS infectious; corresponding to 92.1% (n=129/140) of aseptic meningitis, 1.4% (n=2/140) of encephalitis, 2.9% (n=4/140) of meningoencephalitis, and 3.6% (n=5/140) other viral neurological infections. Echoviruses (E) were isolated most frequently, with 90 cases (64.3%), Coxsackievirus B (CV-B), with 11 cases (7.8%), and 39 cases with EVs untypeable (27.9%). E-6 was the most commonly identified serotype (23.6%; n=33/140), followed by E-30 (20.7%; n=29/140), E-18 (12.1%; n=17/140), CV-B5 (5.7%; n=8/140), E-11 (2.9; n=4/140), E-4 (3.6%; n=5/140), CV-B4 (1.4%; n=2/140), E-9, E-13 and CV-B1 (0.7%; n=1/140). EVs were detected in all the period of the year with the highest rate in the spring and summer months. Data obtained in this study contribute to the knowledge about HEVs circulation implicated in CNS infections over a 9-year period in São Paulo State. Financial support: FAPESP: 2012/50234-5.

IV78 - A NOVEL LAV TETRAVALENT DENGUE VIRUS VACCINE TESTED IN AFRICAN GREEN MONKEYS.

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Arbovax, in collaboration with NC State, has developed a novel strategy to produce a Dengue virus (DV) live, attenuated tetravalent vaccine by creating viral mutants with truncated transmembrane domains. These are host range mutants (HR) and grow well in insect cell culture but poorly in mammalian cells. Thus, these HR viruses are attenuated for growth in mammals. For DV the use of live, attenuated virus vaccines (LAV) yields the best chance of developing: 1) A safe and effective vaccine that will protect against all four DV serotypes 2). Initiate the desired immune response, neutralizing antibodies as well as an effective cellular response and 3). Produce a balanced immune response. Recent work in the dengue field revealed that for DV, neutralizing antibodies are preferentially made against epitopes only found in the native, live-virus configuration. The Arbovax vaccine method creates live virus vaccines for all four serotypes with altered transmembrane domains that are embedded within the virus’s protective outer membrane, so that all virus ectodomains, or outside surfaces that are available to the host’s immune system and are indistinguishable from those of the wild-type Dengue virus. By this method, the best possible substrate for immune response is generated, an attenuated virus with wild-type virus structure. This tetravalent vaccine formulation was tested in African Green Monkeys and found to be safe, immunogenic, and demonstrated limited interference upon vaccination and post challenge. 100% of the animals sero-converted to DV 1, 2, 3 and 4 prior to challenge on day 62. All vaccinated animals showed an anamnestic response of >3 fold increase over control animals. These host range tetravalent vaccine strains contain the homologous non-structural proteins recently found to be critical for the development of a complete protective response in humans. This vaccine is scheduled to move to Phase I clinical trials.

IV419 - EXPERIMENTAL INFECTION IN CYNOMOLGUS MONKEYS (MACACA FASCICULARIS) WITH HUMAN ROTAVIRUS A


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Rotavirus is the most common cause of severe acute diarrhea in infants and young children worldwide. The incidence of rotavirus disease is similar in developed and developing countries but the mortality is higher in the former countries. Their double-stranded RNA consists of 11 segments, which encode six viral capsid proteins (VP1, 2, 3, 4, 6 and 7) and six nonstructural proteins (NSP1-6). Rotavirus is classified into seven serogroups (A-G) based upon the antigenic properties of VP6, an inner capsid protein, of which groups A, B and C are human pathogens. Rotavirus spreads from person to person, mainly by faecal-oral transmission. Detectable rotavirus antigenemia and viremia suggests that
rotavirus escapes from the intestinal tract. In this study, we report the experimental infection of nine infant-
juvenile cynomologus 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 administrated 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 rotavirus infections.

**IV495 - CAMELID NANOBODIES, AN ALTERNATIVE TO DIAGNOSIS HANTAVIRUS INFECTION**


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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 aeroisolized 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 VH or nanobody. This work proposes the use of cameld 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. Amplexils were cloned into PHEN1 phagemid vector and TG1 E. coli strain to construct a VHH immune library with a titer of 2,3 ×1018 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
Oral Presentation

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 multispecific 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
mottle virus). These results were further confirmed by reverse transcription qPCR (qRT-PCR). The expression of these genes is currently being evaluated in a time course assay following virus inoculation. Since it has been described that the silencing of ubiquitination pathway genes enhanced the begomovirus Tomato yellow leaf curl virus infection, the next step of this study will be the silencing of these ubiquitin pathway genes using VIGS (Virus-induced gene silencing). After confirmation of silencing effectiveness, plants will be inoculated with ToCMoV and the resulting phenotype evaluated.

Financial support: Fundo Embrapa/Monsanto, CNPq, INCT-Interações Planta-Praga, FapDF

PIV373 - INFECTIOUS CDNA CLONES OF THE CRINIVIRUS TOMATO CHLOROSIS VIRUS ARE COMPETENT FOR SYSTEMIC PLANT INFECTION AND WHITEFLY-TRANSMISSION

Orílio, A.F., Fortes, I.M., Navas-Castillo, J.


Tomato chlorosis virus (ToCV) is a crinivirus (genus Crinivirus, family Closteroviridae) that causes important emerging diseases in tomato and other crops. ToCV is limited to the phloem, is not transmitted mechanically and naturally is transmitted in a semipersistent manner by the whiteflies Bemisia tabaci, Trialeurodes vaporariorum and T. abutilonea. The ToCV genome consists of two molecules of linear, positive-sense RNA encapsidated into long flexuous virions with a complex structure. Here we present the construction of infectious cDNA clones of the ToCV genome (RNA1 and RNA2) under the control of the CaMV 35S promoter in a binary plasmid. Agroinfiltration of N. benthamiana leaves with clones of both RNAs resulted in systemic infection. Tomato plants also were infected by grafting them with agroinfected N. benthamiana plants, showing the typical symptoms caused by this virus consisting in chlorotic spots on the lower leaves that extend towards the top of the plant and evolves to interveinal yellowing. Furthermore, the viral progeny generated in tomato was transmitted to new tomato plants by B. tabaci. The infectious clones obtained constitute a genetic system that will allow to identify the viral genes involved in replication, movement in the host plant, transmission and pathogenicity by reverse genetics.

PIV394 - CHARACTERIZATION OF DNAJ PROTEINS REVEALS THEIR ROLE DURING PEPPER YELLOW MOSAIC VIRUS INFECTION IN SUSCEPTIBLE HOSTS


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During co-evolution between virus and host, a complex interaction has been developed involving several mechanisms of pathogen attack and host defense. Host defense responses cause up- and downward shifts in gene expression. To understand the process of tomato infection by the potyvirus Pepper yellow mosaic virus (PePYMV), a subtractive library identified several genes as differentially expressed during the early stages of viral infection. Among the induced genes was the one encoding a DnaJ (HSP40) protein. Members of the Dnaj multigene family contain a highly conserved 70-amino acid signature region, the J domain, and assist as chaperones of HSP70s in various cellular processes. The involvement of HSP proteins in the enhancement or inhibition of pathogenesis in a wide range of viral infections has been described. Our own previous data demonstrate that DnaJ induction contributes to the early stages of PePYMV infection. To advance our understanding of the role of this protein during PePYMV infection, the complete sequence of two genes encoding Solanum lycopersicum homologs of DnaJ (SIDj1 and SIDj2) were cloned. Both SIDj1 as SIDj2 proteins have the conserved J, G/F and C-terminal domains but the zinc finger domain is present only in SIDj1. The subcellular localization of SIDj was analyzed by confocal microscopy using a SIDj-GFP fusion. In healthy plants the subcellular localization of SIDj1 and SIDj2 is nuclear and cytoplasmatic while in PepYMV-infected plants, 12 days after inoculation, SIDj1 and SIDj2 are localized only in the cytoplasm. SIDj did not interact directly with any individual viral protein in a two-hybrid assay. It is likely that in the context of infection these proteins interact either with the intermediates of the processing of the viral polyprotein, or indirectly through a bridge protein. Financial support: CNPq, CAPES, FAPEMIG and INCT Planta-praga.

PIV408 - POPULATION GENETIC STRUCTURE OF TOMATO LEAF DEFORMATION VIRUS INFECTING TOMATO CROPS IN ECUADOR AND PERU


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The family Geminiviridae is characterized by a particle morphology of twinned incomplete icosahedra and a genome comprised of circular, single-stranded DNA. Whitefly-transmitted geminiviruses (genus Begomovirus) are responsible for serious agricultural threats in Latin America. We have recently reported the widespread occurrence of a monopartite begomovirus, Tomato leaf deformation virus (ToLDeV), in Ecuador: Here, we determined the genetic structure of ToLDeV populations based on the analysis of 67 full-length genome sequences of isolates collected from Ecuador (determined in this study) and 9 additional sequences of isolates from Peru (previously available from Genbank). Subdivision analysis indicated a markedly genetic differentiation between isolates collected from both countries (FST: 0.42929). Overall, the Ecuadorian subpopulation showed lower genetic variability than that from Peru ($\pi = 0.00853$ and 0.05174, respectively). Interestingly, while the CP, TrAP and Ren genes from the Peruvian subpopulation were about 2.5 times more variable than those from the Ecuadorian subpopulation, its Rep and markedly the AC4 genes were much more variable (about 10 and 18 times more variable than those of isolates from Ecuador, respectively). Neutrality tests (Fu and Li’s $D^*$ and $F^*$) indicated positive selection acting on the AC4 gene of isolates from Peru. However the evidence was weak, since no positively selected sites were detected by the SLAC or PARRIS methods. A single recombination event involving an isolate from Peru as a minor parent was detected by RDP in all 63 haplotypes of isolates from Ecuador analyzed in this study. The contrasting molecular variability levels between isolates of ToLDeV from Peru and Ecuador suggest a more recent foundation of this latter subpopulation. Financial support: FAPEMIG, INIAP, CAPES.

PIV459 - EVOLUTION OF PE-38 GENE IN BACULOVIRIDAE
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Baculoviridae is a family of dsDNA viruses that infects a few orders of insects. They are divided into four genera, Alphabaculovirus (Lepidopteran-specific nucleopolyhedrovirus), Betabaculovirus (Lepidopteran-specific GV), Gammabaculovirus (Hymenopteran-specific NPV) and Deltabaculovirus (Dipteran-specific NPV). It was found a gene in the genome of some baculovirus (BC) that contains a RING-finger domain with ubiquitin-ligase (E3) activity, the pe-38 gene. It has been associated to viral transcription and viral DNA replication. Despite this importance, the evolutionary history of this gene in the BCs family remains unclear. Therefore, the objective of this work was to determine the evolutionary events that shaped the current pe-38 gene distribution among BCs. Initially, the BLAST program was used to search for pe-38 orthologous in BC genomes available in GenBank. We found that pe-38 orthologous were present only in the group 1 Alphabaculovirus and in four related Betabaculovirus. Interestingly, the genome of the Choristoneura occidentalis granulovirus (ChocGV) lacks the pe-38 gene, but presented the flanking upstream region of its in other GV genomes. In this region, we find a gene that has orthologous in NPV genome. This finding may indicate a non-homologous recombination event between the Choristoneura occidentalis granulovirus (ChocGV) and an ancestral NPV took place and that the pe-38 gene present in the NPV may have originated in GVs. To confirm that hypothesis, the phylogeny of the pe-38 gene was reconstructed by using the PhyML program. It was found that the diversity between the GV proteins was greater than the one found in NPV, indicating that the proteins of GVs have been diverging for a longer time. Additionally, we also found that pe-38 gene of BCs showed a significant similarity with a plant gene called makorin, an ubiquitin ligase. Therefore, it is reasonable to assume that pe-38 gene of BCs was acquired from a plant genome by an ancestral GV. Financial Support: CNPq.
West Nile (WNV), of the family Flaviviridae, in wild birds necropsied at the Department of Veterinary Pathology, UNESP, Jaboticabal, SP, Brazil. Fifty two samples of brain, liver, spleen and blood of 52 wild birds were analyzed. Pea-size tissue fragments and blood clots were placed in Trizol (Invitrogen) and stored at -70°C until processed. RNA was extracted by the Trizol manufacturer’s protocol and reverse transcription was carried out to obtain cDNA, which was amplified by Real-Time RT-PCR (One-Step SYBR-Green). MAYV RNA was detected in 10% of the birds tested: 1 crested caracara (Polyborus plancus, brain and spleen); 1 roadside hawk (Buteo magnirostris, spleen); 1 burrowing owl (Speotyto cunicularia, brain); 2 black vultures (Coragyps atratus, one of them in liver, brain, blood and spleen; and another in liver; brain and blood). CHIKV RNA was detected in 11% of the tested birds: 1 crested caracara (Polyborus plancus, brain, liver and blood); 1 roadside hawk (Buteo magnirostris, liver, spleen and blood); 1 burrowing owl (Speotyto cunicularia, brain, liver and blood); 2 black vultures (Coragyps atratus, one of them in liver and spleen and another in liver); and 1 dove (Columba livia, brain, liver and spleen). WNV RNA was detected in 6% of the tested birds: 1 crested caracara (Polyborus plancus, spleen and blood); 1 black vulture (Coragyps atratus, blood and spleen); and 1 toucan (Ramphastos toco, blood, liver and spleen). These results show that important arboviruses such as biocompatibility, high aspect ratio and cell such as West Nile virus (WNV) and Chikungunya virus (CHIKV) have been broadly studied due to their exceptional properties. Carbon Nanotubes (CNT) has been used to develop molecular characterization of CCoV strains circulating in puppies with enteritis by partial “S” gene sequencing. Bottino, F.O., Costa, E.M., Castro, T.X., Cubel Garcia, R.C.N. Universidade Federal Fluminense, UFF, Rua Prof. Hernani Melo 101, São Domingos, Niterói, RJ, Brasil

Canine coronavirus (CCoV) is an important agent of gastroenteritis in puppies. To date, CCoVs are classified in two genotypes, CCoV-I and CCoV-II. Recently, CCoV-II genotype was divided in two subtypes: CCoV-IIa (classical strains) and IIb (TGEV-like strains). The aim of this study was to realize the molecular characterization of CCoV strains detected in 25 fecal samples from diarrheic puppies in Rio de Janeiro. Genomic RNA was extracted using the PureLink™ Spin Column-Based Kit (Invitrogen®). The reverse transcription was performed with random primer (Invitrogen®) and Superscript III enzyme (Invitrogen®). Differential primers directed to the spike (S) gene were used in PCR assays for CCoV genotyping/subtyping: EL1F/EL1R (3538-3883) to amplify CCoV-I whereas S5F/S6R (3486-4179) and CEPol-1/TGSP-2 (20168-20537) for CCoV-IIa and CCoV-IIb. The amplicons were purified and subjected to direct sequencing using BigDye Terminator Cycle chemistry. Nucleotide and amino acid (AA) similarity with Genbank database was assessed using BLAST tool. By RT-PCR, single infection was detected in 16 samples: 6 CCoV-I, 9 CCoV-IIa and 1 CCoV-IIb. Nine samples were positive for more than one genotype/subtype: CCoV-I/IIa (7), CCoV-I/Iib (1) and CCoV-IIa/Iib (1). Sequence analysis of 22/25 strains revealed that they shared high identity with other CCoV prototypes. However, nonsynonymous substitutions were found in these strains that were not described before: two AA changes (residues 1207, 1264) in CCoV-I, 13 in CCoV-IIa (residues 1174, 1218, 1244, 1264, 1265, 1282, 1305, 1334, 1336, 1339, 1359, 1363, 1370) and five in CCoV-IIb (residues 5, 6, 7, 8, 18). The CCoV-IIb strains exhibited the insertion of three nucleotides at the S’end of the S gene which resulted in addition of AA at residue five as also found in UCD-1 strain. These results show that mixed CCoV infections are usual in Rio de Janeiro and further studies are needed to clarify the importance of these AA changes in CCoV evolution. Financial support: FAPERJ, CAPES, CNPq, PROPPI-UFF.

**VV224 - EXPERIMENTAL VACCINE TO BOHV-1 AND BOHV-5 FUNCTIONALIZED TO CARBON NANOTUBES ENHANCES THE IMMUNE RESPONSE IN MOUSE MODEL**


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Bovine herpesviruses 1 and 5 (BoHV-1 and 5) are closely related alphaherpesviruses infecting cattle and co-infection is likely to occur. Both viruses are associated with neurological, respiratory and reproductive disease, causing great economic losses. Vaccination has been the recommended in control programs, although to date there is no vaccine capable of establishing a protective immune response against both viruses. Recombinant proteins have been widely used for production of helpful molecules employed in prevention and treatment of several diseases. Carbon Nanotubes (CNT) has been broadly studied due to their exceptional properties such as biocompatibility, high aspect ratio and cell internalization ability, and CNT functionalized with
antigens have immunogenic potential, as shown in previous studies. In this work, we have used the CNT technology to build experimental immunogens against BoHV-1 and 5. These molecules functionalized or not to the CNT, were used in a prime-boost immunization protocol in C57Bl-6 mice, comparing to recombinants alone or added with alum and inactivated commercial vaccine. Following that, mice TCD4+ and CD8+ lymphocytes activation was analyzed by flow citometry, quantifying the marker CD25. The proteins recognition profile by IgG and IgM from bovines naturally infected with BoHV-1 and 5 was also accessed. Mice immunized with the recombinant proteins functionalized to the CNT plus the adjuvant alum, showed a higher profile of activated CD4+ and CD8+ cells than the other groups. The recombinant proteins were recognized by the IgG and IgM antibodies from bovines naturally infected with both viruses, showing that the CNT doesn't interfere with the recognition profile of the molecules. Since our experimental immunogens were successfully recognized by sera from infected bovines and showed a better cellular response in mouse model, they could be tested as vaccine prototype against BoHV-1 and 5 infections in bovine model in a near future.

**VV270 - VACCINIA VIRUS: TRANSMISSION THROUGH EXPERIMENTALLY INFECTED MILK IN A MOUSE MODEL**


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Bovine vaccinia (BV) is a re-emerging zoonosis caused by Vaccinia virus (VACV) and is involved in several outbreaks in dairy cattle and humans throughout Brazil, which is one of the most important milk producers in the world. Previous studies have described the presence of viable viral particles in milk samples of cows experimentally and naturally infected with VACV. However, it is not known if the VACV infectious particles presented in infected milk could be transmissible. Therefore, the aim of this work was to study the possibility of transmission of VACV by experimentally infected milk. Forty female BALB/c mice with 4 weeks of age were divided in four groups: G1, G2, G3 e G4. The G1 was the negative control group. The mice of the other groups were inoculated orally with 100µL of VACV-GP2. Clinical examination was performed during the 30 days post-infection (d.p.i). Feces and oral swab samples were collected in alternate days, from day 0 to day 30 and then submitted to PCR. Blood and sera samples were collected at days 0, 2, 5, 8, 10, 20 and 30 p.i. for PCR and serology tests. The fecal and blood samples were pooled and analyzed per group. No clinical signs or macroscopic lesions were observed. Sera from mice of groups G2 and G3 showed neutralizing antibodies titers at days 20 and 30 p.i. Furthermore, viral DNA was detected in some samples at different times of collection. Oral swabs positive samples were detected in G3 and G4, in at least one mouse, from days 2 to 10 p.i. Moreover, pooled feces and blood samples were DNA positive, in at least one group, at days 6, 8 and 30 p.i. and at 2, 10 and 30 p.i., respectively. It has been shown that mice could be infected after oral inoculation with VACV contaminated milk, as shown by the DNAmia and fecal positive samples. These partial results suggests that VACV contaminated milk may be able a route of transmission through oral ingestion. Financial support: FAPEMIG, CNPq, CAPES, PROGRAD-UFMG and PRPq-UFMG

**VV327 - INFECTION OF FARMED MARINE SHRIMP WITH WHITE SPOT SYNDROME VIRUS IN THE STATE OF SANTA CATARINA, BRAZIL**

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White spot syndrome is a viral infection responsible for considerable economic damage to the global shrimp-farming industry. Initially, the infection manifests epidemically, with high mortality rates. Later, the infection remains endemic, interfering with the productivity of the tanks. In Brazil’s southern region, the infection was first identified in 2005 and had a significant impact on the production of farmed marine shrimp. This study was conducted to evaluate the presence of white spot syndrome virus (WSSV) both in farmed marine shrimp (Litopenaeus vannamei) and in natural reservoirs on the northern Santa Catarina coast. In the period between 2005 and 2008, 440 samples of different L. vannamei tissues were collected from 12 regularly monitored shrimp farms. The samples were stratified by age, and the collected samples were representative of post-larvae arriving at the farm, of animals with 30, 60, and 90 days of cultivation, and of animals at harvest. Additional samples, independent of regular sampling, were acquired from lots with high mortality rates. In addition, 210 samples of native animals that were present in the reservoirs and harvest channels of the farms, including mangrove and fiddler crabs (Aratus pisoni and Uca mangrove and fiddler crabs (Aratus pisoni and Uca
Group A rotavirus (RVA) infection cause neonatal diarrhea in many animal species worldwide. The genotypes G3, G4, G5, G11, P[6]-Gottfried, and P[7] are commonly identified in piglets. However, several unusual genotypes, such as G1, G2, G6, G8, G9, G10, G12, G26, P[1], P[5], P[6]-M37-like, P[8], P[11], P[13], P[19], P[23], P[26], P[27], P[32], and P[34] have also been identified in pigs. This study was developed to identify the G and P genotypes of 73 wild-type PoRVA strains of Brazilian pig herds. All diarrheic stool samples from suckling piglets collected during 2005 to 2013 were submitted to PAGE showing the high diversity of genotypes circulating in Brazilian marine shrimp and in natural reservoirs in the region and during the period studied.

**VV494 - DIVERSITY OF G AND P GENOTYPES DETECTED IN BRAZILIAN PIG HERDS DURING 2005-2013**


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Group A rotavirus (RVA) infection cause neonatal diarrhea in many animal species worldwide. The genotypes G3, G4, G5, G11, P[6]-Gottfried, and P[7] are commonly identified in piglets. However, several unusual genotypes, such as G1, G2, G6, G8, G9, G10, G12, G26, P[1], P[5], P[6]-M37-like, P[8], P[11], P[13], P[19], P[23], P[26], P[27], P[32], and P[34] have also been identified in pigs. This study was developed to identify the G and P genotypes of 73 wild-type PoRVA strains of Brazilian pig herds. All diarrheic stool samples from suckling piglets collected during 2005 to 2013 were submitted to PAGE showing the high diversity of genotypes circulating in Brazilian marine shrimp and in natural reservoirs in the region and during the period studied.

**VV496 - MOLECULAR DETECTION OF INFLUENZA A VIRUS IN DOGS**


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Influenza A viruses belong to the Orthomyxoviridae family and usually cause respiratory illness in various species, such as humans, domestic poultry, pigs, and horses. The subtype H3N8 is known to cause respiratory disease in equines. However, an influenza A subtype H3N8 has been reported as an emerging respiratory pathogen of dogs in the United States in 2004. This novel virus, called canine influenza A virus (CIV), share ≥96% nucleotide sequence identity to equine influenza A virus subtype H3N8, suggesting transmission between horses and dogs without reassortment with other strains. This report investigated the death of three mongrel dogs with nonspecific clinical signs. One dog was seven years old and was icteric. The other two dogs were about 5 months old and were taken from the street presenting hemorrhagic diarrhea. Pathological lesions of the first dog included hepatitis, pulmonary hemorrhage, and meningoencephalitis. Significant pathological alterations of the other two dogs included depletion of intestinal lymphoid tissue and hemorrhagic enteritis. Fragments of the lungs and kidneys were collected and tested by RT-PCR using the primers M52C and M253R. PCR assays amplified the partial segment 7 of Influenza A virus (244 bp) from all pulmonary tissues evaluated. These data suggest the circulation of CIV in the canine population of Brazil. Since the history of the three dogs is...
unknown, the source of infection either by contact with other infected dogs or horses remains obscure.

VV634 - CHICKEN ANEMIA VIRUS AND AVIAN GYROVIRUS 2 DNA AS CONTAMINANTS IN POULTRY VACCINES

Varela, A.P.M., Santos, H.F., Cibulski, S.P., Scheffer, C.M., Schmidt, C., Lima, F.E.S., Esteves, P.A., Franco, A.C., Roehe, P.M.

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In view of the potential role of vaccines as a source of pathogen dissemination, this study was set up in order to detect AGV2 and CAV genomes in vaccines for poultry. Thirty five largely employed, commercially available vaccines produced by eight different manufacturers against various avian pathogens, and farming were evaluated. Total DNA was extracted from 500 µL of each of the vaccines with PureLinkTM Genomic DNA Mini Kit (Life Technologies). Approximately fifty nanograms of DNA were used in the assays. A quantitative duplex TaqMan® real-time PCR (Wendlant et al.; this event) was performed using AGV2- and CAV-specific primers and probes. Amplification and detection were performed in a StepOneTM Real-Time PCR system (Life Technologies). Copies of AGV2 genomes were detected in 9 of the vaccines evaluated, in amounts which varied from 93 to 156,187 copies/50ng. Regarding CAV, viral genomes were detected in 10 of the vaccines tested, of which three were in fact CAV vaccines and six vaccines to other pathogens. The three CAV vaccines showed distinct numbers of copies of CAV genome, corresponding to 2,175,381; 54,238 and 2,386 genome copies/50ng. The remaining non-CAV vaccines contained between 7 and 173 copies of CAV genome molecules/50ng. Four of the examined vaccines contained DNA of both CAV and AGV2. These results revealed that both CAV and AGV2 genomes may be detected in poultry vaccines. In addition, although CAV contamination of biological has been reported previously, this is the first report of AGV2 DNA as contaminant of vaccines. These findings highlight the need for preventive measures to avoid contamination of vaccines with such viruses. Financial support: CAPES, CNPq, FINEP