HV677 - PRESENCE AND FULL-LENGTH GENOMIC SEQUENCE OF HEPATITIS B VIRUS GENOTYPE E IN A BRAZILIAN PATIENT


1. Gastroenterology, School of Medicine, São Paulo University, FMUSP, Rua Dr enéas de carvalho aguiar; 500 São Paulo
2. Albert Einstein Diagnostic medicine, HIAE, Liver Therapeutic Specialized Center - CETEFI, CETEFI, Clinical Laboratory, Heart Hospital of São Paulo, HCOR, E-mail: monica.viviana@usp.br

Introduction: Hepatitis B virus (HBV) is estimated to cause chronic infections in more than 350 million people worldwide and death in one million per year. Nine HBV genotypes (A-I) have been described so far. Genotype E (HBV/E) is largely distributed in West Africa and has rarely been found in other continents, except for few cases in individuals with African background. However, recent reports found this genotype in a specific community in Colombia and in India. Objectives: The aim of this study was to characterize the complete genome of HBV genotype E infection in a Brazilian patient. Methods: The patient is a man, 66 years old and was born in São Paulo, Brazil. Amplification of the whole HBV genome was performed with P1 and P2 primers described previously. The isolated complete genome (3200bp) was aligned with other previously reported complete genome sequences (n=125) using MUSCLE software. The Bayesian Markov chain Monte Carlo simulation implemented in BEAST v.1.5.4 was applied to obtain the best possible estimates under both relaxed uncorrelated lognormal and exponential molecular clock and using the model of nucleotide substitution (GTR+G+I). Results: HBV complete genome was successfully amplified. The sequence was analyzed for all reported mutations for antiviral resistance and it did not show any of them. After phylogenetic analysis, the complete HBV genome sequence from this patient grouped in a clade with a sequence from Namibia and Argentina. This is the first case of infection by HBV genotype E in a Brazilian native patient. We have recently shown that genotype E was identified in some Africans patients that are followed up in Brazil by some clinicians. This patient lives in Africa and comes to Brazil for medical assistance. As HBV genotype E has apparently spread recently in Africa, it is may become a relevant infectious agent in our country due to the increasing of social and economical relations between or countries. FAPESP 2011/52615-0, FFM.

HV678 - A NOVEL NUCLEOTIDE INSERTION IN S GENE OF HEPATITIS B VIRUS GENOTYPE A1 IN A BRAZILIAN CHRONIC CARRIER


1. Gastroenterology, School of Medicine, University São Paulo, FMUSP, Av Dr Eneas de Carvalho, 500
2. Hospital Israelita Albert Einstein,
HBV is classified into nine genotypes (A-I). In Brazil, genotype A is most frequent, followed by D and F. North, Northeast and Southeast regions have a higher frequency of genotype A while genotype D is the most frequent in the South region. The aim of this study was to determine the HBV genotype and reported the mutations in the S/POL region. The patient is female, 56 years old and was born in Brazil. A fragment of 1306bp partially comprising HBsAg and polymerase coding regions (S/POL) was amplified and sequenced. Viral sequences were genotyped by phylogenetic analysis using reference sequences from GenBank (n=380). The Bayesian Markov chain Monte Carlo simulation implemented in BEAST v.1.5.4 was applied to obtain the best possible estimates and using the model of nucleotide substitution (GTR+G+I). Multiple alignment of partial HBV/S gene (87 to 227 nt) comprising HBV/A1 sequences from Brazil and other countries, which are compared with other sequences of HBV subgenotypes previously reported. After completion of the phylogenetic analysis, the genome sequence of the patient was grouped in a clade with four sequences from Rondonia state. We have identified new mutations in this case: an insertion of a Serine before the position 115, just after changing the nucleotide sequence from Threonine to Asparagine in position 114. The origin of this variant HBsAg was unclear but might occur naturally due to lack of proof-reading activity of rt domain. The major antigenic epitope in the immunodominant loop is called the α-determinant and is composed of residues 124 to 147. This mutation probably does not affect the most relevant antigenic regions of HBsAg, particularly this α-determinant region, where some mutations can interfere with the recognition of HBsAg by anti-HBs antibodies, especially those contained induced by the commonly used vaccines. FAPESP 2011/50562-0, FMM, HCFMUSP.

HIAE, Federal University of São Paulo, UNIFESP, E-mail: monica.viviana@usp.br

The identification of cytokine profile of individuals infected by dengue viruses can be an important instrument to detect patients with a tendency to develop severe illness. In this study, the quantification of cytokines serum levels (IL-6, IL-8, and IL-10) was performed among a group of patients infected by dengue viruses, individuals with clinical suspicion of infection by dengue viruses – but with negative laboratory diagnosis and a healthy control group. We also investigated the frequency of the polymorphism of the genes IL-6 (-634 C/G), IL-8 (-353 A/T), and IL-10 (-1082 G/A), as well...
as the occurrence of the association between the genetic variability and the cytokine serum levels. The ELISA test was used in order to detect cytokine concentrations, while the polymorphisms were investigated using polymerase chain reaction, which was followed by the use of the restriction fragment length polymorphism (RFLP) analysis. The lowest serum level of IL-6 was identified on patients infected with dengue as compared to the control group and the highest levels were described among the disease persons without dengue. The levels of IL-8 and IL-10 were higher both in the infected patients and non-infected patients, but no exclusive profile of these cytokines was associated to any group. The genotypes GG (IL-6 -634 C/G), AT (IL-8 -353 A/T), and AA (IL-10 -1082 G/A) were the most prevalent in all groups, but there was no statistical differences among the genotype and allelic frequencies. The cytokine concentrations were not influenced by the genetic polymorphism. Financial Support: CNPq and UFPA

HV681 - HEPATITIS C VIRUS (HCV) PATIENTS WITH HEPATOCELLULAR CARCINOMA IN BRAZIL: GENOTYPES DISTRIBUTION, CLINICAL IMPLICATIONS AND HCC-ASSOCIATED VIRAL MUTATIONS


University of São Paulo School of Medicine, FMUSP, Av. Dr. Enéas Carvalho de Aguiar, 500, Cérquira César, São Paulo, SP E-mail: joaopaulomoreirapr@gmail.com

Hepatitis C virus (HCV), a major cause of chronic liver disease, frequently progresses to cirrhosis with increased risk of hepatocellular carcinoma (HCC). The present study was undertaken to investigate the distribution pattern of HCV genotypes, clinical implications and HCC-associated virus mutations in patients with HCV/HCC. This study included 129 randomly selected patients with HCV/HCC, who were diagnosed with HCC between 2002 to 2012 at HCFMUSP, São Paulo, Brazil. A fragment of 674bp partially comprising the 5’UTR and Core regions was amplified, sequenced and compared by phylogenetic analysis with reference sequences obtained from the GenBank (n=318). Bayesian phylogenetic analyses were conducted using the MCMC simulation implemented in BEAST v.1.5.3. Among 129 HCC patients, 82.9% were Caucasians, 65.9% were male, mean age was 61.4 (29.8-80.0) years, AFP and ALT means were 987.3ng/mL and 67.1U/L respectively. The frequency of HCV genotypes among HCC patients was: 1b (41.5%), 3a (30.8%), 1a (19.1%), 2b (4.2%), 2c (2.1%), 5a (1.1%) and 2a (1.1%). We determined HCV core gene substitutions at residues 70 and 91 in patients infected with genotypes 1a and 1b. At position R70, 35.9% had a Glutamine (Q) and at position L91, 50.0% had a Methionine (M), respectively. Subtypes 2c and 5a strains were closely associated to strains from Argentina and Africa, respectively. AFP and ALT levels were elevated in this HCC population. The frequency of the different HCV genotypes was similar to previous data from chronic hepatitis C Brazilian patients. HCV amino acid substitutions 70 and 91 in the core region affect the results of
combination therapies of interferon/ribavirin and lead to progression to HCC. A better understanding of these risk factors leads to improved early detection strategies and more-effective therapies for patients with HCV-related HCC. Both viral and host factors may contribute to HCC risk in HCV patients with chronic infection. FFM/HCFMUSP, FAPESP2011/50562-0

**HV682 - INFECTION BY HCV WITH A NEW DELETION OF 69 AMINO ACIDS AT THE MAIN HOMOTYPIC INTERACTION DOMAIN OF CORE GENE IN A CHRONIC INFECTED PATIENT WITH HEPATOCELLULAR CARCINOMA**


School of Medicine, University of São Paulo, FMUSP, Av. Dr. Enéas Carvalho de Aguiar, 500 E-mail: joaopaulomoreira@gmail.com

Hepatitis C virus (HCV) has a 9,600 nucleotides, sRNA(+) genome and displays a high level of sequence diversity. HCV core has been associated in the development of hepatocellular carcinoma (HCC). A Brazilian male patient, 61-years old, Caucasian, and with the following clinical assays results (AFP 55.76 ng/mL; ALP 86 U/L; AST 75 U/L; ALT 101 U/L; GGT 159 U/L and Platelets count 1.04x10^5/mm3) was diagnosed in 2009 with HCC classified as BCLC-A stage. A fragment of 674bp partially comprising the 5'UTR and core regions was amplified and sequenced by automated DNA sequencing. Genotyping was carried out by using a phylogenetic analysis using reference sequences. Patient was infected with HCV subtype 1a. The amplified fragment covered the core region between positions 250 to 674 nt of genome. A new deletion of 207 nucleotides, corresponding to 69 amino acids at positions 49 to 116 in the core protein was detected in the dominant viral population. To our knowledge, this deletion has not been reported before. Many studies reported HCV deletions in different isolated clones in different regions of the HCV genome but not as the major circulating viral population in HCV infected patients. Core contains a number of aminoacid residues essential for assembly and release of the viral particle. This deletion partially included the amino-terminal hydrophilic portion (1aa-115aa) of core protein responsible for multimerization. This region contains the main homotypic interaction domain (82 -102 aa). Previous studies revealed that numerous core residues are essential for infectious virus production, including a significant number in the first 120 positions of the protein but the first 75 N-terminal residues of the C protein could generate nucleocapsid-like particles (NLPs) smaller in size. We can conclude that many smaller viral particles were circulating in this patient and this event may be related to HCC development. Support: FFM, HCFMUSP, FAPESP 2011/50562-0.

**HV683 - STAGE, TREATMENT APPROACH AND VIRAL GENOTYPE IN CHRONIC HEPATITIS B PATIENTS WITH HEPATOCELLULAR CARCINOMA (HCC) FOLLOWED UP IN SÃO PAULO, BRAZIL**

Botelho-Lima, L.S., Moreira, J.P., Paranaguá-Vezozzo, D., Kikuchi, L., Chagas, A., Alencar, R., ONO, S., Sumita,
Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death worldwide. Risk factors for the development of HCC in patients with chronic hepatitis B virus (HBV) infection are being elucidated. Thirty-one HBV/HCC patients were enrolled for this study. A fragment of 1,306 bp (S/POL) was amplified, sequenced and genotyped by phylogenetical analysis using reference sequences. Among HCC patients, 96.7% were cirrhotic patients, 64.5% were Caucasians, 80.6% men and mean age was 56.5 years (range 39-85 years). The mean values of clinical variables were: AFP: 2505.8ng/mL; ALP: 134U/L; AST: 58.9U/L ALT: 44.4U/L; GGT: 134U/L and Platelets: 137.3mil/mm3. HCC tumors were identified by: Screening program–22.6%, Causal Finding–16.1% and Symptoms–61.3%. From 7 cases included within screening program, 28.5% presented an early stage HCC (single nodule<2cm). The majority of patients were BCLC early stage (BCLC-A–54.8%) and applicability of TACE and resection treatments was more frequently: 38.7% and 22.5%, respectively. Furthermore, tumors in BCLC-B and BCLC-C stages were detected in patients with symptoms. The frequency of HBV genotypes among HCC patients was A1 (41.6%), C2 (16.6%), D3 (16.6%), A2 (8.3%), F2a (8.3%) and D1 (8.3%). HBV/A1 and male sex were more prevalent in these patients. HBV/C2, commonly found in Asia, was the second most prevalent genotype. These results agreed with the distribution of HBV genotypes circulating in Brazil, where HBV/A1 is the most prevalent, being predominant in the Northern and Northeastern states. Data demonstrate that the majority of HCC patients are diagnoses within symptoms stage that limits the chance for precocious diagnosis and effective therapy. However, the screening program showed a positive result for detection of very early tumors in HBV patients. According to this result, we concluded that is necessary to invest in the adherence of patients to the screening program. FAPESP 2011/50562-0, FFM and HCFMUSP.

HV684 - DETECTION OF A NOVEL NOROVIRUS RECOMBINANT STRAIN IN AN AFRICAN-DESCENDANT COMMUNITY FROM THE AMAZON REGION, BRAZIL, 2008


Seção de Virologia, Instituto Evandro Chagas. Rodovia BR316, , E-mail: tuliomf@yahoo.com.br

Norovirus, a major cause of acute gastroenteritis outbreaks worldwide, are constantly evolving. This ability is reflected in the speed and efficiency with which these viruses spread and remain in human population. The present study reports the detection of a novel recombination event among norovirus genotypes in Brazil in the year of 2008. Initially the fecal sample (QUI 38F1) was tested for the presence of NoV antigen using the RIDASCREEN® Norovirus 3rd Generation enzyme immunoassay kit. To confirm the immunoassay positive result two RT-PCR methodologies were
used. Region B (ORF1) and region D (ORF2) of NoV genome were amplified by using specific primers and PCR reaction conditions. The sample QUI38F1 yielded positive results with the three methodologies used. Amplicons obtained were purified and directly sequenced in both directions using the Big Dye Terminator Reaction Kit® (v. 3.1) and an ABI Prism 3130xl DNA sequencer. Assignment of the strain to specific NoV genotypes was made according to the Genotyping Tool available online (Noronet) and the phylogenetic analysis was performed using MEGA version 5.05. In order to investigate the possibility of a recombination event in the sample studied, ORF1/2 junction region was amplified with primers Mon431/432 and G2SKR. Phylogenetic analysis carried out with partial polymerase and capsid sequences resulted in QUI38F1 clustering within two different genotypes, GII.7 (ORF1) and GII.20 (ORF2), confirming the results obtained with the genotyping tool. Plot analysis revealed potential recombination of QUI38F1 within two parental strains [GII.7 (Gwynedd) and GII.20 (Leverkusen)] and identified the breakpoint located at 60 nt upstream the ORF1/2 overlap. The present study revealed a novel NoV intergenotype recombinant strain detected in a relatively isolated, African-descendant community living in Northern Brazil. To our knowledge, this is the first description of NoV intergenotype GII.7/GII.20 recombinant strain. The study was funded by the Foundation for Research Support of the State of Pará (Fundação de Amparo à Pesquisa do Estado do Pará - Secretaria de Estado de Desenvolvimento, Ciência e Tecnologia) grant code MS/CNPQ/SECTAM – 001/2006, agreement 032/2007, and by Evandro Chagas Institute, Secretary of Health Surveillance (IEC/SVS), Ministry of Health, Brazil.

HV689 - DETECTION OF HUMAN PAPILLOMAVIRUS TYPE 16 IN CERVICAL ADENOCARCINOMA: CASE REPORT

Buosi, A.S., Gomez, I.C., Souza, N.C.S., Tempaku, P.F., Carvalho, L.V., Caseiro, M.M., Sa-Filho, D.J.

Centro Universitário Lusíada, UNILUS, Rua: Oswaldo Cruz, 179, Boqueirão, Santos - SP – Brazil, 11045-101 E-mail: deiasrf@gmail.com

Background: Human Papillomavirus (HPV) infection is a necessary cause of cervical cancer, and is etiologically associated with a subset of cancers of the anus, oropharynx, penis, vagina, and vulva. Several studies have proposed an association between HPV infection and oesophageal, laryngeal, oropharyngeal, lung, urothelial, breast, cervix and colon cancers. Objective: To investigate the presence of Human Papillomavirus (HPV) DNA in cases of cervical adenocarcinoma. Material and Methods: Four samples were obtained from cases of cervical adenocarcinoma diagnosed and treated at the Hospital Guilherme Álvaro in Santos-São Paulo, Brazil. DNA was extracted from formalin fixed and paraffin-embedded tumor tissues using QIAamp DNA Mini Kit (Qiagen). The quality of DNA extracted was verified by amplifying the human CCR-5 gene. Detection of HPV DNA was performed by PCR using primers GP5 and GP6. HPV positive and negative controls were performed. The presence of DNA was verified by agarose gel electrophoresis. The
sequencing of HPV was performed by ABI PRISM Dye Terminator. The sequence was compared with Genbank database. Results: The genomic DNA from paraffin-embedded tumor tissues presented 2 of 4 positive amplification for the human CCR-5 gene. Cervical adenocarcinoma tested showed positive result for HPV DNA in one sample. The sequence analysis revealed that strain belonged to HPV type 16. Conclusion: Our results are in agreement with some series and showed evidence of HPV DNA in cervical adenocarcinoma.

HV695 - EVALUATION OF CYTOMEGALOVIRUS AS IMPORTANT CAUSAL AGENT IN THE MORBIMORTALITY IN PATIENTS WITH HIV/AIDS, BELÉM, PARÁ


1. UNIVERSIDADE FEDERAL DO PARÁ, UFPA, AV. AUGUSTO CORREA S/N
2. INSTITUTO EVANDRO CHAGAS/ SVS/MS, IEC/SVS/MS, BR 316 KM 7 E-mail: dorotealobato@iec.pa.gov.br

The Cytomegalovirus is considered one of the main infectious agents affecting humans, and also a major cause of morbimortality in immunocompromised patients. This study aims to describe the epidemiology, the clinical and laboratorial aspects of the Cytomegalovirus as important causal agent in the morbimortality in the patients’ HIV/AIDS. The individuals were interviewed using an epidemiological questionnaire, review of the promptuary and blood collection for diagnostic tests serology and Real-Time PCR. It was included 241 blood samples from HIV-infected patients hospitalized in HUJBB/Belém-Pa. The prevalence of cytomegalovirus was 99.6% (IgG+). Gastrointestinal manifestations were more frequent (68%) than the ophthalmic manifestations (24.5%). The positive results of real-time PCR were higher than serology (55.6% RT PCR positive against 2.1% IgM+). The relation between time of treatment with ART and RT PCR positivity showed the highest rates of positivity in the patients who didn’t realized treatment (24.6%), those who realized the treatment less than six months (32.8%) and those who already realized HIV treatment for over ten years (14.2%). It was observed that the RT PCR positivity was higher (55.2%) when CD4+ levels were below 100/mm3, with a reversal of this relation when CD4+ levels were above 200/mm3, when the negative results dominated (38.6%). The CMV was prevalent in the group of low socioeconomic level. It was demonstrated superiority of the RT PCR in relation to the serology concerning early diagnosis of CMV infection. Treatment with ART reduces the incidence of CMV infection. In front of this scenery and due the significant morbidity that cytomegalovirus imposes in HIV-positive patients, it is important the elaboration of a better screening of these individuals in relation to the opportunistic CMV disease. In patients with CD4+ levels below 100/mm3, it is recommended to conduct fundoscopy, serology and real-time PCR for the detection of HCMV.

HV698 - LONG CONTROL REGION GENETIC VARIABILITY OF HPV
16 ISOLATES FROM PARAGUAYAN WOMEN WITH DIFFERENT GRADE OF CERVICAL LESION

Mendoza, L., Picconi, M.A.

Instituto de Investigaciones en Ciencias de la Salud, UNA, IICS-UNA, Rio de la Pla y La Gerenza Facultad de Ciencias - Universidad de la República, UDELAR, Montevideo, Uruguay Servicio de Virus Oncogénicos, Malbrán, Malbrán, Buenos Aires, Argentina E-mail: lauramendozatorres@gmail.com

Human papillomavirus type 16 (HPV 16) plays a cardinal role in the pathogenesis of cervical cancer. HPV 16 has intratypic variants which show different geographical distributions and different oncogenic potentials. This study aimed to characterize the long control region (LCR) genetic variability of HPV 16 isolates from Paraguayan women. Sixty seven HPV 16 positive cervical samples were studied, including 29 low-grade squamous intraepithelial lesions, 29 high-grade squamous intraepithelial lesions, 4 cervical cancer, and 5 samples from women with normal cytology. Specimens were analyzed by polymerase chain reaction-directed sequencing of the LCR. Most variants corresponded to the European branch-E (82%). There were detected 8 HPV 16 variants; 28% Germany-E-G11, 46% G1-E, 3% G10, 1.5% Brazil-B14-Asian-American-AA, 7.5% India-IND8-AA, 1.5% Tanzania-T4-African-Af1, 1.5% Navajo indian-AN12-AA, 6% Amazonian Indian-AM6-AA and 2 new isolates; 1.5% newPYa and 1.5% newPYb, with nucleotide changes at A7752C and A7810T, respectively, which were included in the E branch by phylogenetic analysis. Furthermore, all non-E variants (18%) were detected only in women with cervical lesion, most of them with nucleotide substitutions at binding sites of yin yang 1 (YY1) and nuclear factor 1 (NF1) transcriptional factors. This observation could partly explain the differences in the pathogenic potential of these variants. This is the first report on HPV 16 variant distribution and sequence variability in Paraguay. The characterization of the LCR contributes to better understand the molecular epidemiology, geographical relatedness and pathogenicity of HPV 16 infection.

HV700 - EXACERBATION OF ASTHMA IN PATIENTS WITH RESPIRATORY VIRAL INFECTION


1. Universidade Federal do Rio de Janeiro, UFRJ, Instituto de Microbiologia Paulo de Góes, UFRJ - R. Janeiro

2. Universidade Federal do Rio de Janeiro, UFRJ, Departamento de Clínica Médica, Faculdade de Medicina, UFRJ- R. Janeiro E-mail: rcirlene@ig.com.br

Asthma can be defined as a chronic condition that results from inflammation of the airways of the lungs. The development of asthma emerges from a complex interaction of genetic predisposition and environmental factors with viral infection likely playing a significant role in the effect of environment on asthma inception. Over the last 20 years much effort has been put into clarifying the role that viral respiratory infections play in
the eventual development of asthma. Tests based on the amplification and sequencing of the viral genome have facilitated the understanding of the association between viral infection and asthma exacerbation. The aim of this study was to determine the rates of respiratory virus infections in patients with exacerbation of asthma, treated at the Immunology Service of the HUCFF/UFRJ. Asthma inception or exacerbation was defined as an abrupt or progressive worsening of dyspnea, wheezing, chest pain, cough or a combination of those symptoms. A total of 108 respiratory samples (nasal+oral swabs combined) were obtained from 83 patients between 19 and 80 years of age. The asthma attack was classified by the attending physician as mild, moderate or severe. Samples were analyzed by real time PCR for virus detection. Twenty-six samples (24%; n = 108) were positive for at least one virus: 8 single infection detected with HBoV-2, 6 with HRV, 5 with HAdV, 2 with HCoV (1 229E and 1 HKU1), and 1 with HRSV, HMPV and KIPyV, each. Additionally, co-infections with these viruses were observed 2 samples. The majority of patients with viral infection (53.8%; 14/26) presented moderate/severe episode of asthma with clinical presentation of dyspnea (88.5%; 23/26), wheezing (84.6%; 22/26), and cough (84.6%; 22/26), and duration of symptoms of 7 to 15 days (80.8%; 21/26). These results suggest that in the studied population, viral infections may be associated with exacerbation and/or worsening of asthma attacks. Financial support: CNPq, CAPES, FAPERJ.

**HV708 - POLYOMAVIRUS DETECTION IN SALIVA OF HIV-INFECTED CHILDREN**


1. Universidade Federal do Rio de Janeiro, UFRJ, Instituto de Microbiologia Paulo de Góes, UFRJ - R. Janeiro
2. Universidade Federal do Rio de Janeiro, UFRJ, Faculdade de Odontologia, UFRJ E-mail: gabriella_mendes@micro.ufrj.br

Human Polyomaviruses (HPyVs) are DNA viruses members of the Polyomaviridae family. Primary infections generally occur early in life, are typically subclinical and followed by persistence of the virus in the organism. Reactivation of HPyV infection has been associated to disease in immunocompromised individuals. Despite of its growing importance, the pathogenesis and natural history of HPyVs infection remain unknown. We aimed to detect the excretion of HPyV in the saliva of HIV-infected children in comparison to healthy control children and evaluate the possible association between viral infection and the stage of immunodeficiency. The samples were collected from patients attending the School of Dentistry of the UFRJ. Saliva was obtained from 60 HIV-infected children ranging from 6 to 13 years of age and 60 health children ranging from 7 to 12 years of age. Virus detection and quantitation was done by real time PCR assay. HPyVs were detected in 17 (28.3%) and 6 (10%) of HIV-infected and control children, respectively. A higher frequency of viral infection was observed among HIV-infected children (p = 0.011). Frequency of KIV infection was significantly higher among
immunocompromised children (p = 0.02). No difference was observed for BKV, JCV or WUV. The virus loads were similar in both groups. HPyV was more frequently detected among children with severe immunosuppression (P <0.001). However, no statistically significant correlation between the frequency of HPyV DNA detection and the use of HAART was observed (p = 0.156). In present study, DNA of BKV, JCV, WUV and KIV were detected in saliva samples of both HIV-positive and healthy control children, although the frequency of infection was significantly higher among the HIV-infected subjects. To our knowledge this is the first report of KIV and WUV in saliva samples. These findings suggest that the saliva may be a route of HPyV transmission and the oral cavity could be a site of virus replication and persistence.

HV713 - SHEDDING OF POLYOMAVIRUS IN THE SALIVA OF IMMUNOCOMPETENT INDIVIDUALS


1. Universidade Federal do Rio de Janeiro, UFRJ, Instituto de Microbiologia Paulo de Góes, UFRJ - R. Janeiro
2. Universidade Federal do Rio de Janeiro, UFRJ, Faculdade de Odontologia, UFRJ E-mail: gabriella_mendes@micro.ufrj.br

The human polyomaviruses (HPyV) are small, non-enveloped virions with a double-stranded DNA genome, members of the Polyomaviridae family. These viruses establish persistent, primarily asymptomatic, infections. Although the excretion of HPyV in samples from immunocompetent individuals has been described, the significance of these infections in such individuals has hardly been characterized. The molecular characteristics of HPyVs have been thoroughly analyzed, however much is unknown about their pathogenesis. It has been suggested that for BKV and JCV persistent infection occur in the urinary tract and central nervous system, respectively. Lymphoid tissue has been shown to be the possibly reservoir for WUV and KIV in immunocompetent and immunosuppressed individuals. The epidemiology pattern of HPyV suggests that transmission can occur by direct contact or aerosol. The aim of this study was to investigate and compare the frequencies of HPyVs in the saliva of 291 healthy individuals. The samples were analyzed by real time PCR. A total of 71 samples (24.3%) were positive for at least one of virus: 12.7% (37) for WUV only, 7.2% (21) for BKV only, 2.4% (7) for KIV only and, 0.3% (1) for JCV only. Co-infection of BKV and WUV were detected in 1.7% (5) samples. The mean number of DNA copies was high, particularly for WUV and BKV, indicating active replication of the viruses. Polyomavirus detection was higher among individuals of 15 to 19 years of age (46.0%) and ≥50 years of age (33.3%). WUV was more frequent in individuals of 15 to 19-year-old then decreased in older age groups; BKV excretion on the other hand, peaked in the third decade of life remaining steady thereafter. KIV was detected more often in subjects ≥50-year-old. These findings reinforce the previous hypothesis that the saliva may be a route of transmission of BKV and the oral cavity could be a site of virus replication. Moreover, the data
also show that it might be true for JCV, WUV and KIV as well.

HV717 - HUMAN PAPILLOMAVIRUS (HPV) INFECTION OF AMONG SEXUAL PARTNERS
Rocha, W., Afonso, L., Pesca, L., Carestiato, F., Passos, M. Cavalcanti, S.
Lab Diagn Virologico, MIP, Instituto Biomédico, UFF, Lab Diagn Virologico, Rua Prof Ernani Melo 101, 321, Centro, Niterói 24210-130 Setor de DST, UFF, DST, Campus do Valonguinho E-mail: willker.menezes@gmail.com

Human papillomavirus (HPV) infections of the genital tract are the most prevalent sexually transmitted viruses worldwide. Oncogenic HPV types cause pre-malignant lesions that can progress to cervical carcinoma. In the male genital tract, most HPV infections are sub-clinical and associated with a vicious circle of treatment-reinfection of women. Nevertheless, HPV epidemiological pathways are still poorly understood. The literature suggests that different HPV types can be found among sexual partners, due to site restriction. In our study, we aimed to verify HPV infections in female patients as well as in their sexual partners, to test this hypothesis. The HPV DNA prevalence in women with Cervical Intraepithelial Neoplasia (CIN) was 92.5% compared with 25% for normal women, with a statistically significant difference (p<0.001). In male samples, the HPV DNA prevalence in partners from CIN women was 50% and for normal women partners, it was 17.5%. In the group of CIN women, we observed that 20 couples had HPV DNA in both partners. However, only 50% of the couples shared the same HPV type. In the group of normal women, only 6 couples were simultaneously infected by HPV, and from them only 33.3% had the same virus type. These results may be attributed to differences in local immunity and organization of the genital epithelia of each sex. On the other hand, female lesions may not be the result of re-infection by sexual partners, but rather a true recurrence of a latent infection. Finally, such 40% of agreement among all couples leads us to suggest a redundant process of infection and reinfection, perpetuating HPV in the sexually active population.

HV718 - EFFECTIVENESS OF STANDARD AND DOUBLE DOSE OSELTAMIVIR AGAINST SYMPTOMS AND VIRAL SHEDDING IN PATIENTS WITH PANDEMIC 2009 INFLUENZA A H1N1, NOT RESISTANT
1. Instituto de Ciências Biomédicas - Universidade de São Paulo, ICB-USP, Av Prof Lineu Prestes 1374, São Paulo - SP
2. Hospital Universitário - Universidade de São Paulo, HU-USP,
3. Serv. de Infectologia e Contr. de Infec. Hosp. de Curitiba, SICIH-Curitiba,
4. Núcleo de Pesq. em Geriatria Clín. e Prevenção, NPGCP-SP, E-mail: lucthomazelli@hotmail.com

Of 199 patients screened within 48 hours from onset of symptoms of influenza, thirty-seven patients aged more than 5 years, showing rapid test for influenza A antigen positive, fever
≥38°C and at least one respiratory symptom. They were randomized to receive immediate treatment with standard-dose (75 mg twice daily) or double dose (150 mg twice a day) of oseltamivir twice daily for 5 days if adults. Pediatric patients received equivalent doses suitable for children. Nineteen patients received standard dose and 18 double dose of oseltamivir. We analyzed clinical nasopharyngeal samples, obtained before and after oseltamivir therapy, as well as the questionnaire of signs and symptoms filled in. We isolated the virus in MDCK cell culture, tested for drug resistance by a fluorescence-based neuraminidase inhibition assay and determined quantitative viral loads by Real time reverse-transcriptase polymerase chain reaction (qRT-PCR). The median age was 22 years for standard dose group SDG and 19 for double dose group DDG (interquartile range 6-47 and 7-53 respectively); 42.1% were male in the first group and 50% in the second one. The most common symptoms on the first day of admission beyond fever were cough (100% in both groups), rhinorrhoea (84.2% SDG, 100% DDG), headache (78.9% SDG, 83.3% DDG) and sore throat (47.4% SDG, 55.6% DDG). By day 5 after initiation of treatment no patient had fever ≥38°C, the main symptoms were cough (52.6% SDG, 52.9% DDG), rhinorrhoea (52.6% SDG, 29.4% DDG), sore throat (10.5% SDG, 11.8% DDG) and headache (5.3% SDG, 11.8% DDG). Of 37 samples from the second visit, no one had virus detectable by cell culture, 5/19 (26.3% SDG) and 6/18 (33.3%DDG) had virus detectable by qRT-PCR but in a very low concentration. We found no statistically significant differences in the reduction of viral shedding or time to clearance of virus between the groups. Antiviral-resistant viruses were not recovered.

Financial support: FUSP

HV719 - AN UPWARD TREND IN DNA P16INK4A METHYLATION PATTERN AND HPV INFECTION ACCORDING TO THE SEVERITY OF THE CERVICAL LESION

Moyses, N., Cavalcanti, S., Carestiato, F., Cordeiro, T.

Lab Diagn Virologico, MIP, Instituto Biomédico, UFF, Rua Prof Ernani Melo 101, 321, Centro, Niterói 24210-130 E-mail: natthymoyses@gmail.com

High-risk human papillomavirus (hr-HPV) infection is necessary but not sufficient for cervical cancer development. Recently, P16INK4A gene silencing through hypermethylation have been proposed as an important cofactor in cervical carcinogenesis due to its tumor suppressor function. We aimed to investigate P16INK4A methylation status in normal and neoplastic epithelia and evaluate an association with HPV infection and genotype. This cross-sectional study was performed with 141 cervical samples from patients attending at Hospital Moncorvo Filho, Rio de Janeiro. HPV detection and genotyping were performed through PCR and P16INK4A methylation by nested-methylation especific PCR (MSP). HPV frequency was 62% (88/141). The most common HPV were HPV16 (37%), HPV18 (16.3%), HPV33/45 (15.2%). An upward trend was observed concerning P16INK4A methylation and lesion degree: normal epithelia (10.7%), low grade lesions (20%), high grade (57.1%) and carcinoma.
(93.1%) (p<0.001). A multivariate analysis was performed to evaluate an association between methylation, age, tobacco exposure, HPV infection and genotyping. A correlation was found concerning methylation with HPV infection (p<0.0001), hr-HPV (p=0.01), HSIL (p<0.0007) and malignant lesions (p<0.0001). Since viral infection and epigenetic alterations are related to cervical carcinoma, we suggest that P16INK4A methylation profile may be thoroughly investigated as a biomarker to identify patients at risk for cancer.

HV726 - INHIBITORY EFFECT OF BRAZILIAN CYANOBACTERIA EXTRACTS AGAINST HERPES SIMPLEX VIRUS TYPE 2

Oliveira, R.M., Carvalho, L.R., SantAnna, C.L., Mendes, G.S., Tinga, A.C.C., Romanos, M.T.V.

1. Instituto de Microbiologia Paulo de Góes - UFRJ, IMPG - UFRJ, Bl. I, sala 064 ss - CCS-UFRJ-Ilha do Fundão-Rio de Janeiro/CEP21.941-590

2. Instituto de Botânica-Secretaria do Meio Ambiente-São Paulo, IBt-SMA-SP, Av. Dr. Miguel Stéfano, 3687, Água Funda, São Paulo/CEP 04301-902 E-mail: rebecamdeoliveira@gmail.com

Herpes simplex viruses (HSV) infections are among the most common diseases throughout the world. The incidence and severity of HSV-related pathologies have increased recently and the illness is usually more severe in immunocompromised patients. Several drugs are currently available for the treatment of HSV infections such as acyclovir. However, the emergence of drug-resistant strains of HSV led to a search for alternative antiviral agents that have a wide range of efficacy without serious adverse effects. Thus, new anti-HSV drugs are urgently needed. Cyanobacteria are prolific producers of highly bioactive compounds, some of them displaying interesting antiviral activities. Cyanovirin-N is a protein synthesized by Nostoc ellipsosporum, which, besides inhibiting HIV and influenza virus, blocks HSV-1 entry into cells and prevents membrane fusion mediated by HSV glycoproteins; three Microcystis species showed remarkable activity against influenza virus and, estuarine cyanobacterial extracts are active against HSV-1. In this work, methanolic extracts of the cyanobacterial species Phormidium sp. CCIBt 1018, Merismopedia sp CCIBt 3048 and Geitlerinema unigranulatum CCIBt 971 were evaluated against HSV-2. Antiviral assays were performed on Vero cell cultures, in the presence of the cyanobacteria extracts at non-cytotoxic concentrations. Percentage of inhibition (PI), 50% effective concentration (EC50) and selectivity index (SI) were determined. Phormidium sp. showed PI = 99.2%, with EC50 = 22 µg/mL and SI superior to 22.8; G. unigranulatum, PI = 99.8% with EC50 = 9 µg/mL and SI = 50.5 and no activity was observed when Merismopedia sp. was evaluated. Our results are in agreement with the ones displayed in literature about the cyanobacterial extracts (compounds) inhibitory activity against HSV. Bioactive compounds identification studies will be performed in order to elucidate their structures and mechanism of action. Financial Support: CAPES, CNPq and FAPERJ.
FECTIONS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT): DETECTION AND MONITORING OF ACTIVE INFECTIONS USING ANTIGENEMIA ASSAY AND PLASMA NESTED-PCR


Department of Clin. Med. Faculty of Medical Sciences/UNICAMP, FCM/UNICAMP, UNICAMP, Campinas, SP, Brazil Bone Marrow Transplant Unit, Hemocenter/UNICAMP, HEMOCENTER/UNICAMP, UNICAMP, Campinas, SP, Brazil Department of Clinical Pathology, FCM/UNICAMP, FCM/UNICAMP, UNICAMP, Campinas, SP, Brazil E-mail: prinavas@fcm.unicamp.br

Human Betaherperviruses (CMVH, HHV-6 and HHV-7) cause frequent complications after allogeneic hematopoietic stem cell transplantation (HSCT) and are the major cause of opportunistic infections, increased morbidity and mortality in patients in these patients. To minimize the possible clinical manifestations caused by these herpesviruses in the post-transplant period, it is necessary to use techniques for the rapid diagnosis and early treatment. To standardize the use of antigenemia (AGM) assays in detecting active infection caused by HHV-6 and HHV-7 in patients undergoing HSCT; to monitor prospective patients undergoing HSCT, using antigenemia assays and nested PCR (N-PCR) to evaluate the clinical impact caused by these viruses and coinfection among them. Thirty-five patients undergoing HSCT were monitored weekly, from day 0 until day 100 in the post-transplantation period, using antigenemia assays and N-PCR for the detection of active infections. HHV-6 and HHV-7 antigenemia assays were developed in peripheral blood mononuclear cells with the use of monoclonal antibodies which are specific for these viruses and peroxidase staining. Active HCMV infection detection was performed using a commercial kit (Iq Products) by immunofluorescence staining. Using N-PCR, twenty-six out of the 35 patients monitored had active infections caused by HCMV, HHV-6 and HHV-7, respectively, in 26/35 (74.3%), 13/35 (37.14%) and 19/35 (54.3%) detected by N-PCR and/or AGM. Active infections detection using antigenemia assay for HCMV, HHV-6, HHV-7 occurred, respectively, in 16/35 (45.7%), 19/35 (54.3%) and 22/35 (62.8%). The standardization and development of the HHV-6 and HHV-7 antigenemia assays appear to be effective in the diagnosis of active infections caused by these herpesviruses. However, further studies are required to establish the clinical impact, if any, of immunomudalation due to HHV-6 and HHV-7. Financial Support: FAPESP HV742 - MOLECULAR CHARACTERIZATION OF INFLUENZA VIRUSES COLLECTED FROM YOUNG CHILDREN IN UBERLÂNDIA, BRAZIL - FROM 2001 TO 2010: PRESENCE OF REASSORTMENT BETWEEN 2002 STRAINS

Oliveira, T.F.M.S., Yokosawa, J., Motta, F.C., Siqueira, M.M., Silveira, H.L., Chavéz, J.H., Queiróz, D.A.O.

1. Universidade Federal de Uberlândia, UFU, Av. Amazonas, 2210, Campus Umuarama,
Uberlândia/MG

2. Instituto Oswaldo Cruz , IOC, Av. Brasil, 4365, Manguinhos, Rio de Janeiro/RJ E-mail: thelmao@umuarama.ufu.br

Influenza viruses are important pathogens responsible for respiratory disease worldwide and represent a major threat in public health, causing annual epidemics or even pandemics. A study was conducted about the influenza viruses that circulated in Uberlândia city, in the state of Minas Gerais, Midwestern Brazil, during 2001-2010 seasons. The purposes of the study were to detect these pathogens in young children presenting acute respiratory disease (ARD), to characterize the strains from the identified cases by analyzing partial nucleotide sequences of HA and NA genes (and to compare the strains sequences with the vaccine sequences).

A total of 605 nasopharyngeal aspirates were collected from children under five years old presenting ARD and, by immunofluorescence assay, 40 (6.6%) samples showed to be reactive for influenza viruses: 39 of type A and one of type B. RT-PCR was carried out to amplify segments of HA and NA genes of these viruses. Sequence analyses revealed that 41.0%, 17.9% and 2.6% strains were characterized as belonging to subtypes H3N2, H1N2 and H1N1, respectively. Furthermore, the receptor binding sites were preserved in all isolates, although all of them contained variations in the antigenic sites. Substitutions of specific amino acids residues for sialic acid binding were observed in all 2001-2007 strains. Moreover, extra potential N-linked glycosylation sites were identified in two H3 strains. Some amino acid substitutions that were observed in the neuraminidase gene were not related to the antigenicity. All the H3 isolates detected in the 2001-2003 period were different from the vaccine strain A/Moscow/10/99 that was given in the same period. Constant surveillance of antigenic variants over the years has become vital in order to detect new strains and to determine the need to add them to the flu vaccine in order to reduce the burden that these strains may cause in public health.

HV744- COMPARISON OF DIFFERENT EPIDEMIOLOGICAL METHODS OF DETECTION OF DENGUE VECTOR IN DIVINÓPOLIS, MG


1. Universidade Federal de São João del Rei, UFSJ, Rua Sebastião Gonçalves Coelho, 400 Chanadour - Divinópolis MG

2. Universidade Federal de Minas Gerais, UFMG, Av. Antônio Carlos, 6.627 Pampulha - Belo Horizonte MG

3. Universidade Federal de Juiz de Fora, UFJF, Rua José Lourenço Kelmer, s/n - Campus Universitário São Pedro - Juiz de Fora MG

4. Universidade Federal de São João del Rei, UFSJ, Rodovia MG 443 Ouro Branco MG E-mail: martinellefr@yahoo.com.br

Dengue is principal arboviruses that
affecting humans, becoming a major public health problem. Minas Gerais (MG) is one of the Brazilian States with high endemicity. Divinopolis, a city of MG State, has already notified 131 cases of the disease in 2012, being among the 30th cities with the largest number of cases. The main goal of this work was to carry out an epidemiological study of the vector in Divinopolis, and compare these results with data from the Survey Quick Index of Aedes aegypti (LIRAa). The methodology used in this work is different from that used by LIRAa. The last uses the source present in existing breeding sites in the city to determine the rate of infestation by the vector of dengue, four times in year. In this study, we used traps containing wood vanes manufactured and placed in 44 places, that were distributed in six regions in the city (North, Northeast, Southeast, Southwest, West and Central). Subsequently, eggs of each area were counted and the pallets were submersed in water containing fish meal for 6 days, and these stages L3 and L4 were identified in A. aegypti or A. albopictus. Following, the number of larvae was compared to the number of foci in the same period collected by LIRAa. In the Northeast, in the month of January/2012, 358 larvae were collected in this study and 65 sources of dengue were detected by LIRAa, numbers considered high. In contrast, in the Southeast, in the month of June/2011, larvae were not detected and only three source in the data obtained by LIRAa. These findings were expected since the January/2012 was a rainy season, while June/2011 was a month that had low temperatures (10 °C) and low rainfall. Thus, this study provides contributions to epidemiological studies of dengue and alert to the intensification of preventive and educational especially in regions with a high incidence of the vector. FAPEMIG, CNPq, CAPES, UFSJ

HV746 - DIFFERENTIAL DIAGNOSIS OF FEBRILE ILLNESSES MEASLES, RUBELLA, DENGUE AND PARVOVIRUS B19 IN CENTRAL BRAZIL


1. LABORATORIO DE SAUDE PUBLICA DR. GIOVANNI CYSNEIROS, LACEN-GO, AV. CONTORNO, Nº3556. JD. BELA VISTA. GOIANIA-GO

2. UNIVERSIDADE FEDERAL DE GOIÃS, UFG, RUA 235 - SETOR UNIVERSITARIO - CEP: 74605050 - GOIANIA - GOIÃS - BRASIL

E-mail: angelafarmaceutica@hotmail.com

The incidence of measles and rubella has been decreasing exponentially in Brazil since the implementation of mass vaccination campaigns in the last two decades. No autochthonous measles or rubella case was registered by national surveillance program ever since 2000 and 2009 respectively. In contrast, there is an increasing trend of dengue cases among in adults and children countrywide. The diagnosis of febrile rash illnesses may be difficult and laboratory tests are essential to confirm the etiologic diagnosis and for surveillance purpose. A cross-sectional study was conducted to detect the frequency of IgM antibodies against dengue virus and parvovirus B19 among serum samples from suspected clinical diagnosis of measles or rubella sent to Public Health Laboratory
of Goiás, LACEN-GO in Central Brazil in 2011. The epidemiological surveillance included data of social characteristics, vaccination status and symptoms. The inclusion criteria were: (1) serum samples from patients who fulfilled the criteria of case according to Health Ministry (febrile rash illness with other specific symptoms); (2) samples collected within 28 days of the onset of the symptoms; (3) samples previously tested with IgM negative or indeterminate results for measles and rubella. All samples were stored at -20°C. IgM antibodies against dengue were tested by enzyme linked immunosorbent assay (ELISA) (PanBio). If samples were IgM negative for dengue virus they were also tested for specific IgM antibodies against parvovirus B19 (Biotrin). SPSS 17.0 (SPSS Inc., Chicago, IL) software was used for data analyses. Of 263 samples sent to LACEN-GO, 127 were eligible for the study. Of participants 44.1% were younger than 1 year of age and 43.2% were female. Acute infection by dengue virus and parvovirus B19 were confirmed in 23.6% (95%CI 16.3%-31.3%) and 7.1% (95%CI 3.1%-11.7%) febrile cases respectively. Among 4 indeterminate rubella cases 3 were confirmed by dengue virus infection (according national epidemiological protocol). Laboratory tests provide invaluable contribution for the differential diagnosis of febrile rash in different epidemiological settings. This is the first evidence of parvovirus B19 circulation in Central Brazil. Financial support: Secretaria de Saúde do Estado de Goiás

HV747 - IDENTIFICATION OF AEDES AEGYPTI AND AEDES ALBOPIC-TUS LARVAE IN THE CITY OF BELO HORIZONTE, STATE OF MINAS GERAIS, IN 2011

Miranda, D.P.J.

Universidade Federal de Minas Gerais, Secretaria Municipal, E-mail: dmiranda@superig.com.br

Dengue virus is the most important human arboviral pathogen worldwide. World Health Organization estimates that over 40% of the population is at risk of acquiring dengue and there may 50 million of dengue infection every year. There are two important vectors of Dengue virus (DENV) throughout the world: Aedes aegypti and Aedes albopictus. In Brazil outbreaks of dengue has been associated with the presence of Aedes aegypti, due to its high capacity to spread and adapt to human environment. The introduction of Aedes albopictus in Brazil in 1986 is especially worrying because it can readily transmit major arthropod –borne viruses such as Dengue virus and Chikungunya virus. Belo Horizonte, the capital of Minas Gerais, has been suffering from dengue outbreaks since 1996. Two major outbreaks have already occurred in Belo Horizonte: one in 1998 with approximately 80.000 cases and another one in 2010 with 57.000 cases. This study aimed to identify larvae of Aedes aegypti and Aedes albopictus from oviposition traps displayed in all nine administrative districts of Belo Horizonte city in 2011. To perform this study, oviposition traps were displayed in residential area of Belo Horizonte, for a period of one week, during four times of the year: January, April, July and October of 2011. The eggs from ovitraps were counted and subsequently hatched in laboratory control conditions. After eclosion, each
larva was identified into specie by morphological characteristics. A total of 68,827 larvae were identified. 66,423 (97%) was identified as Aedes aegypti and 2,404 (3%) as Aedes albopictus. The results show that Aedes aegypti is more prevalent than Aedes albopictus. This implicates Aedes aegypti as the major vector responsible for dengue in Belo Horizonte. However, the presence of Aedes albopictus increases the risk of transmission of dengue. Therefore, entomological surveillance must be taken to provide data for control plans of these two vectors.

HV749 - GENETIC VARIABILITY OF HEPATITIS B VIRUS AND HEPATITIS C VIRUS IN CHRONIC CARRIERS WITH AND WITHOUT HEPATOCELLULAR CARCINOMA


2. Hepatologia Hosp. Universitário Clementino Fraga Filho, HUCFF, Rua Rodolpho Paulo Rocco, 255 Cidade Universitária Ilha do Fundão Rio de Janeiro E-mail: araujo.orc@ioc.fiocruz.br

Hepatocellular carcinoma (HCC) is globally the fifth most common cancer. The major risk factors for developing HCC are chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections. Several mutations in these viruses have been previously associated to hepatocarcinogenesis. The purpose of this study is to assess the prevalence of HBV subgenotypes, core promoter (CP) and pre-S mutations, as well as, HCV genotypes and core mutations in chronic carriers with and without HCC. At this moment, 80 patients with chronic HBV infection (15 with HCC) and 58 patients with chronic HCV infection (35 with HCC) referred from HUCFF were enrolled in the study. HBV-DNA and HCV-RNA were extracted from serum samples and amplified by PCR and RT-PCR, respectively. Nucleotide sequences were determined and phylogenetic analysis was conducted using MEGA version 4.1. HBV subgenotypes frequencies in HCC patients were 90% for A1 (9/10) and 10% A2 (1/10), whereas in patients without HCC the distribution was 60% A1 (21/35), 17% A2 (6/35), 3% D1 (1/35), 6% D6 (2/35), 3% D7 (1/35), 6% F2 (2/35) and 6% F4 (2/35). The HBV CP mutations A1762T, G1764A, pre-S F141L mutation and pre-S deletions, were found in 60% (3/5), 60% (3/5), 14% (1/7), and 13% (1/8), respectively. In HBV chronic carriers without HCC, these mutations were detected in 37% (10/27), 41% (11/27), 13% (4/30), 9% (3/33). Among HCV chronic carriers with HCC, genotypes frequencies were 39% for 1a (12/31), 36% 1b (11/31) 26% 3a (8/31), whereas in patients without HCC were 33% 1a (6/18), 56% 1b (10/18) and 11% 3a (2/18). In the HCV 1b genotype isolates, R70Q and L91M were found in 36% (4/11) and in 73% (8/11) of HCC patients, and in 50% (5/10) and in 100% (10/10) of patients without HCC, respectively. Our preliminary results have shown a higher frequency of mutations, previously associated to hepatocarcinogenesis, in HBV chronic carriers with HCC. However, among HCV chronic carriers from our study, this association could not be observed.
HV751 - DIAGNOSIS OF DENGUE VIRUS INFECTION BY DETECTION OF SPECIFIC IMMUNOGLOBULIN A

1. Universidade Federal de Pernambuco, UFPE, Av. Prof. Moraes Rego, 1235 - Cidade Universitária, Recife - PE - CEP: 50670-901

2. Centro de Pesquisas Aggeu Magalhães, CPqAM, Av. Prof. Moraes Rego, 1235 - Cidade Universitária, Recife - PE - CEP: 50670-901

Dengue diagnosis by detection of immunoglobulin M (IgM) has limitations when used in endemic regions, because in case of secondary infection the levels of IgM are lower, sometimes undetectable. Detection of immunoglobulin A (IgA) could be a parameter in the early confirmation of dengue, especially in secondary infections, since it occurs in higher levels in these cases. We performed in house ELISA for IgA detection in serum samples of 78 dengue patients, previously confirmed by IgM and/or RT-PCR. The assay was performed as follows: Microtiterplate was coated with 100µL/well (1µg/100µL) of anti-human IgA diluted in carbonate buffer pH 9.6; blocking was done with 4% bovine albumin in PBS pH 7.2; 50µL of serum diluted 1/100 was added in duplicate, after 1h incubation at 37°C, 50µL of DENV antigen was added and incubated 1h at 37°C. Positive e negative controls were used. Washing was done using PBS-T; 25µL/well of anti-dengue conjugate was added. Tetramethylbenzidine (100µL/well) was used as substrate, followed by the addition of stopping solution. Optical density was read at 450 nm and results were calculated by dividing the mean absorbance of test-samples by the mean absorbance of negative control, samples with results >2 was reactive. Of the 78 individuals, 43 were classified as primary infection and 35 as secondary infections. IgA was detected in 16.3% (7/43) of the primary infection and 65.7% (23/35) of the secondary infections. It was found a sensitivity of 45.2% and specificity of 87.5%. Positive and negative predictive values were 93.3% and 29.2%, respectively. The results have shown that using both, IgM and IgA antibody detection may be very useful and could help in the interpretation of results in the acute disease phase, and also in the early detection of dengue cases, allowing the adoption of preventive measures to prevent the occurrence of severe cases. Financial Support: CAPES

HV752 - STANDARDIZATION OF REAL-TIME PCR TO DIAGNOSE HUMAN CYTOMEGALOVIRUS REACTIVATION AND DETERMINE CUTOFF FOR PREEMPTIVE TREATMENT IN RECEPTORS OF HEMATOPOIETIC STEM CELLS

Department of Clinical Medicine - UNICAMP, SP, Brazil, UNICAMP, BRAZIL Bone Marrow Transplant Unit, Hemocenter - UNICAMP, SP, Brazil, UNICAMP, Brazil E-mail: re_peres@yahoo.com.br

Hematopoietic stem cell transplantation (HSCT) is an important
therapeutic tool for treating malignant and non-malignant disorders, and the human cytomegalovirus (CMV) reactivation occurs in 50-90% of allogeneic transplant recipients. Monitoring of its reactivation is critical for HSCT recipients. While detection of CMV antigenemia is still widely used for monitoring CMV infection and guide preemptive therapy in patients at risk of developing CMV disease, the quantification of CMV DNA in blood by PCR is emerging as an alternative to the antigenemia assay and may soon become the standard for the surveillance of CMV infection in allogeneic HSCT recipients, because it presents advantages over the antigenemia assay. Aim: standardization of real-time PCR to diagnose CMV reactivation and determine cutoff for preemptive treatment in receptors of HSC. The reference standard curve for calibration of CMV copy numbers was constructed inserting the US17 amplicon into a plasmid, using a cloning strategy, and propagated in competent cells. For this construct, plasmid DNA was purified on columns, DNA concentration was determined by measuring using a NanoDrop ND-1000 spectrophotometer and the corresponding copy number was then calculated. The construct was serially diluted in water within a range of 10² to 10⁷ copies/μl. Receiver operating characteristic (ROC) plot analysis will be performing to determine a threshold value of the CMV DNA load by real-time PCR using antigenemia as gold standard. Between 11/2010 and 06/2012 a total of 499 samples was included in the study. The samples came from 35 HSCT recipients. Of these, only 11 (31.5%) had positive antigenemia. Real-time PCR is still being made. The quantification of CMV DNA load using real-time PCR appears to be applicable to the clinical practice and an optimal cutoff value for guiding timely preemptive therapy should be clinically validated in future studies. Financial support: FAPESP

HV753 - DETECTION OF PAPILLOMAVIRUS 16 AND ITS VARIANTS IN A REGION OF HIGH INCIDENCE OF PENILE CANCER IN BRAZIL


1. Instituto Evandro Chagas SVS/MS, IEC, BR 316 Km 07, Leulândia - Ananindeua - Pará.
3. Universidade Estadual Paulista, UNESP, Rua Cristóvão Colombo, 2265, Jardim Nazareth - São José do Rio Preto - SP. E-mail: rodrigosilvestre@iec.pa.gov.br

Introduction. Emerging evidence suggest that penile cancer is in part associated to HPV infection. Laboratory data indicate that HPV DNA is found in until 48% of all penile tumors, and most of these cases correspond to HPV 16. This is a rare tumor location with a global incidence about 0.2% however, is relatively high in developing countries as Brazil where men posses 2.1% of chance to develop this cancer, mainly among the inhabitants in the North of the country. Material and Methods. Eighty-two paraffin embedded biopsies from patients with histological diagnosis of Squamous penis carcinoma, from a reference
hospital of cancer in the city of Belem-Para were submitted to DNA extraction using “QIAamp FFPE”. HPV specific detection was done using the “INNo Lipa HPV” that is able to detect 26 HPV types. The HPV 16 variants were assessed using a primer sets that is able to amplify a 211 bp fragment of HPV 16 E6 gene and was amplified with the same DNA used to HPV identification. The amplified fragments of E6 were directly sequenced and compared with sequences contained in the Gene Bank, the accession No. K02718. The positive samples of HPV 16 variants obtained were classified according Chopjitt et al (2009) using the BioEdit sequence analyzer. Results. From the total 82 samples analyzed, 52 (63.41%) were positive for any HPV type and 14 (26.9%) were positive for HPV 16. The alignment of amplified sequences from E6 gene of HPV 16 revealed 12 samples with 100% homology to the European prototype, one sample was identified as Asian/American G and another samples determined as AF2 variant with substitutions in the positions T109C, G132T, C143G and G145T. Conclusion As observed worldwide, our findings suggest HPV 16 as the viral type most frequently associated with cancer of the penis in our region. Parallel to these findings, the European HPV 16 variants appear to be more commonly associated with penile cancer than other non-European variants in our study.

HV754 - A SOPHISTICATED GIANT: ACANTHAMOEBA POLYPHAGA MIMIVIRUS IS ABLE TO REPLICATE IN HUMAN PBMCs AFTER IFN ALPHA TREATMENT DESPITE THE INDUCTION OF EXPRESSION OF THE ISGS MXA, OAS AND 6/16 MRNAS


Universidade Federal de Minas Gerais, UFMG, Av. Antônio Carlos, 6627 - Pampulha - Cep; 31270-901 - BELO HORIZONTE - MG E-mail: lorena.farmacia@yahoo.com.br

Acanthamoeba polyphaga mimivirus (APMV), the largest virus known, belongs to Mimiviridae family. APMV was first isolated few years ago from a water cooler, in an English hospital during a pneumonia outbreak. Although free-living amoebas are the APMV hosts, recent studies showed that this virus is able to replicate in some murine and human phagocytes. Since APMV is considered a hypothetical human pathogen, this work aimed to investigate if human PBMCs are permissive to APMV replication and the interaction of APMV with the interferon system in peripheral blood mononuclear cells (PBMCs) human. Therefore, human PBMCs were grown in 6 well plates (500,000 cells/well), were treated with 1000 U of IFN alpha for 18 hours and subsequently infected with APMV (M.O.Is: 1, 10 and 50) or directly infected with APMV, without treatment with IFN alpha. Then, infected PBMCs monolayers and supernatants were collected and submitted to titration in amoeba culture and to Real-Time PCR to APMV helicase gene amplification. In addition, Real-Time PCR to verify the expression of mRNAs of interferon stimulated genes (ISGs) were performed. Real-Time PCR and titration results showed that APMV is able to replicate in human PBMCs, even after induction with IFN alpha. Although the presence of the antiviral state was confirmed in treated cells by real time
PCR for ISGs (OAS, 6-16 and MxA), this fact did not showed any influence in virus yield. Analysis of gene expression showed low expression of IFNs mRNAs in PBMCs infected only with APMV, untreated with IFNs. Taken together, our results suggest that APMV is able to replicate in human PBMCs, even after induction with IFN alpha. The large genome of APMV seems to be able to encode genes involved in mechanisms that allows the virus not to stimulate the IFN system. This fact reinforce the theory that mimivirus may be a human pathogen, and could be a reflection of an interaction between the ancestors of APMV and vertebrate hosts.

HV764 - ROTAVIRUS INFECTIONS IN AMERINDIAN CHILDREN FROM WESTERN BRAZIL: CHARACTERIZATION OF UNCOMMON HUMAN G8P[6] GENOTYPE WITH SIMILARITY TO BOVINE AND BAT STRAINS

Luchs, A., Cilli, A., Morillo, S.G., Timenetsky, M.C.S.T.

Instituto Adolfo Lutz, IAL, Av Dr Arnaldo, 355 Centro de Virologia Cerqueira Cesar 01246-902 E-mail: driluchs@gmail.com

Gastroenteritis is the leading problem in indian communities. During national rotavirus (RV) surveillance 2008-2011 G2P[4], G3P[8] and G8P[6] genotypes were detected in children ≤3 years from indigenous villages in western Brazil. The aim of this work was to carry out sequence analysis of the two outer capsid proteins (VP4 and VP7) in order to obtain further information on the genetic relationship between human and animal RV. This retrospective study was conducted with convenient surveillance specimens. RV were detected using ELISA, PAGE, and genotyped by RT-PCR. The genetic relationship was analyzed by sequencing of selected strains: two G2 (IAL-RN191, IAL-RN71094), one G8 (IAL-RN376), two P[6] (IAL-RN376, IAL-RN377), and one G3 (IAL-R2758). IAL G2 RV sequences showed 79%-78.6% similarity compared to animal representative strains. IAL-RN376 G8 sequence shares a clade with bovine and human strains, displaying highest nucleotide identity to African human strains (DRC86-98%, DRC88-97.9%), following by the African bovine NGRBg8 strain (95.1%). IAL-RN376 and IAL-RN377 P[6] sequences showed the highest identity to human R330 strain (99.6%). In addition, IAL P[6] sequences were also closely related to an African fruit bat RV strain (KE4852/07) (94.6%). IAL-R2758 G3 sequence share the highest nucleotide similarity to human strains (98.6-99%), however, demonstrated moderate-to-low nucleotide identity to animal strains (90-70.5%), highlighting two feline strains: Cat2 (89.5%) and BA222 (90%). Taking the risks, this study suggest that a reassortment between bovine RV G8 and bat RV P[6] could be occurred in animal host(s) preceding the transmission to human; nevertheless, also suggest that humans could serve as a RV reservoir, resulting in anthropozoonotic transmission. In indigenous population, an anthropozoonotic transmission is probably fairly frequent once the inhabitants live in close contact with animals together with poor hygienic conditions. Sponsor: PPG-PLSP-CCDSES/SP

HV767 - SEROPREVALENCE FOR HANTAVIRUS IN RURAL WORKERS FROM CORURUPE COUNTY, STATE OF
ALAGOAS


1. Federal University of Alagoas, UFAL, Praça Afrânio Jorge s/n, 57010-020 / Maceió - Alagoas
2. Central Laboratory of Public Health of Alagoas, LACEN-AL, Av. Marechal Castelo Branco, 1773 - Jatiúca, 57030-340, Maceió - Alagoas
3. Faculty of Medicine of Ribeirão Preto, USP, FMRP - USP, Av. Bandeirantes, 3900 - Monte Alegre - CEP: 14049-900 Ribeirão Preto/SP E-mail: patricia_enf2009@hotmail.com

The genus Hantavirus belongs to family Bunyaviridae and includes rodent-borne viruses that are transmitted to humans by inhalation of infectious aerosols from infected rodent excreta, and may cause lethally illness. Since 1993, in Brazil, the hantavirus cardiopulmonary syndrome (HCPS) has been reported in an increasing number of municipalities and regions previously considered free for hantavirus circulation. Nevertheless, in the Northeast region of Brazil data about HCPS is very scanty, and is absent for the state of Alagoas. In the last decades, the landscape from region of south of this state - where is located the Coruripe county - underwent intense alteration because of the sugar cane agroindustry development. These environmental disturbances may alter the frequency of human contact with hantaviral hosts, such as Necromys lasiurus and Oligoryzomys nigripis, and therefore, could result in the emergence of human hantavirus infections. In this sense, the aim of this study was investigate the prevalence of IgG antibodies to hantavirus in rural workers from a Plant in the Coruripe county, conducing a serological survey. Sera of 704 volunteers healthy rural workers were collected and used to detect IgG antibodies against N protein of Araraquara hantavirus (rN ARAV), by enzyme immunoassay (ELISA). The positive samples were then tittered. The prevalence of IgG anti-rN ARAV antibodies were 10.51% (74/704), with titers from 200 to 6,400. Of these, 57 (77.02%) volunteers positive for IgG attested that had never worked out of state of Alagoas. Our findings demonstrate serological evidence of past infections with hantavirus in human from the state of Alagoas, and suggest that there is hantavirus circulation in this state from Northeast Region, Brazil. Financial support: CNPq, CAPES, FAPEAL

HV769 - NO ASSOCIATION BETWEEN HUMAN CERVICAL LESION AND POLYMORPHISMS IN MDM2 AND WAF1 GENE

Amaral, C.M.M., Gurgel, A.P.A.D., Freitas, A.C.

UNIVERSIDADE FEDERAL DE PERNAMBUCO, UFPE, AV. PROFESSOR MORAES REGO, 1235 - CIDADE UNIVERSITÁRIA E-mail: carolinamed3@gmail.com

The product of Double Minute-2 gene, protein MDM2, is a major negative regulator of p53. Polymorphisms in the MDM2 promoter region enhancer the levels of MDM2 transcription. Regulated by p53, the WAF1 gene plays
tumor suppressor function by arrest the cell cycle in G1 phase. Polymorphisms in the WAF1 3'UTR region may cause instability of the mRNA and disrupt the cell cycle. Thereby, polymorphisms in the p53 pathway genes may deregulate the cell cycle and has been associated with the development of many tumor types. Thus, the present study was undertaken to investigate the possible association of polymorphic variants of MDM2 at promoter T309G (rs2278744) and WAF1 at 3'UTR region C590T (rs1059234), with increased susceptibility for cervical lesion in women infected with HPV. The study subjects were Brazilian women HPV infected presenting cervical lesions and women HPV infected without cervical lesions. Detection and typing of HPV were performed by polymerase chain reaction. Genotyping of MDM2 T309G and WAF1 C590T polymorphisms were performed from peripheral blood DNA and cervical smear by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The control group was patients positive for HPV without cervical lesions. There was no significant difference in the distribution of the allele and genotype variants in women without cervical lesions and with cervical lesions for MDM2 and WAF1 polymorphisms. We didn’t observed an increased risk of cervical lesions associated with MDM2 T309G (p=0.09; OR, 2.02; 95% CI, 0.86-4.77) or WAF1 C590T (p= 0.84; OR, 1.12; CI, 0.48-2.71). Although MDM2 and WAF1 polymorphisms have been found to be associated with tumor development, our findings suggested that MDM2 T309G and WAF1 C590T are not correlated with increased of susceptibility to cervical lesions in patients from Northeastern Brazil.

**HV773 - MODULATION OF THE HOST IMMUNE RESPONSES BY DIFFERENT STRAINS OF VACCINIA VIRUS**


Universidade Federal de Minas Gerais, UFMG, Av. Antônio Carlos, 6627 - Pampulha - Belo Horizonte - MG CEP 31270-901 E-mail: lorenafalabella@yahoo.com.br

Fears that Variola virus could be used as an agent of bioterrorism up surged a decade ago, boosting scientific studies looking at the immune mechanisms underlying the protection generated by the administration of Vaccinia virus (VACV). Although such studies have provided large amounts of data on the immunological memory following exposure to the vaccine, the same cannot be said about the understanding of immune responses elicited during the onset of an acute orthopoxvirus' infection. We have evaluated aspects of the cellular immune responses in humans naturally infected by VACV during zoonotic outbreaks taking place in Brazil. Ex-vivo experiments using PBMCs from infected and uninfected individuals revealed a marked virus-induced modulation in macrophages, B and T-CD4+ cell responses. To further look into that we infected BALB/c mice with different VACV zoonotic isolates: the more virulent GuaraniP1 (GP1) and the avirulent Passatempo (PSTV) strains. Ex-vivo analysis of virus-stimulated splenocytes from mice infected with the virulent GP1 revealed CD14+ and B cell modulated responses, similarly to results obtained in the human studies. On the other hand, animals infected with PSTV showed few signals of virus-induced immune
modulation. Likewise, replicative vaccine strains, such as Lister, showed some modulation toward macrophage and B cell responses in mice-infected experiments, whereas the non-replicative MVA showed no signs of immune modulation. Animals infected with the WR strain showed intense signals of immune modulation directed toward CD14+, T CD4+ and B cell responses. Taken together, our results revealed a tendency in which Th2 responses are modulated during acute VACV infections.

HV774 - MOLECULAR CHARACTERIZATION OF HUMAN PARVOVIRUS B19 STRAINS CIRCULATING IN NITEROI/RJ-BRAZIL


1. Hospital Universitário Antônio Pedro, DIP/HUAP, rua Marques do Paran s/n
2. Universidade do Grande Rio, UNIGRANRIO, rua Prof. Jose de Souza Herdy, 1160 - Duque de Caxias/RJ
4. Fundação Oswaldo Cruz - Departamento de Virologia, FIOCRUZ, Avenida Brasil, 4365 - Rio de Janeiro E-mail: re.freire@uol.com.br

Erythrovirus infection is generally acute and self-limiting in immunocompetent people. However, in immunocompromised individuals (HIV-infected patients) the infection is not readily cleared and its long persistence leads to chronic anemia. The infectious erythema or fifth disease is an acute rash illness that occurs mainly in children between 5-14 years of age and it is believed that the rash of erythema infectiosum is the result of the interaction between the virus and the host immune response, since its development is related to the presence of IgM antibodies in serum. Recent studies have shown many more genetic variations among erythrovirus than previously thought, and human strains has been classified in genotype 1(B-19), genotype 2 (A6) and genotype 3 (3a-V9 and 3b-D91.1). The aim of this study was to determinate the genotypes of B19V in patients with erythema infectiosum and in HIV-infected patients attending at Hospital Universitário Antônio Pedro (HUAP/UFF). These samples were collected during outbreaks of erythema infectiosum in Niterói. B19V DNA was extracted from serum samples using QIAamp DNA Blood Mini Kit (QIAGEN) and the nPCR assay was performed using a set of primers P12 /P16 and P13/P16 that amplifies a partial VP1/VP2 region of the B19 genome. Sequencing reactions were performed using the Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems®, CA, US). A total of 21 samples (5 HIV-patients and 16 erythema infectiosum cases) were analysed. The samples from HIV-patients were collected during an outbreak of erythema infectiosum in Niterói (2005-2006). After sequencing we found that four of the five samples of HIV-patients were genotype 1 and one were genotype 3b. All samples of erythema infectiosum cases were genotype 1. This is the first description of genotype 3b in Rio de Janeiro state. These results represents...
The aim of this study was to carry out the molecular analysis of the fragment of the HN gene. Methods: Nasopharyngeal aspirates from 2152 infants and children under five years old, hospitalized at the University of São Paulo Hospital (HU-USP) with acute respiratory illness were collected from 1995 to 2006. The detection of HPIV 1, 2 and 3 were performed by multiplex RT-PCR using specific primers to HN gene, labeled with FAM. The fragment of HN gene was amplified, sequenced and, the molecular analysis was carried out. Results and discussion: A total of 6% (n=135) samples were positive for one of the HPIV, and the HPIV-3 was the most prevalent virus during all years studied, corresponding to 80% (108/135) of positive cases, followed by HPIV-1 with 15% (20/135) and HPIV-2 7% (10/135). The positivity among the years studied ranged from 1% (1/195) in 1996 to 18% (24/154) in 1999. Most mutations observed were silent in all PIVs, however, some amino acids alterations in conserved areas, verified in PIV-3 and PIV-2, and alterations in N-glicosilation sites were observed. Some of these alterations were related with changes in the function of neuraminidas.
C virus (HCV) by parenteral exposure is well documented in the literature, around 40% of infected patients don’t have history of exposure in this way. Therefore, several studies have been conducted to establish other possible transmission routes. Detection of HCV in saliva could be an inexpensive and noninvasive parameter detection and monitoring of hepatitis C. The aim of this study was to investigate the presence of HCV-RNA in serum and saliva of patients anti-HCV positive. A questionnaire was administered to each patient and the reports of complications due to viral infection and genotype were obtained from medical records. Saliva and serum were collected from 54 patients for RNA extraction, performed according to the instructions of Qiamp viral RNA isolation kit (Qiagen). The molecular confirmation of HCV was performed the synthesis of complementary DNA. Then was applied a nested-PCR to the 5NCR region, containing the first reaction primers 939 and 209 and second reaction primers 940 and 211. The study population was 54% of females and 46% of male. Blood transfusion was reported in 38% the cases and 22% of patients didn’t report history of parenteral exposure. The HCV-RNA was detected in 82% (41/50) of anti-HCV positive patients, the genotypes were identified in 70.7% (29/41). Among the different genotypes, type 1 was found in 72.4% (21/29) and type 3 in 27.6% (8/29) of the patients. The HCV-RNA wasn’t detected in any sample of saliva analyzed. The divergent results in the literature about the presence of HCV-RNA in saliva may be explained by the different techniques used for collection, storage, and manipulation of the sample, diagnostic methods and especially the separation of cells saliva, reflecting the need for more studies that may contribute to determining the true role of saliva in the transmission of HCV. Financial support: CNPq. Universal 2009

**HV778 - DISTRIBUTION OF HEPATITIS B VIRUS SUBGENOTYPE F2A IN SÃO PAULO, BRAZIL**


1. School of Medicine, University of São Paulo, São Paulo, FMUSP, Av Dr. Eneas de Carvalho Aguiar, 500
2. Albert Einstein Diagnostic Medicine, HIAE, Av. Albert Einstein, 627/701
3. Federal University of São Paulo, UNIFESP, Rua Sena Madureira, 1500 E-mail: monica.viviana@usp.br

HBV genotype F is primarily found in indigenous populations from South America and is classified in four subgenotypes (F1 to F4), some of them further divided in subgenotypes, such as F1a, F1b, F2a and F2b. Subgenotype F2a is the most common in Brazil among genotype F cases. The aim of this study was to characterize HBV genotype F2a circulating in 16 patients from São Paulo, Brazil. Samples were collected between 2006 and 2012 and sent to the Clinical Laboratory of Hospital Israelita Albert Einstein. A fragment of 1306bp partially comprising HBsAg and DNA polymerase coding regions was amplified and sequenced. Viral sequences were genotyped by phylogenetic analysis using reference
sequences from GenBank (n=206), including 76 classified as subgenotype F2a. Bayesian Markov chain Monte Carlo simulation implemented in BEAST v.1.5.4 was applied to obtain the best possible estimates using the model of nucleotide substitutions GTR+G+I. HBV F2a sequences of patients from São Paulo grouped in two clusters with other sequences from Brazil previously described. One cluster included six sequences with another one also obtained in Brazil and was closer to other branch containing two other new sequences and 4 other sequences from Rondônia. The other cluster of six sequences grouped with another sequence from Brazil and was close to a group with other Brazilian sequences (including one from the present study). Another new sequence from this study grouped isolated from all the others. Since most sequences subgenotype F2a are from Brazil and it is not reported from which state each sequence was obtained, it was not possible to infer its routes of spread. The spreading and the dynamic of subgenotype F2a in Brazil needs the study of a higher number of samples from different regions as it is present through in almost all Brazilian populations studied so far. Supported by IIRS-SBIBAE, FAPESP 2011/50562-0, CNPq, FFM and HCFMUSP.

HV782 - CO-CIRCULATION OF DIFFERENT ARBOVIRUSES DURING DENGE OUTBREAK IN SINOP, MT


1. Universidade Federal de Mato Grosso, UFMT-Sinop, Avenida Alexandre Ferronato, 1200 Sinop, MT

2. Pronto Atendimento Municipal, PAM-Sinop, Avenida das Itaubas, 2795 Sinop, MT

3. Faculdade de Medicina de São José do Rio Preto, FAMERP, Av. Brigadeiro Faria Lima, 5416 São José do Rio Preto, SP

4. Universidade Estadual Paulista, UNESP - Araraquara, Rod. Araraquara-Jaú Km 1 Araraquara, SP E-mail: carlajulia15@hotmail.com

Arboviruses are frequently associated with outbreaks in humans and represent a serious public health problem. Among the Brazilian arboviruses, Dengue virus (DENV), Yellow Fever virus (YFV), Rocio virus, Saint Louis Encephalitis virus (SLEV), Mayaro virus (MAYV) and Oropouche virus are responsible for most of human cases. All these arboviruses usually produce undistinguishable acute febrile illness, especially in the acute phase of infection. In this study we investigated the presence of arboviruses in sera of 180 patients presenting acute febrile illness, during a dengue outbreak in Sinop, state of Mato Grosso. The Duplex-RT-PCR was performed for Flavivirus and Alphavirus genus detection followed by a Multiplex-Nested-PCR, using species-specific primers. The molecular analysis showed that 38 samples were positive to DENV-1, 2 to DENV-4, 1 to SLEV, and 6 to MAYV. These results indicated that during DENV outbreak, different arboviruses co-circulated causing human disease. Thus, it is necessary to have an efficient surveillance system to control the dissemination of these arboviruses in the population. Financial Support:
Mayaro fever is a dengue-like viral disease caused by Mayaro virus (MAYV) (genus Alphavirus; family Togaviridae). Clinical cases and virus isolation have been reported only from South America, where MAYV circulates in an enzootic sylvatic cycle involving tree-canopy-dwelling Haemagogus spp. mosquitoes as vectors and nonhuman primates as natural hosts. The symptoms of Mayaro fever include fever, headache, myalgia, cutaneous rash, and arthralgia. Although the death rate is low, the morbidity caused by the disease, specially the temporary incapacitating arthralgia, could cause an important social, economic and public health impact. In this study we investigated the presence of arboviruses in sera of 180 patients presenting acute febrile illness, during a dengue outbreak in Sinop, state of Mato Grosso. The Duplex-RT-PCR was performed for Flavivirus and Alphavirus genus detection followed by a Multiplex-Nested-PCR, using species-specific primers. MAYV infection was confirmed in 4 cases by RT-PCR followed by nucleotide sequencing. The patients with Mayaro fever presented mainly fever, headache, diffuse body pain and retro-orbital pain. Mayaro fever is important to public health in rural populations, with an increasing incidence of human cases in the Amazonian basin. It is the first time that Mayaro fever is diagnosed in the state of Mato Grosso, in a city of Sinop, located in the north section of the state in a geographical transition zone between savannah and rainforest. Because Dengue fever is endemic in Sinop, and the fact that the Mayaro fever is not well known outside MAYV-endemic regions, the MAYV infections are misdiagnosed. Ever since that some authors consider the possibility of urbanization of the disease, since experimental studies have shown that the virus could infect Aedes aegypti, the occurrence of this virus in Sinop could originate urban outbreaks of Mayaro fever. Financial Support: CNPq
Dengue is a human viral disease transmitted by mosquitoes of the genus Aedes. Infection by Dengue Virus (DENV) constitutes a serious public health problem in tropical countries including Brazil. DENV, member of the family Flaviviridae, genus Flavivirus, is a positive single-stranded RNA, an enveloped virus with four antigenic serotypes: DENV 1, DENV 2, DENV 3 and DENV 4. Infection by DENV causes a disease whose spectrum ranges from clinically asymptomatic to severe clinical forms (hemorrhagic dengue). The objective of this study was to determine the serotypes of DENV in hospitalized patients (n = 256), with clinical suspects of dengue during 2011-2012 in Salvador, Bahia. The viral RNA was extracted from serum samples with QIAamp Viral RNA Mini kits (QIAGEN, USA) for detection and serotyping of DENV by RT-PCR (Reverse Transcriptase - Polymerase Chain Reaction) and nested-PCR, respectively. RT-PCR was performed using primers D1 and D2 which amplify the region corresponding to the junction C/prM of the genome. This result showed that from the total samples (n=256), 100 (39%) were positive for DENV. Each DNA product (fragment of 511 bp) from RT-PCR was next submitted to nested-PCR using serotype-specific primers (TS1-TS4). This result revealed the presence of DENV 2 in 17/100 (17%) samples, DENV 3 in 4/100 (4%) samples and DENV 4 in 79/100 (79%). These findings showed the presence of three serotypes in the population, with high occurrence of DENV 4. The co-circulation of different serotypes could increase the probability of the most lethal clinical manifestation: hemorrhagic dengue. Financial support: PRONEX-DENGUE CNPq and Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB)

HV796 - STUDY AND CHARACTERIZATION OF MOLECULAR HUMAN ROTAVIRUS IN CHILDREN IN SALVADOR, BAHIA


1. Centro de Ciências da Saúde, Universidade Federal do Recôncavo, CCS, Universidade Federal do Recôncavo, Santo Antônio de Jesus, Bahia
2. Hospital Aliança, HA, Salvador, Bahia
3. Laboratório de Virologia, Instituto de Ciências da Saúde, ICS-UFBA, Av. Reitor Miguel Calmon s/n – Vale do Canela. CEP 40.110-100 Salvador, Bahia E-mail: isabelapeixoto@gmail.com

Acute diarrhea is responsible for high morbidity and mortality in the population. It can be associated to virus, bacteria or parasite. Among viruses,
Human Rotavirus (HRV), member of Reoviridae family, is commonly associated to gastroenteritis in children. The viral infection is characterized by vomits, diarrhea and fever with large amounts of viral particles excreted in the faeces. The objective of this study was to analyze the fecal samples during an outbreak of gastroenteritis in children under 5 years from April to July in 2010 in Salvador, Bahia. A total 71 fecal samples were obtained from patients hospitalized with symptom of acute gastroenteritis. The samples were submitted to HRV detection by immunoenzymatic technique ELISA (RIDASCREEN® Rotavirus, R-Biopharm), RT-PCR (Reverse Transcription Polymerase Chain Reaction) and RNA electrophoretic analysis by SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis). The samples analyzed by ELISA, 78% (n=56) were positive for HRV. From these ELISA positive samples, the viral RNA was extracted (QIAmp viral RNA kit, QIAGEN) and submitted to RT-PCR using primers specific Beg9 and End9 (VP7 gene). Subsequently, the cDNA product (1062pb) from RT-PCR was submitted to nested-PCR using serotype-specific primers (RVG9, aAT8, aBT1, aCT2, aDT4, aET3, aFT9), that identified the genotypes G1, G2, G3 and G9. The electropherotype of the genomic RNA analyzed by SDS-PAGE 7,5% showed that all samples were classified as group A. The highest incidence of HRV infection was found in infants under two years of age (60%, 34/56). Regarding the prevalence of HRV, it was observed a higher positive sample in June (33%, 19/56). Concluding, these findings indicate the greater incidence of HRV group A and the circulation of four different genotypes in children under two years old in Salvador, Bahia. Financial support: Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB)

HV802 - RT-SEMI-NESTED-PCR AS COMPARED TO VIRAL ISOLATION IN MOSQUITO CELL CULTURE FOR THE DIAGNOSIS OF DENGUE VIRUS: AN ACCURACY STUDY


1. Universidade do Estado do Pará, UEPA, Rua Perebebuí, nº 2623; Bairro: Marco; CEP: 66087-670 - Belém - PA.

2. Instituto Evandro Chagas, IEC, Rodovia BR-316 km 7 s/n - Levilândia - 67030-000 - Ananindeua / Pará / Brasil
E-mail: nbvale@yahoo.com.br

Dengue virus (DENV -Flaviviridae, Flavivirus) is transmitted by Aedes mosquitoes and is recognized to cause an infectious disease that affects over 100 countries, where more than 2.5 billion of people worldwide are living in risk areas. Therefore, it is considered as an important public health concern. Virus isolation in cell culture is assumed the gold standard technique for DENV detection and serotyping, which it is complex and takes 7-14 days long for a result. Facing this issue, it is encouraged the research for reliable, alternative and faster methods. Hence, we aimed to evaluate the Lanciotti-described RT-Semi-Nested-PCR and compare it to the gold standard technique. For such, we analyzed 872 samples (blood, sera, and viscera) of dengue-suspected patients
admitted at Instituto Evandro Chagas (Ananindeua, Para, Brazil) throughout January to December 2011. According to the cell culture virus isolation, the real prevalence was of the 18.1%. RT-Semi-Nested-PCR correctly identified the serotypes (DENV-1, DENV-2 and DENV-4) and revealed a high specificity of 0.93 (confidence intervals-CI: 95%, 0.91, 0.95), and an acceptable sensitivity of 0.75 (CI 95%, 0.67, 0.82). Positive (0.71) and negative (0.94) predictive values were considered as good and high, respectively. Moreover, the test was associated with a high positive likelihood ratio (11.2) and a moderated negative likelihood ratio (0.27). Therefore, these data demonstrate an excellent diagnosis accuracy of the RT-Semi-Nested-PCR. Thus, taken all data together, we suggest RT-Semi-Nested-PCR as an easy (non-complex), fast (~10 hours) and reliable molecular method with good predictive values (specific and sensible) and should be considered alternatively to virus isolation method for DENV detection. Additional studies considering other epidemiological and methodological circumstances would be helpful to confirm these findings in order to increase the external validity of the individual studies when analyzed together.

**HV808 - GENETIC DIVERSITY OF ROTAVIRUS NSP4 GENE IN TRIÂNGULO MINEIRO REGION, BRAZIL, FROM 2005 TO 2011**


1. Universidade Federal do Triângulo Mineiro, UFTM, Av.Frei Paulino, 30 - Bairro Abadia, Uberaba-MG.

2. Instituto Leônidas e Maria Deane Fiocruz - Amazônia, ILMD/Fiocruz, Universidade Federal do Rio de Janeiro, UFRJ, E-mail: anadulgheroff@gmail.com

Rotavirus A (RVA) is the main cause of acute diarrhea in children worldwide and a vaccine was recently introduced in the Brazilian vaccination program. The virus genome is composed by 11 segments of double-strand RNA. Viral particle is formed by a triple capsid; the outer layer is composed of VP7 and VP4 proteins, which are used for a dual classification system, defining G and P types, respectively. Recently, a new classification system based on characterization of 11 segments of RVA was proposed. Non-structural protein 4 (NSP4) is involved in viral morphogenesis and have an enterotoxin-like activity; until now, 14 NSP4 genotypes (E1-E14) are described. The aim of this study was to investigate the genetic diversity of NSP4 gene from RVA detected in children in the Triangulo Mineiro region, Brazil, period 2005 to 2011. Twenty-one rotavirus samples were selected, submitted to nucleic acid extraction, NSP4 amplification by RT-PCR, followed by sequencing and phylogenetic analysis. Three distinct NSP4 genotypes could be recognized: six samples clustered with NSP4 genotype E1 and were found to be associated with G1P[8], G9P[8] and G12P[8], other 14 samples fell into genotype E2 and were associated with G2P[4], G8P[4], G3Pnon-typed (NT) and one sample was genotyped as E3 associated with G12PNT. Samples from genotypes E1 and E2 split equally into
three distinct clusters; one sample of genotype E2 clustered closely with cow RVA from India and the specimen of genotype E3 grouped with a feline rotavirus. Alignment of deduced amino acid (aa) sequences of NSP4 genes with prototype strains Wa, DS-1 and Au-1 reveals some aa changes, including the region of the cytotoxic peptide. This study provides valuable information for understanding the evolution of RVA strains and subsides for further studies, since NSP4 has also been discussed as a target for vaccine development. Financial support: FUNEP; CAPES; FAPEMIG.

**HV809 - EPIDEMIOLOGIC PROFILE OF PATIENTS ATTENDED IN EVANDRO CHAGAS INSTITUTE WHIT MUMPS VIRUS INFECTION**

Brasil-Costa, I., Polaro, A.A., Souza, W.T., Monteiro, T.A.F.

**Instituto Evandro Chagas, IEC, Rodovia BR-316 Km07 - Levilândia, Ananindeua, Pará E-mail: igorcosta@iec.pa.gov.br**

Mumps virus (MuV), the causative agent of mumps, infects mainly the parotid glands and is transmitted by droplet spread. Approximately, one third of all MuV infections are asymptomatic. The most common symptoms are salivary gland swelling, headache, vomiting, fever, and neck rigidity. Despite being a vaccine-preventable disease, outbreaks continue to occur worldwide. The trivalent measles-mumps-rubella (MMR) vaccine is available to all healthy children between 12 and 15 months of age. In Brazil, a second dose is offered at the age of 4 to 6 years for adequate protection. However, the national vaccination coverage is not full and wild virus continues to circulate. A survey of clinical and epidemiological study was conducted at Evandro Chagas Institute, PA, with 149 patients from 2003 to June 2012, to search for the presence of IgM antibodies against MuV [States of PA (91); RJ (51); and BA (2)]. The association between seropositivity and patient’s information was tested by multiple logistic regression and Fisher’s exact tests using the program BioEstat 5.0. Statistical significance was considered when p<0.05. The results showed that cough, swelling, and anorexia were present twice more often in positive patients, however with no statistical significance (p>0.05). Males group had more positive results (62.5%; p= 0.0478). PA state had more positive cases compared with RJ (p=0.0107); 28.5% (26/91) and 9.8% (5/51) of patients from PA and RJ states were positive, respectively, and only one patient was positive from BA state. In PA, the high rate of positivity could be related with lower vaccination coverage (97%-1st dose and 81%-2nd dose) compared with data from RJ (99.5%-1st dose and 83%-2nd dose). Our data suggest that the increase in vaccination coverage can reduce the circulation of wild MuV preventing outbreaks. It was also found that the profile of patients with MuV infection were mainly male patients presenting symptoms like swelling, cough and anorexia.

**HV810 - NOROVIRUS INFECTION IN CHILDREN WITH GASTROENTERITIS**

Gastroenteritis is a public health problem worldwide, affecting on average each year 700 million children under 5 years old, observed especially in developing countries. Among the viruses that cause gastrointestinal infections, there is the Norovirus (NoV), found in sporadic cases and identified more recently in most outbreaks of acute gastroenteritis worldwide, accounting on average for 80 to 90% of cases. The transmission occurs mainly through food or water contamination and the illness is characterized by a sudden onset of nausea, vomiting, abdominal pain, diarrhea and occasionally, low-grade fever. The NoV classification is based according to their genetic variants in five genogroups (G), of which 3 (GI, GII and GIV) are known to cause infections in humans. Among these, the genogroup GII is regarded as the most prevalent in the world, and genotype 4 accounts for approximately 80% of all infections. Our work confirms that in Brazil, the molecular epidemiology of a NoV shows the predominance the GII.4 strain. From two hundred thirty-three stool samples collected from children under 5 years old with gastroenteritis, NoV infection was detected in 60.08% (140/233), using a commercial immunoenzymatic assay (RIDASCREEN® Norovirus 3rd Generation R-Biopharm, Germany). These samples were from patients at the Aliança Hospital in Salvador, Bahia, during an outbreak from March-June 2009. The median age of them was 1 year and 8 months; with predominance of male gender (56.17%). From the total of positive stool samples, forty-seven (n=47) were submitted to Reverse Transcription-Polymerase chain reaction using the specific primers CAL-32 and MO3-N (first pair) and primers JV-12 and ACAL-36 (second pair) and all samples were positive for this virus. Among these were randomly selected, 28 samples which were subjected to genome sequencing. After the analysis on NCBI / BLAST, we found that all samples exhibited a high degree of similarity with GII.4 strains (95-99% homology). Financial support: Fundação de Amparo à Pesquisa do Estado da Bahia – FAPESB

HV817 - INHIBITORY EFFECT OF A FLAVONOID FROM KALANCHOE DAI-GREMONTIANA AGAINST HERPES SIMPLEX VIRUSES, IN VITRO


Universidade Federal do Rio de Janeiro, UFRJ, E-mail: georgiasaraiva@hotmail.com

Dengue fever is the most prevalent arboviral disease worldwide. Despite this threat for human health, no specific chemotherapy or safe vaccination for dengue virus (DENV) infection is currently available. Therefore, there is a requirement for effective antiviral
agents and therapeutic strategies for DENV infection. Since the first report about the role of heparan sulfate (HS) in the initial interaction for DENV attachment to vertebrate cells, diverse HS-like glycosaminoglycans were evaluated as antiviral agents against DENV. Seaweeds represent a natural source rich in sulfated polysaccharides, compounds mimicking HS. In this study, the anti-DENV-1 activity of six DL-galactans from Cryptonemia seminervis marine alga (crude polysaccharide fraction [P6], polysaccharides purified by treatment with KCl 2M [P7], partial depolymerization [16], subfractions from P16, fractionated by anion-exchange chromatography [P15, P17 and P18]) were evaluated. The experiments were made in C6/36 cell culture, and all six sulfated galactans showed CC50 values higher than 200 μg/mL (maximum concentration tested). Our results show that antiviral activity varied according molecular weight (MW). P6 (332 KDa) and P7 (362 KDa) showed 99.9% of inhibition and after partial reductive hydrolysis there was a decrease in this activity. P15 (231 KDa) presented inhibition of 65%, no activity was observed to P16 (51 KDa) and P17 (60 KDa) and 85% of inhibition was observed to P18 (63 KDa). Despite the low MW, P18 had the highest degree of sulfation of all galactans evaluated. Studies are being conducted to determine the dose-response curve and mechanisms of action. Preliminary results showed that P6 presented an ED50 value of 1.43 μg/mL and P7 of 0.79 μg/mL and selective index values superior to 139.8 and 253, respectively. The results show that sulfated galactan from C. seminervis can be seen as a new pharmacological compound to dengue virus infection treatment.

**HV820 - INHIBITORY ACTIVITY OF DL-GALACTANS FROM CRYPTONEMIA SEMINERVIS MARINE ALGA ON DENGUE VIRUS TYPE 1, IN MOSQUITO CELL**

Cavalcanti, J.F., Colodi, F., Ferreira, L.G., Mendes, G.S., Noseda, M.D., Duarte, M.E.R., Romanos, M.T.V.

**Universidade Federal do Rio de Janeiro, UFRJ, Universidade Federal do Paraná, UFPR, E-mail:** jessica.f.cavalcanti@hotmail.com

Dengue fever is the most prevalent arboviral disease worldwide. Despite this threat for human health, no specific chemotherapy or safe vaccination for dengue virus (DENV) infection is currently available. Therefore, there is a requirement for effective antiviral agents and therapeutic strategies for DENV infection. Since the first report about the role of heparan sulfate (HS) in the initial interaction for DENV attachment to vertebrate cells, diverse HS-like glycosaminoglycans were evaluated as antiviral agents against DENV. Seaweeds represent a natural source rich in sulfated polysaccharides, compounds mimicking HS. In this study, the anti-DENV-1 activity of six DL-galactans from Cryptonemia seminervis marine alga (crude polysaccharide fraction [P6], polysaccharides purified by treatment with KCl 2M [P7], partial depolymerization [16], subfractions from P16, fractionated by anion-exchange chromatography [P15, P17 and P18]) were evaluated. The experiments were made in C6/36 cell culture, and all six sulfated galactans showed CC50 values higher than 200 μg/mL (maximum concentration tested). Our results show that antiviral...
activity varied according to molecular weight (MW). P6 (332 KDa) and P7 (362 KDa) showed 99.9% of inhibition and after partial reductive hydrolysis there was a decrease in this activity. P15 (231 KDa) presented inhibition of 65%, no activity was observed to P16 (51 KDa) and P17 (60 KDa) and 85% of inhibition was observed to P18 (63 KDa). Despite the low MW, P18 had the highest degree of sulfation of all galactans evaluated. Studies are being conducted to determine the dose-response curve and mechanisms of action. Preliminary results showed that P6 presented an ED50 value of 1.43 μg/mL and P7 of 0.79 μg/mL and selective index values superior to 139.8 and 253, respectively. The results show that sulfated galactan from C. seminervis can be seen as a new pharmacological compound to dengue virus infection treatment.

HV824 - MONITORING THE CIRCULATION OF DENGUE VIRUS IN Aedes Aegypti and Aedes Albopictus BY OVITRAPS' INSTALLATION IN OURO BRANCO AND OURO PRETO, MINAS GERAIS

Cecilio, S.G., Magalhães, J.C., Diniz, J.S., Ferreira, J.M.S., Magalhães, C.L.B., Tótola, A.H.

Universidade Federal de São João del Rei, UFSJ, Campus Alto Paraqueba - UFSJ Rodovia MG 443, Km 07 Ouro Branco - MG CEP 36420-00 E-mail: samyracg@msn.com

Dengue is considered one of the most important arthropod borne viral diseases in Brazil. The disease is caused by 4 serotypes of dengue virus, which are transmitted to humans by vectors from genus Aedes. The fast expansion of these insects has been favored by modern urbanization. Ouro Branco and Ouro Preto/MG are endemic in some periods of the year, which indicates a possible adaptation of vectors. In 2011, 48 cases were reported in Ouro Branco (44 autochthonous and 4 imported), and 2 imported cases were reported in Ouro Preto. In 2012, 9 cases have been notified in Ouro Branco (6 autochthonous, 3 imported). In both cities, programs to prevent outbreaks of the disease were put in place. The success of such programs depend on the early detection of cases or evidences to recommend control procedures and prevent further occurrences. Ovitraps with wood vanes and a mosquito-attractive infusion were installed in 32 public schools, 16 of each city of the study. The evaluation was done monthly between September/2011 and May/2012 and the epidemiological data, obtained from the City Health Department, was monitored. Mosquito eggs were counted and hatched in water with fish meal at 26.5°C. The larvae were reared to the fourth stadium and identified according to the species, A. Aegypti or A. Albopictus, and stored at -80°C. From the 445 eggs collected, 274 larvae came to the fourth stadium; 62% of the larvae from Ouro Branco were identified as A. Albopictus and 85.5% of larvae from Ouro Preto were identified as A. aegypti. The larvae were macerated with Biomixer tissue homogenizer and RNA was extracted with RNeasy QIAGEN Kit®. RNA was quantified by NanoVue Plus®. Reverse Transcription PCR and Real Time PCR were performed to detect the viral RNA. No positive samples were detected, which is consistent with epidemiological data. It can be inferred that the control of zoonosis and environmental care in these cities
interfere positively in the circulation of these vectors.

**HV831 - OCCULT HBV INFECTION IN INSTITUTIONALIZED INDIVIDUALS IN THE STATE OF GOIAS, BRAZIL.**


IPTSP/Universidade Federal de Goiás, IPTSP/UFG, Rua 235, s/n. Setor Universitário CEP 74605050
E-mail: tatycinquini@yahoo.com.br

Hepatitis B (HBV) is a major public problem because of the high number of affected individuals and the complication resulting from the acute and chronic forms of infection. Although the diagnosis of HBV infection is made based on the detection of the serological marker HBsAg, recent studies have demonstrated the presence of viral DNA, detected by molecular techniques, in patients with isolated serological profile for anti HBc. This profile corresponds to a particular form of chronic hepatitis B with undetectable HBsAg marker, which has been classified as Occult HBV infection. In order to determine the rate of HBV infection in an institutionalized population, presenting with psychiatric and neurological disorders, in the state of Goias, 334 blood samples were collected and an overall prevalence of HBV infection of 14.1% was observed, with an index of isolated positivity for anti-HBc marker of 5.4%. In this context, this study aimed to survey the DNA/HBV in samples with isolated anti-HBc and HBsAg positive profiles. Twenty-one samples were subjected to viral DNA extraction, followed by genomic amplification by polymerase chain reaction (PCR), for identification of DNA/HBV. Of the total isolated anti-HBc samples, 17/19 (89.5%) were positive for HBV-DNA. Among the samples with HBsAg positive profile, 2/3 (66.7%) were positive for viral DNA. These results highlight the importance of the molecular techniques to confirm HBV infection. Further studies are being conducted in the lab in order to elucidate the HBV infection status of these individuals. Financial support: CNPq

**HV832 - MOLECULAR ANALYSIS OF VP4, VP7 AND NSP4 GENES OF ROTAVIRUS G1 CIRCULATING IN BELÉM AND MARITUBA, PARÁ, BRAZIL**


FUNDACÃO OSWALDO CRUZ, FIOCRUZ, INSTITUTO EVANDRO CHAGAS, IEC, BR 316, Km 07, s/n
E-mail: luanasoares@iec.pa.gov.br

Rotaviruses are major viral agents of acute gastroenteritis and responsible for 36% of hospitalization for diarrhea among children less than five years of age, resulting in 453,000 deaths annually, mostly in developing countries. Rotavirus is a member of Reoviridae family, and its genome consists of 11 double-stranded RNA (dsRNA) which encode 12 proteins. G1 rotavirus is commonly detected in epidemiological investigations, occurring under different prevalence rates. The aim of this study was to analyze the VP4, VP7 and NSP4 diversity genetic of G1 rotavirus circulating in Belém and Marituba, Pará, Brazil, from 1982 to 2008. We selected 83 samples previously characterized as G1 type
and submitted to RT-PCR. It was possible amplification for 63 (75.9%) specimens. Lineages 1 (8/63, 12.7%), 2 (29/63, 46.0%), 3 (18/63, 28.6%) and 9 (8/63, 12.7%) of VP7 gene were detected. The sublineages 2E and 3A were co-predominant detected in 57.1% (36/63) of samples. Three amino acid substitutions (97 [D→E], 147 [S→N] and 218 [I→V]) were observed in VP7 antigenic regions (A, B and C) in samples of lineages 1, 2 and 9. All samples showed P[8] specificity for VP4 gene and lineages 2 (21/63, 33.3%) and 3 (42/63, 66.7%) were detected. Two substitutions (35 [I→V] and 38 [S→G]) occurred in antigenic region of VP4 gene. For NSP4 gene, all samples belonged to E1 type. Phylogenetic analysis of NSP4 gene revealed that occurred changes in amino acid positions 16 (S→P) and 34 (L→P) in all samples and 9 specimens displayed amino acid substitution in NSP4 toxicity residue (aa 131).

Conclusion: This study allowed us to broaden our understanding about genetic diversity and circulation of G1 variants and corroborating the high heterogeneity of this genotype.

Financial Support: CNPq, IEC/SVS/MS.

HV833 - VIROLOGICAL AND HOST FACTORS INVOLVED IN INCREASED DISEASE SEVERITY IN HUMAN RHINOVIRUS INFECTIONS IN YOUNG CHILDREN.


1. Universidade Federal de Uberlândia, UFU, Av. Pará, 1720, Bloco 4C – Campus Umuarama;

2. Instituto de Ciências Biomédicas, ICBIM, Av. Pará, 1720, Bloco 4C – Campus Umuarama; Uberlândia, MG, CEP 38400-902

3. Hospital de Clínicas, HC, Av. Pará, 1720, Campus Umuarama; Uberlândia, MG, CEP 38400-902

4. Instituto de Genética e Bioquímica, INGEB, Av. Pará, 1720, Bloco 2E – Campus Umuarama; Uberlândia, MG, CEP 38400-902

E-mail: loufcosta@yahoo.com.br

Although human rhinoviruses (HRV) are well-known pathogens that cause the common cold, recently they have been investigated in severe respiratory infections. Whether or not disease severity in children younger than five years old may be increased by infection with a second virus or by host comorbidities was investigated. We have tested 434 nasopharyngeal aspirates from children presenting acute respiratory disease for presence of HRV, respiratory syncytial virus (RSV), influenza virus, parainfluenza virus (PIV), adenovirus and human metapneumovirus. HRV was detected in 181 (41.7%) samples: as the only agent in 107 and with a second virus in 74 samples. Moderate-to-severe symptoms were observed in 31.8% (34/107) cases of single HRV infection and in 48.6% (36/74) of co-infections (p=0.0297). These data suggested that presence of a second virus may slightly increase disease severity in HRV infections. Indeed, multivariable analyses showed that co-infections were associated with lower respiratory tract infections (LRTI) (χ²=33.96; p=0.026) and factors of disease severity (χ²=84.05;
p=0.000); However, canonical analyses showed that PIV and especially RSV played a role in the association. In regard to underlying risk conditions, prematurity, congenital heart diseases and non-infections respiratory diseases showed to be associated with disease severity (p=0.0022, <0.0001, and 0.0017, respectively). Although increased severity frequency was higher in children < 12 months old (p<0.05), when considered as the only risk factor the association was not observed (p=0.0731). In conclusion, co-infections with RSV or with PIV, prematurity, congenital heart diseases, and non-infections respiratory diseases have shown to play important role in disease severity in infections caused by HRV.

HV834 - IMPACT OF THE EMERGENCE AND RE-EMERGENCE OF DIFFERENT DENGUE VIRUS SEROTYPES IN THE STATE OF RIO DE JANEIRO


Instituto Oswaldo Cruz, IOC, Av. Brasil, 4365, Manguinhos, 21045-900, Rio de Janeiro, Brazil E-mail: manoelaheringer@yahoo.com.br

The state of Rio de Janeiro has been of great epidemiological importance for introduction and spread of dengue viruses (DENV) and over the last 26 years, was marked by extensive epidemics. The existence of a continuous program of virological surveillance aims to detect and monitor the circulation of DENV serotypes in the state, where DENV-1, DENV-2, DENV-3 and DENV-4 co-circulate. Given the limited options for prevention and control of epidemics, it has been shown that laboratory diagnosis plays an important role in the Epidemiological Surveillance System, by continuous monitoring infections and confirming new cases. In this study a total of 1,621 dengue suspected cases, received by the Laboratory of Flavivirus / Regional Reference Laboratory of the IOC / FIOCRUZ, were analyzed from January 2010 to December 2011. Using three methods, virus isolation in cell culture, RT-PCR and MAC-ELISA, 738 cases (45.5%) of all cases studied were confirmed. The MACELISA confirmed 31.69% (309/975) of the cases, the RT-PCR confirmed with 35.34% (527/1,491) and 20.8% of 1,173 sera inoculated into C6/36 cells were isolated. DENV-2 was the prevalent serotype in 2010. During 2011, DENV-2 was displaced by DENV-1 and the first DENV-4 cases were isolated in the city of Niterói. Our analysis have shown that DENV-1 cases had more chance of increased severity (OR = 1.06 / 95% CI 0.49 to 2.29 / p <0001), moreover, the severe forms were more frequent on children 15 years old and under (OR = 1.8 / 95% CI = 1 to 3.22 / p <0001). From the total of fatal cases confirmed (n = 47), 51.6% were due to secondary infections. Fatal cases were more frequent in children 15 years old and under in 2011 in comparison to 2010. The emergence of DENV-1 in 2011 led to the occurrence of fatal and severe cases in children 15 years old and under and, despite the re-emergence of DENV-1 in 2011, DENV-4 was isolated and spread in the State, causing an epidemic in 2012. Financial support: FAPERJ, CNPq, CAPES and FIOCRUZ.

HV835 - TWENTY FIVE YEARS OF DENGUE: THE EXPERIENCE OF A REGIONAL REFERENCE LABORATORY...
TORY
Oswaldo Cruz Institute, IOC/FIOCRUZ, Av. Brasil, 4365, Manguinhos, Rio de Janeiro E-mail: flaviab@ioc.fiocruz.br

During the past 25 years high dengue activity in Brazil has been evidenced by the large number of cases, in almost all states of the country. DENV-1, DENV-2 and DENV-3 were introduced in Rio de Janeiro, in 1986, 1990 and 2000, respectively. DENV-4 has recently been isolated after 28 years of its first isolation. The introduction of DENV-2 in 1990 caused the first DHF/DSS cases. The introduction of DENV-3 led to severe epidemics in 2002 with the largest number of cases, DHF cases and deaths. In 2007–2008, the country experienced the most severe dengue epidemic in terms of morbidity and mortality and a higher incidence of severe cases in children. From March 1986 to December 2011, 47,346 dengue suspected cases were received in the Laboratory and 41,614 were submitted to one or more routine diagnosis techniques available. A total of 32,374 cases (77.8%) were tested by MAC-ELISA, 25,037 (60.2%) were submitted to virus isolation, 181 cases (0.4%) to HI test and 829 cases (2.0%) to IgG-ELISA. The RT-PCR was performed in 7,441 cases (17.9%) and the NS1 ELISA in 1,124 cases (2.7%). Cases confirmation were 59.7% and 58% in the DENV-1 epidemic occurred in 1986 and 1987, respectively; 52.8% in the DENV-3 epidemic occurred in 2002, 52% in the DENV-2 epidemic in 2008 and 47% and 45% in the DENV-1 occurred in 2010 and 2011, respectively. In the last 25 years, virus isolation and RT-PCR identified the infecting serotype in a total of 4,990 dengue cases. Genomic analysis performed on DENV-2 identified the introduction of a distinct lineage of the Asian/American genotype. In 2009 and 2010, DENV-1 re-emerged and, this serotype has been prevalent in many States of Brazil. The phylogeny has also demonstrated the introduction of distinct lineages of DENV-1. Our experience has shown that the implementation of new laboratorial techniques over the years constituted an important tool for the disease surveillance in Brazil. Financial support: FAPERJ, CNPq, CAPES and FIOCRUZ

HV839 - CO-INFECTION OF DENGUE VIRUS BY SEROTYPES 1 AND 4 IN PATIENT FROM SÃO JOSÉ; DO RIO PRETO, SÃO PAULO
Vedovello, D., Colombo, T., Mondini, A., Nogueira, M.L.

1. Faculdade de Medicina de São José do Rio Preto , FAMERP, Avenida Brigadeiro Faria Lima, 5416 - Vila São Pedro, São José do Rio Preto,
2. Universidade Estadual de São Paulo, UNESP, Rua Cristóvão Colombo, 2265 - Jardim Nazareth São José do Rio Preto, Universidade Estadual de São Paulo - Araraquara, UNESP Araraquara, Rodovia Araraquara - Jatú Km 1, Araraquara, SP E-mail: danvedo@hotmail.com

Dengue is a major public health problem in tropical and subtropical countries. There are four immunologically related
serotypes phylogenetically classified into genotypes. Historically, the State of Sao Paulo, Brazil, has been suffering dengue outbreaks since 1990. The natural co-infection with different serotypes of dengue virus has been observed for many years. We report one case of DENV-1 and DENV-4 co-infection in human serum detected by molecular tests in the sample RP/BR/2012 isolate in São José do Rio Preto, São Paulo, Brazil, on 12 March 2012. The diagnosis was performed by EIA for NS1 protein and did not detect the co-infection, subsequently identified by molecular method. We sequence a fragment of 365 bp (from DENV-1) and 252 bp (from DENV-4) representing the sequences encoding the capside gene (C). This sequences and sequences of DENV 1 and 4 published in genbank were used to alignment of aminoacids and for phylogenetic reconstruction. The phylogenetic analysis indicated that they are classified as genotype 5 and 2 for DENV-1 and DENV-4, respectively. In terms of aminoacids, no significant change was identified in DENV-1 C protein and three aminoacid substitutions were identificated in DENV-4: F5L (Phenylalanine to Leucine in fifth position), R10F (Arginine to Phenylalanine in tenth position), S14F (Serine to Phenylalanine in fourteenth position). The F5L substitution are present only in recent Brazilians strains and can be considered a genetic signature of Brazilian isolates and needs further investigation. Our report demonstrates that co-infection with diferents serotypes of dengue virus can occur naturally and emphasizes the importance of the molecular method in the dengue diagnosis once the EIA for NS1 protein can not identify the presence of two serotypes in a single sampl

HV844 - EXPRESSION LEVELS OF VIRAL SENSOR RIG-I AND ITS REGULATORS RNF125 AND TRIM25 IN HIV INFECTED ADULTS AND CHILDREN


1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, UFRJ, Departamento de Genética; CCS; Bloco A; 2º andar; sala 66; CEP: 21941-590; RJ

2. Instituto de Bioquímica Médica Universidade Federal de Rio Grande, FURG,

3. Faculdade de Medicina Hospital Universitário Gaffrée e Guinle, HUGG, Instituto de Puericultura e Pedriatria Martagão Gesteira, IPPMG - UFRJ, E-mail: alanmessala@gmail.com

RIG-I is a citoplasmatic viral sensor that recognizes viral RNA genome. Its activation culminates in IFN type I production. TRIM25 and RNF125 are ubiquitin ligases that regulate RIG-I. Ubiquitination via TRIM25 is necessary to activate innate response pathway. On the other hand, ubiquitination by RNF125 signals RIG-I degradation via proteassome. Since it is known that RNF125 inhibits HIV-1 replication; that RIG-I recognizes HIV-1 RNA and that IFN type I regulation is important in control/progression of HIV-1 infection we decided to analyze the expression levels of RIG-I, RNF125 and TRIM25 in healthy donors and in HIV infected individuals assessing their progression levels. We performed qRT-PCR from
PBMC of adults: 14 non-infected (NI) adults, 10 progressors (P), 10 non-progressors (NP); and children: 8 healthy, 8 progressors and 4 non-progressors. The adult and children groups were analyzed separately. We found that all 3 genes were more expressed in healthy controls, independent of age. Furthermore, we found that RIG-I was more expressed in P than in NP patients. Moreover we could see an evident relationship among the pattern expression of the three genes studied within NI and P groups. In NI, RNF125 was at least 2 fold more expressed than TRIM25 and RIG-I. In P group, RIG-I was the more expressed gene and RNF125 and TRIM25 were equally expressed. NP and NI had the same expression pattern. We concluded that not only one gene is important to control AIDS progression but the balance among them. Furthermore RNF125 seems to be essential to maintain the innate signalling off when it is not necessary in NI groups and NP. Finally we concluded that RIG-I seems to be important in AIDS progression since is up regulated in P individuals and could then help to predict disease progression.

HV848 - ASYMPTOMATIC GASTROENTERIC VIRUS SHEDDING AMONG CHILDREN THAT ATTEND A DAY CARE CENTER IN GOIÂNIA-GO, BRAZIL


Universidade Federal de Goiás, UFG, Rua 235, Setor Universitário, CEP 74605-050. Goiânia - Goiás - Brasil

Gastroenteric viruses are important etiological agents of childhood acute gastroenteritis. These agents are transmitted by the fecal-oral route, and by person-to-person spread, although environmental contamination could contribute to viral dissemination, with outbreaks frequently occurring in closed institutions such as hospitals, cruise ships and day care centers. Viral excretion by asymptomatic individuals has been reported. In this study, children that attend a day care center in Goiânia, Goiás, Brazil, were monitored for gastroenteric virus detection for a period of three years. For this study, 90 fecal samples, collected from RVA-vaccinated children, were screened for human adenoviruses (HAdVs) using antigen detection kits, by ELISA, polyacrylamide gel electrophoresis (PAGE) and a rapid chromatography test for group A rotavirus (RVA) detection. Sixty-seven samples were also tested for caliciviruses-CVs (noroviruses-NoVs and sapoviruses-SaVs) by RT-PCR, using primers targeting the C region of the viral capsid. Two of 90 fecal samples tested positive for RVA by all three screening tests and third sample, collected from a different child, was positive for adenovirus by both tests used. Five of the 67 (7.5%) samples were positive for CVs. Molecular characterization identified one RVA-positive sample as genotype G2 (VP7 gene) and genotype P[4] (VP4 gene), and the other positive sample remained un-typeable by RT-PCR. Among the samples screened for CVs, four were positive for GII NoVs and one was positive for SaVs. Our data shows RVA, HAdVs, and CVs circulation among asymptomatic RVA-vaccinated children that attend a day care center in Brazil, reinforcing the potential for viral spread in such close settings. Ongoing studies, focusing on
Genomic sequencing and phylogenetic analysis are being conducted in order to better evaluate the epidemiology of gastroenteric viruses among day care children, and also the possible role of asymptomatic children in viral dissemination.

HV850 - Screening for Human Parechovirus in Lymphoid Tissues of the Upper Respiratory Tract


1. Centro de Pesquisa em Virologia - FMRP - USP, CPV - FMRP - USP, Av. Bandeirantes, 3900 - Vila Monte Alegre. 14049-900, Ribeirão Preto - SP

2. Oftalmologia, Otorrinolaringologia, Cirurg da Cabeça e Pescoço, HCFMRP - USP, Av. Bandeirantes, 3900 - Vila Monte Alegre. 14049-900, Ribeirão Preto - SP E-mail: lucianoluna@usp.br

Chronic adenotonsillitis (CAT) is a persistent hypertrophy of tonsils and adenoids, of poorly understood etiology. Several viruses are frequently detected in palatine tonsils and adenoids from patients with chronic adenotonsillitis who undergo tonsillectomy. However, the viral panels used for screening by PCR do not include human parechovirus (HPeV), an emerging picornavirus related with a broad spectrum of diseases, similar to those caused by enteroviruses. In this preliminary study, the presence of HPeV was assessed in respiratory samples and palatine tonsil and adenoid tissue fragments collected from patients with CAT at the University of São Paulo Hospital in Ribeirão Preto, Brazil. A total of 104 samples were tested for HPeV with a two-step real-time RT-PCR assay for the conserved 5'UTR, of which 82 samples (25 nasal swabs, 22 nasopharyngeal washes, 18 palatine tonsils and 17 adenoid fragments) were from 27 patients with CAT, and 22 samples (5 nasal swabs, 5 nasopharyngeal washes, 6 palatine tonsils and 6 adenoid fragments) from 6 patients without CAT as control group. Samples were collected from November 2011 to July 2012 and were previously tested by real-time PCR for a panel of common respiratory viruses. Patients in the study were aged from 1.8 to 13 years. HPeV was detected in 2 samples (1 palatine tonsil and 1 adenoid fragment), both from one patient in the control group (2 year-old female). Further investigation shall be done in a larger number of patients in order to determine the role of HPeV infection in lymphoid tissues in the upper respiratory tract. Financial support: FAPESP, CNPq.

HV851 - Impact of the Emergence and Re-emergence of Different Dengue Virus Serotypes in the State of Rio de Janeiro


Instituto Oswaldo Cruz, IOC, Av. Brasil, 4365, Manguinhos, 21045-900, Rio de Janeiro, Brazil E-mail: manoela.heringer@ioc.fiocruz.br

The state of Rio de Janeiro has been of great epidemiological importance for introduction and spread of dengue viruses (DENV) and over the last 26 years, was marked by extensive epidemics. The existence of a continuous program of virological
surveillance aims to detect and monitor the circulation of DENV serotypes in the state, where DENV-1, DENV-2, DENV-3 and DENV-4 co-circulate. Given the limited options for prevention and control of epidemics, it has been shown that laboratory diagnosis plays an important role in the Epidemiological Surveillance System, by continuous monitoring infections and confirming new cases. In this study a total of 1,621 dengue suspected cases, received by the Laboratory of Flavivirus / Regional Reference Laboratory of the IOC / FIOCRUZ, were analyzed from January 2010 to December 2011. Using three methods, virus isolation in cell culture, RT-PCR and MAC-ELISA, 738 cases (45.5%) of all cases studied were confirmed. The MAC-ELISA confirmed 31.69% (309/975) of the cases, the RT-PCR confirmed with 35.34% (527/1,491) and 20.8% of 1,173 sera inoculated into C6/36 cells were isolated. DENV-2 was the prevalent serotype in 2010. During 2011, DENV-2 was displaced by DENV-1 and the first DENV-4 cases were isolated in the city of Niterói. Our analysis have shown that DENV-1 cases had more chance of increased severity (OR = 1.06 / 95% CI 0.49 to 2.29 / p <0001), moreover, the severe forms were more frequent on children 15 years old and under (OR = 1.8 / 95% CI = 1 to 3.22 / p <0001). From the total of fatal cases confirmed (n = 47), 51.6% were due to secondary infections. Fatal cases were more frequent in children 15 years old and under in 2011 in comparison to 2010. The emergence of DENV-1 in 2011 led to the occurrence of fatal and severe cases in children 15 years old and under and, despite the re-emergence of DENV-1 in 2011, DENV-4 was isolated and spread in the State, causing an epidemic in 2012. Financial support: FAPERJ, CNPq, CAPES and FIOCRUZ.

HV853 - IMMUNOLOGICAL PROFILE AND RISK FACTORS ASSOCIATED TO POSSIBLE OCCUPATIONAL INFECTIONS BY VACCINIA VIRUS


Laboratório de Vírus, Departamento de Microbiologia, ICB-UFMG, UFMG, Avenida Antônio Carlos, 6627, Pampulha, Belo Horizonte, Minas Gerais E-mail: galileuk1@gmail.com

Despite the eradication of smallpox, the orthopoxviruses (OPV) are still a source of concern due to the possible use of Variola virus as a biological weapon, as well as the increase in outbreaks of zoonotic infections caused by OPV in several regions of the world, as by Vaccinia virus (VACV) in Brazil. VACV is the primary component of the smallpox vaccine and infections with this virus can occur after direct contact with animals naturally infected, individuals recently vaccinated or through exposure to the virus in laboratories, as described in Brazil and other countries of the world. The aim of this study was to investigate the immunological profile of workers in a research laboratory, seeking to evaluate the humoral immunity and the risk factors involved in possible occupational infections by VACV. We conducted an epidemiological survey and collected 50 samples of human sera. A total of 18 (36%) men and 32 (64%) women are part the study population, mostly students, beyond teachers and technical assistants, aged 19 to 70 years. It was found that 34
(68%) individuals have direct contact with OPV contaminated material and 29 (58%) forgot, at some time of experimentation, using protective equipment required. Still, 18% suffered an accident with any contaminated material, and of these, 8% had signs and symptoms, and 16% have a history of vaccination, where only 8% have the vaccine take. After diagnosis, it was found that 9 (18%) individuals have anti-OPV neutralizing antibodies. In Brazil, after the smallpox eradication, the vaccination that confers immunity to OPV was discontinued and even researchers and health professionals are not vaccinated. Therefore, it is important to conduct studies of this nature due to the widespread risk that poxvirus offer to human health, as well as its dispersion in laboratory and hospital environments. Financial support: CNPq, FAPEMIG.

HV857 - SEROLOGICAL PROFILE OF EPSTEIN-BARR VIRUS INFECTION IN PATIENTS SUSPECTED OF INFECTIOUS MONONUCLEOSIS IN BELEM, PARÁ, BRAZIL


1. Instituto Evandro Chagas, IEC, Rodovia BR-316 Km07 - Levilândia, Ananindeua, Pará

2. Fundação de Amparo à Pesquisa do Estado do Pará, FAPESP, Travessa Nove de Janeiro nº 1686 E-mail: prodriguesbarros@gmail.com

Epstein-Barr virus (EBV) infection is the major cause of Infectious Mononucleosis (IM), a self-limited syndrome, characterized by the main symptoms: fever, sore throat, lymphadenopathy and pharyngitis. Clinical manifestations may vary according to the immune status of patients infected; therefore, a definitive diagnosis should rely on the assessment of specific markers for EBV infection. The most common serological markers sought to confirm EBV infection in IM are IgM to VCA antigens; however these markers are insufficient to designate an EBV etiology either a primary infection or reactivation. Were tested 20 sera from patients suspicious of IM from Belém, Pará, previously known to be IgM VCA antibody-positive by ELISA, using Immunoblot IgG kit for the following antigens: EBNA-1, p18, p23, BZLF1, p138 and p54. The results were analyzed according to respective symptoms: fever, fatigue, sore throat, lymphadenopathy, exanthema, tonsilitis and hepatosplenomegaly. Statistical analyses were performed with BioEstat 5.0 software, using fisher’s exact test and kappa test. The main symptoms found were: fever (65%), lymphadenopathy (45%) and exanthema (20%). In patients aged 4 years or more, only one EBNA-1-negative result was found, since most children become infected by EBV between 3 years of age. EBNA-1 is the most studied marker to define past EBV infection, although 5% of infected patients have a lack of anti-EBNA-1 response. To assess the absence of EBNA-1, p18 was used as a complementary result (excellent replicability). The sore throat symptom, related to the primary site of EBV infection, was diagnosed only in patients non-reactive to p18 and EBNA-1 (p=0.0035), past markers for EBV infection. Results from the present study suggest that assessment of
serological markers for EBV infection by Immunoblot assay appears to be useful to differentiate between primary infection and reactivation; in addition, sore throat was found to be a major sign of primary EBV infection.

HV859 - SEROEPIDEMIOLOGY OF HEPATITIS B VIRUS INFECTION IN AN INSTITUTIONALIZED POPULATION OF THE STATE OF GOIÁS


Instituto de Patologia Tropical e Saúde Pública, IPTSP, Rua 235 - s/n - Setor Universitário - CEP: 74605050 - Goiânia - Goiás - Brasil E-mail: tatycinquini@yahoo.com.br

The hepatitis B virus (HBV) remains an important health problem worldwide, transmitted mainly by vertical, parenteral and sexual routes. In this context, institutionalized individuals with mental problems constitute a risk group for acquisition of HBV infection due to their low awareness of the risks of viral infection, and because of their prolonged stay in mental institutions. In this sense, this study aimed to determine the HBV infection rate in an institutionalized population with psychiatric disorders and neurological state, located in the State of Goiás, Brazil. Blood samples were collected from 334 participants, and analyzed for the presence of serological markers of HBV infection (HBsAg, anti-HBsAg, anti-HBc) by enzyme-linked immunosorbent assay, using commercial kits. The overall prevalence of HBV infection was 14.1% (47/334), with a rate of occult HBV infection of 5.4% (18/334). Higher infection rates were observed among adult males (36-59 years-old), the elderly (>60 years-old), and also among those individuals that had been institutionalized for over 10 years (p<0.05), when compared to the rest of the population. Only 3.9% (13/334) of the population had serologic evidence of previous vaccination against hepatitis B virus and susceptibility to HBV, characterized by the absence of any serological marker, was observed in 82% of the population. These results demonstrate a high prevalence of HBV infection in the institutionalized population, with a significant rate of occult infection. The data emphasize the importance of adopting preventive measures, with emphasis on HBV vaccination, to control HBV infection in risk groups such as the institutionalized population.

HV866 - LOW PREVALENCE OF SUBTYPE B VARIANT (B'-GWGR) IN THE SOUTHERNMOST REGION OF BRAZIL

Maletich, D., de Medeiros, R., Leite, T.F., Guimarães, M.L., Gräf, T., Pinto, R.A., Almeida, S.

1. Centro de Desenvolvimento Científico e Tecnológico, CDCT-FEPSS, Porto Alegre, RS, Brazil
2. Pós-Graduação em Genética e Biologia Molecular UFRGS, PPGBM-UFRGS, Porto Alegre, RS, Brazil
3. Laboratório de AIDS & Imunologia Molecular FIOCRUZ, FIOCRUZ, Rio de Janeiro, RJ, Brazil
4. Departamento de Microbiologia, Imunologia e Parasitologia, UFSC, Florianópolis, SC, Brazil E-mail: maletich@ig.com.br
The vast majority of HIV-1 subtype B (HIV-1B) sequences present the GPGR motif at the tip of the v3 loop. Epidemiological studies have detected a subtype B variant, which presents the GWGR signature (B'-GWGR), in about 17 to 50% of infections in Southeast Brazil, although the prevalence of B'-GWGR in the southernmost region is not yet clear. So, the present study aimed to trace the temporal trends of this variant in two cities of the Southernmost region of Brazil. Blood samples from 189 HIV-1 treatment-naïve patients were obtained at two distinct time periods in the city of Porto Alegre (PoA), Rio Grande do Sul State: 1998 and 2005-2008. In addition, 98 blood samples were collected between 2004 and 2008, in the city of Florianópolis (FL), capital of Santa Catarina State. Phylogenetic analysis of a 302-base pair fragment of the env gene (C2-V3 region) revealed the presence of 46 (51%) samples of non-B subtypes and 46 (50%) HIV-1B samples from 1998 in PoA, distributed as follows: B'-GWGR (9%), B-GPGR (20.5%) and GXGX (20.5%). In the second studied period, subtype B was detected in 32 samples (33%) and subtype B signatures were as follows: B'-GWGR (6.2%), B-GPGR (14.4%) and GXGX (12.4%). In FL subtype B accounted for 33% of HIV infections and the HIV-1B signatures were distributed as follows: B'-GWGR (11.2%), GPGR (7%) and GXGX (14.3%). HIV-1B molecular signatures were distributed equally within PoA (1998 and 2005-2008) and between PoA and FL. No association could be established between HIV-1B molecular signatures and a specific exposure category in the PoA epidemic. However, the B'-GWGR seems to be related to homosexuals in FL (p=0.012). Southernmost HIV-1 subtype B epidemic seems to be different from that established in the Southern region of Brazil. Our results suggest lower prevalence maintenance of B'-GWGR along the time in PoA and no association between exposure category and B signatures, however an association between B'-GWGR and homosexuals was verified in FL.

HV868 - ASEPTIC MENINGITIS BY COXSACKIEVIRUS B5 IN NEONATE


1. University Hospital of USP, HU/USP, Av. Prof Lineu Prestes, 2565, Cid. Universitária São Paulo, SP, 05508-900

2. Adolfo Lutz Institute, IAL, Av. Dr. Arnaldo, 355 São Paulo CEP: 01246-000 E-mail: helorv@yahoo.com.br

Aseptic meningitis (AM) is considered one of the most common infectious diseases in children, especially those less than 5 years old. Neonatal infections from enteroviruses are fairly common, associated with a wide spectrum of signs and symptoms, which range from a non-specific febrile illness to potentially fatal multisystem disease. Enterovirus genus (EV) is a RNA virus that belongs to the family Picornaviridae, and was originally classified as Poliovirus, Coxsackievirus A and B, Echovirus and Enterovirus, based on their associated pathogenicity. Coxsackievirus B5 (CV-B5) belongs to HEV-B species and is one of the most predominant serotypes in humans being frequently associated with sporadic cases of neurological
diseases and epidemics of AM. The aim of this study was to describe the case of a previously healthy neonate with AM evolution by CV-B5. The newborn patient, male, was taken by his mother to the University Hospital of São Paulo searching for medical care with a history of sneezing, dry cough and fever. From the history reported and the clinical examination, the patient was initially diagnosed with suspected AM and the cerebrospinal fluid (CSF) material was sent to the Adolfo Lutz Institute – Virology Center, Enteric Diseases Laboratory. CSF was inoculated in RD and HEp-2 cell cultures. Viruses isolated were identified by Reverse Transcription Polymerase Chain Reaction and Sequencing and CV-B5 was detected. Especially in neonates and infants, CV-B5 has a more serious evolution, affecting the central nervous system and muscles, causing complications such as AM. Several publications have reported neonates with symptomatic EV infection on day 1 of life, indicating that the infection must have been acquired antenatally - either transplacentally or potentially via ascending infection. This case demonstrates the importance of CV-B5 circulation in the population and the early etiologic diagnosis of meningitis to search for the specific etiological treatment.

**HV869 - ASSAY OF PCR AND CLONING STANDARDIZATION OF HUMAN RESPIRATORY SYNCYTIAL VIRUS GENES**


**Dep. Física - IBILCE - Universidade Estadual Paulista , UNESP, R. Cristóvão Colombo, 2265. Jd**

**Nazareth - São José do Rio Preto**

Human Respiratory Syncytial Virus (hRSV) is the major agent of respiratory infections in children, like bronchiolitis and pneumonia causing deaths around the globe. Its genome is a single strand RNA, with genome organization NS1- NS2 - N - P - M - SH - G - F - M2.1 - M2.2. Several works have been made focusing in diagnosis, inhibitor for the virus, how viral proteins interact with themselves, and so on. However, it is necessary, firstly, to obtain these proteins using DNA recombinant technology. Thus, the aim of this project includes the standardization of PCR amplification and cloning of hRSV genes. The standardization of PCR was performed testing four different DNA Polymerases (taq DNA Polymerase (Recombinant) – Fermentas®; High Fidelity DNA Polymerase (Recombinant) – Fermentas®; Biotools DNA Polymerase – Biotools®; JumpStart Sigma®) following manufacture recommendations. The cloning of the fragments was developed in vector pCR-XL-TOPO (Invitrogen®) and sub-cloningstandardization into expression plasmid pET28a - Novagen® and into two-hybrid plasmids (pGBKKT7 and pGADT7 - Clontech®) were standardized with objective to avaliable proteins for interaction tests. In result, it was successfully obtained amplicons of genes NS1 (438bp), NS2 (393bp), P (744bp), M (789bp), Complete G (912bp), G endodomain (135), G ectodomain (700), F1 (1131bp), F2 (360bp), M2-1 (603bp) and M2-2 (288bp) and better results was obtained with taq DNA Polymerase (Recombinant) – Fermentas® and JumpStart Sigma®. The cloning of NS1, NS2, P, M, G1, G2, F1, F2, M2.1 and M2.2, genes on pCR-XL-TOPO vector was
obtained with success. Into pET28a were subcloned the genes NS1, M2.1, M2.2, P, G1 and G2 and into pGBK7 and pGADT7 were subcloned the genes NS1, G1, G2, M, M2.1, M2.2 and P. Once PCR and cloning standardization is an important and difficult step in diagnosis and protein expression works, the protocols we developed will help the groups who works with hRSV to outcome this first steps.

**HV872 - EVALUATION OF CROSS-REACTIVITY IN ENZYME-LINKED IMMUNOABSORBENT ASSAY (ELISA) TO EPSTEIN-BARR VIRUS (EBV), CYTOMEGALOVIRUS (CMV) AND HUMAN HERPESVIRUS 6 (HHV-6)**


1. Instituto Evandro Chagas, IEC, Rodovia BR-316 Km07 - Levilândia, Ananindeua, Pará
2. Fundação de Amparo à Pesquisa do Estado do Pará, FAPESPA, Travessa Nove de Janeiro nº 1686, Belém, Pará E-mail: igorcosta@iec.pa.gov.br

Viruses EBV, CMV and HHV-6, herpes family, are spread worldwide and persist in the host organism in a latent state, infecting mainly leukocytes. Besides the structure and biology of these viruses, the epidemiology, transmission and clinical manifestations of symptomatic infections are similar, coursing mainly with fever, sore throat and lymphadenopathy. In addition, HHV-6 produces rash as major clinical manifestation. The similarity between the three viruses may lead to diagnostic difficulties in relation to the specificity of routine serological tests. It is important to assess the seropositivity for the virus and compare it with the patient’s symptoms. Were selected 20 serum samples, which were positive by IgM ELISA anti-VCA of EBV (BALF4), and tested by IgM ELISA to CMV and HHV-6, both made from viral lysates. To evaluate the possibility of cross-reactivity was tested in silico homology to the antigenic sites (AS) of the protein BALF4 with the AS of the proteins encoded by HHV-6 and CMV. To research described AS, predict possible AS and test homologies were used immune epitope database, hlapred and Geneious Pro 4.8.5 softwares, respectively. Statistical analyses were performed with BioEstat 5.0 program, using fisher’s exact test. Patients were aged between 10 months and 54 years, average of 17 years. Were found more positive for HHV-6 (90%) than for CMV (35%) (p<0.001). There was no relationship between positive tests with symptoms (p>0.05). Homology was found between proteins BALF4 of EBV, U39 of HHV-6 and gpB of CMV. The homologue AS in BALF4 (ID:114667) and U39 (ID:166736) have been well described, while in gpB had to be inferred. There was greater similarity between AS of EBV and HHV-6. Although these viruses have a structure, biology, transmission path and epidemiology similar, which favors the co-infection, the possibility of false-positive routine test exists due to the similarity between the AS targets of the immune response against these viruses.

**HV875 - ASSOCIATION BETWEEN POLYMORPHISMS IN THE ORGANIC CATIONS TRANSPORTER OCT-1 AND THE FAILURE TO ANTIRETROVIRAL THERAPY AGAINST HIV.**

Dias, J.Z.C., Arruda, M.B., Brindeiro, R.
Tanuri, A., Cardoso, C.C., Aguiar, R.S.

UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, UFRJ, Av. Carlos Chagas Filho, 373, Ilha do Fundão - Cidade Universitária/RJ E-mail: juzdias@gmail.com

The HIV is a pandemic infection with approximately 33.4 million people infected. A total of 6.65 million patients are undergoing HAART (Highly Active Antiretroviral Therapy) and about 10-20% of these patients do not reach therapeutic success, mostly due to the emergence of drug resistant viruses but also because of the host inter-individual variations in drug absorption, activation and metabolism. Since therapeutic success is dependent on the maintenance of drug intracellular levels, the cellular drug transporters are important candidates for pharmacogenetic studies. In this study we investigate the association between 40 polymorphisms in the genes SLC22A1, SLC22A2 and SLC22A3 and ABCB1, coding for the Organic Cation Transporters 1-3 and P-Glycoprotein and antiretroviral failure. For this purpose, we have conducted a case-control study including 219 HIV+ individuals (117 cases of HAART failure and 120 controls for whom first-line therapy successfully reduced HIV-1 viral loads to undetectable levels) selected from the cities of Curitiba and Porto Alegre, in the Brazilian south, and treated for at least 6 months. Genotyping was performed using SNaPshot and the TaqMan OpenArray Platform (Life Technologies). Comparisons between cases and controls were performed by logistic regression models with adjustment for the covariates age and therapy scheme. Our results showed an association between the deletion of a single nucleotide in codon 420 of the SLC22A1 gene (rs3516751) and increased risk of therapeutic failure among heterozygotes (adjusted OR = 2.86; 95% IC 1.47-5.55; p=0.001). We were unable to define allele dose effects due to the complete lack of homozygotes for this deletion. This is the first study designed to investigate the pharmacogenetics of HAART effectiveness in Brazil. Our data describe a clear association between variations in SLC22A1 and HAART failure, suggesting that studies of host genetics are crucial to predict better treatment for HIV+ patients. Financial support: CNPq and FAPERJ.

HV877 - EPIDEMIOLOGICAL STUDY OF ADENOVIRUS ASSOCIATED WITH ACUTE DIARRHEAL DISEASE, DURING 2007-2010, IN MINAS GERAIS, BRAZIL


1. Universidade Federal de Juiz de Fora, UFJF, R. José Lourenço Kelmer, s/n, Campus Universitário-S. Pedro - Juiz de Fora, MG

2. Instituto Oswaldo Cruz , IOC - FIOCRUZ, Av. Brasil, 4365 - Manguinhos - Rio de Janeiro, RJ. E-mail: thaisavr@hotmail.com

Acute diarrheal disease (ADD) is an important cause of child morbidity and mortality in developing countries. Among viruses, enteric adenoviruses are one of the most important etiologic agents of ADD. Human adenoviruses (HAdV) are classified into seven species (A-G) and 54 serotypes. Among them,
serotypes 40 and 41, both of the species F, are the most commonly associated with ADD. Given the importance of this disease in developing countries and the large number of cases without an etiologic agent defined was performed this study in Juiz de Fora, Minas Gerais. Between January 2007 and December 2010 were analyzed 395 diarrheic stools samples. HAdV was detected by PCR and molecular characterization was performed by sequencing and phylogenetic analysis of partial sequences of the hexon gene. For statistical analyzes was used SPSS program, adopting a value of p <0.05 as significant. The HAdV prevalence was 10.9% (43/395). There was no significant correlation between the origin of the sample (hospital X ambulatory) and the occurrence of infection (p=0.152), as well as, in relation to gender of the infected (p=0.393). The majority of positive cases was detected in children up to 24 months of age, showing a significative correlation between the age of the infected individuals and the occurrence of infection (p=0.007). In most cases of infection (36/43), HAdV was the only virus detected, but it has been observed some cases of co-infection with RV (5/43) and NoV (2/43). Phylogenetic analysis of partial sequences of the hexon gene from 35 positive samples revealed that all samples clustered with HAdV species F, serotypes 41, confirming the association of enteric HAdV (EHAdV) with ADD. This survey revealed the presence and circulation of these viruses in Juiz de Fora, MG, in this period as well as its important role in the genesis of ADD, reaveling a great number of diarrreal cases which normally remains unidentified. Financial Support: CAPES, CNPq, FAPEMIG and Propesq-UFJF

**HV879 - PREVALENCE OF ANTI-ORTHOPOXVIRUS ANTIBODIES IN HUMANS IN THE ABSENCE OF BOVINE VACCINIA OUTBREAKS**


Univrasidade Federal de Minas Gerais, UFMG, Av. Antônio Carlos, 6627, Campus Pampulha, CEP: 31270-901. Belo Horizonte, Minas Gerais.

E-mail: polianaofigueiredo@yahoo.com.br

The Vaccinia virus (VACV) circulates in Brazil since the 60s. Infections by this virus affects both cattle and humans and all human cases reported to date are always associated with outbreaks in cattle. In order to analyze retrospectively the immune status against Orthopoxvirus of populations of the Amazon and southeast regions of the country, samples were analyzed from Mantena and Terra Nova do Norte, belonging to the states of Minas Gerais and Mato Grosso, respectively. Sera samples were collected in 1995 and 1996, dates prior to the notification of outbreaks in these regions. The samples were subjected to IgG ELISA and PRNT for the detection of anti-Orthopoxvirus antibodies. Furthermore, molecular tests were performed by real-time PCR for the vgf gene aiming to detect viral DNA on the sera samples. Of the 70 samples tested belonging to the Amazon region, 27% were IgG positive and from those, 27% of positive samples are from people who were not vaccinated against smallpox, or were born after 1977, closing date of vaccination in Brazil. It was found that 6% of the vaccinated population was positive by
PRNT, indicating the efficiency of the immunity generated by the smallpox vaccine. From Mantena rural area, 62 samples were analyzed, with 11% IgG seropositivity and of these, 29% of people are not vaccinated. The PRNT revealed 15% of seropositivity and all positives are vaccinated patients. The molecular test was able to detect viral DNA in some samples thus suggesting a possible DNAemia. The detection of Orthopoxvirus antibodies in unvaccinated individuals indicates a possible silent circulation of VACV in human populations, and seems to be not related to outbreaks of bovine VACV. This data is reinforced by the detection of viral DNA in some samples. Thus, these results support the need for more research on the subject, especially as regards the identification of potential animal reservoirs and new forms of disease transmission.

HV881 - PLASMA LIPIDOMIC EXPRESSION SIGNATURE DISTINGUISHES HEPATITIS C-RELATED HEPATOCELLULAR CARCINOMA AND LIVER CIRRHOSIS


Universidade de Campinas, UNICAMP, Rua Josué de Castro, Campinas-SP CEP 13083-970
Universidade Federal de São Paulo, UNIFESP, Rua Pedro de Toledo, 150 andar, São Paulo-SP CEP 04039-032
E-mail: anampassos@gmail.com

Hepatitis C (HC) is a major cause of hepatocellular carcinoma (HCC). Late diagnosis of HCC represents the main factor for the poor survival of patients. The most widely used biomarker for HCC, alpha-fetoprotein (AFP), has poor sensitivity and specificity and has recently been removed from the American Association for the Study of Liver Diseases (AASLD) guidelines for HCC management. Thus, identification of sensitive and specific biomarkers for HCC diagnosis is an urgent need. In the present study, plasma lipid patterns of patients with HC-HCC and HC-liver cirrhosis (LC) were assessed by performing matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). Plasma samples of 25 patients with HC-HCC and 15 patients with HC-LC were evaluated. Lipids were extracted from plasma using the Bligh-Dyer protocol. The extracts were subjected to MALDI-MS. Data matrix was exported for univariate and multivariate analysis. A total of 2205 ions were initially identified and 7 m/z signals were highlighted as the most important lipids for the discrimination of patients with HC-HCC. The specific lipidomic expression signature generated allows an overall predictive accuracy of 93% of HC-HCC and HC-LC. All 7 peaks showed more than 4-fold change in HC-HCC (P < 0.01). The 7-peak algorithm was able to distinguish the 2 groups at a sensitivity of 96% and a specificity of 87%. MALDI-MS specific peaks signature accurately distinguished patients with HC-HCC from those with HC-LC. The results indicate the potential of this technique and the selected peaks to improve the surveillance of HCC in patients with HC-LC.

HV883 - HUMAN PAPILLOMAVIRUS TYPES 6 AND 11 E6 VARIANTS FROM LARYNGEAL PAPILLOMATOSIS IN BRAZILIAN PATIENTS

Matos, R.P.A., Bonfim, C.M., Mansur,
Recurrence respiratory papillomatosis (RRP) is a disease characterized by benign neoplasms and can occur anywhere within the upper respiratory tract, but the most common lesion site is the larynx. This disease has a bimodal age distribution which forms the basis of its classification as juvenile or adult. The main etiological agent of RRP is Human Papillomavirus virus (HPV), a group of DNA virus of which over 150 types has been identified. HPV-6 and 11 are the most common types found in RRP. HPV stimulates proliferation of the mucosal epithelium leading to the development of papillomas. E7 and E6 oncoproteins from high-risk HPVs induce cell immortalization by interfering in cell cycle regulation. However, the role of E6 and E7 proteins from low risk HPVs are not fully understand as yet and E6 functions might also be related to the clinical course of the RRP. In this study we analyzed genetic variability of the E6 gene in samples of laryngeal papillomas. The complete coding region of E6 was cloned and sequenced in 25 samples: 18 isolates of HPV-6 (72%) and 7 isolates of HPV-11 (28%). A total of three different E6 genomic variants were identified among HPV-6 isolates. Only one identified variant showed an amino-acid change corresponding to 1.3% of the E6 protein. Within the 7 HPV-11 isolates, we identified one genomic variant and two synonymous mutations were detected in this variant. Phylogenetic trees were constructed for both HPV-6 and HPV-11 variants. These enclosed sequences obtained in this study in addition to sequences obtained from GeneBank, including the reference sequences of each genotype. In both phylogenetic trees sequences from Brazil did not group together. We further observed that overall the sequences did not cluster according to their geographical origin neither or the anatomic site of infection. Our data reinforce the hypothesis that HPV-6 and HPV-11 variants are not geographically restricted as observed in HPV-16 and HPV-18. Financial Support: CAPES, FAPESP.
Dengue virus (DENV) (Flavivirus, Flaviviridae) is the most important arbovirus worldwide, being transmitted by Aedes sp. mosquitoes. DENV exists as four different serotypes: DENV-1 to DENV-4. The epidemiological scenario in Brazil is characterized by the co-circulation of four DENV serotypes and the country has the higher number of DENV in Latin America. Minas Gerais state usually present high number of dengue cases and Juiz de Fora, located at Zona da Mata Mineira, had great epidemics of DENV in last years with 11,582 notified cases from 2010 to 2012. The aim of this work was to perform a prospective study of DENV in patients with clinical suspect of DENV infection and naturally infected larvae of A. aegypti obtained in Juiz de Fora, MG. Biological samples were obtained from January to April/2012. Serum samples and pools of larvae (containing up to 50) were used for total RNA extraction. Total RNA was used for cDNA synthesis followed by nested-PCR to detect DENV. From 9 serum samples one was positive for DENV-2, by PCR. This same serum sample was IgG and IgM reactive when tested by Dengue IgG/IgM Test Bioeasy. Using this later test, five other PCR negative serum samples, were IgG/IgM non-reactive. From 56 pools of larvae, two were positive for DENV-1. Pools of mosquito collected in the same period are also going to be tested. The obtained amplicons are going to be sequenced to determine the viral genotype and perform further phylogenetic analysis. This is the first report of DENV surveillance in Juiz de Fora, pointing to co-circulation of DENV-1 and DENV-2 during the last epidemic period. Financial support: FAPEMIG, CNPq, CAPES, UFJF, PROPESQ/UFJF.

HV889 - LIPIDOMIC FINGERPRINTING OF CANCEROUS AND NONMALIGNANT TISSUES IN HEPATITIS C-RELATED HEPATOCELLULAR CARCINOMA: A PROOF OF CONCEPT STUDY


Hepatocellular carcinoma (HCC) is an aggressive liver cancer but biomarkers that can predict natural history of malignant progression are lacking. Lipidomic profiling using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) enables the identification of biomarkers for cancer. The present study explored the lipidome-wide patterns of HCC to identify lipids that could distinguish cancerous and nonmalignant tissues.
Tumor and adjacent nontumor tissues were obtained from a patient with hepatitis C-related HCC. Samples were pulverized in liquid nitrogen before lipids were extracted using the Bligh-Dyer protocol. The extracts were subjected to MALDI-MS analysis in 4 replicates each. Data matrix was exported for univariate and multivariate analysis. A total of 322 ions were initially identified and 10 m/z signals were highlighted as the most important lipids for the discrimination of cancerous and nonmalignant tissues in HCC. All 10 ions presented more than 5-fold change in tumor tissue and were significant at P<0.01. Partial least square discriminant analysis (PLS-DA) could distinctly resolve the 2 groups of replicates assessed. Five peaks were increased in tumor tissue and 5 were decreased. The main classes of the distinguishing lipids were Glycerophosphates [GP10], Glycerophosphocholines [GP01], Glycerophosphoserines [GP03], Acidic glycosphingolipids [SP06], Glycerophosphoinositol [GP06], Flavonoids [PK12], Neutral glycosphingolipids [SP05], and Triradylglycerols [GL03]. This proof-of-principle study suggests that MALDI-MS lipidomic fingerprinting may be a powerful tool for the identification of biomarkers for HCC. In conclusion, these data demonstrated that the lipid fingerprinting of cancerous and nonmalignant tissues in HCC was able to select a number of lipids that should be functionally investigated in the pathogenesis of the disease. MALDI-MS could successfully distinguish tumor and adjacent nontumor tissues in HCC.

HV890 - TEMPORAL PATTERN OF ROTAVIRUS PREVALENCE AND DIARRHEA HOSPITALIZATION, 2005 TO 2011, IN JUIZ DE FORA, MG


Universidade Federal de Juiz de Fora, UFF, R. José Lourenço Kelmer, s/n, Campus Universitário, S. Pedro, Juiz de Fora, MG E-mail: andressasilvino@yahoo.com.br

Rotaviruses (RV) are associated with acute diarrheal disease (ADD) on many occasions become severe, leading many children to hospitalization and death. The high prevalence associated with the fact that only hygienic and sanitary actions would not be enough to decrease the prevalence of ADD caused by RV, pointed to the need of development of the effective vaccine. In March 2006, the Health Ministry of Brazil has included Rotarix® vaccine in National Immunization Program (PNI), with goal of to provide protection against severe disease, thus reducing the number of hospitalization and deaths. The aim of this study was investigate the association of RV disease with official data of hospitalization by ADD. In that way, 705 fecal diarrheic samples, obtained from January 2005 to December 2011, in Juiz de Fora, MG were analyzed. RV was detected after acid nucleic extraction by polyacrylamide gel electrophoresis. The rates of hospitalization for ADD were obtained through Ministry of Health's Hospitalization Data System. In 2005, period preceding introduction of the vaccine in PNI, was observed a higher prevalence of RV associated with major number of hospitalizations for ADD, probably indicating more severe cases of ADD caused by RV. In 2006, year of introduction of Rotarix® in PNI, the
The highest number of hospitalizations was observed. A total of 60 cases occurred in July and 39 cases in August. Beside this, the prevalence of RV was 44.44% (n=20/45) and 34.21% (n=13/38) in July and August, respectively. On the other hand, since 2007, it has not been observed an association between the number of hospital admissions and the RV prevalence, fact that may be related to milder cases of the disease. The lack of association between the RV prevalence and number of hospitalizations by ADD after the introduction of Rotarix® vaccine may indicate the likely effectiveness of vaccination in preventing serious cases of ADD caused by RV. Financial support: CAPES, CNPq, FAPEMIG and Propesq-UFJF.

ELECTROPHORETIC PROFILES OF G- AND P- TYPES OF ROTAVIRUS DETECTED IN SÃO PAULO STATE, BRAZIL


Instituto Adolfo Lutz , IAL, Av. Dr. Arnaldo, 355 - São Paulo- SP

Group-A rotavirus (RV-A) is the most important pathogen of acute gastroenteritis in infants and young children. In developing countries, more than 125 million cases of RV-A infection have been estimated to occur annually. Analysis of mobility of the 11 dsRNA genomic segments of the by polyacrylamide gel electrophoresis (PAGE) yields a characteristic for each strain and has often been used as an indicator of the RV-A diversity. The aim of present study was to determine the frequency of RV-A infection and to detect the diversity of dsRNA genomic electrophoretic pattern from patients with acute diarrhea in the state of São Paulo, from January 2011 to May 2012. Stool samples from 1,277 patients were tested by ELISA, PAGE and multiplex Nested PCR for detection of RV-A. A total of 140 (11.0%) stool samples were RV-A positive: in infant children <1 year of age (36.4%; 51/140), children 1-5 years (57.1%; 80/140), children 6-15 years (3.6%; 5/140), and adults > 15 years (2.1%; 3/140). A total of 106 (75.7%; 106/140) samples were confirmed by PAGE and the dsRNA migration pattern were visualized in 93 samples. Based on migration pattern of dsRNA segments (10 and 11), two distinct groups of electrophoretypes were identified: 83 long profile strains (89.2%) and 11 short profile strains (11.8%). RV-A with long profile were correlated with G3P[8], G9P[8], G12P[8], G3P[3], and G12P[9] genotypes; whereas short profile RV-A were correlated with G2P[4], G3P[6] and G12P[6] types. The long profiles showed a high frequency during the studied period. Recent studies have demonstrated a reduction rate of RV-detection. In addition, there is a need for further detailed analysis of the migration patterns of the 11 dsRNA segments of RV-A, which would have important contributions to the knowledge of the RV-A epidemiology and the evaluation of vaccine programs.

PHYLOGENY OF DENGUE VIRUS TYPE 1 ISOLATED FROM FIELD-CAUGHT VECTORS AND HUMANS REVEALS DIFFERENT LINEAGES OF THE AMERICAN/AFRICAN GENOTYPE

In Brazil, dengue became a major public health problem after DENV-1 introduction in 1986 in Rio de Janeiro (RJ) and in 2009, this serotype re-emerged causing major epidemics in the country. Since then, a virological and entomological program was established for monitoring dengue viruses (DENV) in human sera and vectors and it has constituted an important tool for dengue epidemiology and vector-virus-host interactions studies. Aedes aegypti specimens collected in 1986 (n=120) and in 2001 (n=2,434) in Nova Iguaçu (RJ) and collected in Boa Vista (RR) in 2010 (n=3,705) were pooled and analyzed. Six DENV-1 isolates (three from Ae. aegypti and three from humans) were available. Vector macerates were submitted to conventional RT-PCR and virus isolation. For viral quantification, the RNA from original Ae. aegypti individually macerated was submitted to Real Time qRT-PCR. Sequencing of E gene (1,485 nucleotides) was performed. DENV-1 was identified by virus isolation and RT-PCR during the 1986, 2001 and 2010 entomological surveillances performed in RJ and Roraima (RR) and the Real Time qRT-PCR detected 1.6x10^4 copies/mL of DENV-1 in the macerate of a single Ae. aegypti female naturally infected. The phylogeny demonstrated that DENV-1 isolated from both vector and humans belong to genotype V (Americas/Africa), although the co-circulation of two distinct lineages (lineages II and III) was detected. A higher sequence divergence was observed between lineages II and III, and most amino acid substitutions were observed on domain III from E protein. Moreover, some residues were exclusive to some lineages, and may be predicted to be differentiating the three lineages. The use of molecular techniques combined to virus isolation showed to be important approaches for the surveillance and molecular characterization studies of DENV from field-caught vectors. Financial support: CNPq, FAPERJ, PAPES/FIOCRUZ, FIODEZ.

**HV900 - DETECTION OF FLAVIVIRUS IN THE MOSQUITO VECTOR Aedes Aegypti Collected in an Urban City of Manaus, Amazonas, Brazil**


1. Universidade Nilton Lins, UNL, Av. Professor Nilton Lins, 3259, Parque das Laranjeiras, CEP: 69058-030 Manaus/AM
3. Universidade do Estado do Amazonas, UEA, Av. Djalma Batista, 3578 - Florest CEP 69050-010 - Manaus/AM
4. Universidade Federal de Minas Gerais, UFMG, Av. Antônio Carlos, 6627 - Pampulha, CEP: 31270-901 - Belo Horizonte/MG, Brasil
Arboviruses are viruses transmitted to human and/or animals by arthropods that can cause infections. Arboviruses are mainly present in tropical countries due to the hot and humid climate which are favorable conditions for proliferation of the mosquito vector Aedes aegypti. Among the arboviruses, Flavivirus is of great epidemiological importance as it causes diseases in humans and/or animals. We aimed to identify flaviviruses in pools of A. aegypti collected in the District of Cidade Nova, Manaus / Amazonas state, Brazil. From December 2008 through June 2010, 2,389 females of A. Aegypti were collected in BG-Sentinel traps and distributed in 726 pools. All pools were macerated and kept in Trizol® at -80°C until RNA was extracted. cDNA was obtained using the technique of Reverse Transcriptase-Polymerase Chain Reaction. 574 Pools containing 1-8 mosquitoes were studied. RNA quality was verified in 2% agarose gels and cDNA quality was tested by amplifying the genes nicotinamide adenine dinucleotide dehydrogenase subunit 4 (ND4) and Cytochrome c oxidase II (COII) of the A. Aegypti. For the identification of the genus Flavivirus, primers FL200R and FL100F encompassing the NS5 region were used for PCR. Sera from patients with infection of dengue virus were used as positive controls. Region encompassing the C-prM genes of dengue virus was also amplified using the primers D1 and D2. Eleven pools were nucleotide sequenced by D1 and D2 primers. All were either of genotypes I or II of DENV-4. Studies of molecular epidemiology of DENV provide us a basis for understanding how these viruses evolve genetically in nature.

HV904 - INVESTIGATION OF CULICIDAE AND DENGUE VIRUS IN MONTES CLAROS, NORTHERN MINAS GERAIS, BRAZIL


1. Universidade Federal de Juiz de Fora, UFJF, Rua José Lourenço Kelmer, s/n - Campus Universitário, São Pedro. CEP:36036-900

2. Universidade Estadual de Montes Claros, UNIMONTES, Av. Dr. Rui Braga, s/nº, Campus Universitário. Vila Mauricéia. CEP:39401-089

3. Universidade Federal de Minas Gerais, UFMG, Av. Antônio Carlos, 6627, Pampulha. Belo Horizonte - MG, CEP: 31270-901 E-mail: izabela.biologia@yahoo.com.br

Dengue virus (DENV) (Flavivirus, Flaviviridae) occurs as four antigenically distinct but genetically related viruses named DENV-1 to -4. DENV is transmitted by species from Aedes genus (Culicidae), mainly A. aegypti. DENV-1 to -4 circulate in Brazil and the country has the higher number of DENV in Latin America.
Minas Gerais state usually present high number of dengue cases and Montes Claros is among the five cities presenting the higher number of dengue cases in this state. The aim of this work was to perform a prospective study of Culicidae and DENV in Montes Claros. Mosquitos were collected from February to April of 2012 at UNIMONTES-MG. Adults were collected using Shannon traps and larvae were collected from a transient rainwater pool. Larvae were maintained in BOD incubator until they emerge and mosquitos were identified based on morphological characters. Pools of mosquitos (up to 20) were macerated and total RNA was extracted and used for cDNA synthesis followed by nested-PCR to detect DENV. A total of 4210 mosquitos were collected, including A. scapularis, A. aegypti, A. albopictus, Psorophora albigneu, P. ferox, P. ciliata, P. lanei and Limatus durhamii. The great majority of mosquitos were A. scapularis (>4000). From 257 pools, 47 were initially tested for the presence of DENV and one pool of A. aegypti was positive for DENV-1. The other pools are being tested for DENV and the obtained amplicons are going to be sequenced. Although there is no report of DENV infecting A. scapularis, it is known that different species of Aedes sp are related to DENV transmission. Additionally, A. scapularis has been implicated in Rocio virus transmission and is a competent vector for yellow fever virus. Moreover, these results demonstrate the presence of naturally infected mosquitos with DENV-1 during the last epidemic period in Montes Claros, North region of Minas Gerais.

Financial support: FAPEMIG, CNPq, CAPES, UFJF, PROPESQ/UFJF

HV905 - SEROLOGIC SURVEY FOR ARBOVIRUS IN HUMAN POPULATION OF THE ACRE STATE

Chiang, J.O., Chagas, L.L., Martins, L.C., Rodrigues, S.G., Vasconcelos. P.F.C.,
Instituto Evandro Chagas, IEC, BR-316Km07-Levilândia-Ánanindeua/Pará E-mail: janniferchiang@iec.pa.gov.br

In the literature, little information about arboviruses circulation in the state of Acre, Amazon region was available. The most recent scientific studies related to the circulation of arboviruses in this state occurred in 2004 in the rural municipality of Acrelândia. More recently, between the years 2004-2006, were identified sporadic cases of Oropouche Virus. This state has favorable conditions for the maintenance and spread of these viruses, and this study performed a serologic survey for arboviruses among febrile humans of the Acre state between the years 2011 – 2012. In this period, 382 serum samples were collected in patients with fever and symptoms suggestive of dengue fever. For the serologic survey, the hemagglutination inhibition (HI) test was used for 19 arboviruses, belonging the viral genera as follows; Alphavirus (EEE, WEE, Mayaro and Mucambo); Orthobunyavirus (Guaroa, Tacaiuma, Maguari, Caraparu, Oropouche and Catu) and flavivirus (Yellow fever wild and vaccine strains, Ilheus, Saint Louisencephalitis, Rocio, dengue 1, dengue 2, dengue 3 and dengue 4), while the MAC-ELISA for IgM capture antibody was used for dengue and yellow fever viruses. By HI, 289 (75,6%) serum samples were positive and 93 (24,4%) negative. Among positive samples, 267 (92,4%) were to flaviviruses, 55 (19%)
for orthobunyaviruses and 62 (21.5%) for alphaviruses. It is important to emphasize that each sample can be positive for a single or more viruses from different genera. By ELISA test, just 284 serum samples were tested, and 56 (19.7%) positive for dengue virus, no positive sample was obtained for yellow fever virus. The results of this study, showed the active circulation of the dengue virus in the Acre State and of other arboviruses in specially for those of the flavivirus genus. Financial support: IEC/CNPQ

**HV913 - RAPID IMMUNOCHEMOTAGRAPHIC (IC) TESTS FOR EARLY DIAGNOSIS OF DENGUE VIRUS INFECTION: A PRELIMINARY ANALYSIS**


Oswaldo Cruz Institute, IOC, Avenida Brasil, 4365, Manguinhos, Rio de Janeiro - RJ - Brasil E-mail: moniqueq@ioc.fiocruz.br

Dengue is associated with explosive urban epidemics and has become a major public health problem in many tropical developing countries. The laboratory diagnosis of dengue can be carried out using several approaches, however sensitive and specific assays useful to diagnosis in the early stage of fever are desirable. The NS1 protein, a highly conserved and secreted glycoprotein, is a candidate protein for rapid diagnosis of dengue. Several studies have compared IC technologies with the reference test for dengue. We aimed to evaluate the potential use of 3 commercial rapid IC assays in a panel of 120 serum samples, from the collection of the Flavivirus Laboratory. The NS1 Ag Strip, Early Rapid and SD Bioline are disposables tests using lateral flow. Both NS1 Ag strip and the Rapid Early showed similar sensitivity (66.1%) to confirm dengue cases. A lower sensitive was observed the SD Bioline 52.3%. The detection rate by the NS1 Ag Strip and the Early Rapid were the same (60%) in the presence of anti-DENV IgM, while the SD Bioline was 55%. In this study, the presence or absence of IgM did not influence detection by all kits. However, specificities were 100%, in all assays, based on the analysis of sera of healthy individuals and individuals negative for dengue. No cross-reactivity was observed for the Rapid Early and SD Bioline, nevertheless, the NS1 Ag Strip showed cross-reactivity with one yellow fever vaccine. No differences were observed by the tests NS1 antigen in confirming primary and secondary infections. In this report, the results indicate that the commercially available IC tests have overall low sensitive and high specificity for early and rapid diagnosis of dengue virus infection. It maybe appropriate for use as a screening test consired to other techniques such as RT-PCR in acute samples and/or MAC-ELISA in convalescent samples in routine laboratory and surveillance of dengue disease in public health services. Financial support: FAPERJ, CNPq, CAPES, FIOCRUZ

**HV921 - THE FIRST CASE OF DENGUE SEROTYPE 4 IN CEARÁ, BRAZIL**

1. Laboratório Central de Saúde Pública do Ceará, LACEN-CE, Av. Barão de Studart, 2405 - Aldeota CEP:60120-002

2. Secretaria de Saúde do Ceará, SESA-CE, Av. Almirante Barroso, 600 - Centro Rede Nordeste de Biotecnolgia - UECE, RENORBIO-UECE, Av. Paranjana, 1.700 - Campus do Itaperi - 60740-000 Fortaleza/CE E-mail: izabel.leticia@lacen.ce.gov.br

Dengue virus serotype-4 was first reported in Brazil in a restricted outbreak in Rio Branco, Roraima, 1981. More than 20 years after, it was isolated in patients living in the North region of the country and since then it started to spread to other regions. The State of Ceará reports dengue transmission since 1996, with circulation of DENV-1, DENV-2 and DENV-3 until 2011, year that showed the largest dengue epidemic of the State, with the predominance of DENV-1. In order to detect early the entry of the new serotype, the virological surveillance was implemented. Whole blood or serum samples obtained from acute ill patients were inoculated into C6/36 cells. Isolated strains were identified by indirect immunofluorescence assay using monoclonal antibodies to the four serotypes. Despite DENV-1 epidemic, it was possible to isolate, in March, the first case of DENV-4 in a patient with fatal outcome, who lived in Morada Nova, a city 161 Km far away from the capital, Fortaleza. Another case of classical DENV-4 with full recovery, was isolated in December, from a patient living in Fortaleza. This year, from January to June, 38.852 dengue cases were serologically confirmed from 152/184 (82.6%) counties of the State with the majority of the cases from the capital with 30.437 (78.3%), followed by the city of Maracanaú with 1.195 cases (3%). In relation to severe dengue, 194 cases were reported with 54 DHF and 140 dengue with complications; 16 patients evolved to death, 11 from the capital and five from the other counties. The predominant serotype at the current year is DENV-4 with 95.5% of the isolated. The spread of serotype 4 keeps happening through the cities, what is concern about a severe epidemic for the next year. The vector control should be implemented to avoid a major epidemic and the patients care should be improved to avoid fatal outcomes. Financial support: FUNASA

HV923 - HEPATITIS B VIRUS INFECTION IN A POPULATION OF RECYCLABLE WASTE COLLECTORS, CENTRAL BRAZIL


1. Instituto de Patologia Tropical e Saúde Pública, IPTSP/UFG, Caixa Postal 131, CEP: 74.605-050, Goiânia-GO

2. Faculdade de Enfermagem, FEN/UFG, Caixa Postal 131, Goiânia-GO E-mail: enftamiris@hotmail.com

Hepatitis B virus (HBV) remains a major cause of liver disease worldwide despite vaccination programs implemented over the last decade. Worldwide, it is estimated that 2 billion people are infected with HBV and that more than 350 million are chronically infected. Patients with chronic hepatitis B are at
risk for developing liver cirrhosis and hepatocellular carcinoma. Based on sequence divergence of 8% or greater in the entire genome, HBV has been classified into 10 genotypes (A-J), which have a distinct geographic distribution. Recyclable waste collectors have a lifestyle that is characterized by unfavorable social, cultural, and environmental factors. There is currently very little data on HBV infection in this population. Therefore, the aim of the present study was to investigate the HBV prevalence, risk factors and genotypes in a population of recyclable waste collectors in Central Brazil. A cross-sectional survey was carried out with 431 individuals who were recruited in all 15 recycling cooperatives/associations in Goiania city, Goias state, Central Brazil. All individuals were interviewed, and their serum samples were tested for the presence of HBV serological markers. HBsAg-positive samples were tested for HBV DNA by nested PCR and were genotyped by restriction fragment length polymorphism (RFLP) analysis. The overall HBV prevalence infection was 12.8% (95% CI: 9.8-16.2). A multivariate analysis of risk factors showed that age >30 years and illicit drug use were independently associated with HBV infection. HBV DNA was detected in 2/3 HBsAg-positive samples, in which genotypes D and F were identified. These findings confirm that recyclable waste collectors are at high risk for hepatitis B infection and highlight the importance of having a public health policy that addresses this population. Financial support: CNPq

DENGUE VIRUS IN BRAZIL
Drumond, B.P., Mondini, A., Schmid, D.J., Bosch, I., Nogueira, M.L.

1. Faculdade de Medicina de São José do Rio Preto , FAMERP, Laboratório de Pesquisa em Virologia, FAMERP, São Paulo, Brazil.

2. Universidade Estadual de São Paulo, UNESP, Lab de Saúde Pública. F de Ciências Farmacêuticas. Araraquara.SP Brazil

3. Universidade Federal de Juiz de Fora, UFJF, Laboratório de Virologia, ICB-UFJF, Juiz de Fora, MG, Brasil

4. Cumming School of Veterinary Medicine, Department of Biomedical Sciences. Tufts - North Grafton, MA, USA. Massachusetts

5. Institute of Technology, MIT, Genome Resources in Dengue Consortium. Cambridge, Massachusetts, USA E-mail: betaniadrumond@gmail.com

Dengue virus (DENV) (Flavivirus, Flaviridae) comprises four genetically and antigenically distinct serotypes, named DENV-1 to DENV-4. Brazil is the country with the highest number of dengue cases occurring in the Americas. DENV-1 was the most predominant virus in Brazil in the 80’s when it was replaced by DENV-2 in the 90’s, which was subsequently replaced by DENV-3, in 2000. The four DENV serotypes circulate in Brazil nowadays. Here, we sequenced the genome of 12 isolates of DENV-2 obtained from patients with dengue fever, from São José do Rio Preto, São Paulo, Brazil, in 2008.
These sequences and other genome sequences from DENV-2 viruses were used to perform phylogenetic and molecular evolutionary analyses. Phylogenetic analysis demonstrated that all Brazilian DENV-2 isolates were clustered within the American/Asian genotype being subdivided into three lineages. Analysis of the deduced polyprotein sequences revealed that some amino acids substitutions distinguish those lineages. Four isolates had identical genome sequences. The three lineages of Brazilian DENV-2 grouped strains isolated in different periods: (i) 2007 to 2010, (ii) 2000 to 2006 and (iii) 1990 to 2000. Based on the analysis of envelope gene sequence, those lineages were estimated to be introduced in the country in (i) 2003-2006, (ii) 1998–99 and (iii) 1988-90. Lineage iii did not contain viruses isolated after 2000, what could be a result of surveillance sampling methods or represent a lineage replacement event, by the two other newer lineages. Finally, the sources of exogenous viruses were countries from Latin America, reinforcing the need of genotype surveillance in order to detect and trace virus populations that are circulating in Brazil and Latin America, what is especially important in a scenario of circulation of different DENV serotypes in this region. Financial support: FAPESP, CNPq, INCT-DENGUE, PROPESQ/UFJF, FAPEMIG, CAPES

**HV930 - HEPATITIS A VIRUS (HAV) TRANSMISSION BY BLOOD TRANSFUSION**


**Fundação Oswaldo Cruz, Fiocruz, Av. Brasil 4365, Manguinhos, Rio de Janeiro-RJ Instituto Nacional do Cancer, INCA, E-mail: l_amado@ioc.fiocruz.br**

Hepatitis A infection is the most cause of acute hepatitis and is primarily spread by the fecal-oral route. Viremia appears 1 to 2 weeks before the onset of clinical symptoms, consequently, although a rare occurrence, parenteral transmission of HAV via blood during viremia is possible and has been associated with transfusion of blood. In this report we present a serological and molecular tracing of transfusion-transmitted HAV by a single blood collection. A 39-year-old asymptomatic male volunteer made a whole-blood donation for the Hemotherapy Service of the National Institute of Cancer (INCA), RJ. Twenty days later, the donor became jaundiced and an acute HAV infection was confirmed by detection of IgM anti-HAV and hepatic functions altered. Two patients, who received this donor hemocomponents, were identified. 1) A 27-year-old male with acute leukemia received platelets, two days after the donation. This patient did not develop clinical symptoms of hepatitis and anti-HAV IgM was not detected. 2) A 39-year-old male received the red blood cells. This patient was a chronic hepatitis C and was immunosuppressed after bone marrow transplantation. Ten days post-transfusion, he developed clinical symptoms of acute hepatitis and died twenty-five days after with fulminant hepatitis. Retrospectively, the sera samples collected from the donor at the donation day and from the two patients collected thirty days post-transfusion are tested for HAV-RNA detection by RT-PCR. HAV RNA was identified in
all sera samples evaluated. Analyzed sequences of the HAV RNA revealed that the virus of the three patients were identical, according to the sequences of the region VP1/2A and complete VP1 of the HAV. These results demonstrated a rarely event of transfusion-transmitted HAV evidenced by molecular and serologic tracing, suggesting that additional blood screening tests should be implemented for ensuring product safety to specific groups of immunocompromised patients. Fomento:IOC/Fiocruz

HV931 - DETECTION OF NOROVIRUS, SAPOVIRUS AND ASTROVIRUS IN PATIENTES WITH ACUTE GASTROENTERITIS, FROM FEDERAL DISTRICT (BRAZIL)

Silva, F.G., Anjos, K., Nagata, T., Silva, P.A., Lima, L.M.P.

1. Universidade Católica de Brasília, UCB, EPCT QS 01 - Águas Claras - 71966-700
2. Universidade de Brasília, UnB, Campus Darcy Ribeiro - Asa Norte - 70910-900 E-mail: fabio_silva15@msn.com

Annually 2 billion cases associated with acute gastroenteritis occur worldwide. Since 1994, the Brazilian Ministry of Health, organizes and coordinates the monitoring of acute diarrheal disease, the sentinel surveillance system known as MDDA, in order to detect early outbreaks. The program calls for culture for bacterial isolation and identification, search of intestinal parasites and detection of rotavirus by ELISA. When all these results are negative, the case remains without a proper diagnosis, clinically defined as viral infection. A total of 145 diarrheal samples negative for rotavirus, from MDDA program between 2006 and 2011 were tested. These samples were subjected to a multiplex RT-PCR for detection of astrovirus, norovirus GI, norovirus GII and sapovirus. After extraction of genetic material, RNA was subjected to a RT-PCR, on a subsequent step the cDNA was carried to a multiplex PCR, and finally, using agarose gel electrophoresis, the amplified fragments were separated according to their molecular weight, making it possible to differentiate pathogen which was present in the sample. Among the 145 samples tested, 12 samples were positive (8.3%). In this positive group, 9 (75%) were norovirus GII, 2 (17%) were sapovirus and 1 (8%) astrovirus. The age group most affected was of individuals under 12 years old and higher prevalence occurred during the drought period. We found that the peak of infection occurred in July extending to September, months that are within the period of drought. It was possible to prove the movement of the three pathogens in this study, which reinforces the need for further investigation of different gastroenteric viruses in Federal District than Rotavirus, also shows that the multiplex PCR used in this study might be suitable to be used in laboratorial routine practice. Financial support: Universidade Catolica de Brasilia and LACEN/SES/DF

HV932 - DEVELOPMENT OF A REAL-TIME PCR- BASED SYTEM FOR RAPID DETECTION AND QUANTIFICATION OF HEPATITIS DELTA VIRUS (HDV) IN THE WESTERN AMAZON REGION, BRAZIL.

Hepatitis delta is a serious infectious disease that causes severe inflammation in hepatocytes and rapidly progressive and caused by Hepatitis Delta Virus (HDV). HDV is classified as HBV subvirus satellite because it is unable to infect in the absence of HBV, it requires HBsAg. The HDV RNA genome is of very small circular approximately 1700nt and negative polarity. The western region of the Brazilian Amazon area considered highly endemic for HDV. We selected 35 patients from Rondônia and Acre with anti-HDV positive. In quantitative RT-PCR kit was used TaqMan PCR Master Mix with probes labeled with FAM/TAMRA primers that amplify a fragment corresponding to HDAg-L. For standardization of quantitative PCR two standard curves were constructed using serial dilutions of the cloned fragment linearized with EcoRI and another transcribed into RNA with T7 RNA polymerase, both produced "in house". Thereafter dilutions were subjected to quantitative RT-PCR to obtain the standard curves, the following tests in triplicate intra-assay and inter-assay four times in consecutive days. Standard curves were produced and showed a detection limit of 1.9 million to 19 copies/mL for the linearized and 8.4 million to 84 copies/mL for the RNA transcript. We generated two linear regression curves relating the number of copies/mL x Ct, with a coefficient of determination R²=0.93 and slope -3.3 (p <.05) and R²=0.99 and slope -2.9 (p <.05) of efficiency linearized and transcribed into the RNA, respectively. The reproducibility of the test was observed by the coefficient of variation produced by each dilution tested and the specificity of the test by not obtaining a signal in any of the negative controls added in each race. A molecular approach described in this study is important and immediate impact on public health, particularly for chronic patients in the region favoring confirmation of the diagnosis as well as the orientation therapy to be instituted. Financial support: UNIR/FIOCRUZ/CNPq/IPEPATRO/CEPEM

HV934 - OCCULT HEPATITIS B VIRUS INFECTION IN HIV-1-POSITIVE, TREATMENT-NAÏVE PATIENTS IN CENTRAL BRAZIL


1. Instituto de Patologia Tropical e Saúde Pública, IPTSP/UFG, Caixa Postal 131, CEP: 74.605-050, Goiânia-GO

2. Instituto Oswaldo Cruz, IOC/FIOCRUZ, Rio Janeiro, RJ E-mail: marina.poliveira@hotmail.com

Two billion people have been infected with hepatitis B virus (HBV) and more than 400 million are chronically
Worldwide, there may be 3 to 6 million HIV-infected people living with chronic HBV. HIV-HBV coinfection increases the morbidity and mortality beyond those caused by either infection alone. People coinfected with HIV have higher levels of hepatitis B viremia, have faster progression to chronic hepatitis B, and have a higher risk of cirrhosis and hepatocellular carcinoma. Occult HBV infection, a peculiar form of chronic infection, is characterized by the presence of HBV DNA in serum and/or liver in HBsAg-negative individuals. Occult HBV infection is relevant in different clinical contexts and its prevalence varies from 0% to 89% among HIV-infected patients. This study aimed to assess the prevalence of HBV occult infection in HIV-infected, treatment-naive patients in Goiania-Goias, Central Brazil. HBV genotypes were also investigated. A cross-sectional study was conduct among HIV-infected, treatment-naive patients attended at a reference hospital in Goiania city. The participants (n=505) were tested for serological markers of HBV infection. HBV DNA was detected by a semi-nested PCR and quantified using the real-time PCR TaqMan technology. The amplicons were genotyped by nucleotide sequencing. Of 73 anti-HBc/anti-HBs reactive samples, 7 were HBV DNA-positive. Among the 26 anti-HBc only reactive samples, 9 was HBV DNA-positive. These results were confirmed by repeating both the DNA extraction and amplification procedures, thereby establishing the occult HBV infection rate at 16.2%. As expected, low HBV DNA levels were found in these patients (mean: 6.59 x 102 copies/mL). HBV genotypes A (62.5%, A1), F (25%, F2) and D (12.5%, D2 and D3) were identified. These findings revealed a high prevalence of occult HBV infection and the predominance of genotype A (A1) in HIV-infected, treatment-naive patients in Central Brazil. Financial support: FAPEG

HV935 - INCIDENCE AND CLINICAL ASPECTS OF CONGENITAL CYTOMEGALOVIRUS (CMV) INFECTION IN THE ILHEUS, BA
Marin, L.J., Cardoso E.S.C., Jesus, B.L.S., Françaiso, M.F.S., Santana, J.G., Júnior, H.M.C., Raiol, M.R.
Universidade Estadual de Santa Cruz, UESC, Rodovia Ilhéus Itabuna, km 16, Salobrinho, Ilhéus, Bahia, cep 45662900 E-mail: lajumarin@hotmail.com

The cytomegalovirus (CMV) infection is highly prevalent in humans and it is associated with lower levels of education and incomes. The virus can be vertically transmitted to the newborn by different ways. The CMV has been recognized as a major cause of congenital infection (prevalence between 0.2 to 3%). In addition, it has been demonstrated that 90% of congenital infected infants have no symptoms at delivery, but it has been shown that they can present later disorders, including neurologic problems and hearing loss. In this study, we investigated CMV infection in infants delivered at Santa Helena Hospital of Ilhéus, Bahia. In addition, we pretend to verify clinical aspects of CMV infection and to observe neurological disorders for four years. This study will be conducted from July 2010 to December 2013. The study was approved by the UESC Ethics Committee. The mothers or responsible of all newborns are asking
to sign a written informed consent. Saliva and urine samples have been collected at delivery. The samples have been analyzed by nested PCR and the positives will be confirmed by another PCR using new samples of saliva and urine obtained until 3 weeks of life. Sociodemographic and clinical data have been obtained by standardized questionnaire. Until now, it was possible collect samples of 1.825 newborns and we had 18 positive result (incidence 1%). These children will be analyzed until 4 years to verify possible late disorders and hearing loss. We consider that it is necessary to carry out continuous studies in order to define the main risk factors for congenital infection. These studies could help to define best program in public health to prevent vertical transmission. Besides that, the identification of a congenitally infected newborn infant soon after birth is very helpful to early intervention in order to avoid or minimize the potential involvement of the central nervous system, sensorineural hearing loss, developmental delay, or motor abnormalities.

HV939 - CORRELATION OF RS 12979860 AND RS8099917 IL28B POLYMORPHISMS IN BRAZILIAN PATIENTS WITH HEPATITIS C TO PREDICT TREATMENT RESPONSE


Hospital Israelita Albert Einstein, HIAE, Av. Albert Einstein, 627 E-mail: robertas@einstein.br

Genetic variation in the IL28B gene region on chromosome 19 was identified for predicting sustained viral response of patients chronically infected with genotype 1 HCV infection. The most strongly associated single nucleotide polymorphism (SNP) for treatment response, rs12979860, has also been shown to be significantly associated with spontaneous hepatitis C clearance. In addition, other SNPs were described, such as rs8099917. In order to evaluate the combined genotyping of IL28 polymorphisms rs12979860 and rs8099917 in our population, we performed genotyping of the two sites using TaqMan custom designed probes (Applied Biosystems) on an ABI7500 instrument and the ABI TaqMan allelic discrimination kit from Applied Biosystems. We analyzed 538 samples from HCV patients of a private hospital in São Paulo during the period from December 2010 to April 2012. The results of both SNPs were compared to find a correlation of better and worse response according to the genotype. We found an agreement of 65.2% between the results (25.3% better response, 35.5% intermediate and 4.5% worse response), and 34.7% discordant results. The discordant results were composed by 3.3% totally divergent results and 31.4% heterozygous result for one SNP (21.9% CT for rs12979860 and 9.5% GT for rs8099917). The heterozygous patients for rs12979860 had 21.7% rs8099917 TT genotype (worse response) and 0.2% rs8099917 GG genotype (better response). According to literature, patients with heterozygous genotype of rs12979860 benefit from further determination of rs8099917. Based on this finding and the high rate of rs12979860 heterozygous patients with discordant results in our
cohort, despite the good correlation of the studied SNPs (65.2%), we suggest that the combined determination of both SNPs will improve treatment decisions in a considerable number of Brazilian chronic HCV patients. Financial support: Hospital Israelita Albert Einstein

HV947 - DENGUE VIRUS TYPE 1 GENOTYPE V WAS CIRCULATING IN RIBEIRÃO PRETO, SÃO PAULO, IN 2011
Soares, A.M., Amarilla, A.A., Aquino, V.H.
Faculdade de Ciências Farmacêuticas de Ribeirão Preto, FCFRP-USP, Av. do Café, s/nº. - Campus Universitário - Ribeirão Preto - SP - 14040-903
E-mail: dri.msoares@gmail.com
Dengue is the most important mosquito-borne viral disease worldwide. Dengue virus (DENV) is a member of the genus Flavivirus and family Flaviviridae. DENV includes four antigenically related viruses named dengue virus type 1 to 4 (DENV 1-4). Each of the four serotypes is able to cause disease with a full spectrum of clinical manifestation. DENV-1 is composed of five genotypes: genotype I is represented by viruses of Taiwan and Thailand; genotype II is represented by viruses of Thailand; genotype III is represented by strains of Malaysia; genotype IV is represented by viruses of Southeast Asia, South Pacific and Australia and genotype V is represented by viruses of America, Africa and Southeast Asia. Considering that Ribeirão Preto is an endemic city for dengue, it is of great importance to monitor the circulating viruses, which would allow the identification of new serotypes or subtypes that could be related to most severe cases of the disease. Phylogenetic analysis also allows a better assessment of the pattern of migration and evolution of these viruses. In this study, four dengue viruses isolated in Ribeirão Preto in 2011 and confirmed by real-time RT-PCR and by indirect immunofluorescence were included. The partial gene sequence of the nonstructural protein 5 (NS5) was amplified by RT-PCR, purified and sequenced. Sequences were analyzed and aligned with the BioEdit and the MEGA 5.05 software. Based on the alignment, a phylogenetic tree was constructed using the Neighbor-joining method. The phylogenetic analysis showed that DENV-1 isolated in Ribeirão Preto correspond to genotype V of DENV-1 and are closely related to other Brazilian DENV-1 isolates.

HV961 - HIV-1 MOLECULAR EPIDEMIOLOGY IN URUGUAIANA/RS: MONITORING THE DISPERSION OF SUBTYPE C IN A BRAZIL-ARGENTINA BORDER TOWN
Gräf, T., Junqueira, D.M., Tamayo, A., Almeida, S.E.M., Pinto, A.R.
1. Universidade Federal de Santa Catarina, UFSC, Florianópolis/SC - 88040-900 - Brasil
2. Fundação Estadual de Produção e Pesquisa em Saúde, FEPPS, Av. Ipiranga, 5400, 3º Andar - Porto Alegre/RS - CEP: 90610-000
3. Serviço de DST/aids de Uruguaiana, E-mail: akograf@yahoo.com.br
The epidemic of HIV/AIDS in Brazil faces its worst scenario in Southern region, where the highest AIDS incidences and mortality rates due
to HIV-1 infections are reported. Furthermore, while in most of the regions of Brazil subtype B is dominant, in the capital cities of Southern region HIV-1 subtype C is highly prevalent with evidences of a slow dispersion through vicinities countries (e.g., Argentina and Uruguay). In the view of subtype C epidemic expansion, the current study investigated the HIV-1 molecular features in Uruguaiana, a Brazilian border city with Argentina. Blood samples from 26 HIV-1 infected patients from the local reference hospital were collected in FTA card. After sample processing, nested-PCR amplification and sequencing of the HIV-1 gp120-C2/V3 region were performed. Sequences were aligned using MusSEL and phylogenetic analyzes were performed by Maximum Likelihood algorithm with GTR+G+I nucleotide substitution model. HIV-1 subtype C was observed in 9 (43%) patients, subtype B in 11 (52%) and subtype D, a rare HIV-1 form in Brazilian epidemic, in 1 (5%). Patients were mainly infected by heterosexual intercourse and no correlation between subtypes and exposure categories were observed. The results presented here show a significant prevalence of subtype C in the border of Brazil and Argentina, a geographical region without previous studies in HIV-1 molecular epidemiology. Uruguaiana is roughly located halfway between Porto Alegre and Buenos Aires and is an important monitoring point of the Brazilian and Argentinian epidemics. In conclusion, the partial results presented herein indicate a significant expansion of subtype C epidemic in a border city among Brazil and Argentina with possible influences to the HIV-1 epidemic in South America. Financial support: FAPESC

HV967 - RISK BEHAVIORS AND PREVALENCE OF HTLV-1 INFECTION IN FEMALE SEX WORKERS IN GOIÂNIA-GOIÁS, BRAZIL


1. INSTITUTO DE PATOLOGIA TROPICAL PUBLICA / UFG, IPTSP/UFG, AV. UNIVERSITÁRIA S/N, S. UNIVERSITÁRIO

2. Faculdade de Enfermagem, Universidade Federal de Goiás, FEN/UFG, S. Universitário -

3. Instituto Oswaldo Cruz, Fiocruz, IOC, Rio Janeiro, RJ, Brazil E-mail: dlceu@hotmail.com

Human T-cell lymphotropic virus type 1 (HTLV-1) is a retrovirus associated with T cell leukemia/lymphoma in adults (ATL) and tropical spastic paraparesis (TSP) or HTLV-1 associated myelopathy (HAM / TSP). HTLV-1 transmission occurs by sexual, parenteral and vertical paths. Female sex workers are a vulnerable population to parenteral and sexually transmitted infections since they have high risk behaviors such as illicit drug use and unprotected sex. This study aimed to determine the HTLV-1 infection prevalence and risk behaviors in a population of female sex workers (FSW) in Goiânia city, Goiás, Central Brazil, using the respondent driven sampling methodology. From May 2009 and June 2010, 402 FSW were interviewed about demographic and risk characteristics for HTLV infection. Blood samples were collected from all females and were tested by enzyme-
linked immunosorbent assay (ELISA) for the presence of HTLV antibodies (anti-HTLV1/2). Reactive samples were tested for confirmation by polymerase chain reaction (PCR). PCR products were purified and directly sequenced. Nucleotide sequences obtained were subjected to phylogenetic analysis.

The mean age of the study population was 27.5 years (SD: 9.1 years). Most (67.1%) were single and 47.3% had 10 to 12 years of formal education. One third of female sex workers reported illicit drug use (34.1%), though only 2.7% reported injecting drug use, 51.9% had more than seven sexual partners in the last week and 36.3% did not use condom with their steady sexual partners. Some women reported to recruit their clients in more than one type of venue, being nightclubs (41%), bars (27.7%) and streets (25%) predominant. Three of the 402 samples were found to be positive by ELISA and, when subjected to the detection of HTLV-DNA for the tax and LTR regions, only one was positive for HTLV-1, resulting in a prevalence of 0.25% (CI 95%: 0.0-1.6). The virus isolate was classified as Transcontinental subgroup of the HTLV-1 Cosmopolitan subtype. Although the findings of this study showed high frequencies of risk behaviors, a low prevalence of HTLV-1 was found among female sex workers in Goiânia city.

**HV970 - PREVALENCE OF OCCULT HEPATITIS B VIRUS INFECTION IN HIV PATIENTS**


Universidade Federal de Pernambuco, UFPE, Av. Prof. Moraes Rego, 1235 - Cidade Universitária, Recife - PE - CEP: 50670-901 E-mail: jeffersonalmeida83@yahoo.com.br

The hepatitis B virus (HBV) coinfection in human immunodeficiency virus (HIV) patients is common, because these viruses present similar transmission routes. Therefore, the liver disease has become the main cause of morbidity and mortality in HIV/HBV coinfected patients with more prolonged survival, due to advancement of highly active anti-retroviral therapy. The occult HBV infection is defined as the presence of HBV-DNA in the liver, followed by detectable or undetectable HBV-DNA in serum, of HBsAg negative individuals with viral load usually very low (<200 IU/mL). The aim of the study was to investigate the prevalence of occult HBV infection in HIV patients attended at Infectious Diseases Outpatient Clinic at the Clinics Hospital, Federal University of Pernambuco, Recife, Brazil. The serological markers HBsAg, anti-HBc total and anti-HBs were screened by enzyme immunoassays kits (Bio-Rad Laboratories, France) and for detection and quantification of HBV viral load in the plasma was used a real time PCR kit (Qiagen, Germany). Ninety patients were analyzed, 43 (48%) were female and 47 (52%) were male, mean age 36.76 ± 10.65 years. The anti-HBc total was identified in 20.11% (19/90), of which 31.58% (6/19) had only this marker, 63.16% (12/19) exhibited anti-HBs and 5.26% (1/19) had the HBsAg positive. Others 27.77% (25/90) had only the anti-HBs, suggesting the vaccination history for hepatitis B. It was emphasized that 50% (45/90) of patients did not show any of the serological markers evaluated and they were considered susceptible to virus infection. The HBV-DNA was detected in 25.56% (23/90), of which 95.65%
(22/23) in HBsAg negative patients. Among these cases, 50% (11/22) had high viral load (4,447,725 ± 9,055,188 IU/mL) and the others it were not quantified (<3,000 IU/mL). Thus, only the HBsAg negative patients with low viral load were considered as cases of occult HBV infection, revealing a prevalence of 12.22% (11/90), of which was similar than reported in the states of Rio de Janeiro and São Paulo (5% to 14%). Besides helping the clinical management of HIV/HBV coinfected patients, the presented data had a negative impact on the transmission of the virus, especially in cases of negative serology with the presence of HBV-DNA. Financial support: National Council for Scientific and Technological Development (CNPq).

HV978 - DEVELOPMENT OF DIAGNOSTIC TEST FOR THE DETECTION AND QUANTIFICATION OF HUMAN HERPESVIRUS 1 AND 2 IN CSF FOR REAL TIME PCR


Universidade Federal de Minas Gerais, UFMG, Av. Antônio Carlos, 6627 E-mail: danilobretas@yahoo.com.br

Meningitis is a disease with worldwide distribution, affecting people in all parts of the globe. The main etiologic agents of this disease are viruses, bacteria and fungus. Viral infections are the main causes of infection in the central nervous system (CNS) around the world. In Brazil, on average, there are reported probable viral meningitis 11,500 cases/year. However, for most cases there is no identification of the agent. Human Herpesvirus 1 (HHV-1) and Human Herpesvirus 2 (HHV-2) are responsible for about 2% of cases of acute viral meningitis. Meningitis by HHV-1 and HHV-2 cause more neurological complications than most other viral meningitis, since about 30% of all patients develop CNS complications. The objective of this work is to use real-time PCR for development of a diagnostic test for detection and quantification of HHV 1 and 2 in cerebrospinal fluid (CSF) of patients with clinical suspect of viral meningitis. Primers and a probe were designed for the viral polymerase gene, inside a region with 100% genetic similarity between HHV 1 and HHV 2. The viral isolate HHV-1 EK and HHV-2 (ATCC VR 590), were used for initial primer tests, and also for fragment amplification, which was used for cloning in plasmid pGEM®-T Easy. After obtaining this control plasmid it was sequenced and used to measure the efficiency of the reaction. To test the analytical sensitivity a matrix that mimics the CSF with known amounts of viral load was used. The reaction used in this test showed high efficiency (114%). When an analytical sensitivity test was performed, we observed that the test is very sensitive with a detection limit up to 1 PFU/µl. So this test shows potential for use in the health system, with the aim of reducing mortality and morbidity of meningitis caused by HHV1 and HHV-2.

HV979 - PANDEMIC INFLUENZA A H1N1 2009 VIRUS INFECTION IN PREGNANCY IN CEARÁ

1. Rede Nordeste de Biotecnologia - UECE, RENORBIO-UECE, Av. Paranjana, 1.700 - Campus do Itaperi - 60740-000 Fortaleza/CE

2. Laboratório Central de Saúde Pública do Ceará, LACEN-CE, Av. Barão de Studart, 2405 - Aldeota CEP:60120-002

3. Secretaria de Saúde do Ceará, SESA-CE, Av. Almirante Barroso, 600 - Centro Universidade Federal do Ceará - Medicina Cariri, UFC-Cariri, E-mail: carolbperdigao@gmail.com

Influenza season in Ceará occurs in the first semester of the year. It is known that during influenza epidemics, pregnancy constitute a high-risk group related to morbidity, with an increased risk of adverse outcomes (e.g., spontaneous abortion, preterm delivery). This study aimed to describe the epidemiological and clinical features of the pregnant women with Influenza A/H1N1/09pdm virus infection in Fortaleza, Ceará from January to June 2012. Of 518 individuals with an influenza-like illness that had nasopharyngeal swab collected, 154 were pregnant women. From those, 53 (34.4%) had laboratory confirmation for Influenza A/H1N1/09pdm virus infection by qRT-PCR, 15 (28.3%) were outpatients and 38 (71.7%) were hospitalized. Of the 53 patients, 05 (9.4%) were in the first, 20 (37.7%) were in the second, 25 (47.2%) were in the third trimester of pregnancy and 3 (5.7%) unknown. The age pregnant women ranged from 14 to 37 years old, mean 24.8 years. The virus was detected in 11 cities with predominance in the capital (67.9%). Only 12 (22.6%) pregnant women had been previously vaccinated against influenza. The most prevalent symptoms were fever and cough, 41 (77.3%), dyspnea, 38 (71.1%) and coryza, 34 (64.2%). Seven patients had some co-morbidity associated such as asthma, smoking, lung disease, hypertension and diabetes. One case, a new sample collected 7 days after treatment with oseltamivir continued positive, what might be a resistant strain. Four women were admitted to intensive care unit, with one maternal death and a stillbirth. The pregnant woman who died presented with respiratory insufficiency, kidney and liver failure due to virus infection. While they were hospitalized, five women underwent preterm cesarian delivery. Two children were born infected with H1N1 virus, characterizing vertical transmission. The results of this study corroborate the findings in the literature where complications such as premature birth, stillbirth and death in pregnant women were reported. Financial support: FUNCAP, FUNASA

HV983 - HPV GENOTYPES DISTRIBUTION IN MEN WITH PENILE CANCER FROM TWO DIFFERENT GEOGRAPHIC AREAS IN BRAZIL


1. São Paulo State University, IBILCE (UNESP), 2265, Cristóvão Colombo street. São José do Rio Preto, SP, Brazil. 15054-000

2. School of Medicine of São José do Rio Preto, FAMERP, Brigadeiro Faria Lima Avenue. São José do Rio Preto, SP, Brazil. 15090-000

3. A.C. Camargo Hospital, 109, Prof. Antônio Prudente Street. São
Human papillomavirus (HPV) infections are associated to different genitourinary tumors. Until now about 120 HPV genotypes had been described infecting the urogenital area, classified as high or lower risk based on their association with tumor development. Squamous cell cancer of the penis (SCCP) is a rare and understudied tumor which, in the last years, had been associated to HPV infections. It were studied 99 SCCP samples from patients of two different geographic areas, whose population have distinct socioeconomic patterns. One group is from São Paulo (SP group, 39 samples) the richest state of the country and the other group is from Pará (PA group, 60 samples), a state which has lower socioeconomic indicators. The mean age is 64.7 for SP group and 60.9 for PA group. The prevalence of HPV in single or mixed infections is higher in PA group (81.67%) than in SP group (64.10%), as well as the rate of multiple infections, mainly with high risk genotypes (43.3% and 23.1% respectively). It was found a diverse set of HPV genotypes in the PA group (HPV6, 11, 16, 18, 33, 52, 53, 58 and 68) while in SP only three genotypes were found (HPV6, 11 and 16). The tumor stage was also observed. Patients in PA group show a more advanced stage. While in SP group only 12.8% of the tumors are in the stage IIIa, IIib or IV, in PA group 48.3% of the tumors are in these stages, indicating a worst prognostic. These data suggests that much more HPV genotypes, mainly high risk genotypes, are circulating in Pará than in São Paulo and they may contribute to the higher prevalence and worst prognosis of SCCP in the Pará. The differences in the HPV genotype circulation between populations from São Paulo and Pará prompts for more studies about HPV circulation among different populations. These data may be useful in development of guidelines for the implementation of more efficient and less expensive vaccination programs. Financial support: FAPESP.

HV994 - THE USE OF SALIVA SAMPLES FOR DETECTION OF CYTOMEGALOVIRUS (CMV) DNA FOR NEONATAL SCREENING OF CONGENITAL INFECTION

Santana, J.G., Oliveira, J.S., Souza, G.O.S., Almeida, L.S., Jesus, B.L.S., Cardoso, E.S.C., Marin, L.J.
Universidade Estadual de Santa Cruz, UESC, Campus Soane Nazaré de Andrade, km 16 Rodovia Ilhéus-Itabuna E-mail: jgs.biomed@yahoo.com.br

The CMV congenital infection is a important problem of public health in the world. Identification of a congenitally infected newborn infant soon after birth before hospital discharge is very helpful in planning to appropriate early diagnosis, follow up and intervention regard the potential involvement of sensorial hearing...
loss, motor abnormalities and other problems. The aim of this study is evaluated the usefulness of saliva from newborns infants as a sample of neonatal screening of congenital CMV infection as compared with urine when both are processed by PCR. Newborn with any gestational age that was possible to collect saliva and/or urine sample within 3 weeks of life were enrolled in a public maternity of Ilheus city, Bahia, from July of 2011 until now and was approved by the UESC Human Research Ethics Committee and written informed consent was obtained from the mothers. Infants for whom the presence of viral DNA was confirmed by PCR in at least two saliva or urine samples obtained before 3 weeks of birth were considered with CMV congenital infection. Urine and saliva samples were attempted to be obtained from the 750 infants, when the CMV DNA were detected by PCR, the presence of the virus was confirmed in follow up urine and saliva samples. It was not possible to obtain a urine sample from 489 of 750 (65,2%) newborns because difficulties such contamination with meconium, a delay in establishing abundant diuresis, urine leakage from the colleting bags, perineal cutaneous irritation and excessive infant handling. Among 750 infants successfully screened for CMV, 10 (1,33%) were congenitally infected, all asymptomatic until here. Comparison of the proportions of CMV excretion did not show a difference when the PCR was applied only in saliva samples, 6/489 (1,23%) and in both samples, saliva and urine, 4/261 (1,53%). We conclude that in a screening of CMV infected infants, the easily obtained saliva samples are as useful as urine to identify DNA of CMV by PCR.

HV1000 - ANALYSIS OF THE EPSTEIN-BARR VIRUS LATENT MEMBRANE PROTEIN 1 (LMP1) COMPLETE GENE SEQUENCE IN EBV+ HODGKIN'S LYMPHOMA AND DESCRIPTION OF AFRICAN-RELATED NEW LMP1 VARIANTS CIRCULATING IN BRAZIL

Garcia, A.C., Guimarães, A.P., Guiretti, D., Stefanoff, C.G., Hassan, R.,
Instituto Nacional de Câncer, INCA, Praça Cruz Vermelha, 23 - Centro - 20230-130 - Rio de Janeiro - RJ E-mail: aruanagarcia@gmail.com

Epstein-Barr virus (EBV) is a transforming herpesvirus. LMP1 codifies the viral oncoprotein, being a very variable gene with potential selection of oncogenic variants. Hodgkin's lymphoma (HL) is associated with EBV in ~50% in Brazilian S.E region, expressing LMP1 in all cases. LMP1 molecular variability has not been completely understood. We aimed to perform an analysis of the complete LMP1 sequence, including coding and promoter regions from HL-associated virus and non neoplastic controls (NNC). LMP1 gene (1624pb) was analyzed from 27 EBV+ HL and 4 NNC. EBV was detected by EBER-ISH. Sequences obtained in an ABI3130xl sequencer were submitted to phylogeny analysis with Mega4 software. Reference sequences: B95.8, Med+/-, China 1/2, CAO (Asian), Alaska, Raji (African) and A, B, C and D (European) variants. Phylogenetic reconstruction of the promoter identified B95.8 and D/CAO variants as distinct lineages. Within B95.8 clade, 48% of sequences clustered around B95.8 and 45% around Raji (2 samples with outside position). In the coding region, 93.5%
sequences clustered around B95.8, including 3 Med+/-, 2 B-like, 3 B95.8/A-like, 6 Raji-like sequences and a distinct branch containing 14 sequences more similar to each other than to the others in the clade. Two sequences clustered together with Asian variants. The 14 unclassified sequences exhibited consistent amino acid replacement at E2D, H3L, L25I, V43I, D46N, S57A, I63V, I124V (unique), L126F, M129I, L151I, I152L (u), G212S, H213N, E214Q. Most sequences (12/14) exhibited Raji-like promoter. We have extended the picture of LMP1 molecular variability, describing for the first time the entire LMP1 gene in Brazilian EBV isolates, the circulation of African-related variants outside Africa, and a potential new LMP1 variant. The role of immune escape in the shaping of LMP1 variability in Brazil, and the oncogenic role of these variants deserve further studies. Financial Support: INCT para Controle do Câncer, INCA, CNPq/CAPES.

HV1002 - CIRCULATION OF DENGUE VIRUS SEROTYPES 1 AND 4 IN MATO GROSSO, BRAZIL IN 2012

Zuchi, N., Heinen, L.B.S., Santos, M.A.M., Viniski, A.E., Kinoshita, S., Mussi, A.D., Dezenagrin, R.

1. Universidade Federal de Mato Grosso, UFMT, Av. Fernando Correa da Costa, n.2367 Bairro Boa Esperança Cuiabá, MT

2. MT-Laboratório, MT-Laboratório, Rua Thogo da Silva Pereira, n.63, Centro, Cuiabá, MT E-mail: nzuchi@hotmail.com

Dengue virus (DENV) is an arthropod-borne Flavivirus transmitted mainly by Aedes Aegypti. In the past decade, approximately 70% of dengue cases in the Americas occurred in Brazil. The aim of this study was to verify the serotype frequency of DENV in the serum of 600 patients with acute febrile illness in Mato Grosso (MT) by virus isolation in C6/36 cells followed by indirect immunofluorescence assay using serotype-specific monoclonal antibodies against dengue and yellow fever viruses. Our preliminary results have shown 125 positive samples: 30.3% (112/369) for DENV-4 in Cuiabá (80/112), Várzea Grande (23/112), Poconé (4/112), Acoralizal (2/112), Nossa Senhora do Livramento (2/112) and Nobres (1/112), and 3.5% (13/369) for DENV-1 in Sinop (6/13), Sorriso (4/13), Pontes e Lacerda (1/13), Tangará da Serra (1/13) and Várzea Grande (1/13). Among positive patients, 98.4% and 1.6% are residents in urban and rural areas, respectively. There was no gender predominance (50.4% [63/125] female and 49.6% [62/125] male) however age correlation was observed, being dengue fever more frequent between 13-25 years (27% [12/125]; 13-25y 41.6% [52/125]; 26-40y, 21.6% [27/125]; 41-60y, 20.8% [26/125] and >61, 4% [5/125]). Clinical manifestation of dengue included hyperthermia 74.4% (93/125), myalgia 66.4% (83/125), headache 61.6% (77/125), retroorbital pain 37.6% (47/125) and petechiae 24% (30/125). Previous history of similar disease was present in 17.6% (22/125). According to occupation, 33.7% (30/92) were children/students, 30.8% (24/78) general services workers, 60% (6/10) college level professionals, 33.3% (4/12) health care professionals, 35.7% (5/14) commerce employee, 40% (4/10) retired, 28.5% (4/14) public
employee, 18.1% (2/11) housewives, 33.3% (2/6) informal workers and 60% (3/5) unemployed. DENV-4 was identified in MT for the first time in 2012, but the origin of the isolates is unknown. As 66.1% (244/369) of samples from clinically characteristic acute febrile patients were negative for DENV, further studies are necessary to identify other arboviruses possibly circulating in Mato Grosso. * Project supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq.

HV1003 - THE INFLUENCE OF HLA CLASS I PROFILE ON AIDS PROGRESSION OF HIV-INFECTED PATIENTS FROM THE SOUTHERNMOST STATE OF BRAZIL

Matte, M.C.C., Medeiros, R.M., Santos, B.R., Simon, D., Jobim, M., Chies, J.A.B., Almeida, S.E.M.

1. Fundação Estadual de Produção e Pesquisa em Saúde, FEPPS, Av. Ipiranga, 5400
2. Universidade Federal do Rio Grande do Sul, UFRGS, Av. Bento Gonçalves, 9500
3. Hospital Nossa Senhora Conceição, GHC, Av. Francisco Trein, 596
4. Universidade Luterana do Brasil, ULBRA, Avenida Farroupilha, 8001
5. Hospital de Clínicas de Porto Alegre, HCPA, Rua Ramiro Barcelos, 2350
6. Universidade Feevale, , RS-239, 2755 E-mail: mcrisstaia.matte@gmail.com

Variations in immune response genes have been investigated to explain the heterogeneity observed in the clinical course of HIV-1 infection. HLA class I molecules, such as HLA-A and HLA-B, and non-classical HLA class I genes, as HLA- G seems to play an important role on the modulation of HIV disease progression. The main objective of this study was to investigate the influence of HLA-B alleles, HLA-A*03, HLA-A*11 and 14bp insertion/deletion and +342 G/C HLA-G polymorphisms on aids progression of HIV-infected patients from southernmost state of Brazil. From 3,300 medical records of HIV+ patients reviewed retrospectively in Infectology Service of Nossa Senhora Conceição Hospital between January and July of 2011, 98 patients with well defined criteria of clinical progression to AIDS were included in the study (21 rapid progressors, 29 long-term nonprogressors and 48 cronic progressors). HLA-B alleles, HLA-A*03 and HLA-A*11, and HLA-G polymorphism were identified using molecular techniques. To evaluate the influence of genotypes and alleles in the AIDS progression statistical tests as Kaplan-Meier curves and log-rank test and multivariate Cox regression analyses were performed. Despite the admixture observed in Porto Alegre population, a higher frequency of African-derived individuals were found in LTNP group (p=0.010). Our results support the role of HLA-B homozygote genotype on rapid aids progression (p=0.006). Also, we suggest an influence of HLA-G on aids progression, since a lower median time to aids was observed for the alleles associated with a higher expression of the molecule (C and del alleles). None of the classical genetic factors described in the literature...
as markers for rapid (HLA-B*53 e HLA-B*35) or slow (HLA-B*57, HLA-B*27) progression to AIDS showed an independent influence on this group. The identification of genetic markers in different populations with different ethnic origin could contribute for a better comprehension of the HIV-1 pathogenesis.

HV1006 - MAYARO, DENGUE 1 AND 4 IN PATIENTS WITH ACUTE FEBRILE ILLNESS IN MATO GROSSO, BRAZIL

Zuchi, N., Santos, M.A.M., Mondini, A., Nogueira, M.L., Dezengrini, R.

1. Universidade Federal de Mato Grosso, UFMT, Av. Fernando Correa da Costa, n.2367 Bairro Boa Esperança Cuiabá, MT
2. Faculdade de Medicina de São José do Rio Preto, FAMERP, Av. Brigadeiro Faria Lima, 5416, Vila São Pedro São José do Rio Preto, SP
3. Universidade Estadual Paulista Julio de Mesquita Filho, UNESP-Araraquara, Rod. Araraquara-Jaú Km 1, Bairro Machados, Araraquara, SP
4. MT-Laboratório, MT-Laboratório, Rua Thogo da Silva Pereira, n.63, Centro, Cuiabá, MT E-mail: nzuchi@hotmail.com

Arthropod-borne viruses are frequent in tropical areas, representing a significant problem for human health. Mayaro (MAYV) is an Alphavirus belonging to Togaviridae family, transmitted by Haemagogus janthinomys and Aedes aegypti mosquitoes. MAYV is enzootic in tropical South America, where it is maintained in sylvatic cycles involving wild primates and birds, and it is endemic in the Amazon region, being reported during human outbreaks in Tocantins, Goiás, MatoGrosso do Sul and Pará. Patients with acute febrile illness usually are diagnosed clinically with dengue in Mato Grosso. We collected 600 serum samples from patients with acute febrile illness suspected of having dengue infection between the period of January and July 2012. A total of 35 samples were tested by multiplex RT-PCR with genera-specific primers (Alphavirus, 433 bp; Flavivirus, 988 bp) followed by multiplex semi-nested RT-PCR for dengue virus (DENV) serotypes (DENV-1, 472 bp; DENV-2, 316 bp; DENV-3, 659 bp and DENV-4, 222 bp), other Flavivirus (YFV, 253 bp; WNV, 195 bp and SLEV 232 bp) and Alphavirus (MAYV, 270 bp; AURAV 98 bp; EEEV, 124 bp; WEEV, 208 bp and VEEV, 400 bp). Amplicons were submitted to sequencing to confirm virus specificity. We found 3/35 positive samples for Mayaro virus in Várzea Grande and Nossa Senhora do Livramento; 3/35 for DENV-1 in Cuiabá and Poconé and, 24/35 for DENV-4 in Cuiabá, Várzea Grande, Pontes e Lacerda, Cáceres and rural area of Poconé. MAYV generally causes a mild febrile illness clinically mistaken with dengue fever, with a short viremic period and recovery in 3 to 5 days. These aspects, combined with absence of routine laboratorial diagnose in Mato Grosso state may contribute to the lack of detection of MAYV circulation. This is the first description of this agent in neighboring cities of Cuiabá. Further studies are necessary to understand the magnitude of Mayaro infection in Mato Grosso and the possibility of co-circulation with other arboviruses. Project supported by Conselho Nacional de Desenvolvimento Científico e
HV1007 - VIROLOGICAL FEATURES OF HEPATITIS DELTA AND B VIRUSES AMONG PATIENTS WITH CHRONIC INFECTION FROM RONDÔNIA STATE


1. FUNDAÇÃO OSWALDO CRUZ RONDÔNIA, FIOCRUZ RO, RUA DA BEIRA, 7671, LAGOA
2. CENTRO DE PESQUISA EM MEDICINA TROPICAL DE RO, CEPEM, AV. GUAPORE, 215, LAGOA
3. LABORATÓRIO DE GASTROENTEROLOGIA E HEPATOLOGIA, USP, E-mail: deusilene@fioicruzb.br

BACKGROUND AND AIMS - In the Brazilian Amazon delta hepatitis is a serious public health problem with prevalence exceeding 85% in patients with chronic hepatitis. The state of Rondônia in the western Brazilian Amazon is considered highly endemic for hepatitis B and Delta, but little is know about genetic diversity of B and D viruses circulating in this region. The aim of this study was characterize HDV and HBV genotypes in HBV/HDV chronic hepatitis cases from Rondonia state.

METHODS - Serum samples isolated from seventeen anti-HDV positive patients were included in this study. HBV genotypes were characterized by phylogenetic analysis of a 1306 bp fragment partially comprising surface and polymerase genes and HDV genotypes by analysis of a 403 bp fragment comprising part of the L-HDAg. RESULTS - HDV RNA was detected in all samples and genotype 3 was identified among them. Fourteen also showed HBV DNA detectable and HBV genotypes were characterized in 13; among these samples HBV subgenotypes A1 (38%), D2 (8%), D3 (46%) and F2a (8%) were found.

CONCLUSION – This study confirm that HDV genotype 3 is the most prevalent genotype in Amazon region and it is not associated to a specific HBV genotype. The genotypic characterization of HBV and HDV shown in this study is important for understanding the evolution of chronic patients in the region favoring the elucidation related to disease progression. Financial support: FIOCRUZ RO, SUS.

HV1009 - INVESTIGATION OF NOROVIRUSES IN FECAL SAMPLES RECOVERED OF CHILDREN WITH ACUTE GASTROENTERITIS FROM MANAUS, AMAZONAS IN 2010


1. UNIVERSIDADE DO ESTADO DO PARÁ, UEPA, Trav. Perebebuí, 2623 - Marco - 66087-670 - Belém / Pará / Brasil
2. INSTITUTO EVANDRO CHAGAS, IEC, Rodovia BR-316 km 7 s/n - Levilândia - 67030-000 - Ananindeua / Pará / Brasil

E-mail: costasamya@hotmail.com

Acute gastroenteritis (AGE) is a worldwide public health problem. Nowadays, the noroviruses (NoVs) are recognized as the major cause of outbreaks of nonbacterial AGE in humans, commonly reported in closed
communities such as hospitals, schools, and cruise ships. The genome of NoVs is composed of a non-enveloped capsid with a single-stranded, positive-sense RNA that encoding three open reading frames (ORF-1, -2 and -3). The goal of this study was to investigate the role of NoV in cases of AGE and determine the circulating genotypes in children under 10 years old, from Manaus in the year of 2010. Two methods were used for NoVs detection: the enzyme immunoassay (EIA) and the reverse transcriptase-polymerase chain reaction (RT-PCR) using primers specific for B region (polymerase). Positive samples by RT-PCR were sequenced using primers that target a partial region (D) of the genome that encodes the main capsid protein. Amplicons obtained by RT-PCR were purified and sequenced using the Big Dye Terminator Reaction Kit® (v. 3.1) and the ABI Prism 3130xl DNA sequence. Sequences were edited and aligned using the Bioedit and the phylogenetic analysis was performed using MEGA version 5.05. A total of 171 stool samples were sent by the LACEN of Manaus to the Evandro Chagas Institute in 2010. NoVs was detected in 39.2% of the samples being 34.5% by EIA and 28.6% by RT-PCR. Of the 12 positive strains sequenced, GII.4 variant 2010 was the most prevalent, found in 91.7% (11/12) of the cases and GII.7 (8.3%) in one sample. These results corroborate with other obtained in different places, like in Rio de Janeiro where NoVs was detected in 35.1% of the cases and the most prevalent genotype was the GII.4 variant 2010. Thus, detection methods associated with molecular characterization important tools to contribute with the establishment of a continuous genotype surveillance of NoVs in Brazil, which may help in the future in the formulation of a possible vaccine. Financial Support: Instituto Evandro Chagas, Secretaria de Vigilância em Saúde, Ministério da Saúde.

HV1022 - ANALYSIS OF ANTIVIRAL ACTIVITY OF THE HEMOLYMPH FROM LEPIDOPTERA (MEGALOPYGIDAE)

Carvalho, N.D., Giovani, D.N.S., Moraes, R.H.P., Mendonça, R.M.Z., Mendonça, R.Z.

Instituto Butantan, IBU, Av Vital Brasil, 1500. São Paulo/SP. Brasil E-mail: nathalia@ig.com.br

Several studies have shown the presence of active properties in the hemolymph of arthropods, some of which are of interest for the development of new pharmacological drugs. However, relatively little data are available on molecules from insects with antiviral activities. In this study, the effects of supplementation of infected culture with hemolymph from larvae of Lepidoptera were investigated. Citotoxicity and genotoxicity were evaluated and no adverse effects were observed in culture after hemolymph addition (up to 5%). The effect of hemolymph on virus growth was measured on confluent monolayers of infected cells with measles virus, influenza virus (H1N1) (enveloped virus) and picornavirus (non enveloped virus). The cultures were observed daily for evidence of cytopathic effect. The analyses of the viral titer demonstrated that the addition of 1% of hemolymph decreased significantly (p=0.002) the virus titer. The antiviral protein responsible for this activity was isolated and purified by gel filtration chromatography using a gel
filtration column system (Superdex 75) and further fractionated using a Resource-Q ion exchange column system. Experiments with the purified protein led to a 32-fold reduction in influenza virus production, 64-fold reduction in measles virus production and a 256-fold reduction in picornavirus production. Heating and freezing seem to have no influence over its antiviral activity. Also, the protein does not display virucidal activity and does not act on receptors on the cell membrane. The observations suggest an intracellular mechanism of action and that the protein may act as a constitutive agent that affects the innate antiviral immune response. Financial support: FAPESP 10/52434-6

HV1023 - RECOMBINANT ANTIGEN-BASED ELISA FOR DETECTION OF ANTI-DENGUE IgM FROM BLOOD SAMPLES COLLECTED IN FILTER-PAPER


1. Fundação Oswaldo Cruz, CPqRR/ Fiocruz, Avenida Augusto de Lima, 1715, Barro Preto, BH, Minas Gerais

2. Universidade Federal de Minas Gerais, UFMG, Avenida Antônio Carlos, 6627, Pampulha, Belo Horizonte, Minas Gerais E-mail: andreiacursino@yahoo.com.br

Dengue fever is an infection carried by mosquitoes and caused by any of four related Dengue virus (DENV) serotypes belonging to the family Flaviviridae. The clinical manifestations range from asymptomatic to severe characterized by hemorrhagic fever and shock syndrome. The indirect enzyme linked immunosorbent assay (ELISA) is one of the most used serological tests for dengue diagnosis whereby immunoglobulin M for acute phase or immunoglobulin G for phase convalescent. The objective of this study was to detect IgM anti-dengue in blood samples collected on filter-paper obtained from peripheral blood using an ELISA based on recombinant antigens and compare its results to a commercial kit. For this study 48 blood samples were collected from suspected dengue infected patients from Caratinga (MG), between 6º and 87º days after the onset of symptoms in the period from November 2010 to July 2011. Samples of whole blood collected on filter-paper were submitted to a recombinant protein IgM-ELISA and serum samples were tested with a commercial Kit (IgM ELISA anti-dengue Human do Brasil). The ELISA plates were coated with DENV recombinant proteins, and blood samples collected on paper-filter were diluted in PBS-T (Phosphate buffered saline + 0.05% of Tween 20) and BSA 0.1%. Reading of the ELISA was made at 450nm. Forty-three (89.6%) samples were concordant in both methods, 7 positive and 36 negative. Only 5 (10.4%) samples were discordant, IgM-ELISA sensitivity was 87.5% and specificity was 92.5%. Therefore the ELISA performed with samples collected on paper-filter has a good correspondence between the results from serum samples. Thus, the ELISA based on dengue recombinant proteins showed be efficient for dengue diagnosis. Financial support: CNPq, CAPES, DECIT/MS, FAPEMIG e PRONEX-Dengue and INCT-Dengue.
**HV1026 - VALIDATION OF REAL TIME PCR FOR HTLV-1 PROVIRAL LOAD IN PERIPHERAL BLOOD NONNUCLEAR CELLS**

Rosadas, C., Cabral-Castro, M.J., Peralta, J.M., Puccioni-Sohler, M.

Universidade Federal do Rio de Janeiro, UFRJ, Rua Professor Rodolfo Paulo Rocco 255, 3 andar, Serviço de Patologia Clínica E-mail: carolrosadas@gmail.com

HTLV-1 infection remains asymptomatic in the majority of infected individuals. However it may cause a neurological chronic disorder called as HTLV-1 associated myelopathy (HAM/TSP). The laboratory diagnosis is based on immunoassays such as ELISA and Western blot. These techniques have some disadvantages in indeterminate results, immunosuppressed patients and in neonatal infections. In such cases, the molecular biology techniques appear as an alternative. The real-time quantitative PCR (qPCR) quantify the proviral load, which may help to assess disease progression. Although qPCR has a high sensitivity and specificity it is still an in house technique. Thus prior validation is essential before the implementation of this technique on laboratorial routine. The aim of the study is to validate a TaqMan qPCR assay for HTLV-1 proviral load detection in peripheral blood mononuclear cells based on a conserved region of tax gene. DNA of TARL-2 cells were used to prepare the standard curve. The range of the TARL-2 standard was 5x10⁴ to 5 copies of virus/rxn. The samples were evaluated in triplicate on two consecutive days. To evaluate the limit of detection a sample containing only one copy of the target gene was added and analyzed in triplicate. Nineteen patient samples reactive for HTLV-1 by ELISA and one negative sample were included in the study. To evaluate the intra- and inter-assay variation one sample was tested 20 times in two consecutive days. All positive samples presented gene amplification. The negative sample did not presented amplification of tax gene, but presented amplification of the reference gene. The limit of detection was 1 copy/rxn. The qPCR efficiency, slope and correlation coefficients (r²) were all acceptable (presenting at least 98,58%, -3,298 and 0,993, respectively). The assay gave coefficient of variation for the Ct values of less than 1,53% and 1,93% for intra and inter assay, respectively. This assay is able to reliably quantify proviral load. Financial support: FAPERJ

**HV1030 - OVEREXPRESSION OF ANXA1 IN PENILE CARCINOMAS POSITIVE FOR HIGH RISK HPVS**


1. Instituto de Biociências, Letras e Ciências Exatas/UNESP, IBILCE/UNESP, Rua Cristovão Colombo, 2265- São José do Rio Preto, São Paulo

2. Laboratório Inst. Anatomia Patológica e Citopatologia IAPC, Faculdade de Medicina de São José do Rio Preto, FAMERP, Av. Brigadeiro Faria Lima, 5416. Vila São Pedro. CEP: 15090-000. S. J. do Rio Pr

3. Hospital A.C. Camargo, Rua Professor Antônio Prudente, 211. CEP: 01509-010. São Paulo-SP.
4. Departamento de Radiologia e Oncologia Básica/USP, USP, Av. Dr. Arnaldo, 255. CEP: 01246-903. São Paulo-SP. E-mail: macal131@gmail.com

The incidence of penile cancer varies between populations but is rare in developed nations. Penile cancer is associated with a number of established risk factors and associated diseases including phimosis with chronic inflammation, human papillomavirus (HPV) infection, poor hygiene and smoking. In penile carcinomas the most common HPV types are HPV 16 and HPV 18 being that HPV 16 is most prevalent in North America, Europe, South America and India. HPV 18 is the second most common HPV type identified in squamous cell carcinoma samples, but it has low prevalence. HPV contributes to tumorigenesis predominantly through the action of viral oncoproteins E6 and E7, which regulate the expression of known genes like p53 and pRb. The objective of this study was to identify genes related to penile carcinoma. The detection of HPV infection was analyzed in 47 penile squamous cell carcinoma samples. HPV DNA was detected in 48.9% of penile squamous cell carcinoma cases. High-risk HPV infections were present in 42.5% of cases and low-risk HPV infections were detected in 6.4% of penile squamous cell carcinomas. The RaSH approach identified differential expression of Annexin A1 (ANXA1), p16, RPL6, PBEF1 and KIAA1033 in high-risk HPV positive penile carcinoma; ANXA1 and p16 were overexpressed in tumoral cells. ANXA1 and p16 proteins were significantly more expressed in the cells from HPV-positive penile carcinoma as compared to HPV-negative tumors (p<0.001). We suggested the p16 could be a marker for penile carcinoma, confirming the diagnosis of malignant penile lesions infected with high risk HPVs. Overexpression of ANXA1, which has anti-inflammatory, antipyretic and antihyperalgesic activities and is associated with various physiological processes including cellular differentiation, cell proliferation and signal transduction, was demonstrated in penile squamous cell carcinoma samples and its protein expression is strongly associated with high risk HPV infection. Financial support: FAPESP

HV1031 - DETECTION AND GENOTYPING OF SAPIVIRUS IN FECAL SPECIMENS OF CHILDREN WITH ACUTE GASTROENTERITIS FROM MANAUS - AM, BRAZIL


INSTITUTO EVANDRO CHAGAS, IEC, Rodovia Br 316 Km 07 Levilândia, Ananindeua-PA E-mail: tammykathlyn@gmail.com

Sapovirus (SaV) is an important pathogen of acute gastroenteritis (AGE) in humans. As member of the Caliciviridae family, this non-enveloped virus has a single-stranded positive-sense RNA genome of approximately 7.5 kb. SaV strains can be divided into five genogroups (G) of which GI, GII, GIV, and GV infect humans, and can be further divided into many genotypes. In general, this pathogen is associated with sporadic cases of AGE in young children and elderly people. It is also related with
outbreaks in day care centers, nursing homes and hospitals. Symptoms include diarrhea with watery stools, vomiting, and fever. The transmission occurs by fecal-oral route, by aerosol and by consumption of contaminated food or water. Fecal samples collected from January/2010 to May/2011, of children with AGE, previously negative for rotavirus and norovirus, were tested for the presence of SaV. It was used Reverse Transcription-Polymerase Chain Reaction (RT-PCR), with the primers 289/290, specific for human calicivirus. The products obtained were visualized in an agarose gel and samples that showed specific amplicons of 331 bp were considered positives. The sequencing of positive samples was performed using primers 289/290 (polymerase region) and SLV5317/5749 (capsid region) with Big Dye Terminator Kit and the results compared with sequences registered in the GenBank. SaV was detected in 6/131 samples tested (4.5%). Two samples were sequenced and classified as GI.2 and GI.1. The positivity rate detected in this study (4.5%) was similar to the ones obtained in researches conducted in Belém-PA (4.9%) and Australia (4.1%), in the years of 2010 and 2006, respectively, however lower than the registered in India (10.2%) in 2008. This is the first report concerning the detection and characterization of SaV in Manaus-AM. Considering few studies conducted in Brazil relating to SaV, further researches are required to elucidate the real epidemiological importance of this pathogen.

HV1033 - SCREENING OF ANTIVIRAL ACTIVITY OF ORGANISMS FROM THE MARINE ENVIRONMENT AGAINST THE BOVINE VIRAL DIARRHEA

VIRUS, SURROGATE MODEL FOR THE HEPATITIS C VIRUS
Bastos, J.C.S., Kohn, L.K., Padilla, M.A., Berlinck, R.G.S., Fantinatti-Garboggin, F., Arns, C.W.

1. Universidade de São Paulo, USP - São Carlos, Avenida Trabalhador são-carlense, nº 400, Bairro Centro. CEP 13566-590 - São Carlos
2. Universidade Estadual de Campinas, Unicamp, Cidade Universitária "Zeferino Vaz" Distrito de Barão Geraldo 13083-970 Campinas E-mail: jusantiago_farmacia@yahoo.com.br

The hepatitis C virus (HCV) causes chronic hepatitis, which can progress to liver cirrhosis and hepatocellular carcinoma. There is no vaccine available and treatment has limited effectiveness. The marine environment is a promising source for new antiviral drugs, because of its wealth and diversity, it has been little explored. This study evaluated the antiviral activity of 400 extracts from invertebrates and microorganisms from the marine environment against bovine viral diarrhea virus (BVDV), a surrogate model for HCV. For this, we performed the evaluation of antiviral activity of each extract (50 µg/mL), through its ability to inhibit citopathic effect against to BVDV virus (100 TCID50/50 µL) after 72 hours of incubation. The results were obtained through the observation of citopathic effect and the inhibition percentage was calculated through the MTT assay. The extracts that showed protection percentage greater than or equal to 80% were considered potentially active, and selected for further tests. 400 extracts were tested, and the results
showed that 5% were active against BVDV. For the extracts obtained from fungi, 4.2% were active, from bacterial 6.9% were active, from ascidian 0% were active, and from sponge 4.8% were active. The active extracts will be evaluated on which phase of the viral replicative cycle they operate. For this, different tests will be performed. In conclusion, the organisms of the marine environment represents a source of compounds with potential antiviral activity and, against the BVDV, the extracts obtained from bacterial showed the best results, generating the greatest amount of active extracts. Financial support: Capes

HV1035 - AN OVERVIEW OF DENGUE FATAL CASES DURING 25 YEARS BY A REGIONAL REFERENCE LABORATORY


Instituto Oswaldo Cruz, IOC, Av Brasil, 4365 E-mail: bianca_santis@yahoo.com.br

In Brazil, dengue became a public health problem after the DENV-1 introduction in 1986, by the State of Rio de Janeiro. Here, we aimed to perform a retrospective analysis on dengue fatal cases occurred in the past 25 years, received in the Flavivirus Laboratory, IOC/FIOCRUZ, Regional Reference Laboratory for the Brazilian Ministry of Health. Cases records were analyzed and all data, such as patients’ personal information, signs and symptoms, sample collection were input on the Laboratory’s Database. Cases were submitted to the different techniques according to its availability over at that time. From April 1986 to December 2010, a total of 688 suspected dengue fatal cases were analyzed and 27.9% (192/688) was confirmed. Cases were received from 14 Brazilian States, however 76.9% were from Rio de Janeiro. Cases were more frequently received and confirmed from February to May. No differences were observed on cases confirmed on males (51.6%) or females (48.4%), however children ≤15 years old were the most affected (29.1%, 49/168 of the fatal cases confirmed). Fifty percent of the deaths occurred on defervescence. Commonly observed signs and symptoms in the confirmed cases included fever 87.2%, myalgia (52.4%), headache (47.5%), vomiting (46.3%) and prostration (43.9%). Shock was observed in 30.5% of the cases, non-characterized bleeding in 29.3%, low platelet count in 23.8%, hypotension in 20.1% and abdominal pain in 16.5%. DENV-2 was identified in 60.2% of the cases. On 2002 and 2009, most of the fatal outcomes were due to primary infections (71% and 57%, respectively), however in 2007 and 2008, fatal cases were due to secondary infections (61% and 69%, respectively). The data obtained in this analysis may help the elucidate the role of dengue on fatal outcomes during distinct epidemiological scenarios, where different serotypes were introduced, emerged and re-emerged in an endemic country such as Brazil. Financial support: FAPERJ, CNPq, CAPES and FIOCRUZ

HV1038 - PREVALENCE AND QUANTIFICATION OF THE HEPATITIS C VIRUS (HCV) IN PATIENTS FROM BLUMENAU-SC

Herkenhoff, M.E., Ferreira, P.S., Rodakiewicz, S.M., Gaulke, R., Branco,
Hepatitis C is an infectious disease affecting primarily the liver, caused by the hepatitis C virus (HCV). The virus is a small, enveloped, single-stranded, positive-sense RNA virus. It is a member of the hepacivirus genus in the family Flaviviridae. The infection is often asymptomatic, but chronic infection can lead to scarring of the liver and ultimately to cirrhosis, which is generally apparent after many years. HCV is spread primarily by blood-to-blood contact associated with intravenous drug use, poorly sterilized medical equipment and transfusions. An estimated 130–170 million people worldwide are infected with hepatitis C. Many infected people suffered chronic hepatitis from that time onwards and urgently needed treatment and care. The virus is transmitted by exposure to infectious blood or body fluids such as semen and vaginal fluids, while viral DNA has been detected in the saliva, tears, and urine of chronic carriers. Besides genotype, quantitative analysis of HCV infection is extensively used for monitoring disease progression and treatment. Real-time PCR has engendered wide acceptance for quantification of hepatitis B virus (HCV) DNA in the blood due to its improved rapidity, sensitivity, reproducibility, and reduced contamination. The aim of this study was to determine the HCV prevalence in a population in Blumenau, Santa Catarina state, southern Brazil, in January/2010 to July/2010. We obtained a sample of 172 individuals, which was used the blood serum to estimate the prevalence and the number of viral copies. We have used the Real-time PCR method to indenitify the virus and estimate the number of viral copies in each positive individual. After that, we have made a statistical analysis using the Z-test with 5% of significance. This study has showed a prevalence of 54.65% on the total sample, 51% on the male population and 59.72% on the female population. The number of viral copies was showed an average of 827,067,624 copies/ml in the male population and 752,058,587 copies/ml in the female population, there is no statically difference in those two populations, male and female, determined by the Z-test (5% of significance). In conclusion, our study have showed a significant prevalence by this virus in the population that was studied, and that are not a difference on the number of copies between the male and the female population. Financial Support: Genolab.
the viruses of this genus have been associated with disease in humans. In infection by arboviruses the fever is the principle clinical manifestations observed. Objective: Laboratorial investigation for arboviruses of the genus Flavivirus in patients with acute febrile syndrome, residents in the municipality of Parauapebas, state of Pará, from August 2010 to March 2011. Material and methods: Were obtained 159 sera samples during this period. For the detection of total antibodies and IgM antibodies was used the Hemagglutination Inhibition (IH) test and immunoenzymatic assay (ELISA) respectively for 8 Flaviviruses (Yellow Fever virus - YFV, Dengue virus - DENV (serotypes 1, 2, 3, 4), Saint Louis encephalitis virus - SLEV, Rocio virus - ROCV and Ilheus virus - ILHV); 100 blood samples of patients with up five days after the onset of clinical symptoms were inoculated for attempts of viral isolation in cell line clone C6/36 (Aedes albopictus). Results: Of the 100 blood samples tested were isolated 12 (12%) of DENV-1, one (1%) of the DENV-2 and 87 (87%) were negative. Of the 159 sera tested by HI, 45 samples (28.3%) were negative, and 114 (71.7%) were positive for the viruses of the genus Flavivirus. The 114 positive samples by HI were tested by ELISA for DENV and YFV, with 82 (72%) positive samples for DENV and 32 negative. The 32 negative samples were tested by ELISA for other flaviviruses, where only one (3.1%) was positive for SLEV. Conclusion: A high prevalence of antibodies to flaviviruses were detected in febrile patients living in the municipality of Parauapebas-PA, however most of the infections were caused by DENV and also the possibly circulation of the SLEV. Financial supports: CNPQ/IEC/VALE

HV1040 - IDENTIFICATION OF CALOMYS FECUNDUS AS THE TRULY RODENT RESERVOIR OF LAGUNA NEGRA HANTAVIRUS IN NORTH WESTERN ARGENTINA


1. INEVH “Dr. Julio I. Maiztegui”, , Monteagudo 2510, Pergamino, Argentina

2. Cátedra de Genética de Poblaciones y Evolución, UNC, Córdoba, Argentina

3. Laboratorio de Investigaciones Ecológicas de las Yungas,, LIEY, San Miguel de Tucumán, Argentina

4. Dirección Provincial de Programas Sanitarios, , San Salvador de Jujuy E-mail: pininoemi@yahoo.com.ar

Laguna Negra virus (LNV) was first identified as etiologic agent of Hantavirus Pulmonary Syndrome (HPS) in Paraguay in 1997, and was isolated from Calomys laucha rodents. In 2000, LNV was found in Argentina and Bolivia from human and rodent (Calomys callosus) samples and in 2012 in Brazil (human and Calomys callidus samples). LNV sequences obtained from HPS cases and rodent samples from North Western Argentina (Jujuy province) were compared with LNV sequences from other regions; we also performed the molecular identification of rodent reservoirs trapped in the same area in 2000 and 2011. Human samples with a positive IgM serology and rodent samples with positive IgG by ELISA using Maciel virus antigen were analysed to detect
viral RNA. Mitochondrial DNA was used to confirm morphological field identification of rodents captured in 2011 and 2000 (previously identified as C. callosus); all positive rodents corresponded to C. fecundus. Viral RNA was amplified by nested RT-PCR with specific primers for S (447 bp) and M (513 bp) segments. Phylogenetic analysis was carried out by Neighbor Joining method and bootstrap support calculated, as implemented in MEGA 5.0. For the S fragment, all Argentine strains showed differences < 1% (nucleotides) and were identical in aminoacids. Jujuy strains showed less than 4% difference when compared with reference strain (Paraguay) and Brazil strain (Bolivia strains were not available at GenBank). At present, LNV was identified in four different reservoir species of the Calomys complex: C. laucha (Paraguay), C. callosus (Bolivia), C. callidus (Brazil) and C. fecundus (Argentina); only molecular DNA mitochondrial analysis could determine the proper identification of this cryptic species. However, they are infected with variants of the same hantavirus. It is not common that a hantavirus infects different rodent species, and we agree with the hypothesis that LNV had infected a common ancestor to the genus. Financial support: Fondos concursables Focanlis 2010

HV1045 - DETECTION OF HUMAN CYTOMEGALOVIRUS (HCMV) IN GLIOBLASTOMA MULTIFORME (GBM)


1. Universidade Federal do ABC, UFABC, Rua Santa Adélia, 166.

Bairro Bangu. Santo André - SP - Brasil. CEP 09.210-170

2. Hospital das Clínicas da Faculdade de Medicina da USP, FMUSP, Av. Dr. Enéas de Carvalho Aguiar, 255 - Cerqueira César - 05403-000 - São Paulo E-mail: santos.claudiaij@gmail.com

The Human Cytomegalovirus (HCMV) is an ubiquitous infectious agent, present in almost 90% of the world’s population. In healthy individuals, the infection is normally asymptomatic, however, in cases of immunosuppression the virus can be a life threatening agent. Recent findings suggested a relationship between HCMV and cancer. The virus has been detected in different cancer types, specially in glioblastomas, the most malignant kind of glial tumors. The studies of the HCMV role in tumor progression are rapidly advancing and additional work needs to be done to confirm the viral presence in tumors and identify possible viral proteins involved in malignity. The aim of this work was to detect the HCMV genome in tumor biopsies obtained from glioblastoma patients at the Hospital das Clínicas de Sao Paulo, Brazil. We employed three different techniques: conventional PCR (cPCR), real time quantitative PCR (qRT-PCR) and semi-nested PCR to detect HCMV in peripheral blood and lesions of glioblastomas patients. The viral genome was only successfully detect by semi-nested PCR using primers for the variable region of the viral glycoprotein gB. Using a HCMV-BAC the detection limit of the techniques was 9 copies/ul in semi-nested PCR, 6800 copies/ul in cPCR and 20 copies/ul in qRT-PCR, indicating that semi-nested PCR is a more sensitive
In this case, 20 glioblastomas samples were tested and 9 (45%) were positive for the gB region. Four (44%) of the 9 positive patients had also detectable HCMV in their peripheral blood. Sequencing analysis of the gB demonstrated the presence of different gB genotypes in the tumors and we are analyzing the gB genotypes in peripheral blood of the patients. We are currently performing experiments in order to verify the viral gene expression in the glioblastomas samples. Our results confirm the presence of HCMV in glioblastomas indicating a possible relationship of the virus with tumor malignity.

HV1046 - CHARACTERIZATION OF GENOTYPES AND MUTATIONS IN HEPATITIS B VIRUS RESISTANT TO ANTIVIRAL DRUGS IN PATIENTS WITH HBV IN THE STATE OF AMAZONAS, BRAZIL

Galvão, R.S., Braga, W.S.M., Castilho, M.C., Vasconcelos, H.L., Rocha, J.M., Silva, S.S., Oliveira, C.M.C.

Introduction: The Amazonian region is characterized as one with the highest incidence of hepatitis B (HBV) worldwide. In the Amazonas State, the gutters regions of the rivers Jurua, Purus and midst Solimões are considered to have the highest hepatitis B endemicity. Besides these areas being known for their highest endemicity, very little is known about the virological and clinical information for providing adequate treatment. The genotypic characterization and the search for mutations associated with resistance to therapy are important for the monitoring of viral dynamics and treatment. This study aims to characterize the HBV genotype as well as the mutations associated with primary resistance to therapy from patients with HBV in the state of Amazonas.

Methodology: Blood samples from 77 treatment-naïve HBV patients with viral load > 250UI/mL (COBAS TaqMam Hepatitis B virus) were collected. The PreS and S regions of the surface gene were amplified by polymerase chain reaction (PCR) and nucleotide sequenced in the ABI 3130 xl automatic sequencer (Applied Biosystems). The sequences were edited and aligned using a BioEdit Sequence Alignment Editor, version 7.1. The phylogenetic relationship of the S gene fragment sequences was determined using Molecular Evolutionary Genetics Analysis (MEGA), version 5.19. The Tamura–Nei algorithm was used, employing the neighbor–joining method. The phylogenetic groups were evaluated by the bootstrap test (1,000 bootstrap replicates). Mutation analysis was performed using the web site www.hepseq.org/public/web_front_main.php and hivdb.stanford.edu/hbvseq/deselopment/hbvseq.html.

Results: Of the 77 samples analyzed, 53 (68,8%) were positive for HBV DNA. The following genotypes were obtained in 44 HBV DNA samples analyzed: 21 (47,7%) A, 7 (15,9%) D,
6(13,6) F, 3(6,8%) C and 1(13,6) G. One mutation S202G was observed in the reverse transcriptase region. This mutation is related to resistance to antiviral Entecavir in a patient with HBV genotype D undergoing treatment since 3 years. Conclusion: The HBV genotype A was prevalent in this study population confirming other studies performed in the region. Of interest, the detected mutation S202G resistant to the use of Entecavir suggests that it is important to sequence the RT region for providing adequate treatment. Financial support: CNPq, DN DST, Aids e HV/MS, CAPES

HV1049 - PREVALENCE AND QUANTIFICATION OF THE HEPATITIS B VIRUS (HBV) IN PATIENTS FROM BLUMENAU-SC

Herkenhoff, M.E., Rodakiewicz, S.M., Ferreira, P.S., Gaulke, R., Branco, L.C., Pitojovanciv, A.K., Remualdo, V.R.

1. Genolab, Genolab, Rua floriano peixoto, 425 - Centro-Blumenau cep: 89010-500
2. Universidade do Estado de Santa Catarina, UDESC, Av. Luís de Camões, 2090, Bairro Conta Dinheiro, Lages/SC E-mail: marcos.herkenhoff@gmail.com

Hepatitis B virus (HBV) infection is a public health problem worldwide. It is estimated that approximately 3 billion people have been exposed to HBV, of whom more than 350 million are chronically infected. The virus, a species of the genus Orthohepadnavirus, which is likewise a part of the Hepadnaviridae family of viruses, is a major cause of chronic liver disease worldwide. The virus is transmitted by exposure to infectious blood or body fluids such as semen and vaginal fluids, while viral DNA has been detected in the saliva, tears, and urine of chronic carriers. Real-time PCR has engendered wide acceptance for quantification of hepatitis B virus (HBV) DNA in the blood due to its improved rapidity, sensitivity, reproducibility, and reduced contamination. The aim of this study was to determine the HBV prevalence in a population in Blumenau, Santa Catarina state, southern Brazil, in January/2010 to July/2010. We obtained a sample of 158 individuals, which was used the blood serum to estimate the prevalence and the number of viral copies. We have used the Real-time PCR method to indentify the virus and estimate the number of viral copies in each positive individual. After that, we have made a statistical analysis using the student test with 5% of significance. This study has showed a prevalence of 24,5% on the total sample, 29,35% on the male population and 18,19% on the female population. The number of viral copies was showed an average of 25016079,22 copies/ml in the male population and 2870525,583 copies/ml in the female population, there is no statically difference in those two population, male and female, determined by the student test (5% of significance). In conclusion, our study have showed a significant prevalence by this virus in the population that was studied, and that are not a difference on the number of copies between the male and the female population. Financial Support: Genolab

HV1052 - GENOTYPIC RESISTANCE PROFILE OF HUMAN INFLUENZA A VIRUS TO NEURAMINIDASE INHIBITORS IN CITIES OF NORTH AND
NORTHEAST REGIONS OF BRAZIL DURING 2012 SEASON


1. Evandro Chagas Institute, IEC, Highway BR-316 km 7 s/n - Levilândia - Ananindeua / Pará / Brazil
2. Nucleus of Tropical Medicine-UFPA, Belém, PA, Brazil, NMT/UFPA, Avenue Generalíssimo Deodoro, 92 - Umarizal - Belém / Pará / Brazil E-mail: raimundopinho@iec.pa.gov.br

Introduction. Influenza virus causes millions of deaths and hospitalizations worldwide every year. In Sporadic infections, annually epidemics or pandemics, antiviral drugs become the principal means for managing the disease specially neuraminidase inhibitors (NAI). However due to the viral genetic evolution some viral strains acquire point mutations in the Neuraminidase (NA) codifying gene that drive some amino acid substitutions leading to drug resistance. Objectives. Describe the occurrence of mutations in NA encoding gene from Influenza A virus, which may be related to (NAI) resistance in circulating strains at the North and Northeast of Brazil in the period between January and June 2012.

Material and Methods. From the period described 1204 samples were selected based on the clinical symptoms of acute respiratory infection (ARI) with up to five days of evolution. Viral RNA was purified and RT-PCR was performed with specific primers to amplify NA gene sequence of Influenza A (H3N2 and H1N1 pdm). RT-PCR fragments were direct sequenced in the ABI3130XL (Applied Biosystems). Results. We had 151 positive samples to Influenza A, 100 (66.2%) for (H1N1pdm) and 51 (33.8%) for (H3N2). Partially analyzed Nucleotides sequences from 18 samples of the subtype N1 and 11 N2 demonstrate in two samples N1 from Fortaleza and Manaus the substitution His275Tyr that confers high Oseltamivir resistance, low sensitivity to Peramivir and still sensible to Zanamivir. Genotyping of N2 samples showed the following amino acid substitutions: Asn329Thr, Ser334Ile, His347Gln, Ser367Asn and Lys369Thr that were not associated to NAI resistance. Conclusion. The 2009 pandemic period alert us for Influenza monitoring through surveillance of circulating strains and mutations in the viral genome that can be associated with resistance to antiviral drugs. These are the first reported cases of Influenza A virus circulation H1N1pdm resistant to oseltamivir in the North and Northeast. Financial support: National Council of Scientific and Technological Development - CNPq.

HV1054 - LCR GENETIC VARIABILITY AND PHYLOGENY OF LOW-RISK HPV IN LARYNGEAL PAPILLOMATOSIS


1. Faculty of Medicine of Ribeirao Preto, Sao Paulo University, USP, Bandeirantes Avenue, 3900 - Monte Alegre 14049-900 - Ribeirão Preto - SP.
2. Sao Paulo State University ,
Recurrent respiratory papillomatosis (RRP) is a benign disease associated with Human Papillomavirus (HPV) infection, particularly low-risk HPV types 6 and 11, and is characterized by the formation of papillomas mainly in the larynx. Replication and transcription of HPVs depend on the interaction of host cell and viral transcription factors with the non-coding region of the virus called Long Control Region (LCR). This region encloses diverse cis-regulatory elements central for its activity. It has been predicted that nucleotide substitutions in the LCR region leads to differences in the malignant potential among HPV variants. This study aims to expand the knowledge concerning genetic variability of HPV types commonly found in laryngeal papillomas, and to further identify the impact of nucleotide variations in the LCR region upon HPV transcriptional activity. HPV detection was conducted by PCR using the PGMY09/11 primer system followed by Restriction Fragment Length Polymorphism (RFLP) genotyping. We analyzed 11 biopsy specimens of juvenile laryngeal papillomatosis and 9 of adult laryngeal papillomatosis. HPV-6 was found in 14 (70%) samples and HPV-11 in 6 (40%) samples. Cloning and sequencing of the HPV-6 LCR showed insertions and substitutions of nucleotide in this genomic region. A total of six genomic variants were identified. Phylogenetic analysis of the LCR complete genomic region of 14 Brazilian HPV-6 isolates, in addition to 77 Slovenian and 12 African HPV-6 isolates previously described, and the HPV-6a, HPV-6vc and the HPV-6b prototype reference isolates revealed a tree with two separated clusters. The sequences of this study were grouped in branches into non-prototypic closely related HPV-6a and HPV-6vc-related genomic variants. We will further analyse the transcriptional activity of these samples in order to verify if changes in this highly variable region are involved in regulating the expression of genes involved in cell cycle control and differentiation.

HV1059 - HTRA1 EXPRESSION IN HIGH-RISK HPV-POSITIVE PENILE TUMOR SAMPLES AND CELL LINES


1. UNESP, São José do Rio Preto, IBILCE/UNESP, Rua Cristóvão Colombo, 2265, São José do Rio Preto/SP

2. Center for Translational Research in Oncology, ICESP, Av. Dr. Arnaldo, 251 - Cerqueira César - São Paulo A.C. Camargo Hospital, Department of Pathologic Anatomy, A.C.Camargo Hospital, Rua Prof. Antônio Prudente, 109, Liberdade, São Paulo

3. National Institute of Science and Technology - HPV Institute, INCT HPV, E-mail: bru.stuqui@gmail.com

The Human Papillomavirus is the...
The most prevalent virus among sexually transmitted infections and it is associated with various malignancies. The E6 and E7 viral oncoproteins of high-risk HPVs are the main responsible for cell homeostasis alteration and immortalization. One of the mechanisms used in cell transformation by E6 protein is the interaction of its carboxy-terminal domain, known as PDZ, with PDZs domains presents in some cellular proteins, triggering them to degradation. A protein that is associated with various pathological conditions and has PDZ domain is the protease HTRA1. The aim of this study was to evaluate the HTRA1 gene expression in HPV-positive penile tumor samples and high-risk HPV positive cell lines. The HTRA1 was selected from a microarray where it was observed low expression of this gene in cell line positive for HPV 16 (HF698). In our laboratory, we evaluated HTRA1 expression by qPCR in high-risk HPV positive penile tumor samples compared to normal samples, where we observed low HTRA1 expression in 70% of the samples analysed. After gene validation in tumor samples, we evaluated the HTRA1 mRNA expression in high-risk HPV-positive cell lines: HF698, SiHa, CasKi and HF698 transfected with pCMV6/HTRA1 expression vector. The gene presented low expression in HF698 cell line like previous microarray results. However, when the HF698 line was transfected with pCMV6/HTRA1 vector this gene expression was significantly increased. The lines SiHa and CasKi also showed low expression for this gene. The HTRA1 protein expression in transfected cell line with pCMV6/HTRA1 vector was confirmed by Western blotting. These results suggest that HTRA1 expression is low in high-risk HPV positive cells. The HTRA1 overexpression effect will still be measured in HF698 cell line by viability, cell proliferation and apoptosis assays. Financial Support: CAPES, FAPESP

**HV1075 - COMPARATIVE GENOMIC ANALYSIS BETWEEN AFRICAN AND BRAZILIAN HBV ISOLATES: THE ROLE OF AFRICAN COUNTRIES IN THE DISSEMINATION OF HBV/A/A1 IN BRAZIL**


1. Fundação Oswaldo Cruz, FIOCRUZ, Av. Brasil, 4365 - Manguinhos, Rio de Janeiro - CEP: 21040-360
2. Ministério da Saúde - República de Angola, E-mail: barbaravl@ioc.fiocruz.br

It is estimated that 2 billion people have been infected with hepatitis B virus (HBV) worldwide and more than 400 million people are at risk of developing cirrhosis and hepatocellular carcinoma due to chronic infection. Based on a genomic sequence divergence in the entire genome exceeding 7.5%, HBV strains have been classified into 8 genotypes, denoted A (HBV/A) to H (HBV/H). It has been established that HBV/A1 is one of the most prevalent genotype in Africa, especially in southern and eastern coast. In previous studies we have shown that HBV/A1 is the main genotype circulating in Brazil. Studies conducted in isolated Afro-Brazilian communities demonstrated that these communities have almost exclusively HBV/A1, suggesting that it was introduced by the slave trade. The aim of this study is to compare HBV/A1 isolates from different African
regions with Brazilian isolates in order to investigate, throughout genetic identity, which African countries have contributed to dissemination of HBV/A1 in Brazil. A comparison among samples from African countries and Brazilian isolates may help to establish possible routes of HBV/A1 spread. For this purpose, 50 samples, previously classified as HBV/A1 by RFLP analysis, from different Brazilian regions were selected. Until this moment, 20 samples were amplified for HBV complete genome by PCR assay. Fourteen HBV/A1 samples were successfully sequenced for HBV entire genome directly from PCR products. Sequences were compared with HBV/A1 samples available in GenBank/NCBI. Phylogenetic analysis demonstrated that Brazilian sequences are more closely related to Asian/East African sequences than with sequences from other African regions (genetic distance values: 0.02 versus 0.03). These preliminary results suggest that HBV infected slaves brought to Brazil came mostly from the East African coast during the slave trade. However, further studies are necessary to confirm this hypothesis and to estimate which African countries have contributed to the spread of HBV/A1 in Brazil. Financial support: IOC/FIOCRUZ

HV1076 - HEPATITIS B AND DELTA VIRUS PREVALENCE AMONG BLOOD DONORS IN LUANDA, ANGOLA


1. Fundação Oswaldo Cruz, FIOCRUZ, Av. Brasil, 4365, Manguinhos - Rio de Janeiro-RJ - CEP: 21040-360

2. Universidade Federal do Rio de Janeiro, E-mail: savassi@ioc.fiocruz.br

Hepatitis deltavirus (HDV) is associated with hepatitis B virus (HBV) infection and is frequently related to more severe disease than that due to the underlying HBV monoinfection. HDV is a subviral pathogen of humans, a satellite of HBV that depends on the envelope protein of HBV for its assembly and propagation. HDV is spread in the same way as HBV, mainly through parenteral exposure. The virus is highly endemic in Mediterranean countries, Middle East, Central Africa and northern parts of South America. Worldwide, more than 350 million people are considered to have chronic HBV infection, and 15 – 20 million of these individuals are thought to be coinfected or superinfected with HDV. Although Africa is considered an endemic region for this infection, for many countries, there is no available data in the literature. The aim of this study was to investigate seroprevalence of HDV in blood donors from Luanda, Angola. A total of 213 samples were submitted to HBsAg detection. All HBsAg reactive samples were subsequently tested for the detection of anti-HDV and, finally, anti-HDV reactive samples were tested for HDAg detection. Serological tests were performed by ELISA. HBsAg presence was detected in 19.2% (41/213) of blood donors samples. Nine out 41 (22%) HBsAg positive were also reactive to anti-HDV, 34.1% (14/41) were anti-HDV negative and 43.9% (18/41) were in the gray zone. All positive and gray zone samples were tested for HDAg and this antigen was found in 2 samples, suggesting that these individuals are actively infected. Both HDAg samples were in the gray zone in ELISA for anti-HDV detection.
Molecular analysis of the HDV positive samples are in progress. Our results show a still high prevalence of HBV in Angolan general population and also describes a high seroprevalence of HDV among these HBsAg carriers. Studies about HDV epidemiology in sub-Saharan countries are scarce, these data contributes for a better understanding of HDV circulation in African continent. Financial support: CNPq, Fiocruz

HV1084 - DENV-2 DETECTION IN UMBILICAL CORD FROM A DENGUE FATAL CASE


INSTITUTO OSMALDO CRUZ, IOC, AV. BRASIL, 4365, MANGUINHOS E-mail: pricgn@ioc.fiocruz.br

In Brazil more than seven million cases of dengue have been reported and currently the four serotypes circulate in the country. Although dengue is endemic in several regions of Brazil, there are few reports of dengue infection in pregnant women and the consequences for the fetus. This study reports a fatal outcome due to dengue resulting in maternal and fetal death. In November of 2010 a pregnant woman aged 23 years old, was admitted to the maternity of Miguel Couto Municipal Hospital, in Rio de Janeiro complaining of abdominal pain, vomiting and diarrhea, fainting and inaudible fetal heartbeat. The ultrasound revealed a single fetus with no active movements, little or absent amniotic fluid and placenta with reduced dimensions. The fetus was 32/33 weeks presenting placental abruption with apparently early detachment. During the caesarean there was a large extravasation of blood in cavity, deterioration of clinical picture, hypothermia, anuria, hemodynamic instability, mydriasis, absent reflexes and death. Tissues of spleen, placenta and umbilical cord were paraffin-embedded to investigate dengue virus. The RNA was extracted from sections of 5 um and RNA extraction was performed using the kit Purelink FFPE RNA Isolation Invitrogen. Real time PCR and RT-PCR was performed. DENV-2 was detected by both methods only in the umbilical cord. Considering that dengue infection during pregnancy may represent a risk to mother and concept measures of epidemiological surveillance and virological diagnosis should be implemented to this specific group especially in dengue endemic areas. Financial Support: CNPq and FAPERJ

HV1087 - MOLECULAR TRACKING OF LAMIVUDINE RESISTANCE MUTATIONS IN HBV SUBPOPULATION FROM HIV/HBV COINFECTED PATIENTS BY PYROSEQUENCING

Spitz, N.T.D., Lago, B.V., Moraes, M.T.B., Gomes, S.A., Soares, C.C.

Fundação Oswaldo Cruz, FIOCRUZ, Av. Brasil, 4365 - Manguinhos, Rio de Janeiro - CEP: 21040-360 E-mail: nataliastd@gmail.com

Due to similar routes of transmission, human immunodeficiency virus-1 (HIV-1) and hepatitis B virus (HBV) coinfection remains one of the most frequent comorbidities with a significant impact related to liver disease. Approximately 10% of the HIV-infected population has concurrent chronic HBV. Both can lead to chronic disease, and neither can
be eradicated with the use of current therapies. Reverse-transcriptase (RT) is an important enzyme for the replication of both viruses and for this reason it is used as a target in antiretroviral therapy. Lamivudine (LAM) is a nucleoside analogue which inhibits RT and is used in treatment against HBV and HIV. However, its clinical benefit has been compromised by the emergence of resistant viral strains carrying specific mutations in HBV and HIV RT genes. In HBV, the primary LAM-resistance mutation (rtM204V/I) affects viral replication and compensatory mutations (rtL180M, rtV173L) that partially restore replication efficiency are often co-selected. The aim of this study is to evaluate by pyrosequencing, the incidence of LAM resistance mutations in both viruses. At the moment, only was made the evaluation of HBV mutation and 26 samples were analyzed. rtM204V/I were successfully analyzed in 21 samples. Mixed population (wild type -wt- and mutant strains) was found in 81% (17/21) of samples. Only rt204V mutant population was found in 14.3% (3/21) and in 4.7% (1/21) of samples only wt strains were found. Compensatory mutation rtL180M was investigated in 14 samples and only wt strains were found. RtV173L was studied in 13 samples. Only wt strains were found in 85% (11/13) and mixed population was found in 15% (2/13) of the samples. Pyrosequencing is a very sensitive technique that may be useful in detecting and quantifying subpopulations of resistant viruses, that may be not be detected by other methods. It can be useful in predicting the appearance of mutant strains that can derail the treatment, improving the outcome of therapy. Financial support: Fiocruz, CNPq/PIBIC

HV1091 - SEROPREVALENCE OF HEPATITIS DELTA VIRUS (HDV) AMONG HEPATITIS B VIRUS (HBV) CHRONIC CARRIERS FROM CAMPO GRANDE- MATO GROSSO DO SUL, CENTRAL BRAZIL.


1. Fundação Oswaldo Cruz, FIOCRUZ, Av. Brasil, 4365, Manguinhos - Rio de Janeiro-RJ - CEP: 21040-360
2. Universidade Federal do Mato Grosso do Sul, UFMS, E-mail: savassi@ioc.fiocruz.br

Hepatitis delta virus (HDV) is a subviral pathogen of humans, a satellite of hepatitis B virus (HBV) that induces severe acute and chronic liver diseases. This agent depends on the envelope protein of HBV for its assembly and propagation, so it can only infect an individual who has coexistent HBV, either after simultaneous transmission of the two viruses or via superinfection of an established HBV carrier. HDV infection has a worldwide distribution and it is estimated that 15 – 20 million individuals are anti-HDV positive. This virus is highly endemic in Mediterranean countries, the Middle East, Central Africa and northern parts of South America. The Brazilian Amazon is known as an endemic area for this infection and it represents a significant public health problem, but few studies have assessed its prevalence in non-Amazonian regions in the country. The Amazon River region is located in the northern part of
Brazil and includes areas of 7 Brazilian states (Acre, Amazonas, Amapá, Pará, Rondônia, Roraima and Mato Grosso). The aim of this study was to evaluate the seroprevalence of HDV among HBsAg chronic carriers from Campo Grande - MS, West Central region, located outside of the Brazilian Amazon Basin. A total of 125 samples from HBV chronic carriers (HBsAg positive) were enrolled in this study. All samples were submitted to anti-HDV detection by ELISA and reactive samples were subsequently submitted to HDAg detection. Antibodies anti-HDV were found in 4.8% (6/125) of the samples, and HDAg was found in only one sample, suggesting that this individual is actively infected. Molecular analysis are in progress. These data reflects HDV circulation outside the Brazilian Amazon Basin and highlights the importance of epidemiological studies in different Brazilian regions. Clinical and epidemiological studies are still needed to clarify the presence and the role of HDV infection in Brazil. Financial support: CNPq, Fiocruz.

HV1093 - INFLUENCE OF POLYMORPHISMS IN GENES OF THE INNATE IMMUNE RESPONSE IN THE COURSE OF HIV-1 INFECTION

de Medeiros, R., Matte, M.C.C., Mirandolli, T.B., Araújo, L.A.L., Lunge, V.R., Melo, M.G., Almeida, S.E.M., Chies, J.A.B.

1. Universidade Federal do Rio Grande do Sul, UFRGS,
2. Fundação Estadual de Produção e Pesquisa em Saúde - RS, FEPPS-RS,
3. Grupo Hospitalar Nossa Senhora da Conceição Porto Alegre - RS,
4. Universidade Luterana do Brasil - RS, ULBRA - RS,
5. Universidade Feevale, Feevale,
E-mail: medeirosrubia@gmail.com

Variations in innate immune response genes have been associated with different AIDS progression. In HIV infection, TLRs (Toll-like receptors) recognize molecules of the virus present inside the infected cell; i.e., TLR7 and TLR8 recognize the viral RNA, and TLR9 the proviral DNA. This study investigated the influence of TLR7, TLR8 and TLR9 polymorphisms in the course of HIV-1 infection in patients from Southernmost Brazil. From 3,300 medical records of HIV+ patients, 98 individuals were defined as rapids, slow and chronics AIDS progressors. TLR7(Gln11Leu), TLR8(Met1Val) and TLR9(T-1237C and G1635A) polymorphisms were determined by PCR and restriction enzymes cleavages. To evaluate the influence of genotypes in the progression to AIDS Survival Analysis tests and Cox regression corrected by the presence of protective alleles CCR5del32 and HLA-B27/57 were performed. Kaplan-Meier curves to polymorphism -1237T/C in TLR9 revealed a significant association between C allele carriers and longer time (10 years) to AIDS progression when compared with T allele carriers (6 years). Moreover, this association was also reproduced in the multivariate Cox regression (0.616 Hz, 95% CI 0.379 to 1.003, p <0.05), including age and ethnicity as variables. However, adjusting the model to the presence of the protective alleles CCR5del32 and HLA-B27/57 the association
was lost. For the polymorphism +1635G/A in TLR9, when analyzing the variation within ethnic groups, statistically significant association of allele A (1.966 HZ, 95% CI 1.052 3.674, p<0.05) with rapid AIDS progression was observed in European-derived patients. No significant results for TLR7 and TLR8 polymorphism and AIDS progression were shown. Our data highlights a relationship between the investigated polymorphisms in TLR9 gene and progression to AIDS. In addition, the results suggests the genetic background is important in HIV-1 infection, although several genes and variants responsible by this behavior remain to be identified.

**HV1095 - EVIDENCE OF MULTIPLE INTRODUCTIONS OF HIV-1 SUBTYPE C IN RIO DE JANEIRO**


Instituto Oswaldo Cruz - Fundação Oswaldo Cruz, IOC - FIOCRUZ, Av. Brasil, 4365. Manguinhos - Rio de Janeiro - RJ - Brasil. CEP: 21040-360 E-mail: edsonod@ioc.fiocruz.br

Subtype C is the most prevalent HIV-1 clade worldwide and has spread very efficiently in the southern states of Brazil. Phylogeographic studies indicate that the subtype C epidemic in southern Brazil was initiated by the introduction of a single founder virus probably through Parana at some time point between 1960 and 1980. Recent studies demonstrate an increasing prevalence of this subtype in the southeast and central-west Brazilian regions. Little is known, however, about the genetic characteristics and spatial dynamics of subtype C viruses circulating in those regions. The objective of this study was to trace the origin of the HIV-1 subtype C viruses circulating in the state of Rio de Janeiro. Thirty HIV-1 subtype C samples isolated in this state between 2003 and 2011 were compared with subtype C pol sequences of Brazilian and African origin previously described. Phylogenetic and phylogeographic analyses were conducted using Maximum-likelihood and Bayesian methods. These analyses reveal that there have been multiple independent introductions of the HIV-1 subtype C clade in Rio de Janeiro, as signified by the presence of more than 20 phylogenetically distinct lineages. Most (>90%) subtype C viruses circulating in Rio de Janeiro originated in the southern Brazilian states. Five independent subtype C lineages, however, probably originated from southern and eastern African countries. We also find evidence that a few subtype C lineages were locally disseminated throughout the state. These results indicate a massive influx of HIV-1 subtype C strains from southern states into Rio de Janeiro and also demonstrate the importance of Rio de Janeiro as a route of entry of new viral strains from Africa to Brazil. Financial support: CAPES.

**HV1099 - DETECTION OF DENGUE VIRUS OF SEROTYPE 4 AND GENOTYPE I IN AEDES AEGYPTI FROM MANAUS CITY, BRAZIL**


1. Instituto Nacional de Pesquisas da Amazônia, INPA, Av. André Araújo, 2936, Aleixo Manaus – AM. CEP: 69060-001
2. Instituto Leônidas e Maria Deane / Fundação Oswaldo Cruz, ILMD-FIOCRUZ, Rua Teresina, 476. Adrianópolis, Manaus – AM. CEP: 69.057-070 E-mail: jonaidias@yahoo.com.br

Dengue epidemics have been reported in Brazil since 1985. In Manaus, a large city in the Brazilian Amazonic region, dengue is hyperendemic with all 4 serotypes simultaneously causing human disease and an increase of DHF/DSS cases as well as the number of fatalities. Two dengue serotype 4 virus (genotypes I and II) have been found in Manaus in the last 5 years. Herein, we report for the first time in Brazil, dengue serotype 4 virus of genotype I infecting a mosquito. This study was part of a dengue virus surveillance performed in Tancredo Neves, District of Manaus, 2008-2010, where 3240 mosquitoes were captured, identified and grouped into 324 pools. RNA extracts of mosquito pools were tested by a Reverse Transcription-Polymerase Chain Reaction (RT-PCR), followed by a Nested PCR, assays for detection and identification of flaviviruses. Flavivirus amplicons of putative dengue serotype 4 were obtained from 1 pool of Aedes aegypti. The nucleotide sequence of the amplicon, with ~980 pb showed it was dengue serotype 4 of genotype I, a virus previously only found in Asia but described in Manaus since 2008. This virus was probably introduced into Manaus as a consequence of the extensive trading of Manaus city with Asian countries and our results show that it has been transmitted by Aedes aegypti.

HV1103 - IMMUNOGENICITY DETERMINATION OF THE INFLUENZA VIRUS VACCINE/2011 IN THE POPU-
influenza B strain studied (68%) in the period pre-vaccination. The positive values were considered for dilution above 1:40. The negative ones for dilution below and including 1:40. 179 samples analyzed, the Influenza Vaccine/2011 stationary title was high in the study population, in any of the three components of the vaccine cultures. It was noted, therefore, that the influenza A/California/7/2009 (H1N1), A/Perth/16/2009 (H3N2) and B/Brisbane/60/2008 had already been circulating in the population before administration of the vaccine. Brazil has continental dimensions and great variety of climate, which influences the respiratory viral outbreaks. It is necessary to evaluate a change at the time of vaccination in Maceió or even in Northeast of Brazil. Financial support: Lacen/AL.

HV1104 - DETECTION OF ADENOVIRUS IN HOSPITALIZED CHILDREN WITH ACUTE RESPIRATORY INFECTION IN THE STATE OF PARA, BRAZIL


2. Instituto Evandro Chagas, IEC, BR 316, Km 7. Ananindeua - Pará E-mail: rebeccablack_15@hotmail.com

Introduction: Acute respiratory infections (ARI) remain a major cause of morbidity and mortality worldwide especially in children under five years old and in less developed regions such as north of Brazil. As viruses are important etiologic agents of such infections, it is necessary to monitor these pathogens such as adenovirus (AdV). The objective of this study is to detect adenovirus in children hospitalized with acute respiratory infection in Pará. Material and methods: From July 2009 to June 2010 were randomly selected, with a sampling error of 5%, 178 samples of nasopharyngeal aspirate or combined oral plus nasal swab collected from children aged zero to five years old, hospitalized with respiratory infection without pandemic H1N1 influenza virus. The study of AdV was performed on samples from 21 districts of the Pará State by polymerase chain reaction in real time, followed by sequencing of positive samples. Results: In total, 18.54% (n = 33) of samples were positive with no prominence in any age group. The positive samples represented 100% (n = 2) of the cases referred from Parauapebas, 31.25% (n = 5) of the Ananindeua and 19.55% (n = 26) of Belém. During the months of February to May 2010 were identified 61% (n = 20) of positive cases, more frequently (30.3%, n = 11) in March 2010, however, no difference (p = 0.6663) between the frequencies of July to December 2009 (21.31%, n = 13) and January to June 2010 (17.39%, n = 20). Among the positive samples were identified AdV-3, AdV-5 and AdV-6. Conclusion: In Pará State, the AdV were present in approximately 20% of children aged zero to five years admitted with acute respiratory infection without pandemic H1N1 influenza virus, demonstrating the importance of monitoring this virus in order to prevent outbreaks of unusual types. Financial support: Fellowship Program for Scientific Initiation of UFPA (PIBIC-UFPA/FAPESPA/CNPq).
HV1105 - HYPERENDEMIC CIRCULATION OF DENGUE VIRUS IN SAO JOSE DO RIO PRETO IN THE PAST TWO YEARS


1. Universidade Estadual Paulista Júlio de Mesquita Filho, UNESP, Rua Cristóvão Colombo, 2265, Jardim Nazareth. São José do Rio Preto

2. Faculdade de Medicina de São José do Rio Preto, FAMERP, Av. Brigadeiro Faria Lima, 5416 - Vila São Pedro, São José do Rio Preto, SP

3. Secretaria de Saúde de São José do Rio Preto, SP, E-mail: taty_ec@hotmail.com

Dengue is the most common arboviral infection worldwide and it is caused by four distinct serotypes (DENV 1-4). Sao Jose do Rio Preto (SJRP) has been presenting a hyperendemic circulation of DENV since 2008, when three serotypes started circulating in the city. This is a report of DENV transmission in SJRP from October 2010 to June 2012. We used serum samples of suspected and confirmed DENV patients provided by the Health Secretariat to profile DENV circulation. The viral surveillance was performed using Multiplex RT-PCR with Flavivirus generic primers based on the non-structural protein (NS5), followed by Nested assays with species-specific primers for the identification of DENV 1-4. We examined 46 samples in 2010, 431 in 2011 and 76 until June 2012. There were 378 cases officially confirmed in SJRP from October 2010 to June 2012. Our PCR results showed that DENV-1 was the main circulating serotype in both years. We examined 551 samples and 383 were positive for DENV (341 were positive for DENV-1, 21 for DENV-2, 20 for DENV-4). A co-infection of DENV-1 with DENV-4 was detected in one patient. DENV-1 was the first and only serotype to cause autochthonous DENV cases in SJRP from 1990 to 1998. Only in 2008, DENV-1 was detected again in SJRP. It was also the main serotype in the 2011 outbreak. DENV-2 was introduced in the city in 1998 and DENV-4 in 2011. Thus, we show that three different serotypes of dengue have been detected in the city in different proportions and the impact of this hyperendemic circulation should be further evaluated for epidemiological purposes.

HV1109 - HPV 6 IN ONE CASE OF INVASIVE CERVICAL CANCER: ANALYSIS OF BIOMARKERS


1. Fundação Oswaldo Cruz, Fiocruz, Av. Brasil, 4.365 - Manguinhos, Rio de Janeiro, Brazil

2. Johns Hopkins University, JHU, Baltimore, EUA

3. São Paulo University , USP, Av. Dr Enéas Carvalho Aguiar, 470 São Paulo, Brazil

4. Instituto Fernandes Figueira Institute , IFF, Niterói, Brazil

5. OHI University Hospitals, , Columbus, OH, USA
6. Laboratório Fonte de Medicina Diagnóstica, FONTEMED, E-mail: sergioafilho@gmail.com

Single low-risk HPV infections in high-grade lesions are rare. Some studies have reported low-risk HPV DNA in high-grade lesions but they are often co-infected with high-risk HPV types. HPV E6 and E7 viral oncoproteins can form specific complexes with tumor suppressor proteins that are capable of changing mechanisms of the cell cycle, modifying the expression of cellular proteins. p16INK4a, Ki-67, and more recently MCM-2 proteins have been associated with tumor aggressiveness, showing overexpression in high-grade lesions and invasive cervical cancer (ICC). Conversely, p53 has often been associated with low expression in ICC. The goal of this study is to report an unusual case where a single infection with low-risk HPV (type 6) is observed in an invasive cervical cancer sample and to analyze the expression of p16, Ki-67, MCM-2 and p53 in this rare case. One invasive cancer specimen was analyzed by means of immunohistochemistry for p16, Ki-67, MCM-2 and p53. HPV DNA was detected by PCR following the genotyping by sequencing. Additionally, INNO-LiPA and PapilloCheck Kit were used in order to confirm the single HPV type infection. The single HPV 6 infection was confirmed by the three techniques: automatic sequencer, INNO-LiPA and PapilloCheck. The markers related to proliferation, Ki-67 and MCM-2, were overexpressed with a mean of 65% and 35% positive cells per field, respectively. The literature has reported high p16 positivity but low-level expression of p53 in ICC. Curiously, in this case p16 was reported negative, while p53 was overexpressed showing a mean greater than 90% of positive cells per field. Consideration should be given to alternate pathways leading to virally induced carcinogenesis. Other factors such as polymorphic or epigenetic events may play a role in an association with cervical cancer. LIPMED - IOC – Fiocruz; CAPES; Fogarty International Center/US – NIH. Pós-Graduação em Biologia Celular e Molecular – IOC – Fiocruz.

HV1116 - ISOLATION OF DENGUE 4 IN SAO JOSE DO RIO PRETO, SÃO PAULO, BRAZIL


1. Universidade Estadual Paulista Júlio de Mesquita Filho , UNESP, Rua Cristóvão Colombo, 2265, Jardim Nazareth. São José do Rio Preto

2. Faculdade de Medicina de São José do Rio Preto , FAMERP, Av. Brigadeiro Faria Lima, 5416 - Vila São Pedro, São José do Rio Preto, SP E-mail: taty_ec@hotmail.com

DENV-4 had a brief circulation in Brazil in 1982 in the Northwestern region of Brazilian Amazon in a focal epidemic. No further cases of infection had been registered in the country until 2008, when the virus was detected in three patients, who had no international traveling history, in Manaus. DENV-4 reemerged in the country in 2010 in the municipalities of Boa Vista and Cantá in Roraima State, spread to different geographic regions of Brazil. In the present work about DENV-4 transmission in SJRP from 2009 to 2012, we used serum samples of suspected and confirmed
DENV patients provided by the Health Secretariat to profile DENV circulation. The viral surveillance was performed with Multiplex RT-PCR using Flavivirus generic primers based on non-structural protein (NS5), followed by Nested assays with species-specific primers and cultured cells of Aedes albopictus for the identification and confirmation of DENV-4. We examined 383 positive samples for DENV and 20 were positive for DENV-4. Due to the availability issues, only 14 have been subjected to specific RT-PCR reaction for the envelope gene for DENV-4. Fragments were purified from PCR mixtures and sequenced using the BigDye v3.1 (Applied Biosystems, USA) in a ABI3130 automatic sequencer. The nucleotide sequences were analyzed using the DS Gene 2.0 Software and were confirmed as envelope gene for DENV-4. The reemergence of DENV-4 should be a concern for health authorities since there are evidences that the replacement of a dominant circulating genotype is associated with the rising of a previously rare lineage.

**HV1121 - IN VITRO ANTI-ROTAVIRUS ACTIVITY ASSESSMENT OF CELASTRACEAE FAMILY CONSTITUENTS AND EXTRACTS USING MA-104 CELLS**


1. Universidade Federal de Ouro Preto, UFOP, Campus universitário Morro do Cruzeiro, s/n, cep 35.400-000, Ouro Preto, MG

2. Universidade Federal de Minas

Gerais, UFMG, Av. Antônio Carlos, 6627, Pampulha, Belo Horizonte, Minas Gerais

3. Universidade Federal de São João del Rey, UFSJ, Praça Frei Orlando, 170, Centro - São João Del Rei - MG, 36307-352 E-mail: bibo@ef.ufop.br

Rotavirus targets mainly children and infants from developing countries. It accounts for 30-50% cases of diarrhea and about 1.9 million deaths annually worldwide. Celastraceae family extracts and constituents were evaluated due to their medical potential as an infusion using MA-104 cells. Austroplenckia populnea and Maytenus gonoclada ethanolic and ethyl-acetate extracts of leaves branches and roots were assessed, in vitro, for their toxicity and antiviral activity. Studies associated their biological activity with triterpenes which was confirmed to be present in all tested extracts. Cytotoxicity was assessed using MTT method; thus CC50 was estimated as the concentration capable of reducing the cell viability in 50%. Extracts toxicity ranged from 25 to 152,7 µg/mL. Crude extracts dilutions, below CC50 threshold, were used for the antiviral evaluation. MTT was also used to determine cell viability. All experiments were done in triplicate and the viral infection happened prior to the extract exposure. 96 well plates were incubated at 37°C in a 5% humidified CO2 chamber for 72 hours. Results analysis expressed the concentration capable to inhibit 50% of viral induced death (CE50) these values ranged from 1701 to 24,74 µg/mL. All extracts, when tested in higher concentrations, presented activity against the Rotavirus. To determine whether they were safe or not we used...
SI, which is calculated as it follows: CC50/CE50. Only 3 extracts presented values above 1, being the highest 1,59. In this study we could assert that three of the tested compounds have potential to serve as antiviral drugs, new experiments could try to isolate molecules from this extracts in search of a more active and less toxic substance.

HV1123 - COMPARATIVE ANALYSIS OF BIOMARKERS IN DETECTING ADVANCED STAGES OF INVASIVE CERVICAL CANCER


1. Fundação Oswaldo Cruz, Fiocruz, 2. Johns Hopkins University, JHU, 3. Instituto Fernandes Figueira, IFF, 4. Ohio Medical University, OMU, 5. Laboratório Fonte Medicina Diagnóstica, INCTHPV, Universidade de São Paulo, USP, E-mail: sergioafilho@gmail.com

Cell cycle protein expression plays an important role in the pathology and clinical diagnosis of cervical cancer. However, few studies have attempted to correlate the use of these biomarkers with the clinical progression of the tumor. Objective: to analyze the expression of Ki-67, MCM-2, p53 and p16 in cervical cancer and to search for a differential expression that can assist in the assessment of clinical tumor staging according to FIGO classification. Methods: two blocks of Tissue Micro-Array containing 87 cervical samples from patients with invasive cervical cancer (ICC) and 43 controls were analyzed. Sociodemographic, behavioral and clinical characteristics of patients were obtained from the medical records. HPV DNA detection was done by PCR and in situ hybridization. By means of immunohistochemistry the markers were analyzed. Statistical analysis was performed by STATA 10.1. Results: a strong association (p<0.005) in advanced tumor stages (III and IV) was observed in women over 55 years, with more than four pregnancies and without school education. The prevalence of HPV DNA by PCR in the ICC was 94.3%. The most prevalent types found in the ICC were HPV16 (67.5%), followed by HPV33 (12.0%) and HPV35 (3.6%). An increase (p<0.05) expression of Ki-67, MCM-2, p53 and p16 was found in ICC compared to controls. Ki-67 and p16 showed strong expression in advanced disease, FIGO III and IV (p=0.008 and p=0.023, respectively). There was no association between the expression of p53 and MCM-2 with the tumor staging. Women with HPV16 tended to be younger (50.9 years; SE 1.9) compared to women with other types (59.9 years; SE 2.8), suggesting that HPV16-infected women develop cervical cancer earlier than the others. In conclusion, we found that only Ki-67 and p16 intensity was associated with the stage of the ICC. The most prevalent types found were HPV16, 33 and 35 suggesting that further studies should be considered for implementation of vaccination against HPV in Brazil. LIPMED - IOC – Fiocruz; CAPES; Fogarty International Center/US – NIH. Pós-Graduação em Biologia Celular e Molecular – IOC – Fiocruz.
HV1127 - SELECTION PRESSURE ANALYSIS OF THE HCMV TEGUMENT PROTEINS

Stangherlin, L.M., Braz, A.S.K., Silva, M.C.C.

Universidade Federal do ABC, UFABC, Rua Santa Adélia, 166. Bairro Bangu, Santo André - SP - Brasil. CEP 09.210-170 E-mail: lmsbotucatu@gmail.com

Human citomegalovirus is a betaherpesvirus present in 50 to 80% of the human population around the world. HCMV can cause important diseases in immunosuppressed individuals such as AIDS patients and transplant recipients. The viral particle is composed of a double strand DNA coated by an icosahedral capsid made of proteins, which is surrounded by a tegument layer comprised of proteins and RNAs. The capsid and the tegument are enclosed by a lipid envelope containing glycoproteins. The viral tegument contains 20 to 25 different types of proteins, present in many copies. Studies about occurrence of selection pressure in HCMV proteins are scarce, specially in the tegument proteins. In the present work we investigated sites of positive selection pressure in the pp71(UL82), pp65(UL83) and pp28(UL99) tegument proteins. We searched for sequences in databases through the BLAST tool and sites under selection pressure were analyzed considering changes in codons and amino acids. Our results so far demonstrated that positive selection occurs more frequently on the UL99 ORF that encodes the pp28 protein, responsible by the final acquirement of the envelop. In addition sites of positive selection in the pp28 are concentrated at the C terminal region of the protein.

The low frequency of positive selection at the amino terminus is probably due to the fact that this region of the protein is important for virus assembly and therefore must be conserved. These results suggest that the C terminal region of pp28 that is under selective pressure can have an important function that confers advantage for the viral life cycle.

HV1129 - ISOLATION, IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF DENGUE VIRUS FROM THE DEMAND OF PATIENTS WITH FEVER, SERVED IN INSTITUTO EVANDRO CHAGAS

Vasconcelos, N.K.N.S.
E-mail: kissnogueira@gmail.com

Mosquitoes infected with Dengue virus (DENV – Flaviviridae, Flavivirus). Antigenically, four serotypes can be distinguished within DENV species (DENV 1-4) and are all known to cause similar clinical presentations, ranging from asymptomatic infection, dengue fever, and also severe cases, such as dengue hemorrhagic fever and dengue shock syndrome (DHF/DSS). In order to report the current DENV serotypes circulations and their genotypes at the metropolitan area of Belem (Pará state, Brazil), we sought to isolate DENV from human samples (blood and sera) and genetically characterize them. Human samples were derived from patients admitted during 2010-2012 period by the Medical Care Sector from Instituto Evandro Chagas (Ananindeua, Pará, Brazil). Therefore, one hundred samples from symptomatic patients (up to the five day of onset) were included in the study. Ten patient of them were selected for nucleotide sequencing, and phylogenetic analysis.
the describe the genotype circulating. Moreover, information from all patients were also analyzed regarding gender, age, place of living, employment, and clinical symptoms. Hence, we obtained 100% of DENV isolation confirmed by immunofluorescence assay, from which 21% were DENV1, 41% DENV2, and 38% DENV4. Afterwards, we hate partially sequenced four DENV1, two DENV2, and four DENV4 isolates. Phylogenetic analysis revealed DENV1 strains as belonging to genotype V (Americas/Africa), whereas DENV2 strains were considered as a distinguished lineage within the American/Asian genotype, and DENV4 strains were included into genotype II. Altogether, we identified the main circulating serotypes at the metropolitan area of Belém as well as indicated their genotypes that are more likely to be infecting this population.

HV1140 - THREE-DIMENSIONAL MODELS OF NS3 PROTEASE CONTAINING PRIMARY RESISTANCE MUTATIONS TO PROTEASE INHIBITORS FROM CHRONICALLY INFECTED HCV PATIENTS


1. Depto Clínica Médica, Universidade Federal do Rio de Janeiro, HUCFF-UFRJ, R. Rodolpho Paulo Rocco, 255, 9o andar, Serv de Hepatologia, Ilha do Fundão, RJ

2. Instituto Nacional para Pesquisa Translacional, INCT-INPeTAm/CNPq, Av. Carlos Chagas Filho, 373, CCS, bl G, sl G1-050, Ilha do Fundão, RJ

3. Instituto Biofísica, Universidade Federal do Rio de Janeiro, IBCCF-UFRJ, Av. Carlos Chagas Filho, 373, CCS, bl G, sl G1-050, Ilha do Fundão, RJ

4. Instituto Federal de Educação, Ciência e Tecnologia do RJ, IFRJ, Rua Pereira de Almeida, 88, Pça da Bandeira, RJ

5. Instituto Biologia, Universidade Federal do Rio de Janeiro, IB-UFRJ, Av. Carlos Chagas Filho, 373, CCS, bl A, sl 121, Ilha do Fundão, RJ E-mail: luisa@biof.ufrj.br

New compounds are being considered for anti-HCV therapy, mainly NS3/4A protease inhibitors (PIs). Previously, we have analyzed the genetic diversity of HCV NS3 protease from patients treated with peg-interferon (PEG-IFN) and ribavirin (RBV). We have positively correlated the outcome of the treatment to some characteristics of the predominant NS3 virus sequence. Also we have found reported resistance mutations to the PIs (telaprevir and boceprevir) in 3 (4.4%) of the 68 patients, at least one of the mutations V36L, T54S and V55A in naïve as well as during treatment with PEG-IFN and RBV. We aimed to identify possible resistance mutations to new PIs by means of three-dimensional (3D) prediction models. The NS3 protease region from HCV were amplified and sequenced from patients serum obtained before and during treatment with PEG-IFN and RBV. The sequences were analyzed using the softwares Geneious version 4.7.5 and MEGA version 4.1. The 3D NS3/4A models were predicted by comparative molecular modeling using software MODELLER and analyzed using software PyMol.
The genetic diversity and phylogenetic analysis were assessed using H77 as the reference sequence and the comparative modeling was done using 2FM2 as the reference structure. In 3D models we can observe some amino acid alterations near Serine-139. This is one of those amino acids from protease active site suggesting that it can interfere at PI and NS3/4A protease active site ligation and consequently interfering in treatment outcome. We plan to investigate docking of these new protease inhibitors in these predicted models. This approach may suggest which patients will not benefit from treatment with these drugs. Moreover, we are performing next generation sequencing to assess viral quasispecies.

HV1142 - EVALUATION OF HAV RAPID TEST FOR DIAGNOSIS AND EPIDEMIOLOGICAL PURPOSES


Fundação Oswaldo Cruz, Fiocruz, Av. Brasil, 4365 - Manguinhos, Rio de Janeiro E-mail: wanessa_cristiny@hotmail.com

Hepatitis A is an acute infectious disease transmitted by fecal-oral route. The diagnostic routine is performed by detection of anti-HAV IgM antibodies in serum samples through EIA tests. To facilitate the diagnosis and epidemiological studies, it would be reasonable the use of a rapid test that is ease of implementation, not requiring the use of equipment and the result is generated within minutes using a visual reading. This feature would be relevant to bypass many difficulties mainly in regions with difficult access and in outbreaks. The aim of this study was to evaluate the accuracy of a commercial rapid test (SD Bioline HAV IgG/IgM®) for diagnosis and prevalence of hepatitis A. In this study 256 samples were tested, 152 were serum samples collected from patients (mean age of 23 y/o) involved in a hepatitis A outbreaks; and 111 were plasma samples obtained from whole-blood donors used to establish the accuracy of the rapid test for epidemiological study. Anti-HAV IgM and IgG were detected in these samples by rapid test and the results were compared with EIA results (standard gold). Among samples from outbreak, anti-HAV IgM were detected using rapid tests and the results were also compared with EIA results. For anti-HAV IgM, the rapid test showed a sensitivity of 95.5% (95% IC 0.84 to 0.99) and a specificity of 100% (95% IC 0.54 to 1.0). While for anti-HAV IgG it was observed a sensitivity of 77.8% (95% IC 0.84 to 0.99) and a specificity of 100% (95% IC 0.54 to 1.0). In the samples from blood donors it was observed for anti-HAV IgG a sensitivity of 18.3% (95% IC 0.037 to 0.32) and a specificity of 100% (95% IC 1.0 to 0.88). The high sensitivity and specificity achieved in the diagnosis of acute hepatitis A (IgM anti-HAV) by rapid test suggest that this assay could be used as an alternative tool for diagnosis of this infection and that it is especially applicable during outbreaks. However, the low sensitivity and specificity detected for anti-HAV IgG indicates that it is not appropriate test for prevalence studies of HAV.

HV1144 - REPORTED DENGUE CASES IN THE CITY OF CARUARU-PE, FROM 2001 TO 2010

Albuquerque, A.C.C., Bezerra, J.S.L., Silva, M.R., Paiva, M.H.S.
Background: Dengue is currently the most important arboviral disease that affects humans and is a major public health problem worldwide. Objective: To evaluate epidemiological data on dengue in the city of Caruaru-PE, from 2001 to 2010. Methods: Data regarding reported and confirmed dengue cases from 2001 to 2010 were obtained from the Municipal Health Department of Caruaru-PE. Collected data were: reported and confirmed dengue fever cases, according to the month of onset symptoms, age, sex and deaths due to dengue. They were stored and analyzed in Excel 2007 and results were presented as graphs and tables. Results: The most problematic years regarding dengue cases, from the period 2001 to 2010, in the City of Caruaru-PE, were: 2002, 2003, 2007 and 2010. Regarding these four years ago, the number of reported cases exceeded 34.4%, 28.7%, 73% and 81.5% of confirmed cases. Females and aged 30 years or older were the most committed. In the study period there were nine deaths due to dengue, in which five occurred in 2007. Conclusions: The years 2002, 2003, 2007 and 2010 were years of epidemic disease in Caruaru-PE, however in 2002 there was a greater number of confirmed cases of the disease. The months from February to May were when most occurring signs and symptoms of the disease, probably due to more rainfall. In Caruaru, despite several educational campaigns to eradicate the Aedes aegypti mosquito, dengue cases are still observed in the city because it is an endemic disease in the region. For dengue control, epidemiological surveillance should be strengthened, increasing the predictive ability of risk factors and early detection of disease outbreaks as well as improve the quality of actions against the vector. Financial support: Secretaria de Saúde de Caruaru-PE

HV1145 - MOLECULAR EPIDEMIOLOGY OF HIV-1 AND HIV-2 EPIDEMICS IN CAPE VERDE

Araújo, I.I.M.P., Guimarães, M.L., Bello, G., Vicente, A.C.P., Morgado, M.G.

1. Universidade de Cabo Verde, UNICV, Praça António Lereno - Praia, Santiago - Cabo Verde CP 379C
2. Instituto Oswaldo Cruz/Fiocruz, LabAids - IOC/Fiocru, Av. Brasil, 4365 - Pav. Leonidas Deane Sl. 413 Manguinhos, Rio de Janeiro - RJ
3. Instituto Oswaldo Cruz/Fiocruz, Lab. Genética Molecu, Av. Brasil, 4365 - Pav. Leonidas Deane Sl. 607 Manguinhos, Rio de Janeiro - RJ E-mail: isabel.araujo@ioc.fiocruz.br

Cape Verde is an archipelago of 10 islands located in Western Africa. Serological studies indicate the co-circulation of both types of HIV in Cape Verde, with a predominance of HIV-2 to the mid-90s, to later be overcome by HIV-1. The objective of this study was to characterize the molecular profile of the epidemics and identify the predominant HIV-1 and HIV-2 subtypes...
circulating in Cape Verde. A total of 89 HIV-positive samples (HIV-1 = 60, HIV-2 = 29) from patients living in Cape Verde collected from 2004 to 2011 were analyzed. Molecular subtyping was based on the pol (PR/RT) region and subtype determination was performed by phylogenetic inferences and bootscan analysis. Among the 60 HIV-1 patients, 70% were female and 28% were male, the mean age of this group was 37 years (SD=15.4 years). Among the 29 HIV-2 patients, 62% were female and 34% male, the mean age was 49 years (SD=16.1 years). On the basis of pol sequence analysis, the 60 HIV-1-positive specimens were classified as subtypes G (35.0%), CRF02_AG (33.3%), A (8.3%), F1 (6.7%), URF_CRF02/CRF06 (6.7%), B (3.3%), C (3.3%), CRF05_DF (1.7%) and CRF49_cpx (1.7%). All HIV-2-positive specimens belonged to subtype A. According to this analysis, the HIV epidemic in Cape Verde is dominated by HIV-1 subtypes G, CRF02_AG and A, and HIV-2 subtype A, similar to the molecular epidemiologic scenario observed in some West African countries.

HV1164 - SEQUENCE ANALYSIS OF HEPATITIS C VIRUS AND ITS IMPLICATION TO THE MOTHER-TO-CHILD TRANSMISSION

Dias, T.T., Passini, S.S.S., Zarife, M.A.S., Reis, M.G., Silva, L.K.

1. Gonçalo Moniz Research Center (Fiocruz-BA), CPqGM/Fiocruz-BA, Rua Waldemar Falcão, 121, Candeal - Salvador/BA CEP: 40296-710

2. Professor José Maria de Magalhães Netto Reference Maternity , MRPJMMN, Rua Marquês de Maricá, s/nº. Pau Miúdo. Salvador/BA. CEP: 40320-350

3. Central Public Health State Laboratory, Lacen-BA, Rua Waldemar Falcão, 123 – Candeal – Salvador/Bahia. CEP: 40296-710

4. Northeast Biotechnology Network, Renorbio, Av. Paranjana, 1.700 - Campus do Itaperi. UECE. Fortaleza/CE. CEP: 60740-000 E-mail: tamitdias@gmail

Mother-to-child transmission (MTCT) is the main pathway of hepatitis C virus (HCV) infection in children. The aim of this study was to evaluate the molecular basis of the HCV MTCT. A cross-sectional study was conducted in the Prof. Jose Maria Magalhaes Netto Reference Maternity Hospital, Salvador-BA and blood from mother and from the umbilical cord was screened using an anti-HCV rapid test. HCV-RNA detection was performed for diagnostic confirmation. Different subgenomic regions of the HCV (5UTR, E1/HVR1, E2) were amplified for sequence analysis. From a total of 3,254 screened pregnant women, six (0.18%) tested HCV-RNA detectable in the serum (Amplicor, Roche). MTCT was identified in two newborns (33.3%), showing evidence of HCV intrauterine transmission. This was confirmed through phylogenetic analyses. Viral sequences from mother and newborn showed high similarity between each other in all regions, including the E1/HVR1. It was also shown a high similarity between the isolates from the two mothers who transmitted the HCV to their newborns. This may
戊型肝炎：HV

2012年10月 - 伊瓜苏市，巴拉那州，巴西

XXIII巴西病毒学大会及
VIIMercosur病毒学会议

Human Virology: HV

2012年10月 - 伊瓜苏市，巴拉那州，巴西 - 海报 - 人类病毒学

HV1167 - 监测和监测在巴西2011
到2012年流感病毒株对抗


1. Instituto Oswaldo Cruz - Fundação
Oswaldo Cruz, IOC - FIOCRUZ, Av. Brasil, 4365 Manguinhos, Rio de Janeiro, RJ - Pav. HPP - B105 CEP: 21040-360

2. Laboratório Central de Saúde
Pública do Rio Grande do Sul,
LACEN-RS, Av Ipiranga, 5.400 –
Bairro Jardim Botânico – Porto Alegre/RS CEP: 90610-000

3. Laboratório Central de Saúde
Pública de Minas Gerais, LACEN-
MG, Rua Conde P. Carneiro, 80,
Gameleira, Belo Horizonte MG -
FUNED - CEP: 30510-010

4. Laboratório Central de Saúde
Pública de Santa Catarina, LACEN-
SC, Rua Felipe Schmidt, 788,
Centro, Florianópolis, SC. CEP: 88010-002 E-mail: paolabmrj@gmail.com

抗病毒药物代表了防止流感的重要线。当

指示病毒基因中存在可变性。识别共

同的病毒双原体可导致疫苗候选抗原

识别和开发中和抗体并防止传播。财

政支持：CAPES, PRONEX PNX0017/2009.

Antiviral drugs represent an important
do line of defense against influenza. As

virtually all current circulating strains

of influenza are resistant to M2 proton
channel blockers, neuraminidase
inhibitors (NAI) represent the main
class of drug in clinical use. The NAI
oseltamivir (OST) has been widely
used since the 2009 pandemics, and
increasing reports of OST resistance
have been registered thereafter. In
Brazil, we have been studying influenza
virus in a population in which resistance
was likely to emerge, individuals
that underwent hospitalization, that
are immunocompromised or have
comorbidities. In our previous letter,
we have described that OST resistant
strains of pandemic influenza A virus
(H1N1pdm09) were not detected in
2009-2010 – suggesting that if this
variant was circulating in our country,
it would be at very low frequency.
Here, we describe our continuous
surveillance for NAI resistant strains of
H1N1pdm09 southern, southeastern,
northeastern and north regions of
Brazil from 2011 (n = 142) to 2012 (n =
100). And the large majority of samples
were from southern (82.39 % in 2011
and 76.25 % in 2012) region of Brazil.
Neuraminidase gene was sequenced
by Sanger or by pyrosequencing for
residues associated with NAI resistance
or decreased sensitivity. Viruses were
also isolated in cell culture to perform
functional assays of NAI resistance. We
found that the vast majority of samples
were sensitive to OST, with an median
IC50 value of 2.23 ±1.2 nM. However,
in states at southern region of Brazil
in which influenza circulation is more
intense due to their geographical
localization at a temperate region, we
were able to detect H1N1pdm09 virus
with decreased sensitivity or resistant
to OST. In 2011 at the RS state, we found
1 virus carrying the S247N mutation,
and other 25 sequences with the V106I mutation, specially the former change has been associated with decreased sensitivity to OST in H1N1pdm09 virus. More recently, in 2012 at the SC state, we detected a sample with the SNP associated to OST resistance, H275Y. Due to the confirmation of a resistant virus, according to the WHO criteria, we investigated retrospectively, in samples not tested for OST resistance, the presence of the H275Y mutation. Indeed, we detected in 2009 at the RS state another H275Y mutation. Altogether, this report expands our laboratory-based surveillance for NAI resistance and confirms low frequency of OST-resistant virus detected in throughout the world.

HV1176 - COMPARATIVE ANALYSIS BETWEEN TWO MOLECULAR ASSAYS FOR THE DIAGNOSIS OF ACUTE DENGUE IN BRAZIL


1. Universidade Federal do Rio de Janeiro, Rio de Janeiro, UFRJ, Cidade Universitária, Rio de Janeiro, RJ

2. Universidade Federal do Rio de Janeiro, Rio de Janeiro/Macaé, UFRJ/Macaé, Departamento de Ensino, Universidade Federal do Rio de Janeiro, Macaé Laboratório Central de Saúde Pública Noel Nutels, LACEN, Rua do Resende, 118, Rio de Janeiro E-mail: marcelomeneses@micro.ufrj.br

Dengue virus (DENV) belongs to the genus Flavivirus, family Flaviviridae, and is represented by four distinct serotypes – DENV-1, 2, 3 and 4. The virus is associated with outbreaks of Dengue Fever, which is an important urban disease and became a major public health issue in many tropical Countries in the world. Currently, the World Health Organization estimates that 50-100 million people are infected annually with DENV worldwide with an estimated 5% cases of dengue hemorrhagic fever (DHF) and 0,5% deaths occurring. The laboratory confirmation of dengue infection is mainly performed by virus isolation, IgM anti-NS1 antibodies (serology) and viral genome detection (RT-PCR). Virus isolation, despite being the gold standard methodology, has a low sensitivity and high cost. The techniques currently used for the typing methodology of DENV at the Public Health Central Laboratory of Rio de Janeiro (LACEN-RJ) are virus isolation and conventional RT-PCR for the envelope glycoprotein. In this study, we intended to test a new commercial kit, the SimplexaTM Dengue Kit (Focus Diagnostics) developed for use in a Real-time RT-PCR platform (3M Integrated Cycler), and compare the results to the methods used at the LACEN-RJ. All the samples tested were from LACEN’s sera collection. The sera were previously screened using an ELISA NS1 Test (Panbio) and all positive samples that were negative by RT-PCR methodology were selected. We analyzed 47 samples and observed that SimplexaTM Dengue Kit was able to detect 34 samples (72%), typifying 5 samples as DENV-1 and 29 as DENV-4. We conclude that the Real-time RT-PCR tested in this study was successful in detecting and typifying samples that were negative for the current methodologies used. The data reinforces the use of Real
Time technology for more accurate detection and typing of DENV in the Reference Center. Financial support: CAPES, FAPERJ, CNPq, INBEB

**HV1179 - PREVALENCE AND VACCINATION AGAINST HEPATITIS B AMONG FEMALE SEX WORKERS IN GOIÂNIA, CENTRAL BRAZIL**


1. Faculdade de Enfermagem/UFMG, Faculdade de Enfermagem/UFMG, Pça Universitária Hospital das Clínicas/UFG, HC/UFG, S. Universitário, Pça Universitária Secretaria Municipal de Saúde de Goiânia-GO, SMS-GO, Secretaria Municipal de Saúde de Goiânia-GO, SMS-Jataí-GO, Jataí-GO

2. Laboratório de Virologia/IPTSP/UFMG, IPTSP-UFG, S. Universitário, Pça Universitária E-mail: marcosdeminas@yahoo.com.br

Female sex workers (FSW) are at high risk for hepatitis B virus (HBV) infection, that in turn has been cause of acute and chronic hepatitis, cirrhosis and liver cancer. In Brazil, there is few data on HBV infection among FSW. Thus the present study estimated the hepatitis B virus infection prevalence, compliance with and response to hepatitis B vaccine among female sex workers in Goiânia-GO, Central Brazil. Between May 2009 and June 2010, a total of 402 women were interviewed and tested for detection of HBV markers (HBsAg, anti-HBc total and anti-HBs) by ELISA (Hepanostika Uniform Organon Téknika B.V., Boxtel, Holland). Hepatitis B vaccination was offered to FSW found susceptible for HBV infection, and the vaccine doses were administered at their workplace. An overall HBV infection prevalence of 16.4% was found: 4 (1.0%) showed positivity to HBsAg, 65 (16.2%) to anti-HBc, and 164 (40.8%) to anti-HBs. 109 (27.1%) women showed positivity to anti-HBs only, suggesting previous hepatitis B vaccination. 227 FSWs (54.5%) were susceptible for hepatitis B, and 170 (75%) accepted the first vaccine dose. The second and third vaccine doses were administered in only 97 (57%) and 68 (40%) FSW, respectively. In 60 women, blood samples were available for quantitative detection of anti-HBs. Of them, 88.3% developed protective titers of anti HBs. The geometric mean titers of anti-HBs was 256.4 mIU/mL. These results reinforce the importance of Public Health programs including health education, health promotion. The low frequency of FSWs immunized and the low compliance with HBV vaccination highlight the need for strategies aimed to reach this population at high risk. Financial support: CNPQ

**HV1181 - IDENTIFICATION OF CIRCULATING DENGUE VIRUS TYPE 4 (DENV-4) IN RIO DE JANEIRO, BRAZIL AS A RESULT OF THE SURVEILLANCE SYSTEM**


1. Universidade Federal do Rio de Janeiro, Rio de Janeiro, UFRJ, Cidade Universitária, Rio de Janeiro, RJ
2. Universidade Federal do Rio de Janeiro, Rio de Janeiro/Macaé, UFRJ/Macaé, Departamento de Ensino, Universidade Federal do Rio de Janeiro, Macaé Laboratório Central de Saúde Pública Noel Nutels, LACEN, Rua do Resende, 118, Rio de Janeiro E-mail: renatacampos@micro.ufrj.br

In Brazil, DENV-4 was first detected during a short period in 1982 in the Amazon region and again only in 2008, in three patients without any traveling history. At the Rio de Janeiro State, DENV-4 was detected in 2011, during an outbreak of DENV-1 in Niterói City. Since the first reports about the circulation of DENV-4 in Rio de Janeiro, surveillance systems have supported the implementation of laboratorial methodologies capable of quickly characterizing the four dengue serotypes. According to the surveillance program, 10% of the samples from patients presenting fever for 5 days or less are directed to the State Central Laboratory (LACEN-RJ) for serological tests for the detection of NS1 Dengue virus protein, anti-Dengue IgM, virus isolation in C6/36 cells and serotype identification by RT-PCR. As a result of this surveillance, the first case of DENV-4 was detected in the City of Rio de Janeiro one day after the symptoms started in December 2011. The second case, about 26 Km distance from the first case, was attended with three days after the symptoms initiated and the sample was sent to LACEN in January 2012. The two cases were positive for NS1 ELISA, and both RT-PCR and RT-PCR Real Time identified DENV-4. The nucleotide sequencing of the structural polyprotein was performed for both samples. The two sequences had 99.3% homology, and also presented homology superior to 99% when compared to samples isolated from Roraima/2010, São Paulo/2011, Colombia and Venezuela in 2005, 2006 and 2007. This data suggests that the DENV-4 virus detected in the City of Rio de Janeiro is related to the virus that is circulating in Latin America. It is also important to note that the screening methodology based on NS1 detection for sentinel samples followed by PCR typing allowed an increase in sensitivity of virus isolation and serotype identification in the scenario of DENV-1 co-circulation. Financial support: CAPES, FAPERJ, CNPq, INEBEB.

HV1182 - INCIDENCE OF NOROVIRUS, ASTROVIRUS AND ADENOVIRUS INFECTION AMONG CHILDREN WITH ACUTE GASTROENTERITIS IN PORTO VELHO-RONDONIA, BRAZIL

Amaral, M.S.

CEPEM/ Fiocruz Rondônia, Fiocruz, Av.Guaporé 215, Bairro Lagoa, Porto Velho/RO E-mail: sandra.amaral@hotmail.com

Acute gastroenteritis is a common disorder in young children. It is associated with dehydration, a leading cause of hospital admissions in industrialized nations and a major source of mortality in developing countries. Enteric viruses have been identified as the most significant etiological agents of the disease, with four categories of viruses being considered clinically relevant: Group A rotavirus, norovirus, adenovirus, and astrovirus. With the exception of Rotavirus, whose importance has been well established in the medical community due to its high prevalence and worldwide impact, little is known
about the epidemiology of the other three groups of viruses. This study aimed to determine the incidence of infection the norovirus, adenovirus and astrovirus in children 0-6 years of age admitted with acute gastroenteritis to a public children’s hospital in the city of Porto Velho, Rondonia. Between the periods of February 2010 and February 2012 a total of 591 stool samples from children were analyzed for the presence of adenovirus type F40/41, using the enzyme immunoassay (ELISA) and confirmed by PCR methodology. Detection of norovirus and astrovirus was accomplished by RT-PCR using primers specific for each viral type. There were 98 cases (98/591) of enterovirus infection detected, with infection rates of 8.9% (53/591), 5.2% (31/591) and 2.3% (14/591) for norovirus, astrovirus, and the adenovirus respectively. 9.1% of the children were co-infected with norovirus and astrovirus. There was a higher incidence of infection in children ages 0-24 months. All children had typical symptoms associated with enteroviral infection, including diarrhea, vomiting and fever. Bloody stool was found in 16.9% (6/31) of the norovirus infected children, in 19.3% (6/31) of astrovirus infected and in 21.4% (3/14) of adenovirus infection. Viral infection was detected mainly in the months of February through May, the period corresponding to the rainy season in Porto Velho. A higher incidence of norovirus was detected in the periods of March and April of 2010 with 20.4% (20/98) of cases, with lower rates in the subsequent year. The data presented here may contribute to a better understanding of the role of enteroviral infection in the pediatrics population of Porto Velho, Rondonia and may be important in the strategic planning of control of the disease in this region. Financial support: Instituto Oswaldo Cruz-Fiocru-RÓ and CNPq.

HV1184 - EXPRESSION OF VARIABLE AND CONSERVED REGIONS OF CAPSID PROTEIN OF NOROVIRUS GII/4

Oliveira, L.M., Nagata, T.

Universidade de Brasília, UnB, Campus Darcy Ribeiro E-mail: layssamiranda@gmail.com

Norovirus genome consists of a single-stranded RNA which encodes six non-structural and two structural proteins. The capsid protein (VP1) gene contains variable and conserved region in its sequence. This study proposed the production of antibody of the variable and conserved regions of VP1 of norovirus GII/4 through the prokaryotic expression system. Protein expression in E.coli system of both regions was confirmed by Western blotting which showed high expression level of both after induction of 2 hours with IPTG. By solubility test the variable region was shown to be insoluble, hence denaturing condition was used for purification. These results confirmed that the system chosen for expression and purification was effective and it was expected for the development of immunoassay using these partial capsid proteins. Financial Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-CAPES.

HV1185 - A RETROSPECTIVE SEROLOGICAL SURVEY OF HANTAVIRUS INFECTIONS IN THE COUNTY OF CÁSSIA DOS COQUEIROS, STATE OF SÃO PAULO, BRAZIL
In recent years, hantavirus infections producing severe diseases have obtained an increased attention from public health authorities from the countries of Eurasia to the Americas. Brazil has reported 1,300 cases of hantavirus cardiopulmonary syndrome (HCPS) from 1993 to 2010, with about 80 of them occurring in the northeast of the State of São Paulo, with 48% fatality rate. Araraquara virus was the causative agent of HCPS in the region. Considering that hantaviruses causing human disease in the Americas were unknown until 1993, we have looked for hantavirus infections in the population of Cássia dos Coqueiros county, northeast of the State of São Paulo, Brazil, before this time. This county has about 2,800 inhabitants and an economy based on agriculture, including cultivation of Brachiaria decumbens grass. The grass seeds are an important rodent attraction, facilitating transmission of hantavirus to man. Four HCPS cases were reported so far in the county. In this study, 1,876 sera collected from 1987 to 1990 were tested for IgG to hantavirus by IgG-ELISA, using the N recombinant protein of Araraquara virus as antigen. Positive results were observed in 89 (4.7%) samples, which were all collected in 1987. The positivity among urban inhabitants was 5.3%, compared with 4.3% among those living in rural areas. Our results showed that hantavirus infections occurred in Cássia dos Coqueiros, completely unrecognized, even before hantaviruses were described in the Americas.

HV1187 - LABORATORY DIAGNOSIS BY PCR OF INFECTION CAUSED BY ORTHOPOXVIRUS IN BRAZILIAN SAMPLES IN THE PERIOD OF 1999 TO 2011

Gomes, G.M., Lancelloti, S.R., Barreto-Vieira, D.F., Barth, O.M.

E-mail: gabriellagomes@ioc.fiocruz.br

The Poxviruses are one of the most feared virus in the history of virology. The Poxviridae family comprises viruses that infect mammals, insects and brids. Among the genus belonging to this family four have the capacity to infect humans. The Orthopoxvirus which the Smallpoxvirus, the virus Vaccinia (used in vaccine production), Monkeypox, Cowpox, among others, is the most important genus related to diseases in humans and animals (Oliveira, 1994). The Poxviruses are one of the largest viruses already described. The viral particles can reach about 400 nm in length. These viruses have a complex morphology; the genome comprising a DNA molecule of linear single-stranded helix, and the conserved sequences stored in the central region (in Orthopoxvirus) (Schatzmayr & Barth 2005). This work was carried out with samples from several Brazilian states received by de Laboratório de Morfologia e Morfogênese Viral during a period of 12 years (1999 – 2011). A total of 104 samples (human and bovine) were subjected to the technique of polymerase chain reaction (PCR) for detection of Orthopoxvirus infection.
71% of bovine samples (74 samples) and 29% of human samples (30 samples). Among the bovine samples were obtained 46% of positivity (34 samples) while in human samples while the percentage positive was 77% (23 samples). According to the data analyzed during the entire period of study we concluded that the number of humans and animals infected with poxviruses has become constant. Thus we emphasize the need to use a rapid and accurate method of diagnosis, and that allows the identification and classification of infectious agents reliably, as well as its use as an epidemiological monitoring tool of viral dissipation in our country.

HV1189 - NORWALK VIRUS LIKE PARTICLES ASSEMBLY IN PLANTS USING VIRAL SUPPRESSOR PROTEINS OF POSTTRANSCRIPTIONAL GENE SILENCING

Souza, A.C., Bonnet, R.M.V., Inoue Nagata, A.K., Lacorte, C., Nagata, T.

1. Universidade de Brasília, UNB, Departamento de Biologia Celular, Laboratório de Microscopia Eletrônica.

2. Universidade Católica de Brasília, UCB, Campus Avançado Asa Norte SGAN 916 Módulo B B Avenida W5 - CEP: 70790-160

3. Bras Embrapa Hortaliças, CNPH, Km 09 BR 060, Rodovia Brasília-Anápolis, Caixa Postal 218, CEP 70359-970

4. Embrapa Recursos Genéticos e Biotecnologia, CENARGEN, Parque Estação Biológica - PqEB - Av. W5 Norte (final) , CEP 70770-917 Brasília E-mail: anitapatos@gmail.com

The virus like particles (VLPs) of norovirus (NV) is mainly produced by baculovirus expression system, however, this system is expensive as other cell culture systems. Aiming to use alternative system with high throughput quality but reduced cost, VLPs of NV was expressed using plants in transient manner. The transient expression of NV VLP in plant using binary vector has the disadvantage of low yields by RNAi machinery. To improve the production efficiency, the co-expression of plant virus suppressor proteins of post-transcriptional gene silencing (PTGS) was applied. Three candidates of PTGS suppressors: 126K of Pepper mild mottle virus (PMMov), Hc-Pro of Brugmansia suaveolens mottle virus (BsMoV) and AC2 of Tomato severe rugose virus (ToSRV), were co-expressed together with green fluorescent protein (GFP) using binary vector (pGreenII)-Agrobacterium infiltration system and evaluated their increasing abilities of GFP accumulation in plant. The 126K protein of PMMoV showed better GFP accumulation effect, and it was selected to NV VLP production. The NV capsid (CP) was also cloned in binary vector pBINPLUS and Agro-infiltrated with the construct of 126K of PMMoV in Nicotiana benthamiana. Three days after the infiltration, the leaves were harvested and VLPs were purified with sucrose gradient. The NV CP expression was confirmed by Western Blotting and the successful formation of NV VLPs by transmission electron microscopy. The antigenicity of these VLP will be evaluated by injecting these VLPs preparations in animal models in further study.
HV1191 - LOOP MEDIATED ISO-THERMAL AMPLIFICATION (LAMP) FOR DETECTION OF MAYARO VIRUS


Instituto Leônidas e Maria Deane/ Fiocruz Amazônia, ILMD/FIOCRUZ, Rua Teresina, 476, Adrianópolis, Manaus, AM, Brasil E-mail: victor@amazonia.fiocruz.br

The molecular diagnostic is dominated by polymerase chain reaction (PCR), although new techniques are emerging, such as Loop-mediated Isothermal Amplification (LAMP). This method does not require sophisticated equipment for thermal cycling or detection process, since the reaction occurs isothermal conditions, usually carried out in 1 hour. The positive reaction can be identified directly in a tube by naked eye, thus reducing the risk of cross-contamination. Mayaro virus (MAYV) is an RNA genome arbovirus which belongs to Alphavirus genus (Togaviridae family) that can cause a febrile syndrome in humans, characterized by rash and severe arthralgia, which can persist for months, being more disabling than Dengue. The virus is endemic to the Amazon region, where it can be detected in patients, vectors and animal reservoirs. The aim of this study was to evaluate the applicability of a methodology based on LAMP to detect the MAYV genome. All GenBank available MAYV complete sequences were used in an alignment for LAMP target selection. In order to standardize the initial reaction conditions a synthetic control was constructed carrying the selected target downstream of a T7 RNA polymerase promoter site. The limit of detection for the MAYV LAMP method was determined by testing serially 10-fold diluted copies of the synthetic control. Each dilution point was double-checked for accuracy by a MAYV real-time PCR previously developed by our group. Under the conditions tested so far, and with regards to detection of the amplification products by color change or electrophoresis on agarose gels, stained with Gel Red and observation under UV light, our results demonstrated a detection sensitivity about 103 copies per reaction. These indicate that the LAMP technique can be a useful tool for molecular diagnosis of MAYV virus, especially in resource-poor settings. Financial support: PPSUS / FAPEAM

HV1192 - MULTIPLEX REAL-TIME PCR FOR QUANTIFICATION OF EPSTEIN-BARR VIRUS, CYTOMEGALOMAVIRUS AND PARVOVIRUS B19 IN HUMANS


1. Instituto Leônidas e Maria Deane Fiocruz Amazônia , FIOCRUZ/ ILMD - AM, Rua Terezina, 476. Adrianópolis. Manaus - AM. CEP: 69057-070

2. Hospital Universitário Getúlio Vargas, HUGV, Av. Apurinã, N 4, Praça 14 de Janeiro, CEP: 69020-170, Manaus - AM E-mail: georgevillarouco@hotmail.com

Infections caused by Cytomegalomavirus are involved in complications with opportunistic infections, as Epstein-Barr virus, which may began indirect effects on host immune system in immunosuppressed patients, resulting an ample range of pathologies and clinical manifestations.
As well as, the Human parvovirus B19 can cause asymptomatic infections. We develop a multiplexed real-time PCR with high sensitivity and specificity to Epstein-Barr; Human parvovirus B19 and Citomegalomavírus viruses in humans. The sequences of each virus were selected from GenBank database and aligned for each target with purpose to select conserved regions for primers and probe design. The selected sequences were then used for synthesize a plasmid containing the chosen targets as an insert and was use as positive control encompassing both virus selected target regions. Due the assay developed to detection in humans, we also insert the target B-Actin as internal control positive. We calculate the DNA concentration based on size and amplicon sequence used for knowledge of the exact copy numbers of targets. The TaqMan Fast master mix was used in all qPCR amplifications with recommended cycling parameters and a volume of 2µL of template DNA in 20µL reaction, the PCR were performed with amplification efficiency approximately 101.18% in mean. According to the standard curve results and if considered Ct <37 as positive, the assay has a limit of detection for 2 copies (mean 36.5, 36.9 and 36.3 for EBV, B19V and CMV, respectively), the internal control B-Actin present in mean Ct = 36.1. We report the development of a sensitive and specific method for detection both viruses in multiplex real-time PCR in humans, the assay described here will help as a tool for viruses detection and assisting in the control other diseases, as autoimmune diseases, as well as, in cases of coinfection. Finacial Support Fundação de Amparo à Pesquisa do Estado do Amazonas, Conselho Nacional de Desenvolvimento Científico e Tecnológico and Instituto Leôndidas e Maria Deane Fiocruz Amazônia.

HV1193 - ANALYSIS OF THE EFFICIENCY OF THE TECHNIQUES IMMUNOFLUORESCENCE, “IN HOUSE” RT-PCR AND REAL TIME RT-PCR FOR DETECTION OF IN HUMAN PARAINFLUENZA VIRUS


UNIVERSIDADE FEDERAL DE SÃO PAULO, UNIFESP, R. Pedro de Toledo, 781. 15° andar - CEP 04039-032 E-mail: sheilaparmezan@hotmail.com

Sensitive detection of respiratory viruses is important for early diagnosis of infection. Highly sensitive methods are needed to detect respiratory virus infections in patients with few or no symptoms. Human Parainfluenza Virus (HPIV) is a common cause of viral infection in patients with hematologic hematopoietic stem cell transplantation (HSCT). The present study analyzed the diagnostic methods of immunofluorescence (DFA), “in house” Reverse Transcription-PCR (RT-PCR) and Real Time RT-PCR for HPIV 1, 2, 3 and 4 detection in patients with acute respiratory symptoms attended in a Sao Paulo tertiary hospital. Included patients were those who had a clinical picture of acute respiratory infection or possibly asymptomatic patients who have contact with patients presenting with infection. These patients were evaluated by an infectious disease physician who came into contact with the laboratory staff when there were suspected cases. We studied 202 nasal washes from patients (mean of 45 year of age, variation of 5 to 80
years) attended between March 2008 to December 2009 in a hematology ward or outpatient HSCT of São Paulo hospital. Among analyzed samples 4% and 10% were positive for HPIV by “in house” RT-PCR and real time RT-PCR, respectively, all positive samples by “in house” RT-PCR were positive in real time RT-PCR. Immunofluorescence did not result positive among samples. Considering the real time RT-PCR as gold standard sensitivity, specificity and accuracy of the “in house” RT-PCR method were 36% [95% confidence interval (CI) 19.7 – 50.0], 100% (95% CI 97.9 – 100), 93% (95% CI 88.7 – 93.5), respectively. This study retrospectively examined the detection of HPIV for three different diagnostic methods and pointed to the need highly sensitive methods to detect respiratory virus infections in immunocompromised patients for better understand the impact of parainfluenza viruses among high risk patients.

HV1194 - MOLECULAR CHARACTERIZATION OF BK AND JC POLYOMAVIRUSES DETECTED IN URINE SAMPLES OF RENAL TRANSPLANT PATIENTS AND HEALTHY INDIVIDUALS FROM THE SOUTH OF BRAZIL

Comerlato, J.

1. Universidade Federal do Rio Grande do Sul, UFRGS, Rua Sarmento Leite 500, Porto Alegre, CEP 90050-170, Rio Grande do Sul/Brasil

2. Instituto de Pesquisa Veterinária Desidério Finamor, IPVDF, Eldorado do Sul, CEP 92990-000, Rio Grande do Sul/Brasil

3. Universidade Feevale, Feevale, RS

The human polyomaviruses JC (JCV) and BK (BKV) are widespread in the human population. Following the primary infection, viral reactivation may lead to nephropathy and graft rejection in renal transplant patients (RTPs). This study was carried out to access the presence and the phylogenetic analysis of BKV and JCV DNA in urine samples collected from RTPs and healthy individuals in Porto Alegre, Rio Grande do Sul. Ninety-two samples from RTPs and 88 samples from control group were collected and submitted to a nested-PCR. Sequencing and molecular characterization was performed of selected positive samples. A significant higher frequency of BKV was found in RTPs (65.2%) in comparison to the control group (32.9%). JCV was equally detected in the RTPs (45.6%) and in the control group (36.4%). Phylogenetic analysis of both BKV and JCV amplicons show that all subtypes of BKV were found, whereas for JCV, four different groups are described (1, 2, 3 and 4). BKV is most closely related with RTPs than healthy individuals, whereas for JCV this association is not observed. The molecular detection proposed by this article could be an alternative tool to control of viruria in RTPs preventing the graft lost through the immunosuppressive therapy modulation. The phylogenetic analysis demonstrated circulation of different JCV and BKV variants in the study population which was described for the first time in the South of Brazil.

HV1195 - HEPATITIS A OUTBREAK IN A SEASIDE TOWN IN RIO DE JANEIRO
Mendes de Oliveira, J., Pinto, M.A., Miagostovich, M.P., Lewis, L.L., Melgaço, J.G., de Paula, V.S., Gaspar, A.M.C.

1. Instituto Oswaldo Cruz/IOC/FIOCRUZ, Av. Brasil 4365, Manguinhos - Pavilhão Helio e Peggy Pereira

2. Universidade Federal Fluminense, UFF, Laboratório de Desenvolvimento Tecnológico em Virologia, Laboratório de Virologia Comparada e Ambiental, Ambulatório de Hepatites Virais, E-mail: jackie@ioc.fiocruz.br

The incidence of hepatitis A virus (HAV) infection in Brazil has declined in recent years, with a progressive shift of the average age of infection towards late childhood and adulthood. Even though the global improvement of sanitation levels in Brazil, HAV still causes periodic outbreaks. In May 2012, approximately 150 cases of acute hepatitis A were reported in Mangaratiba, a seaside town in Rio de Janeiro state. Contamination of the public water supply was initially suspected by local public health care, which reported an increasing number of sanitation levels in Brazil, HAV still causes periodic outbreaks. In May 2012, approximately 150 cases of acute hepatitis A were reported in Mangaratiba, a seaside town in Rio de Janeiro state. Contamination of the public water supply was initially suspected by local public health care, which reported an increasing number of acute hepatitis A cases covering several localities at the First District of the town. We carried out investigations in order to characterize the etiological agent, identify the source of infection, and implement appropriate control measures. Sera from 63 symptomatic individuals and 86 asymptomatic contactants were tested for detection of HAV antibodies and RNA. Water samples, collected by the local epidemiological surveillance from 18 spots including public water supply and alternative sources, had no HAV detected by RT-PCR, after concentration by the Katayama’s method. Phylogenetic analysis of the VP1/2A region of HAV genome linked the outbreak to the IA HAV genotype, which was detected in 22 (88%) out of 25 sera from a subset of anti-HAV IgM positive patients. The detection of the IB genotype in seven individuals (three symptomatic and four asymptomatic, without epidemiological link) suggests the co-circulation of these two subgenotypes of HAV during the outbreak. The primary source of transmission of the virus could not be confirmed. However, the identification of acute cases of HAV asymptomatic infection (anti-HAV IgM negative, HAV RNA positive) among household contacts of patients with recent past infection (anti-HAV IgM positive) shows the secondary spread of the virus during the outbreak. Financial support: CNPq; IOC/Fiocruz.


Fundação Oswaldo Cruz, FIOCRUZ, Av. Brasil, 4365, Rio de Janeiro -RJ E-mail: moyramp@ioc.fiocruz.br

Nowadays, commercial molecular methods are available for Hepatitis B virus DNA (HBV DNA), but these methods are very expensive to be introduced in limited resource laboratories. This study aims to develop a real time PCR for quantification of HBV DNA among sera samples using taqman methodology. A total of 62 sera samples (32 HBsAg reactive and
30 HBsAg not reactive) was employed for validation of the method. DNA was extracted using a commercial kit (High Pure Viral Nucleic Acid kit, Roche Diagnostics, USA) according to manufacturer’s instructions. For HBV DNA quantification, primers and probes were constructed for surface region of HBV genome (position 593 to 672 nt) and amplified by taqman methodology using iCycler equipment (Biorad). The standard curve was constructed by cloning a sample from HBV Quantification Panel (OptiQuant, Acrometrix) that contains 2 x 10^7 copies HBV DNA/mL. A ten fold serial dilution of this curve (viral load varying from 10^-1 to 10^-10 copies per reaction) was employed in five PCR runs in a total of 40 reactions. After that, standard curve and sample volume, and melting temperature (TM) were evaluated. As results, standard curve presented linear regression coefficient of 0.997 and the method was able to detect until 20 copies of HBV DNA per reaction. A good reproducibility and specificity were observed based on coefficient of variation and absence of signal in negative controls, respectively. Best reaction conditions were observed when 1µL of standard curve, 5 µL of DNA and TM of 62°C were used. Using optimized protocol, HBV DNA was detected among 17 of 32 HBsAg reactive samples giving a median viral load of 1.51 X 10^7 copies HBV DNA/mL. HBV DNA was not detected among 30 HBsAg not reactive samples. Homemade real time PCR was effective for detection and/or quantification of HBV DNA and can be a tool for HBV diagnosis in limited resource laboratories.

**HV1215 - DIFFERENTIAL DIAGNOSIS OF ACUTE RESPIRATORY DISEASE IN PATIENTS PRESENTING INFLUENZA LIKE ILLNESS, BY REAL TIME PCR**


1. Instituto Adolfo Lutz, IAL, Av. Dr. Arnaldo 355, CEP 01246/902, São Paulo, SP,

2. Brasil Centers for Disease Control and Prevention, CDC, 1600 Clifton Road, N. E. Atlanta, GA 30333 E-mail: ttterezinha@uol.com.br

**INTRODUCTION** – The pandemic influenza occurred due to the influenza virus of type A (H1N1) pdm09 emerging has changed the respiratory viruses investigation scenarios. Taking into account the impact of acute respiratory diseases worldwide the development of laboratory methodologies towards to detect non influenza respiratory viruses is urged globally. **OBJECTIVE** - To improve the differential diagnosis of acute respiratory disease towards to provide public health answers facing respiratory disease outbreaks. **MATERIAL AND METHODS** - In the present study we selected 108 respiratory secretions collected from individuals presenting influenza like syndrome by the three National Influenza Network Sentinel Units (two located in São Paulo city and one in Guarulhos, SP) during January-June 2010. These samples has already presented negative results for influenza viruses type A and B, adenovirus, respiratory syncytial virus, parainfluenza viruses 1, 2, 3 by indirect immunofluorescence (IF) the routine assay to perform the differential diagnosis of acute respiratory illness. These negative samples were submitted
to a non-influenza respiratory virus test kit containing primers and probes for adenovirus (ADV), rhinovirus (RVs), respiratory syncytial virus (RSV), human metapneumovirus (hMPV), parainfluenza 1, 2, 3 (PV1, PV2, PV3) by using the Real Time PCR protocol developed at the Centers for Disease Control and Prevention. RESULTS - Of the 108 samples collected from individuals presenting acute respiratory illness symptoms, presenting negative results, 49 (45.37%) were positive by Real Time RT-PCR. The following etiologic agents has been identified: RSV 16 (32.60%); PV1 3 (6.10%); PV2 6 (12.30%); PV3 8 (16.30%); ADV 10 (20.40%); RVs 6 (12.30%). In addition, two samples has shown mix infection: ADV+RVs; RSV+RVs. This methodology also enable us to identify the etiology of acute respiratory disease in individuals that has been vaccinated with influenza seasonal vaccine; in these individuals rhinovirus and adenovirus infections has been detected. DISCUSSION - This study demonstrates the high sensibility of Real Time RT-PCR to attend the differential diagnosis; in addition to provide quickly answer to the public health authorities towards to apply the control and prevention measures timely. Financial support: Adolfo Lutz Institute, Secretariat of Health of São Paulo State, Ministry of Health of Brazil, Centers for Disease Control and Prevention, Atlanta, GA.

HV1219 - EVALUATION OF RAPID TEST FOR DETECTION OF HEPATITIS B VIRUS ANTIBODY (ANTI-HBS)


Viral Hepatitis Laboratory, Oswaldo Cruz Institute, LHV/FIOCRUZ, Laboratory of Technological Development in Virology, LDTV/FIOCRUZ, Clementino Fraga Filho Hospital, UFRJ, Federal University of Tocantis, UFTO, E-mail: leninhamedina@gmail.com

Being able to test for the presence of immunity against hepatitis B virus (HBV) could be important for prevention of this disease. Rapid test for antibodies against HBV (anti-HBs) detection would be very useful for large scale community studies, since there is no need of special equipments and result can be released in few minutes. The objective of this study was to evaluate the performance of commercial rapid test for anti-HBs detection in serum samples of individuals from high and low HBV endemicity. A total of 565 sera samples from individuals referred to Viral Hepatitis Ambulatories from Rio de Janeiro and healthy individuals from North and Southeast region of Brazil were included. Most of the individuals were female (53%) and mean age was 39.9 years (±18.9). Anti-HBs was detected using commercial enzyme immunoassay (EIA) (ETI-AB-AK-3, Diasorin, Italy) and rapid test (Imuno-Rápido anti-HBsAg, Wama, Brazil). The same sample volume was employed in both assays (100µL) whereas time of execution was 280 minutes to EIA and 20 minutes to rapid test. As results, anti-HBs was detected in 177 and 80 samples by EIA and rapid test, respectively. On the other hand, anti-HBs was not detected in 388 and 385 samples using EIA and rapid test, respectively. These results gave 82.3% of concordance, 99.2% of specificity and 45.2% of sensitivity of rapid test compared to EIA.
sensitivity was evaluated only among anti-HBs reactive samples comparing antibody levels, sensitivity increased to 86% showing more concordant results among samples presenting anti-HBs titers higher than 100UI/mL. It is concluded that rapid tests presented high specificity in this population, but the method was mainly sensitive among samples presenting high anti-HBs levels.

HV1221 - EVALUATION OF SEROPROTECTION AND SEROCONVERSION OF HIV-1 POSITIVE INDIVIDUALS VACCINATED WITH TRIVALENT INACTIVATED INFLUENZA

Martorelli, A., Fintelman-Rodrigues, N., Sacramento, C. Q., Grinsztejn, B., Camacho, L., Santini-Oliveira, M., Souza, T. M. L.,

Fundação Oswaldo Cruz, FIOCRÚZ, Rua Leopoldo Bulhões, 1480, Manguinhos, Pavilhão HPP, sala B109B, Rio de Janeiro.
E-mail: andressa.cbio@gmail.com

Individuals infected with human immunodeficiency virus (HIV) have an higher risk of being affected by serious diseases, such as respiratory virus infections, including the influenza virus. It has been described controversial clinical outcomes from these patients infected with pandemic influenza virus infection. Although the immunosuppression of these individuals may affect their ability to response to active immunization. Vaccination against influenza still represents the primary way of reducing the impact of this virus. Due to the circulation of pandemic influenza virus A/H1N1, A/H3N2 and B viruses, current vaccine composition include antigens from these three agents in its formulation. Thus, analysis of the impact of trivalent inactivated influenza vaccine (TIV) in HIV-1-infected individuals merits further studies. A cohort of 119 HIV-1-infected individuals with controlled viremia received two single doses of the TIV with 21 days interval. The anti-hemagglutinin titers of their sera was evaluated at the baseline, 21 and 42 days after vaccination. About 61%, 70% and 28% of individuals were already protected for pandemic influenza A/H1N1, A/H3N2 and B, respectively. Relative to the seroconversion after vaccination, we found that at day 21 86.1% of the individuals seroconverted for A/H1N1 and that at day 42, it reached 93.6% of seroconversion. In regard to A/H3N2 rates of seroconversion were 80.3% at day 21 and 81.8% at day 42. Finally, with respect to Influenza B, 67% and 70.3% of the individuals seroconverted 21 and 42 days after vaccination, respectively. Altogether, our results indicate that two doses of the TIV were more effective after for A/H1N1 virus. Further studies are being carried out to investigate the sustainability of the immune response.

HV1223 - GENOTYPING AND MOLECULAR CHARACTERIZATION OF STRUCTURAL GENES FROM SPECIE A ROTAVIRUS CIRCULATING IN NORTHERN BRAZIL BEFORE AND AFTER ROTARIX® VACCINE INTRODUCTION

Farias, Y.N., Soares, L.S., Mascarenhas, J.D.P., Gabbay, Y.B., Linhares, A.C., Leite, J.P.G.

2. Laboratório de Rotavírus, Seção de Virologia, SEVIR, SVS/MS, Br 316 Km 07 S/N - Levilândia - Ananindeua, Pará. E-mail: yasmin.farias@ioc.fiocruz.br

Specie A Rotavirus (RVA) is the leading cause of acute diarrhea in children under 5 years old, responsible for 453,000 deaths each year. The RVA genome is composed by 11 segments of double-stranded RNA (dsRNA), which encodes six structural (VP1-4, VP6-7) and six nonstructural proteins (NSP1-6). Recently, was proposed a new classification system based on whole-genome sequence analysis, providing important information about the genomic diversity of the RVA, such as reassortment events and interspecies transmission. This study aimed to characterize genes that encode for six VP’s from RVA genotypes G1, G2, G4 and G9 circulating in Northern Brazil, before and after Rotarix® vaccine introduction and has been approved by the Committee in Ethics and Research from the IEC. A total of 22 fecal specimens were selected between 1994 and 2010 from children hospitalized due to acute diarrhea, being 12 samples from pre and 10 post vaccine period. The viral dsRNA was extracted, reverse transcribed (RT), amplified by polymerase chain reaction (PCR) and the amplicons were sequenced. The sequences obtained were aligned using the BioEdit, compared to sequences available in GenBank and at phylogenetic analysis was performed using MEGA software. Based on the phylogenetic analyses, 45% (10/22) of analyzed strains were classified as G1P[8]; 36% (8/22) as G9P[8]; 14% (3/22) as G2P4 and one sample was classified as G4P[6]. The strain HSE005 showed close relationship with porcine origin prototype for the VP3 and VP6 genes, presenting M1-I3 genotypes, respectively. The G1 and G9 strains were related with R1-C1-M1 genotypes, for the VP1, VP2 and VP3 genes, respectively; and G2 strains were classified as R2-C2-M2. The current work is one of the few studies about molecular characterization of all structural RVA genes in Northern Brazil. These results help to increase the knowledge on the genomic diversity of RVA, aiming detect new variants and possible antigenic changes, whose potential effect on vaccine effectiveness should be studied. Financial support: CNPq, IEC, IOC-FIOCRUZ.

HV1224 - DEVELOPMENT OF A SENSITIVITY AND COST-EFFECTIVE REAL TIME PCR: MEASURING A WIDE RANGE OF HBV DNA CONCENTRATIONS


1. Instituto de Pesquisa em Patologia Tropical, IPEPATRO, Rua da Beira, 7671, Lagoa, Porto Velho-RO.

2. Centro de Pesquisa em Medicina Tropical, CEPÉM, Av. Guaporé, 215, Lagoa, Porto Velho-RO

3. Fundação Oswaldo Cruz Rondônia, FIOCRUZ-Rondônia, Rua da Beira, 7671, Lagoa, Porto Velho-RO. E-mail: alcione.m@hotmail.com

The quantification of HBV viral load is indispensable to start the treatment of patients and the following up of them, so quantitative assays must measure a wide range of viral DNA concentrations. For this reason,
the aim of this study was develop a sensitivity and low cost in house Real Time PCR method. A fragment with 109 bp was cloned and serial diluted to standard curve construction. The calibration of the HBV - DNA values was performed against OptiQuant® HBV-DNA Quantification Panel, AcroMetrix Europe B.V.). Specifically, serial dilutions of the standard ranging from 2 to 2x10^6 were tested. Based on a linear regression, a conversion formula was calculated for the in-house measurements (copies/mL) to the international standard units (IU/mL). The correlation between AcroMetrix kit and in house assay was performed by Pearson´s test, using GraphPad 5.0 to fit regression lines between IU/mL and copies/mL. The following equation was obtained: \[ \log(IU/ml) = -0.5249 + 0.6618 \log_{10}(\text{copies/mL}) \], consequently 1UI/ml = 6,21 copies/ml. These findings suggest that the performance of in-house assay is equally as well as the commercially available kit. To test assay’s sensitivity we used samples 30 negative from Rondonia blood bank and 26 indeterminate for HBsAg and 40 positives for HBsAg and HBeAg to Ambulatorio Specializes of Rondonia Viral Hepatitis tested previously by ELISA. They were performed again using AcroMetrix and the in house assay. The negative (n=30) and positive (n=40) samples were confirmed in both methods (AcroMetrix and in house) and unresolved cases (n=26) were identified as positive samples by AcroMetrix and also by in house test. These initial data suggest a 100% of sensitivity. The method used in this study suggests a lower final cost and it can be used as acid nucleic test to resolve indeterminate cases. On the same way it can be a tool for management of chronic HBV patients in Amazonic region. Consequently, the validation of this in house assay is the initial step for implementing on the blood banks’ trials and clinical routine. Finanical support: Sistema Único de Saúde (SUS); CNPq.

HV1225 - MOLECULAR CHARACTERIZATION OF NOROVIRUS GII.4 VARIANTS IN BRAZIL, 2005-2010
Fiocruz, Instituto Oswaldo Cruz, Fiocruz, IOC, Av. Brasil, 4365, CEP: 21040-360, Manguinhos, Rio de Janeiro. Pav. HPP, sl B203 E-mail: juliafior@ioc.fiocruz.br

The genus Norovirus belongs to Caliciviridae family and is divided into 5 genogroups (G), which GI, GII and GIV are described infecting humans. The GI and GII are the most prevalent, containing the the greatest diversity of genotypes described, and the GII.4 responsible for the majority of outbreaks worldwide. The norovirus (NoV) are non-enveloped, with icosahedral symmetry virus, its genome is composed by ssRNA with approximately 7.5 kb, divided into 3 open reading frames (ORF). ORF1 encodes non-structural proteins, ORF2 encodes structural protein VP1 that composes the viral capsid and ORF3 encodes structural protein VP2. The aim of this study was characterize the different genotypes and variants of GII.4 of NoV circulating in different Brazilian states and regions during the years 2005-2010. For this purpose were selected 265 stool samples from cases of acute gastroenteritis received in the Laboratory of Comparative and Environmental Virology, through
spontaneous demand, and previously diagnosed as NoV by polymerase chain reaction preceded by reverse transcription (RT-PCR). For the molecular characterization was performed partial sequencing of two regions of the NoV genome, region D for genotyping and P2 subdomain for characterization of variants GII.4. By sequencing the region D, 213 samples were characterized as GII (80.4%) and four GI (1.55). It was not possible characterize 48 samples (18.1%). Were detected the genotypes GL.2, GL.5, GL.8, GII.4, GII.6, GII.7, GII.12, GII.16, GII.17 e GII.20, and the most prevalent was the GII.4 (79%), found in 13 of the 15 states evaluated, followed by GII.6 (13%). This is the first description of the circulation of GL.5, GII.12, GII.16, GII.17 and GII.20 in Brazil. Posteriorly, by sequencing the region P2 were characterized five variants GII.4 called 2003, 2004, 2006a, 2006b and 2010, and the most prevalent was 2006b (54.4%), followed by 2010 (21.9%) and 2006a (17.5%). The characterization of a subcluster consisting of 22 samples within the 2006b variant suggests the emergence of a new variant. The high genetic diversity of NoV circulating in Brazil, as well as the possibility of the emergence of new variants demonstrates the need to establish a national network of surveillance that would make available information regarding the geographical and temporal spread of these viruses as it does other countries. Financial Support: IOC, CNPq, CGLAB/SVS-MS.

**HV1227 - SEVERE METAPNEUMOVIRUS INFECTIONS AMONG IMMUNOCOMPETENT AND IMMUNOCOMPROMISED PATIENTS**

Sinohara, J., Watanabe, A., Carraro, E., Granato, C., Bellei, N.
(70.8%) in 2008. Besides, HCoV-229E displayed a marked autumn seasonality while HCoV-HKU1 and HCoV-OC43 predominated in winter. Dyspnea was more associated with HCoV-229E infections (66.6%) and cyanosis was reported only in HCoV-OC43 infections. Our data provide an insight into the epidemiology knowledge of HCoVs among different subsets of patients, supporting the notion that HCoVs have different circulation trends and play an important role among patients with comorbidities. Financial support: FAPESP (nº09/17307-6 and 09/54640-5)

HV1231 - PAIRED ANALYSIS OF RNA DETECTION OF HEPATITIS C VIRUS (HCV) IN DRIED BLOOD SPOT (DBS) AND SERUM SAMPLES FOR REAL-TIME PCR


Fundação Oswaldo Cruz, Fiocruz, Av. Brasil, 4365 - Manguinhos, Rio de Janeiro E-mail: miss.maques@gmail.com

Several studies have demonstrated the importance of DBS samples as a good alternative specimen once it represents a non-invasive blood collection with no need of phlebotomist. Moreover, samples can be storage and sent to other laboratories without freezing. The present study aims to compare two commercial enzyme immunoassay (EIA) for anti-HCV detection among DBS samples. Ten individuals were selected and gave paired sera and DBS samples, where 5 were anti-HCV reactive and 5 were anti-HCV negative among sera samples by commercial EIA 1 (HCV Ab, Radim). DBS samples were assayed by EIA 1 increasing sample volume ten-fold compared to serum. Using commercial EIA 2 (Murex Anti-HCV kit, Abbot), manufacturer’s instructions and sample volume (five and nine-fold increase compared to sera) were evaluated. Using EIA1, 100% of concordance for anti-HCV detection was observed among sera and DBS. Using EIA2 among DBS samples according manufacturer’s protocol gave 80% of concordance compared to sera results, where two false negative results were obtained (mean OD value=1.13). When sample volume was increased 5 and 9- fold compared to sera samples, 100% of concordant results among sera and DBS samples were observed. Mean OD values for anti-HCV positive samples were 3.63 and 3.98 using five and nine fold increase, whereas mean OD values for negative samples were 0.070 and 0.226 using five and nine fold increase. It is concluded that both EIAs can be used for anti-HCV detection among DBS samples, but sample volume has to be increased. Financial Support: FAPERJ, CNPQ.

HV1237 - HEPATITIS A AND E VIRUS INFECTIONS IN WESTERN BRAZILIAN AMAZON


1. Universidade Federal Fluminense, UFF, Rua Professor Ernani Melo, 101, Centro, Niterói, RJ CEP: 24210-130

2. Instituto Oswaldo Cruz, (IOC/Fiocruz), Av. Brasil, 4365 - Manguinhos - Rio de Janeiro
To compare the epidemiologic profiles of hepatitis A virus (HAV) and hepatitis E virus (HEV) infections in Western Brazilian Amazon, the prevalences of HAV and HEV infections were studied in two Amazonian populations: (a) toddlers and preschoolers who live in a small border town (Acrelândia) and (b) preschool children, schoolchildren and adults living in an agricultural settlement (Granada) distant 30-50 km from Acrelândia, Acre, Brazil. A total of 1508 serum samples (1103 from the urban area and 405 from the rural area) were obtained in a population based survey conducted in 2007 and tested for anti-HAV antibodies. For HEV prevalence investigation, a sample of 364 individuals was randomly selected and tested for IgG anti-HEV. Anti-HAV was detected in 47.7% and 81.5% subjects from Acrelândia and Granada, respectively, whereas IgG anti-HEV was detected in only 10 individuals (2.8%). The anti-HAV prevalence of children aged five or less from both urban and rural areas was only 40-50%, increasing to 80% among those aged 10 or more. Seropositive IgG anti-HEV samples where all from subjects living at the rural area with ages ranging from 17 to 90 years old (mean ± standard deviation, 54.5 ± 2.8 years). HAV infection was shown to be highly prevalent in both population groups from the western Brazilian Amazon, although the anti-HAV prevalence observed among children has been lower than the one expected for endemic areas. This fact corroborates with several studies that have been shown a large proportion of Brazilian children under the age of five susceptible to HAV infection, even from low socioeconomic groups. On the other hand, in spite of being transmitted by similar routes, HEV infection was uncommon. The anti-HEV IgG prevalence observed was consistent with those found in other Brazilian studies that characterize the country as a non-endemic area for HEV infection. Financial support: CNPq, Finep, FAPERJ

HV1239 - PICORNAVIRUSES IN HYPERPLASTIC LYMPHOID TISSUES OF CHRONIC ADENOTONSILLAR DISEASE


Chronic adenotonsillar diseases are frequent otorhinolaryngologic disorders due to chronic inflammation of adenoids and palatine tonsils. They frequently lead to surgery, for removal of hypertrophic tissues. Although diverse respiratory viruses can be detected in adenotonsillar tissues of patients with chronic adenotonsillar diseases, the role of these agents as triggers of chronic inflammation in those tissues is poorly understood. Human rhinoviruses (HRVs) together with the closely related human enteroviruses (HEVs), both belonging
to the Picornaviridae family, cause most of the acute respiratory illnesses in humans and are frequently detected in adenoids and palatine tonsils from patients with chronic adenotonsillar diseases. This study was done to characterize the replicative status and sites of replication/persistence of picornaviruses in adenoids and palatine tonsils from patients with adenotonsillar diseases. To assess the picornavirus replication activity in adenoids and palatine tonsils, the viral loads of HRV/HEV and presence of anti-sense RNA species were determined by qPCR in 121 patients with chronic adenotonsillar disease, without symptoms of acute respiratory infections. The sites of viral detection were determined by immunohistochemistry (ISH) and in situ hybridization (ISH). The 5' UTR PCR fragments obtained were sequenced, which allowed for identification of species. Of 121 enrolled patients, respectively 50% and 55.4% had palatine tonsils and adenoids positive for picornaviruses. HRV was more frequently detected in adenoids while HEV was more common in palatine tonsils. ISH with antibody for VP1 capsid protein indicated the presence of virus capsids within the lymphoid tissue, in the lymphoid follicles of palatine tonsils, and also in epithelial cells from adenoids. The high frequency of detection of picornavirus genomes and protein expression in tissues from patients with chronic adenotonsillar diseases, suggests that these agents may play important roles in the pathogenesis of these frequent diseases. Financial support: CAPES, FAPESP,CNPQ

PARTICLES EXPRESSION IN BACULO-VIRUS SYSTEM


1. Universidade de Brasília, UNB, Departamento de Biologia Celular, Laboratório de Microscopia Eletrônica, CEP 709 Laboratório Central do Distrito Federal, LACEN-DF, R Sgan Q., 601 Lotes O e P Asa Norte - Brasília - DF CEP: 70830-010

2. Universidade Católica de Brasília, UCB, Campus Avançado Asa Norte SGAN 916 Módulo B B Avenida W5 - CEP: 70790-160

3. Bras Embrapa Hortaliças, CNPH, E-mail: dosanjos.karol07@gmail.com

The family Caliciviridae consists of viruses belonging to five genera: Sapovirus, Norovirus, Lagovirus, Vesivirus and Nebovirus. In Brazil it is known that the most causative gastroenteritis viruses are rotavirus and norovirus, whereas the occurrence of sapovirus is sporadic. The sapovirus genome is linear, positive-sense, single-stranded RNA, of approximately 7.5kb that is polyadenylated at the 3' terminus. Sapporo virus is divided into, at moment, seven genogroups (GI-GVII) and, causes acute gastroenteritis in human, porcine and mink hosts. This work aimed to produce virus-like particles (VLPs) of sapovirus GI isolated form human diarrheic stool sample in Brasília, DF, Brazil, for physicochemical study of VLPs and future use of vaccine. For this purpose, firstly, the partial capsid protein (P domain) was expressed by
Gateway system (Invitrogen) using pENTR 2b and subsequently pDEST 17 and two rabbits were immunized to produce antisera in order to confirm VLPs expression by Western blotting. Then, Baculovirus expression system was used for sapovirus-like particles production. The expression gene cassette containing complete capsid gene and downstream regions were subcloned into pFastBac1 after PCR. Subsequent bacmid was transfected to insect cells (Trichoplusia ni, Tn5) using Bac-to-bac system (Invitrogen). The VLPs were purified by CsCl gradient centrifugation. The possible sapovirus-like particles were observed by transmission electron microscopy, showing the abundant spherical particles with the expected particle size of 30 nm in diameter by negative staining with 2% phosphotungstic acid solution. This purified preparation now is analyzing by Western blotting using antiserum against P domain previously described.

HV1246 - SEQUENCING AND MODELING OF THE ENVELOPE PROTEIN OF A YELLOW FEVER VIRUS STRAIN (BEAR378600) ISOLATED IN THE BRAZILIAN AMAZON


1. Instituto Evandro Chagas, IEC, Rodovia BR-316 km 7 s/n - Levilândia - 67030-000 - Ananindeua / Pará / Brasil

2. Universidade Federal do Pará, UFPA, Rua Augusto Corrêa, 01 - Guamá. CEP 66075-

110. Caixa postal 479 E-mail: edivaldojunior@iec.pa.gov.br

The yellow fever virus (YFV) belongs to the family Flaviviridae, genus Flavivirus. In Brazil, the YFV is mainly transmitted by the mosquito species Haemagogus janthinomys. According to the World Health Organization, approximately 200,000 yellow fever cases and 30,000 deaths are reported annually worldwide. YFV is endemic in tropical areas of Africa and Americas (Central and South) and the number of cases have increased due to deforestation, urbanization and climate changes. The molecular modeling of proteins is considered a crucial step in the development of new drugs, since there are no specific treatments for this disease and vaccine adverse events have been reported. The objective of this study was to elucidate the three-dimensional (3D) model of the E of YFV strains isolated in the Amazon region. The complete genome of the BeAr 378600 strain, isolated in 1980 from Haemagogus sp. in Uruaçu state of Goiás was completely sequenced using the GSFLX 454 (Roche). The 3D modeling of the E protein was performed using the software MODELLER with seven reference models (1OAN, 1P58, 1TG8, 1TGE, 3C6D, and 3G7T 3P54), and validation performed using software Procheck. The post translational analyzes using the BeAr 378600 E gene coding sequence, demonstrated high similarity with other YFV proteins previously studied. The modeling showed a high quality three-dimensional stereochemistry and globally residue-residue geometry with 99.5% of the waste accepted regions. Multiple sequencing alignment carried out among BeAr378600 and several other YFV E gene sequences revealed
high similarity with strains isolated in the Amazon region. No mutations in key regions of the protein E were observed, as well as no amino acid changes were detected in important regions of the E protein. Due to the high 3D model quality (stereochemistry quality exceeding 99%) presented to the BeAR 378600 YFV strain, this model can be further used in studies on the development of potential inhibitors for the YFV.

HV1247 - DETECTION OF ANTIBODIES WITH POTENTIALLY NEUTRALIZING ACTIVITY IN NATURAL CHRONIC HCV INFECTIONS

Marins, R.S.Q.S., Moraes, M.T.B., Lewis-Ximenez, L.L., Gomes, S.A.

1. Laboratory of Molecular Virology, Oswaldo Cruz Institute, IOC - FIOCRUZ, Brasil Avenue, 4365 - Manguinhos, Rio de Janeiro, Brazil

2. Laboratory of Viral Hepatitis, Oswaldo Cruz Institute, IOC - FIOCRUZ, Brasil Avenue, 4365 - Manguinhos, Rio de Janeiro, Brazil E-mail: rmarins@ioc.fiocruz.br

Hepatitis C Virus (HCV) affects more than 170 million people and causes chronic hepatitis in about 70% of cases, with progression to cirrhosis, hepatic failure, and hepatocellular carcinoma. The envelope glycoproteins E1 and E2 are the natural targets of neutralizing antibodies responses but are also the two of the most variable HCV proteins. Several neutralizing antibodies that target HCV epitopes have been described in the literature. One of these regions, encompassing amino acids (aa) 412 to 423 of the E2 protein, is the target of several neutralizing monoclonal antibodies described in the literature. To determine the immunogenic characteristic of this small well conserved amino acid sequence (412-419) inside this important neutralizing region and to detect the presence of antibodies reacting with this small 412-423 sequence in natural infection, an in house enzyme linked immunosorbent assay (ELISA) was developed and tested against serum samples from chronic HCV infected patients. Twenty-five serum samples from chronic HCV patients and 25 samples from healthy individuals who tested negative for anti-HCV antibodies were used to establish the reactivity of these sera with the small 412-419 synthetic peptide. Cutoff value was established calculating mean absorbance value of HCV negative sera plus three times the standard deviation. A serum anti-peptide 412-419 previously obtained in a immunized rabbit was used as positive control. Two of the 25 sera derived from chronic HCV patients reacted specifically with this small peptide. These results indicate that the selected epitope was able to induce humoral immune responses during the natural infection in a small proportion (8%) of the studied patients. Further studies were necessary to associate the presence of these antibodies with progression of liver diseases. The reasons why human anti-E2 antibodies raised against HCV natural infection do not neutralize a HCV infection is not well understood and may be related to either genetic variations among viral population, low titers of anti-E2 antibodies or interference among neutralizing and non neutralizing antibodies. Could antibodies detected
in the present study protect against a severe hepatitis? If yes, the small E2 conserved peptide may be used as a therapeutic vaccine against HCV infection. Financial support: CNPq and Fiocruz.

HV1252 - A SINGLE DOSE OF INACTIVATED HEPATITIS A VACCINE PROMOTES MEMORY CELL RESPONSE


1. Universidade Federal Fluminense, UFF, Instituto Biomédico, Niterói-RJ

2. Instituto Oswaldo Cruz, IOC, Av. Brasil, 4365, Manguinhos-RJ Biomanguinhos, Bio-Fiocruz, Av. Brasil, 4365, Manguinhos-RJ E-mail: jmelgaco@ioc.fiocruz.br

Although T cells are known to have important functions in immune protection against viral diseases, only a few studies have addressed the cellular basis of immunity to hepatitis A virus (HAV) in humans. In order to further identify the roles of HAV-specific T cell responses, we investigated the generation of cellular immunity after HAV vaccination in a time elapse study in a small study population. Ten subjects received two doses of inactivated hepatitis A vaccine (0-6 months). For monitoring the antigen-specific immune response, blood samples were collected before the 1st dose (T0), 6 months after the 1st dose (T1) and 24 months after the 2nd dose (T2). Cell responses was measured by proliferation assay (CFSE), memory phenotype CD4+/CD8+/CD22+/CD45RO+ and cytokine production (IL-10, IFN-y, TNF-a) to identify antigen-specific lymphocytes in vaccinees. Results of the proliferation assays showed HAV specific cellular response soon after the 1st dose application. There was an increase in the proliferation index (PI) 2.65 fold in comparison with the PI obtained with control samples (T0) (p<0.05), which remained elevated (2.95, p<0.05) 24 months after the 2nd dose (T2). There was no statistical difference (p>0.05) between PI rates induced by lymphocytes obtained after the 1st (T1) and 2nd doses (T2) of vaccine. Although no statistical difference had been observed with regard to memory cell phenotype expression (p>0.05), an increase in CD8+ production could be detected with a positive correlation between 1st and 2nd dose (p<0.05; r2=0.70). Moreover, an increased in IL-10, TNF-a and IFN-g cytokines production could be detected after 1st and 2nd dose, with a positive correlation between IL-10 (T2) and IFN-g (T1) production (p<0.05; r2=0.60), IL-10 (T1) and TNF-a (T1) production (p<0.05; r2=0.54), TNF-a (T1) and TNF-a (T2) production (p<0.05; r2=0.70). Inactivated hepatitis A vaccines have been licensed in multiple countries, protecting against the disease with nearly 100% efficacy. However, their use have been limited by cost considerations. One approach for reducing the cost of its implementation would be the use of a single dose schedule like the one adopted in Argentina. Results of this study suggest that a single dose of inactivated hepatitis A vaccine is able to promote memory cell response. Financial Support: Capes, Faperj, CNPq Virologia Humana.
HV1253 - HTLV-1 INFECTION IN ASYMPTOMATIC PERUVIAN PEOPLE

Mayta, E.M.H.

1. Major National University of San Marcos Biological Sciences, UNMSM, Lima, Peru

2. Universidade de Sao Paulo, USP-ICB-II, Sao Paulo, Brasil

Universidade de Sao Paulo - Facultade de Medicina Humana, USP, Sao Paulo, Brasil E-mail: egmahuatuco@yahoo.com.br

Human Lymphotropic Virus Type 1 T cells (HTLV-1) is a delta-retrovirus that causes a severe lymphoproliferative disorder to CD4+ T cells. It causes leukemias, neurodegenerative diseases, inflammation myelopathy associated, uvieitis with strong unique support epidemiological and clinical characteristics that distinguish them from other similar ones. It is a complex RNA virus with a single strand of positive sense; its genome expresses unique proteins with oncogenic potential. The aim of this study was to determine the presence of HTLV-1 in asymptomatic persons. We evaluated 78 samples of peripheral blood by venipuncture with vacuum extraction system (vacutainer) to individuals attending the Health Campaign at the San Fernando Health Center (Augustino, Lima-Peru). Samples were processed at the Laboratory of Clinical Virology and Molecular FCB UNMSM to obtain the serum by centrifugation and subsequently used for the serological diagnosis of HTLV-1. We used the ELISA HTLV-I/II BIOKIT and confirmed results by Western blot HTLV Innolia I / II Innogenetics. We obtained 7.7% positive samples to HTLV-1. The confirmatory test showed intense bands (3 +, 1 +, + / -), the positive control bands do not show gag specificity (p19 I / II and p24 I / II) and two env bands (gp 46 I / II and gp 21 I / II). In addition, the env gp 46-I bands are specific to HTLV-I while env gp 46-II bands to HTLV-II. Of those positive samples for HTLV-1, 1.3% were male and 6.4% were women. Also, we found that HTLV-1 was prevalent in infected individuals from 26 to 60 years old. In determining the place of birth of seropositives of HTLV-1, it was found one person from Junin locality and five people from Lima. Our results indicate that HTLV-1 virus is circulating mostly in healthy individuals. Some studies reveal that HTLV-1 infected people remain asymptomatic. Also, HTLV-1 is present in endemic areas, as well as Lima, in people of economically active ages with female gender predominance. Keywords: HTLV-1, asymptomatic, HTLV-1 antibodies. Financial support: CSI-UNMSM

HV1260 - RECOMBINANT OF HEPATITIS B VIRUS (HBV) GENOTYPES A AND G DETECTED AFTER A FOLLOW UP OF A PATIENT IN THE CHRONIC PHASE OF AN OCCULT HBV INFECTION


1. Lab de Virologia Molecular, Instituto Oswaldo Cruz/Fiocruz, LVM, IOC/FIOCRUZ, Av. Brasil, 4365, Manguinhos, Rio de Janeiro, Brasil

2. Lab de Hepatites Virais, Instituto Oswaldo Cruz/Fiocruz, LAHEP, IOC/FIOCRUZ, Av. Brasil, 4365, Manguinhos, Rio de Janeiro, Brasil
Chronic hepatitis B virus (HBV) infection is a serious global health problem and an important cause of morbidity and mortality in endemic areas. Eight HBV genotypes (HBV/A to HBV/H), have been identified. HBV genotypes and their subgenotypes have distinctive geographical and ethnic distribution around the World. In Brazil, HBV/A, HBV/D (worldwide) and HBV/F (Native from Indians of the New World) are the most prevalent, rarely occurring HBV/G. Genotype G is found in the USA and Europe. Occult HBV infection is defined by detectable HBV genome in the absence of surface antigen (HBsAg, the main serological marker of active infection). The cause of an overt HBV infection becoming an occult one is unknown. In this study, a recombinant of HBV A/G isolated from a patient with occult HBV infection is reported. The patient was a 23-year-old Brazilian male with classical acute infection (seropositive for HBsAg, HBeAg and anti-HBc in 10/2008. After six months, all serological markers became negative except anti-HBc but HBV DNA was detectable in low titles <10^4 copies/mL. This patient was monitored for HBV markers for about two years with a total of 14 serial serum samples available. Genomic regions of S, pre-C and C genes were PCR amplified, followed by nucleotide sequencing. The first and second last sample were cloned and 16 and 10 clones of each sample were analyzed, respectively. PCR products were also subjected to pyrosequencing in order to verify the presence of mixture of genotypes. Possible recombination was analyzed by SIMPLOT program. Based on phylogenetic analyses of the S, pre-C/C genes and pyrosequencing results of the first six serial samples and clones from the first sample, all amplicons clustered on a branch within subgenotype A2, however, analysis of the last two samples along with their clones showed mixtures of genotypes A and G and suggested recombination A/G for S region. The possibility of the development of occult HBV infection after acute hepatitis B, leads to a discussion of the need to expand monitoring to patients with undetectable HBsAg and without seroconversion to anti-HBs, through molecular tests for detection of HBV DNA prior to outpatient release. In this study we identify a recombinant HBV A/G in the chronic phase whereas only HBV/A was detectable in the acute phase. These data should encourage further epidemiological and virological investigations on the clinical evolution of co-infected (HBVA/G) patients.

Financial support: FIOCRUZ

HV1281 - HUMAN PAPILLOMAVIRUS IN CANCER OF DIVERSE HEAD AND NECK SUBSITES

Betiol, J.C., Sobrinho, J.S., Costa, M.C., Rossi, L., Costa, H.O., Villa, L.L., Sichero, L.

1. Instituto do Câncer do Estado de São Paulo, ICESP, Av. Dr. Arnaldo, 251 - Cerqueira César - São Paulo - SP - CEP: 01246-000
2. Instituto do HPV, INCT-HPV, Rua General Jardim, 618, 3º andar sala 32, São Paulo - SP
3. Departamento de Radiologia - FMUSP, DR-FMUSP, Av. Dr. Enéas de Carvalho Aguiar, 255 E-mail: julio.betiol@icesp.org.br

Head and neck cancer (HNC) is the sixth...
Human Virology: HV

The most common neoplasia worldwide, and HPV infection is causally associated with a subset of these tumours. We sought to characterize HPV genome diversity in paraffin-embedded samples from individuals diagnosed with HNC. Extracted DNA was amplified using a very sensitive PGMY/GP+ nested PCR protocol. Adequacy of DNA was assessed by beta-globin gene PCR. Amplicons were cloned and sequenced for HPV genotyping. To date, we have analyzed DNA derived from 62 patients. These included laryngeal (n=50), oropharyngeal (n=4), vocal cord (n=6) and other sites (n=2) specimens. The overall HPV prevalence was 38.71% (24/62). HPV positivity was higher among oropharyngeal samples (75%) followed by laryngeal (38%) and vocal cord (16.6%) specimens. Seven positive samples were genotyped as yet. The most prevalent type was HPV-83 (42.86%; 3/7) followed by HPV-18 (28.57%; 2/7), HPV-16 (14.3%; 1/7) and HPV-51 (14.3%; 1/7). Our preliminary results indicate a different pattern of HPV type-specific infection among HNC tumors, once studies performed worldwide observed HPV-16 prevalence of up to 90% in some head and neck subsites. Our study will provide epidemiological evidences of HPV types distribution among HNC in samples from São Paulo, data that is actually lacking in our country and are important for the establishment of effective governmental policies, reaffirming the emergent necessity of vaccine implantation in Brazilian health programs in order to reduce the bound of tumors in our population.

Financial support: FAPESP 11/09616-9, 12/01513-9 and 08/57889-1; CNPq 573799/2008-3; Ludwig Institute for Cancer Research Area: Virologia

Humana Tema: Papilomavírus Humano Arquivo: Betiol et al.,2012 Apresentador: Julio Cesar Betiol

HV1293 - VARIABILITY IN THE S GENE SEQUENCE OF HEPATITIS B VIRUS FROM CHRONIC CARRIERS WITH SIMULTANEOUS POSITIVITY OF HBSAG AND ANTI-HBS SEROLOGICAL MARKERS

Ferreira, A.C., Pinho, J.R.R., Mendes-Corrêa, M.C.J., Nastri, A.C.S.S, Soares, M.C.P., Gomes-Gouvêa, M.

1. Instituto de Medicina Tropical - USP, IMT/USP, Av. Dr. Éneas Carvalho de Aguiar nº 500 Prédio 2, 2º andar
2. Faculdade de Medicina da Fundação do ABC, FMABC, Hospital das Clínicas da Faculdade de Medicina da USP, HC/FMUSP
3. Instituto Evandro Chagas, IEC, E-mail: ariana86carolina@hotmail.com

HBV clearance is classically characterized by the emergence of anti-HBs antibodies that may be detected by serological tests after Hepatitis B surface antigen (HBsAg) clearance, although it is produced early in the course of infection. Detection of HBsAg and anti-HBs concomitantly comprise a peculiar serological profile in the chronic hepatitis B context, which may be related with the selection of HBsAg variants that escape from immune response. This study aimed to assess the relationship between the coexistence of HBsAg and anti-HBs in chronic hepatitis B carriers and the presence of HBsAg variants. Serum samples from chronic HBV carriers both seropositive for HBsAg
and anti-HBs (case group=16) and only seropositive for HBsAg (control group=21) were analyzed in this study. HBV S gene sequences were obtained by direct sequencing and its amino acid changes were identified by comparison with consensus sequences belonging to the different HBV genotypes. HBV genotypes A and D were found in both groups, genotype F was detected only in one sample from case group. HBV/A was the most prevalent in both groups, but HBV/D was found more frequently in the control group (48% vs 18%). HBV variants with amino acid mutations within the HBs sequence were found more frequently among control group (76% vs. 63%) and these mutations were more common in N and C-terminal regions of the S protein. Variants with mutations at the major hydrophilic region of the HBsAg (AA 99–160) were also found more frequently among individuals from control group (48% vs 19%), however none of these mutations were located in the second 'a' determinant loop (AA 139 -148) or were previously related with anti-HBs escape. In conclusion, these results shows that the simultaneous positivity of HBsAg and anti-HBs in chronic HBV carriers studied was not related with a selection of HBV escape mutants. The higher variability of the S gene observed among strains isolated from control group may be related with the higher frequency of genotype D in this group, but it was not related to a lower sensitivity of the kit in detecting these variants. Financial support: FAPESP (2010/50081-9 and 2010/51208-2)

HV1294 - MAYARO VIRUS IN PATIENTS FROM ACRE STATE, BRAZIL (2010)

Sabino, G.F., Mondini, A., Colombo, T.E., Nogueira, M.L.

Universidade Estadual Paulista "Júlio de Mesquita Filho", UNESP, Faculdade de Medicina de São José do Rio Preto, FAMERP, E-mail: guitosabino@gmail.com

Arboviruses usually have very similar symptoms in mild or severe cases, such as fever, malaise, headache, nausea, vomiting, arthralgia, myalgia among others. This wide range of symptoms may lead to inaccurate diagnosis and an increase in unreported cases of several diseases in official health data. This situation usually occurs in regions where there are outbreaks caused by enzootic circulation of arboviruses. The implementation of surveillance systems and an improvement in diagnostic expertise has allowed the detection of new human cases of arboviruses in regions where their presence was previously underestimated. Therefore, arboviruses remain largely unknown or not reported in the regions where refined diagnosis is not available. Our goal was to detect dengue (DENV) and other arboviruses in patients that presented febrile illness and searched health facilities for medical care. We analyzed medical records of 400 patients from the Hospital de Base of São José do Rio Preto, São Paulo (Brazil) whose clinical diagnosis was dengue. The analysis of these patients from São José do Rio Preto suggests that 84.75% of the febrile illnesses might be caused by other agents than DENV. Actually, only 14.5% of the patients were DENV positive and 0.75% presented unknown ethiology. These results raise concerns on the parameters that have
been used for arbovirus diagnosis. The great similarity of the symptoms in diseases caused by arboviruses requires better analyses, training of health professionals and tests that present high sensitivity and specificity. In order to improve the health policies needed to strengthen surveillance, the identification of vectors and reservoirs, the development of effective diagnostic tests and strategies to control diseases are extremely important to uncover arboviruses that hide under dengue umbrella. Financial Support: INCT-Degue/CNPq; FAPESP; CAPES; PRONEX;

HV1295 - ARBOVIRUS CASES HIDDEN UNDER DENGUE UMBRELLA IN NORTHWEST REGION OF SÃO PAULO STATE


Faculdade de Medicina de São José do Rio Preto, FAMERP, Universidade Estadual Paulista “Júlio de Mesquita Filho”, UNESP, E-mail: guitosabino@gmail.com

Arboviruses usually have very similar symptoms in mild or severe cases, such as fever, malaise, headache, nausea, vomiting, arthralgia, myalgia among others. This wide range of symptoms may lead to inaccurate diagnosis and an increase in unreported cases of several diseases in official health data. This situation usually occurs in regions where there are outbreaks caused by enzootic circulation of arboviruses. The implementation of surveillance systems and an improvement in diagnostic expertise has allowed the detection of new human cases of arboviruses in regions where their presence was previously underestimated. Therefore, arboviruses remain largely unknown or not reported in the regions where refined diagnosis is not available. Our goal was to detect dengue (DENV) and other arboviruses in patients that presented febrile illness and searched health facilities for medical care. We analyzed medical records of 400 patients from the Hospital de Base of São José do Rio Preto, São Paulo (Brazil) whose clinical diagnosis was dengue. The analysis of these patients from São José do Rio Preto suggests that 84.75% of the febrile illnesses might be caused by other agents than DENV. Actually, only 14.5% of the patients were DENV positive and 0.75% presented unknown ethiology. These results raise concerns on the parameters that have been used for arbovirus diagnosis. The great similarity of the symptoms in diseases caused by arboviruses requires better analyses, training of health professionals and tests that present high sensitivity and specificity. In order to improve the health policies needed to strengthen surveillance, the identification of vectors and reservoirs, the development of effective diagnostic tests and strategies to control diseases are extremely important to uncover arboviruses that hide under dengue umbrella.

HV1302 - SEVERE MYOCARDITIS AFTER ENTEROVIRUS INFECTION IN A YOUNG WOMAN

Camargo, C.N., Perosa, A., Granato, C.F.H., Bellei, N.

Universidade Federal de São Paulo, UNIFESP, Rua Pedro de Toledo, 781. 15andar E-mail: claricencamargo@gmail.com

Although the majority of human EV infections remain asymptomatic, these
viruses are associated with diverse clinical syndromes, ranging from minor febrile illness to severe and potentially fatal pathologies, including aseptic meningitis, encephalitis, myopericarditis, acute flaccid paralysis, and severe neonatal sepsis-like disease. Enteroviral infection of the heart has been noted in a significant proportion of cases of myocarditis and dilated cardiomyopathy. The presence of enterovirus RNA at stages of disease after acute infection and correlation of enterovirus replication with worse clinical outcome suggests continued replication of the virus is involved in the progression of the disease. In 2012, a young woman was admitted to Sao Paulo Hospital in the 5th months after her puerperal period. Physical examination and cardiac laboratory evaluation revealed the diagnostic of myocarditis and a low ejection fraction (<40%). An initial etiology supposed for the case was puerperal myocarditis but an infectious diseases consultation was required to evaluate the patient. Myocarditis caused by EV was diagnosed when the virus was detected in nasal swab and antibodies to Coxsackie B virus were detected in serum. Patient was discharged after 10 days of hospitalization with a final diagnosis of viral myocarditis and heart failure and is under treatment at the chronic heart disease ambulatory. Early recognition of heart failure and adequate diagnostic testing for cardiotropic viruses is important to understand the impact of these viruses among community. Financial support: CAPES, FAPESP 2009/17384-0

PAULO CITY
Camargo, C.N., Bellei, N.
Universidade Federal de São Paulo, UNIFESP, E-mail: claricencamargo@gmail.com

Enteroviruses (EVs) are among the most common viruses infecting human beings worldwide and can induce non typical respiratory illnesses in infants or adults, including upper respiratory tract infections but also lower respiratory tract infections, resulting in bronchitis, bronchiolitis, and pneumonia. The aims of our study were to investigate the occurrence of EV in hospitalized and non hospitalized patients with respiratory symptoms. The nasal secretion was collected from 253 hospitalized (96 children and 61 adults) and 281 community patients (128 children and 153 adults). Samples were tested by Real time RT-PCR to detect EV nucleic acids. Positive samples for EV were detected in 3.9% samples (21/534), in which, 5.7% (16/281) outpatients and 2% (5/253) were hospitalized patients. All hospitalized patients were children, the majority of inpatients positive cases occurred in children up to 1 year old. EV positive infections identified in 21 patients correspond 5.2% (5/96) of hospitalized children, 4% (5/128) and 7.2% (11/153) non-hospitalized children and adults respectively. The main clinical manifestations related by patients were fever, coryza, cough, myalgia and sore throat besides dyspnea among inpatients. Most EV positive cases occurred during autumn and winter. In conclusion, our result highlighted the importance of EV as a causative agent of severe respiratory infection young children but is rarely observed among community adults.

HV1303 - HUMAN ENTEROVIRUS INFECTION IN COMMUNITY AND HOSPITALIZED PATIENTS IN SÃO PAULO CITY
Financial support: CAPES, FAPESP 2009/17384-0

HV1309 - CHARACTERIZATION OF HIV-1 SUBTYPES AND DRUG RESISTANCE MUTATIONS AMONG INDIVIDUALS INFECTED WITH HIV IN CAMPINAS/BRAZIL

Neto, D.F.L.N., Aoki, F.A., Arns, C.W.

Universidade Estadual de Campinas, UNICAMP, Instituto de Biologia Rua Monteiro Lobato - n° 255 E-mail: danielviro@gmail.com

In order to describe HIV-1 subtypes and drug resistance mutations in Brazil, blood samples from 764 patients infected with HIV-1 collected from 2001 to 2008 were genotyped. Of these, 126 samples were from newly diagnosed, antiretroviral (ARV)-naïve patients and 27 from ARV-treated patients. Partial pol and gag region sequences were used to identify drug resistance mutations and to conduct phylogenetic analysis for subtype determination. The results indicated that 43% patients harbored subtype C viruses, 35.2% carried subtype CRF-BC virus and 18%. Among patients with no prior exposure to ARVs, mutations associated with resistance were detected in five patients: three (2.4%) patients had reverse transcriptase (RT) inhibitor mutations and two other patients had the protease (PI) inhibitor associated mutation M46I. PI mutation V77I was found in 42 of subtype C isolates. Of 27 ARV-treated patients, 22 (81.5%) harbored at least one nucleoside reverse transcriptase inhibitors (NRTI), a non-NRTI (NNRTI) and/or a PI mutation. The most common NRTI resistance mutation was M184V/I (74.1%). Frequency of thymidine analog mutations was relatively low (25.9%). With regard to NNRTI mutations, G190S/A was the most frequent mutation, which might be a preferred mutations for subtype C. Brazil's HIV epidemic continues to be dominated by Subtype A FSU. The prevalence of transmitted drug resistance is low, but has the potential to increase with increasing use of ARVs.

HV1311 - EPIDEMIOLOGICAL FEATURES OF HUMAN CORONAVIRUSES SPECIES AMONG BRAZILIAN PATIENTS


1. Clinical Virology Laboratory, Discipline of Infectious Diseases, Department of Medicine of The Sao Paulo Federal University, Sao Paulo, SP, Brazil. E-mail: taticabeca@yahoo.com.br

2. Universidade Federal de São Paulo, UNIFESP, Rua Pedro de Toledo, 781 15° andar E-mail: taticabeca@yahoo.com.br

Human coronaviruses (HCoVs) cause upper respiratory illness and occasionally lower tract disease in susceptible populations. Five HCoVs are known: OC43, 229E, SARS-CoV and the recently identified NL63 and HKU1. Since there is little knowledge on the epidemiological features among the different HCoVs species, we conducted a comprehensive study by analyzing the non-SARS HCoVs on 1.137 respiratory samples from subsets of patients from Sao Paulo, Brazil, between 2001 and 2010. Subjects were 50 asymptomatic and 1.087 presenting...
acute respiratory infections: 465 patients from community (adults and children), 410 at-risk patients (renal transplanted patients, children with heart diseases and patients under stem cell transplantation program) and 212 hospitalized patients (adults and children). To identify the HCoVs in samples, species-specific real-time RT-PCR assay were performed. Human coronaviruses were detected in 88 out of 1,137 (7.7%) of the samples. The most frequently detected HCoV species were NL63 (50.0%) and OC43 (27.3%). HCoV-NL63 was the species most frequently associated with children, both from the community (100%) and presenting heart diseases (50%), while there was a high rate of HCoV-229E detection among renal transplant patients (44%). Patients in stem cell transplantation programs were more frequently infected with HCoV-OC43 (47%). There were inter-seasonal differences in the detection frequencies, with HCoV-229E being predominant in the year 2004 (61.5%) and HCoV-NL63 (70.8%) in 2008. Besides, HCoV-229E displayed a marked autumn seasonality while HCoV-HKU1 and HCoV-OC43 predominated in winter. Dyspnea was more associated with HCoV-229E infections (66.6%) and cyanosis was reported only in HCoV-OC43 infections. Our data provide an insight into the epidemiology knowledge of HCoVs among different subsets of patients, supporting the notion that HCoVs have different circulation trends and play an important role among patients with comorbidities. Financial support: FAPESP (n°09/17307-6 and 09/54640-5)

HV1312 - INCIDENCE OF CASES OF DENGUE 4 (DENV - 4) IDENTIFIED IN THE STATE OF PERNAMBUCO, DURING THE PERIOD FROM JANUARY TO JUNE OF 2012


Laboratório Central Dr. Milton Sobral, LACEN/SES-PE, Rua Fernandes Vieira, s/n, Boa Vista, Recife-Pe E-mail: licixea@hotmail.com

Infections caused by dengue virus (DENV) is one of the most important diseases transmitted by arthropod-borne, about 50 to 100 million people worldwide are infected each year and 500,000 live in the risk areas. The increase and spread of dengue cases in the state of Pernambuco in 2012, may be due by introduction of DENV-4. In this study, the serotypes of the dengue virus were identified, isolated and the affected counties have been mapped. Some samples were isolated by viral isolation in C6/36 cell culture, others have been screened by the technique of NS1 ELISA, and then serotyped by RT-PCR. Of the 254 reagent samples tested by the NS1, 109 (42.9%) were positive by RT-PCR where all four serotypes were identified as 14 (5.5%) were DENV-1, 2 (0.8%) were DENV-2, 4 (1.6%) were DENV-3 and 89 (35%) were DENV-4. Of the 504 samples tested by virus isolation only 37 (7.4%) were identified as DENV-4. Of the 504 samples tested by virus isolation only 37 (7.4%) were identified as DENV-4. Of the 504 samples tested by virus isolation only 37 (7.4%) were identified as DENV-4. Of the 504 samples tested by virus isolation only 37 (7.4%) were identified as DENV-4. Through these two methods the serotypes were mapped across the state of Pernambuco. However, despite the four serotypes are circulating in the state, there was a predominance of DENV-4, where its introduction is a recent event and the population was more susceptible to infection. The knowledge
of these data is important to trace the involved population's epidemiological profile and to adopt effective control measures.

HV1314 - MOLECULAR EPIDEMIOLOGY OF NOROVIRUS IN CHILDREN


1. Universidade Federal do Recôncavo da Bahia, UFRB, Av.Carlos Amaral, 1015 - Santo Antônio de Jesus - BA. CEP: 44.570-000

2. Universidade Federal da Bahia, UFBA, Av. Reitor Miguel Calmon s/n - Salvador - BA. CEP 40.110-100

3. Hospital Aliança, HA, Av Juracy Magalhães Jr, 2096 - Salvador - BA. CEP 41920-900 E-mail: fabianalopes.ufrb@gmail.com

Viral gastroenteritis is one of the most common diseases of humans and it is estimated to occur in more than 700 million cases in children under 5 years old. The common viral agent that causes gastroenteritis outbreaks worldwide is Norovirus (NoV). The viral transmission is mainly by the fecal-oral route via person to person or food and water contaminated. NoV, a member of Caliciviridae family, is a RNA virus, classified into five genogroups (GI to G IV) from of which GII is the most prevalent in humans. The objective of this study was to identify and to characterize the NoV during an outbreak from March-July 2010 from children under 5 years old. The commercial immunoenzymatic assay (ELISA RIDASCREEN® Norovirus 3rd Generation R-Biopharm, Germany) and Reverse Transcription-Polymerase chain reaction (RT-PCR) were performed to detect NoV in stool samples. From the total of two hundred and six samples, NoV was detected in 26.21% (54/206), where the median age was 1 year and 6 months, using an ELISA test. From the total of ELISA positive stool samples, twenty-three were submitted and confirmed the presence of NoV by RT-PCR using the primers CAL-32/MO3-N and JV-12/ACAL-36. Then, fifteen of them (15/23) selected at random were subjected to sequencing. After the analysis on NCBI/BLAST, it was found that all samples exhibited a high similarity to GII.4 strains (96-99% homology). Concluding, the acute gastroenteritis outbreak in children during 2010 confirmed the presence of NoV GII.4. Financial support: Fundação de Amparo à Pesquisa do Estado da Bahia – FAPESB

HV1318 - THE ROLE OF EAST-WEST BALANCE IN THE SOUTHERN HEMISPHERE INFLUENZA VACCINE RECOMMENDATION

Born, P.S., Bentancor, G.B., Siqueira, M.M., Motta F.C.

INSTITUTO OSWALDO CRUZ/FUNDAÇÃO OSWALDO CRUZ, IOC/ FIOCRUZ, AV BRASIL, 4365 RJ 21040-360 E-mail: priscilaborn@yahoo.com.br

Influenza infections are the principal cause of severe respiratory disease, affecting individuals worldwide on a yearly basis. The vaccination is the
main strategy for infection control, but the vaccine needs to be updated every season to avoid mismatches between vaccine prototypes strains and those circulating in the population. The choice of vaccine prototypes is driven by a comparison of viruses circulating throughout the year in the Northern or Southern Hemisphere (SH). Historically, the greatest amount of virus isolates come from Oceania, particularly Australia, which raises the question if the samples present in the SH vaccine recommendations are closer to the viruses from that region, therefore, promoting less protection to virus in South America (SA). Aiming to evaluate this question, we compared influenza A(H3) hemmaglutinin sequences from Australia and Brazil throughout 2004-2011 with respective vaccine prototypes by year. The sequences were aligned using the MEGA v5.1 software and the comparison between samples and prototypes were carried-out using JTT algorithm (1000 bootstrap replicates). During this period the most important prevalence of A(H3) in SA was detected in 2004, 2007 and 2011, just after the years were recommended vaccine prototypes showed less identity with community isolates in Brazil. Our results demonstrated divergence among the geographic distinct groups during most influenza seasons evaluated when samples were compared with vaccine prototypes. These differences were remarkable in 2006 and 2009, when samples circulating in Australia were closer to vaccine strains in comparison to Brazilian ones. In this analysis we could identify differences between vaccine and circulating strains along two distinct regions in SH. In addition, we demonstrated a simple method to generate data that, combined to serological results, allow a rapid analysis of influenza vaccines annually administrated in South Hemisphere. Financial support: DECIT/MS, IOC/Fiocruz, CAPES

HV1319 - DISTRIBUTION OF IL28B SINGLE-NUCLEOTIDE POLYMORPHISMS IN PATIENTS WITH HEPATITIS C VIRUS INFECTION

Pelegrini, A., Passos, A.M., Granato, C.F.H.

2. Universidade Federal de São Paulo, UNIFESP, Rua Pedro de Toledo, 781, 15º andar, Vila Clementino, São Paulo E-mail: andreia.pelegrini@grupofleury.com.br

Hepatitis C virus (HCV) infection is a global health problem that affects a significant population worldwide. HCV causes chronic hepatitis, which may progress to liver cirrhosis and hepatocellular carcinoma. Factors related to the virus, host, environment, and their interplay have an important role in determining the disease progression. Recently, the single-nucleotide polymorphisms in the interleukin 28B gene (IL-28B SNPs) have been associated to virological response to interferon-based therapy, but it remains unclear whether IL28B SNPs influence the severity and progression of liver disease. The aim of this retrospective study was to assess the distribution of IL28B genotypes in HCV patients and investigate a possible impact of the polymorphisms on laboratorial findings. A total of 45
patients were enrolled. Genotyping of the rs12979860 SNP and plasma HCV viral load were determined using real-time quantitative RT-PCR assays. Blood and biochemical tests were performed using standard methods. Most patients were males (62.2%), and the overall mean age was 53.1 years (range 29 - 67 years). The distribution of IL28B genotypes was 15.6% for the CC genotype, 66.7% for the CT genotype, and 17.8% for the TT genotype. Differences in mean serum value of AST, ALT, total bilirubin, AFP, platelets, and HCV viral load were observed when CC group and non-CC group were compared; however, there were no statistical significant differences between them. Some studies have indicated the involvement of the CC genotype at rs12979860 in both spontaneous and treatment-induced control of HCV infection. Our results demonstrated a low frequency of the CC genotype and there were no evident association between the polymorphism and laboratory-detectable abnormalities. As a preliminary study, the small number of samples influenced our results and further information can contribute for a better understanding of the role of IL28B SNPs in the progression of the HCV infection.

HV1320 - DETECTION OF HEPATITIS B SURFACE ANTIGEN (HBsAg) IN A NEONATE BORN TO A HBV VACCINATED WOMEN: A CASE REPORT


2. Universidade Federal de São Paulo

Paulo, UNIFESP, Rua Pedro de Toledo, 781, 15º andar, Vila Clementino, São Paulo E-mail: andreia.pelegrini@grupofleury.com.br

Hepatitis B is a vaccine-preventable disease that has been estimated to have infected over 2 billion people worldwide. The likelihood of progression to chronic infection is inversely related to age at the time of infection. Around 90% of infants infected perinatally become chronic carriers, unless vaccinated at birth. In Brazil, the hepatitis B vaccination is recommended for all infants, regardless of the HBsAg status of the mother, and the first dose is administered preferably within 12 hours of birth. In this case report, we describe the detection of hepatitis B surface antigen (HBsAg) in a neonate born to a HBV vaccinated woman. Furthermore, we discuss the proper interpretation of the serological results for the correct diagnosis. A serum sample of a 3-day-old neonate was sent to our laboratory for screening for evidence of HBsAg. The sample was reactive in the Roche Modular E170 assay (reading/cut-off: 2.39/1.0) and was confirmed by neutralization in the HBsAg Confirmatory Test. After six days, a second sample was sent for screening for evidence of HBsAg, anti-HBs, and anti-HBc markers. HBsAg and anti-HBc results were negative and anti-HBs were positive (reading/cut-off: 447.0/10 UI/L). The woman presented negative evidence of HBsAg, anti-HBs, and anti-HBc markers. Analyzing all the serological markers results, the presence of HBsAg in the neonate sample represented a possible cross-reactivity with vaccine antigens, since the mother did not presented a prior evidence of infection and consequently...
the mother-to-child transmission was excluded. The development of serological assays to detect HBsAg and other hepatitis B markers has played a major role in the diagnosis of infection. This case highlights the importance of not assessing the HBV status of the neonate before the completion of the course of vaccination since serological profiles can at times be ambiguous and thus additional evidences are widely useful for the proper interpretation of the results.

HV1322 - COMPARISON OF LABORATORY TECHNIQUES FOR HUMAN RESPIRATORY SYNCYTIAL VIRUS IN CLINICAL SAMPLES OF OUTPATIENT CHILDREN AND BONE MARROW TRANSPLANTED ADULTS WITH SUSPECT ACUTE RESPIRATORY INFECTION TREATED AT SAO PAULO HOSPITAL


1. UNIVERSIDADE FEDERAL DE SÃO PAULO, UNIFESP, – Rua Pedro de Toledo, 781, Vila Clementino – SP, CEP: 04039-032 – Brazil

2. Universidade Estadual do Centro Oeste, Unicentro, Guarapuava, Paraná, Brasil E-mail: lucianamoreira10@hotmail.com

Human respiratory syncytial virus (HRSV), an important agent in the acute respiratory tract infections of immunocompromised patients, accounts for more than 50% of the deaths of hematopoietic stem cell transplant patients. Infection control and clinical management rely on the prompt diagnosis of suspected cases. This study was performed to evaluate an improved diagnostic flow for the detection of HRSV among patients of the Haematopoietic Stem Cell Transplant program (HSCT) using a direct immunofluorescence assay (DFA), immunochromatographic point-of-care RSV Bio Easy® (PC) assay and a polymerase chain reaction assay used as the gold standard. Laboratory surveillance guided by viral seasonality according to community surveys among children was also evaluated. A total of 230 nasal wash samples, 102 from HSCT patients and 128 from children, revealed a viral detection rate of 14.1% for children and 18.6% for HSCT patients. An overall concordance of 84.6% was obtained among the three methods, and 88.4% for DFA and PCR. For the samples collected 5 days after the onset of symptoms, PCR exhibited the highest sensitivity. We conclude that the low sensitivity of the tested immunocromatographic assay do not support its use on routine practice. For the children group, DFA was considered sufficient for epidemiological surveillance. Routine laboratory surveys based on DFA and a combination of both DFA and RT-PCR methods for HSCT high-risk patients provided the best diagnostic flow for HRSV diagnosis among these patients. Financial support: CNPq/FAPESP

HV1324 - MOLECULAR EPIDEMIOLOGY OF G9 ROTAVIRUS GENOTYPE INFECTION AMONG CHILDREN IN NORTH REGION, BRAZIL FROM 1999 TO 2011


Instituto Evandro Chagas, IEC, Rodovia Br-316-Km07, s/n,
Levilândia, Ananindeua, Pará
Núcleo de Medicina Tropical - UFPa, NMT-UFPa, E-mail: sylvia guerra@iecpa.gov.br

Rotavirus (RV) is the most common etiological cause of acute gastroenteritis in infants and young children worldwide, representing a significant cause of morbi-mortality among children aged less than five years old. Rotavirus belongs to the genus Rotavirus, family Reoviridae, with a genome consisting of 11 segments of double-stranded RNA (dsRNA) that encodes 12 proteins. Two proteins, VP7 and VP4, independently elicit neutralizing responses and define different genotypes of RV, G and P, respectively. At least 27 G-types and 35 P-types have been described. The VP7 gene encodes 326 amino acids and carries six antigenic regions. The G9 genotype is one of the most frequent genotype with distinct genetic and molecular characteristics. Currently, the phylogenetic analyses of G9 genotypes confirm the existence of 6 lineages. The aim of this study was to characterize VP7 gene of RV G9 strains detected in north region, Brazil, between 1999 and 2011 from children with acute. The dsRNA viral of 35 samples was extracted from fecal suspensions and submitted to reverse transcription and then amplified by PCR. The products were submitted to a sequencing reaction and thereafter phylogenetic analysis was performed. The phylogenetic analysis demonstrated that all G9 strains grouped into lineage III, showing great similarity and being very conserved. The VP7 sequences had high identities among themselves ranging from 96.8% to 100%, and have shown major divergences when compared to strains from Acre state (2005) and strains from 2010 and 2011 years, which showed modifications in the amino acid residue located in the antigenic region A (residue 100 aa). The phylogenetic analysis of G9 genotype that is currently spread on a global scale, seems very important mainly if we consider the present post-rotavirus vaccine introduction scenario when possible emergent new strains may pose a challenge to rotavirus vaccination. Financial support: CNPq, Instituto Evandro Chagas.

HV1327 - ASSESSMENT OF ARBOVIRUS IN MOSQUITOES COLLECTED FROM URBAN/FOREST TRANSITION AREAS OF SÃO JOSÉ DO RIO PRETO, SÃO PAULO AND SINOP, MATO GROSSO (BRAZIL)

Ozanic, K., Parra, M., de Carvalho, C.P.T., Vedovello, D., Machado, D.C., Nogueira, M.L., Bronzoni, R., Mondini, A.

1. Faculdade de Medicina de São José do Rio Preto, FAMERP, Av. Brigadeiro Faria Lima, 5416 - Vila São Pedro - 15090-000
2. Universidade Estadual Paulista, UNESP, Rod. Araraquara-Jaú Km 1 Machados 14800-901 - Araraquara, SP
3. Universidade Federal do Mato Grosso, UFMT, Av. Fernando Corrêa da Costa, nº 2367 - Bairro Boa Esperança, Cuiabá - MT - 7806 E-mail: katiaozanic@gmail.com

Arboviruses are zoonoses that depend on animal specimens to replicate and spread within the environment. In terms of public health, the most important arboviruses are the ones transmitted by mosquitoes since they
are usually found in cities and forests alike. It is possible to investigate viral circulation patterns within the vectors through mosquito collection. Our goal was to assess the presence of arboviruses in mosquitoes collected at urban/forest transition areas of São José do Rio Preto, São Paulo and Sinop, Mato Grosso (Brazil) from October 2011 to April 2012. The specimens of several species were grouped in 141 pools according to date of collection, site, gender and genus/species. Viral RNA was extracted using TRIZOL and the pools were tested with a Hemi-Nested-Multiplex-RT-PCR that uses generic and specific primers for Flavivirus and Alphavirus. Seventy-seven samples (54.6%) were analyzed at the moment. One pool collected in September 2011 was positive for Culex flavivirus, which was confirmed by sequencing of NS5 region. This preliminary data is in accordance with what was found in the city of São José do Rio Preto in 2007 and 2008, when Culex flavivirus was circulating within the city. It is likely that this virus has established a continuous circulation in the region and the reflex of its circulation may be the hampering of the transmission of other flaviviruses. Financial support: CNPq (480945/2010-1)

HV1328 - MOLECULAR CHARACTERIZATION OF DENGUE VIRUS SEROTYPE 1 IN SÃO JOSÉ DO RIO PRETO - SP

Biselli, J.M., Vedovello, D.

Faculdade de Medicina de São José do Rio Preto, FAMERP, Av. Brigadeiro Faria Lima, 5416, São Pedro, São José do Rio Preto, SP, 15090000 E-mail: joicebiselli@famerp.br

In 2010, Brazil registered more than one million probable cases of Dengue in consequence of the recirculation of DENV1 and Sao Jose do Rio Preto (SJRPP), SP, had the largest Dengue outbreak, with DENV1 as the most important agent after over 10 years without its detection in 2008. The introduction of new serotypes/genotypes is the main risk factor for Dengue outbreaks; however, it is not clear if the outbreaks reported in Brazil have occurred due to clade replacement, population susceptibility or secondary infections. Thus, DENV1 samples collected between 2009-2012 in SJRP are under investigation of Envelope (E) gene sequence to identify predominant genotypes and eventual clades of DENV1 that have contributed to the rise of DENV1 infection in SJRP. This study included serum samples sent to the Laboratorio de Pesquisa em Virologia of FAMERP for Dengue diagnosis, with 414 positive samples for DENV and 368 for DENV1. Since virus isolation in cell lines can lead to selection of virus strains, we decided to sequence the complete E gene directly from serum of infected patients. A PCR strategy was optimized in order to amplify a 1855 bp fragment of DENV1 genome and the samples will be sequenced. Until now only four DENV1 samples have been subjected to sequence analysis of E gene. After specific RT-PCR, purified PCR amplicons were sequenced using the BigDye v3.1 in ABI3130 automatic sequencer (Applied Biosystems). Derived DENV1 nucleotide sequences were aligned using Accelrys Gene 2.5 software (Accelrys) with previously published E gene sequences from GenBank and the sequences were confirmed as DENV1 E gene. Phylogenetic analysis performed using Mega 5.05 software showed that
these four samples of DENV1 belong to genotype V and are grouped in a same clade. We will sequence up to 100 samples from every year with DENV1 circulation. This study can be an important tool for monitoring the introduction and propagation of viruses and to predict their potential epidemiological consequences. Financial support: INCT/CNPq-Dengue; FAPESP; CAPES; FAMERP/FUNFARME; Secretaria Municipal de Saude.

**HV1331 - RESISTANCE TO NS5A ANTIVIRAL AGENTS IN THERAPY-NAÏVE BRAZILIAN PATIENTS WITH HEPATITIS C VIRUS INFECTION**

Peres da Silva, A., de Almeida, A.J., Lampe, E.

1. Universidade Federal do Estado do Rio de Janeiro, UNIRIO, Rua Mariz e Barros, 775 - Tijuca, Rio de Janeiro - CEP: 20270-004
2. Fundação Oswaldo Cruz, Fiocruz, Av. Brasil, 4365 - Manguinhos, Rio de Janeiro - CEP: 21040-360 E-mail: peres_allan@yahoo.com.br

The current therapy to Hepatitis C virus (HCV) infection are suboptimal especially in patients infected with HCV genotype 1 and are poorly tolerated because of its side effects. Major researches efforts focused on new therapeutic approaches are based on NS3/4A protease and NS5B polymerase inhibitors which are currently in most advanced clinical development. Among the nontraditional targets, NS5a protein has shown potentially active across different HCV genotypes and promising antiviral efficacy in clinical studies. However, several resistance variants to NS5a inhibitors have been described both in in vivo and in vitro studies and may represent an important factor that limit the effectiveness of therapy with direct-antiviral agents (DAAs). In this context, the aim of this study was to analyze the genetic variability of NS5a gene in HCV genotype 1 isolates circulating in our region. A total of 26 samples from therapy-naïve Brazilian patients chronically infected with HCV subtype 1a were studied. Viral RNA was extracted and the region encompassing the NS5a gene (6258nt-7602nt) was reverse-transcribed and PCR-amplified and submitted to direct sequencing. Nucleotide sequences were analyzed by multiple alignment using the MEGA 4.0 program and deduced amino acid sequences were inferred by the same program. The analysis revealed the presence of variants in two Brazilian samples, BR137 and BR157, at sites Y93 (Y93H) and H58 (H58P), which confers high resistance to the inhibitor Daclatasvir (BMS-790052). These mutants were not related to occur in European therapy-naïve patients with HCV subtype 1a infection, corroborating to the fact that analysis of viral sequences from different geographical regions may show significant differences in the frequencies of resistance to new DAAs inhibitors. Information on patterns of resistance to new DAAs may be determinant for future decisions on how to combine drugs to achieve an optimal antiviral effect. Financial support: Capes-Papes V/ CNPq

**HV1334 - HEPATITIS D VIRUS SCREENING IN PATIENTS WITH ACUTE OR CHRONIC HEPATITIS B VIRUS INFECTION IN RIO DE JANEIRO, BRAZIL**
Hepatitis D virus (HDV) is a defective virus that requires the presence of hepatitis B virus (HBV) to complete its replicative cycle. HDV infection is associated with more severe form of hepatitis and increased risk of progression to complications such as cirrhosis and hepatocellular carcinoma. In Brazil, the Amazonian Basin is an endemic area for HDV infection, however, few data is available for others regions in the country. This study evaluated the seroprevalence of HDV in patients with acute or chronic infection with HBV followed at the Viral Hepatitis Ambulatory, Rio de Janeiro, Brazil, between 2006 and 2011. A total of 370 samples from patients presenting hepatitis B surface antigen (HBsAg) positivity were tested for the presence of serum anti-HDV antibodies using the commercial assay ETI-AB-DeltaK-2 (Diasorin, Italy), according to the manufacturer's instructions. Reactive or indeterminate results were retested in duplicate to confirm the result. Patients’ samples with anti-HDV positive were tested by PCR to amplify a fragment of delta antigen (HDAg) genomic region. HDV RNA positive samples were submitted to direct nucleotidesequencing and phylogenetic analysis using MEGA v. 5.0 software package to characterize HDV genotype. Our study population consisted of 243 males and 127 females, with a median age of 43 years (1 – 82 years), being 138 patients with acute HBV infection and 232 with chronic. Six patients were positive for anti-HDV antibodies (acute HBV, n = 1; chronic HBV, n = 5), one of the patient with chronic HBV infection had detectable HDV RNA. The phylogenetic analysis showed that the HDV sequence clustered within genotype 3. In conclusion, despite the HDV seroprevalence found to be low in our cohort, this results highlighted the importance of HDV infection investigation in non endemic areas.

Financial support: FIOCRUZ

HV1337 - EPIDEMIOLOGIC IMPACT OF HEPATITIS D (DELTA) IN THE MUNICIPALITY OF GUAJARA-MIRIM/RO

Justiniano, R.L., Santos, A.O., Vieira, D.S.

1. Oswaldo Cruz Foundation RO, Brazil, FIOCRUZ RO, RUA DA BEIRA, 7671, LAGOA
2. CENTRO DE PESQUISA EM MEDICINA TROPICAL DE RO, CEPEM, AV. GAPORE, 215, LAGOA

Delta virus (HDV) infection is highly pathogenic and transmitted in the presence of surface antigen of the virus causing hepatitis B (HBsAg). The transmission is given by a co-infection or superinfection. In areas endemic for hepatitis B, the HDV infection represents a serious public health problem, and the state of chronic HBV (HBsAg positive) constitutes the main factor for the epidemiological spread of HDV, which occurs, for example among the native populations of the Brazilian Amazon, Peru and Venezuela and, in certain areas of Africa. The municipality of Guajará-Mirim located in the western Brazilian Amazon, belonging to the state of Rondônia an estimated population of 41.933 inhabitants, considered the eighth most populous

Fundação Oswaldo Cruz, FIOCRUZ, Av. Leopoldo Bulhões, 1480. Manguinhos - Rio de Janeiro - RJ
E-mail: vmarques@ioc.fiocruz.br
county in the state has characteristics of mixing of various races with the native (indigenous culture), resulting in a population Amazon typically with the predominance of "shifting cultivators" and a strong presence of mixing with immigrants from the border (Bolivia). This study evaluates the epidemic incidence of HDV infection in this city through exploratory and descriptive research. Data were obtained through the Program STD/HIV/AIDS and Viral Hepatitis of the Municipal Health Guajará-Mirim RO-2010 - 2011, by which it was estimated 138 cases of HBV infection and 12 cases of co-infection with HDV. The most affected age group was the between 20 and 39 years of age, with 66% of the total, and the male was the most affected by HDV, registering 55.8% of cases. By these data we can see the need to program or improve measures that can reduce the incidence and prevalence rates of Hepatitis Delta. Financial support: SUS.

HV1344 - COMPARISON OF DIRECT FLUORESCENCE ASSAY AND REAL-TIME PCR FOR THE DETECTION OF INFLUENZA VIRUS A AND B IN IMMUNOCOMPROMISED PATIENTS

Perosa, A., Watanabe, A.S.A., Ricci, E., Guatura, S., Bellei, N.

Universidade Federal de São Paulo, UNIFESP, Rua Pedro de Toledo, 781 - 15o andar E-mail: anaperosa@gmail.com

During 2009 H1N1 pandemic, morbidity was high in Brazil and hospitalizations resulting from severe respiratory disease due to suspected influenza infection were of concern during the subsequent years. According to health authorities, during 2011 influenza A virus caused eight deaths in São Paulo state, but this year number of cases and deaths has been increased. The aim of this study was evaluate de influenza virus A and B prevalence among samples collected from hospitalized patients with severe acute respiratory infection (SARI) and from outpatients who presenting with influenza-like illness (ILI) received at Virology Laboratory of São Paulo Hospital. Influenza A and B were investigated by CDC Real-time PCR (RT-PCR) with minimal modifications to include influenza B. During 2011, we analyzed 169 respiratory samples (nasal swabs/washes) and influenza prevalence was 21.3%. In 2012, we received 103 respiratory samples and influenza prevalence was 30.1%. During 2011, influenza was detected in 4.8% (1/21) of patients with SARI (only seasonal influenza A) and in 29.5% (33/112) of ILI patients, of whom 60.6% (12/33) were influenza B, 36.4% (12/33) were influenza A H3N2 and 3% (1/33) H1N1pdm/09. On the other hand, 2012 influenza detection was 36.1% (13/36) in hospitalized patients and 42.9% (9/21) in ILI patients, out of this 89% (8/9) were influenza A. We documented that influenza is responsible for mild and severe respiratory infections and accounted for a large proportion of hospitalizations for SARI during 2012 influenza season. Financial support: CNPQ, FAPESP

HV1345 - THE USE OF SOCIAL NETWORKING FACEBOOK AS A TOOL FOR DISSEMINATION OF SCIENTIFIC KNOWLEDGE IN VIROLOGY

Silva, G., Vedovello, D., Ozanic, K., Nogueira, M.
Faculdade de Medicina de São José do Rio Preto, FAMERP, Av. Brigadeiro Faria Lima, 5416 - Vila São Pedro - 15090-000 E-mail: gislaine.cds@gmail.com

The use of social networks like Facebook, Orkut, MySpace or Twitter crossed the personal relationships and became a great way to Internet marketing and dissemination of knowledge. For dissemination of exclusive scientific knowledge there are networks like ResearchGate, that includes more than 35,000 Brazilian researchers, BiomedExperts, SciLink, and others. These networks are restricted to a specific audience and they aren’t used by the remainder of the population. The Facebook has become the larger tool of relationship in the world and are widely used by scientific research groups worldwide. By having the largest number of users this may contribute to the access and disclosures of scientific papers, closer relationship between the Brazilians laboratories and the others labs in the world. Using the search engine of the Facebook we found 42 groups in the social network (restricted or open) that have specific content of Virology and has over 2300 members. Among them we can highlight our group page Laboratório de Pesquisa em Virologia - FAMERP (http: // www. Facebook . com / groups / 205746489469432). This group is the largest in number of members, totaling 405 people, consisting of undergraduate and postgraduate students, teachers of Secondary and Higher Education and renowned scientific researchers. In the LPV group there are members of all regions of Brazil; 260 (64,3%) members are from the Southeast Region, 9 (2,2%) are from the Southern Region, 6 (1,4%) are from the Midwest, 5 (1,2%) from Northern and 3 (0,7%) from Northeast. There are also members of the U.S. and the others countries, 12 (2,9%) and 112 (27,7%) members did not specify the place of residence/ origin. Created in June 2011 the our group on Facebook aims to disseminate information in the scientific area, promote discussions, and facilitate the exchange of manuscripts (within the area of human virology, plant and animal) published in Brazil and abroad. From January to July 2012 more than 114 articles were shared and discussed among the group members. The tool also allowed the dissemination of courses and events related to this science – Virology. This shared of information can have a positive effect making when learning becomes more enjoyable.

HV1348 - PREVALENCE OF INFLUENZA VIRUS IN HOSPITALIZED PATIENTS WITH SEVERE ACUTE RESPIRATORY INFECTION AND OUTPATIENTS WITH INFLUENZA-LIKE ILLNESS DURING 2011 – 2012 IN SÃO PAULO HOSPITAL

Perosa, A., Camargo, C., Guatura, S., Bellei, N.

Universidade Federal de São Paulo, UNIFESP, Rua Pedro de Toledo, 781 - 15o andar E-mail: anaperosa@gmail.com

During 2009 H1N1 pandemic, morbidity was high in Brazil and hospitalizations resulting from severe respiratory disease due to suspected influenza infection were of concern during the subsequent years. According to health authorities, during 2011 influenza A virus caused eight deaths in São Paulo state, but this year number of cases and deaths has been...
increased. The aim of this study was to evaluate the influenza virus A and B prevalence among samples collected from hospitalized patients with severe acute respiratory infection (SARI) and from outpatients who presented with influenza-like illness (ILI) received at Virology Laboratory of São Paulo Hospital. Influenza A and B were investigated by CDC Real-time PCR (RT-PCR) with minimal modifications to include influenza B. During 2011, we analyzed 169 respiratory samples (nasal swabs/washes) and influenza prevalence was 21.3%. In 2012, we received 103 respiratory samples and influenza prevalence was 30.1%. During 2011, influenza was detected in 4.8% (1/21) of patients with SARI (only seasonal influenza A) and in 29.5% (33/112) of ILI patients, of whom 60.6% (12/33) were influenza B, 36.4% (12/33) were influenza A H3N2 and 3% (1/33) H1N1pdm/09. On the other hand, 2012 influenza detection was 36.1% (13/36) in hospitalized patients and 42.9% (9/21) in ILI patients, out of this 89% (8/9) were influenza A. We documented that influenza is responsible for mild and severe respiratory infections and accounted for a large proportion of hospitalizations for SARI during 2012 influenza season. Financial support: CNPQ, FAPESP

HV1358 - DETECTION OF NOROVIRUSES IN FECAL SAMPLES OF CHILDREN WITH AND WITHOUT ACUTE GASTROENTERITIS FROM RIO BRANCO, ACRE


Instituto Evandro Chagas, IEC, Rodovia Br 316, Km 07 E-mail: leiteevandro2@yahoo.com.br

Noroviruses (NoVs) are the main cause of diarrheic outbreaks non-bacterial origin, transmitted primarily by fecal-oral route, through contaminated water and food or by person to person contact. The NoVs belong to Caliciviridae family and the main symptoms caused by these agents are vomiting and diarrhea. Populations of developing countries are more susceptible to diarrhea by this virus and studies that demonstrate the etiology of these infections are important to guide public policies of prevention and control. The objective of this investigation was to detect NoVs in fecal specimens collected from children under five years old, during three expeditions carried out in Rio Branco, Acre, in February, April and June of 2012. The fecal specimens were collected from children attended in the Emergency unit (UPA) of the I and II district. The samples were initially tested by enzyme immunoassay (EIA) and after by reverse transcriptase-polymerase chain reaction (RT-PCR) using primers Mon 432-434/431-433 specific for NoVs genogrupos GI and GII, respectively. Of the 277 samples collected, a positivity of 11.9% (33/277) was observed to NoVs for at least one technique, among which, 8.7% (9/103) was collected in February, 3.8% (4/106) in April and 29.4% (20/68) in June. Therefore, the viral detection decreased from January to April, with considerable increase of the positivity in June. Furthermore, molecular characterization will be done for NoVs genotypes identification. NoVs have been identified as relevant etiological agent of diarrhea in many places. In Belém (35.4%-171/483)
and São Paulo (29.2%- 26/89), NoVs infections were also detected in high percentage in cases of diarrhea among hospitalized children less than five years old. This is the first report about NoVs detection in Rio Branco, Acre and demonstrates the epidemiologic importance of this virus in that region. Financial Support: IEC/SVS/MS.

HV1360 - STRUCTURAL FEATURES OF HIV-1 C2-V3-C3 REGIONS OF GP120: SELECTIVE PRESSURE AND DIVERSITY BETWEEN CLADES B AND C FROM BRAZIL


Fundação Estadual de Produção e Pesquisa em Saúde, FEPPS/RS, Av. Ipiranga, 5400 - Porto Alegre/RS - CEP: 90610-00 E-mail: leonardo_luvison@hotmail.com

In Brazil, HIV-1 subtypes B and C have accounted for the majority of infections. Nearly half of the infections caused by the subtype B strain are due to viruses with the unusual GWGR motif in the V3 loop of the envelope (Env) gene. Understanding how and why inter- and intra-subtype differ in Env is necessary to tackle the genetic diversity of HIV-1 in vaccine design and treatment. For this purpose, all available gp120 C2-V3-C3 (HXB2 6816-7380) for clades B and C from Brazil were downloaded from the Los Alamos National Laboratory HIV Sequence Database and GenBank. Additional sequences obtained from samples collected in Porto Alegre, Brazil, were included in the data set totalizing 297 subtype B and 166 subtype C sequences. Shannon entropy was estimated for each individual amino acid position in order to reflect the variability of that position across all sequences. Positive selection was assessed using the Maximum Likelihood approach implemented in SLAC and FEL in the DataMonkey package. The sequences of subtype B are from the North (6.6%), Northeast (8.2%), Midwest (14.8%), Southeast (43.8%) and South (26%) regions of the Brazil. The sequences of subtype C are mostly from South region (94.4%). Our analysis among Brazilian subtypes B and C exhibits that the structural domain encoded in the C3 region overlapping sites under positive selection, suggesting a convergent evolution of these clades. Examination of dN/dS ratios in V3 revealed much higher diversifying selection in subtype B than in subtype C. It is believed that C clade V3 domain lacks sites of strong positive selection due the formation of a cluster of hydrophobic residues (I307, I309, and F317). The analysis of subtype B GWGR amino acid frequencies show that position I309 tends to not preserve specific hydrophobic residue (>94%). The impact on the fitness of virus might be relevant, since that V3 may be more exposed in the GWGR viruses serving as an antibody-mediated neutralization target. Our data show structural differences of the Brazilian subtype B compared to subtype B analysis worldwide.

HV1362 - THE LARGEST OUTBREAK OF DENGUE IN THE STATE OF CEARÁ


1. Laboratório Central de Saúde
Pública do Ceará, LACEN-CE, Av. Barão de Studart, 2405 - Aldeota CEP: 60120-002

2. Secretaria de Saúde do Ceará, SESA-CE, Av. Almirante Barroso, 600 - Centro Rede Nordeste de Biotecnologia - UECE, RENORBIO-UECE, Av. Paranjana, 1.700 - Campus do Itaperi - 60740-000 Fortaleza/CE E-mail: briciamt@yahoo.com.br

Dengue virus infections are a major concern in developing countries, especially those located in subtropical and tropical areas, as Brazil. The disease affects, approximately, 100 million people/year. In the state of Ceará in Northeast Brazil, dengue epidemics have been described since 1986, initially with involvement of DENV-1, followed by DENV-2 in 1994, DENV-3 in 2002, and DENV-4 in 2011. Twenty-six years of endemic dengue were evaluated by the number of cases reported and confirmed. Data collection was based on the Epidemiological Bulletins of dengue published by the Health Department of the State of Ceará. Among the analyzed period, 5 epidemic peaks were reported in 1987, 1994, 2001, 2008 and 2011. Of these, the highest number of cases was observed in 2011, with 56,714 cases with laboratory confirmation, with 457 cases of DCC and 174 cases of DHF. The total number of DCC and DHF were behind only the data for year 2008. The incidence was of 670.98 per 100,000 inhabitants, with predominance of children and young adults. Secondly, the year 1994 had 47,789 confirmed cases with an incidence of 732.31 and with predominance of serotype DENV-2. In the year 2011, was detected serotype DENV-1 mainly (98.7%), and serotypes DENV-4 and DENV-3 (0.9% and 0.4% respectively). The highest number of cases observed in 2011 was probably due to recirculation of DENV-1 in recent years, having had similar prevalence in the epidemic of 1987. The DENV-1 circulated widely in Ceará by the year 2002. The population born after this period was susceptible to the virus that was circulating again. Only one genotype of DENV-1 has been circulating since the first epidemic reports in Brazil. However, two different lineages of DENV-1 genotype 5 have been found around the country, so as in Ceará. Financial support: FUNASA.

HV1381 - RT-PCR AMPLIFICATION OF DENGUE VIRUS RNA USING A MAGNETIC EXTRACTION METHOD

Barboza, M.M.O., Araújo, F.M.C., Perdigão, A.C.B., Cruz, J.N.M., Lima, D.M., Pires Neto, R.J.

1. Universidade Federal do Ceará, UFC, Rua Alexandre Baraúna, 949 - Rodolfo Teófilo - CEP 60430-160 - Fortaleza - CE

2. Laboratório Central de Saúde Pública, LACEN-CE, Av. Barão de Studart, 2405 - Aldeota, Fortaleza-CE

3. Universidade de Fortaleza, UNIFOR, Av. Washington Soares, 1321, Edson Queiroz E-mail: morganabiologia@gmail.com

Dengue virus is an RNA virus belonging to the Flaviviridae family and the etiologic agent of today’s most important arthropod-borne disease. It is estimated that about half of the world’s population is at risk of contracting the disease. It is transmitted by the bite of the female Aedes aegypti
mosquito. The disease can be caused by any of the four serotypes (DEN1-DEN4) and possesses a spectrum of clinical presentations ranging from undifferentiated fever to dengue hemorrhagic fever and dengue shock syndrome. Laboratory confirmation of dengue depends on the time-of-sample collection. Viral isolation, serological tests and molecular methods are currently available for diagnostic of dengue infection. The polymerase chain reaction preceded by reverse transcription (RT-PCR) is an important molecular method for early diagnosis of dengue infection. However, for a good yield in the RT-PCR is of paramount importance that the RNA extraction method is suitable. Our aim is to evaluate the performance of magnetic extraction method followed by One-step RT-PCR. We used serum samples from twenty patients known to be positive for DEN-1 by viral isolation and immunofluorescence technique. The magnetic extraction of viral RNA was performed with Biomerieux NucliSense miniMAG kit following the manufacturer's recommendations. For RNA amplification in one step we used the QIAGEN OneStep RT-PCR kit. The primers used were described by Rico-Hesse (1990) and corresponds to the E/NS1 junction region. The amplified fragments were separated on 2% agarose gel plus 5ul etidium bromide at a voltage of 103 for 1 hour. The bands were visualized under ultraviolet light. Of the 20 samples tested, only 9 (45%) had the E/NS1 region amplified. We conclude that the magnetic extraction method was suitable for RT-PCR of dengue viruses but a greater number of samples are necessary to better evaluate performance.

**HV1382 - PREVALENCE OF ANTI-HBC ALONE IN PATIENTS TREATED AT THE SEROLOGY LABORATORY OF THE AMBULATORY SPECIALIZING IN VIRAL HEPATITIS IN THE CENTER FOR RESEARCH IN TROPICAL MEDICINE IN 2010 AND 2011, RONDÔNIA, BRAZIL**


1. Fundação Oswaldo Cruz de Rondônia, FIOCRUZ Rondônia, Porto Velho, Rondônia, Brazil
2. Fundação Universidade Federal de Rondônia, UNIR, Porto Velho, Rondônia, Brazil
3. Research Center for Tropical Medicine, CEPEM, Porto Velho, Rondônia, Brazil
4. Tropical Pathology Research Institute, IPEPATRO, Porto Velho, Rondônia, Brazil

The world has two billion people infected with hepatitis B, and of these about 360 million are chronic carriers. HBV is a DNA virus belonging to the family Hepadnaviridae and preferentially infects hepatocytes. Detection of anti-HBc alone in the absence of anti-HBs HBsAg and corresponds to a serological profile that is unknown how the clinical importance, and can mean past infection without seroconversion or with decreasing to undetectable levels of anti-HBs. This profile can also be observed in the phase of the window in the cases of acute hepatitis B resolution. However, this serological profile can also be suggestive of occult hepatitis B infection with HBsAg undetectable, because of a low amount of virus in the blood or mutations intrinsic to the virus. Therefore it is important
to characterize the epidemiology of the prevalence of anti-HBc alone serological tests in patients attended in Serology Laboratory Specialized of Viral Hepatitis Ambulatory in Rondônia. To that end, we consulted the results of examinations of patients seen at the Serology Laboratory of Viral Hepatitis Ambulatory between January/2010 and December/2011. 4645 examinations were consulted where 483 patients as anti-HBc alone were selected for study. In 2010 found 175 patients, 66% male, with higher incidence in the age group of 40-60 years and 77% living in the capital. In 2011 found 308 patients, 53% male, with higher incidence in the age group of 40-60 years, 55% living in the capital and 45% inside the state. These results corroborate other studies on the prevalence corresponding to a high prevalence of anti-HBc alone in Serology Laboratory of Viral Hepatitis Ambulatory in Rondonia. A solution to solve this frame anti-HBc alone is the test nucleic acid amplification (NAT), which can detect a possible occult HBV infection and to elucidate the molecular mechanisms that cause this serological profile. Financial support: IPEPATRO, CEPEM/SESAU, FIOCRUZ RONDÔNIA e UNIR

HV1392 - DETECTION OF POTENTIALLY NOVEL FLAVIVIRUSES IN MOSQUITOES OF THE BRAZILIAN PANTANAL


1. Fundacao Oswaldo Cruz, FIOCRUZ, Avenida Brasil 4365, Rio de Janeiro, RJ, 21045-900, Brasil

2. Centers for Disease Control and Prevention, CDC, 3150 Rampart Rd, Fort Collins, CO 80521, USA
E-mail: pauvolid@ioc.fiocruz.br

The Brazilian Pantanal hosts large concentrations of diverse wildlife species, and therefore this region is a hotspot for arbovirus studies in South America. A recent study reported serological evidence of various arboviruses, including West Nile virus and Ilheus virus (ILHV). To extend this study, we captured 3111 adult mosquitoes of 16 species from the Nhecolandia sub-region of Pantanal during 2009 and 2010. Mosquito pool homogenates were assayed for infectious viruses in C6/36 and Vero cell monolayers and also tested for flavivirus RNA by a group-specific Real-Time RT-PCR using a SYBR-green detection platform. In addition to a single isolation of Ilheus virus from Aedes scapularis, several unidentified flaviviruses were detected by Real-Time RT-PCR from Mansonia pseudotitillans and Culex chidesteri. Amplicons in the NS5 gene region of the 11 kb flavivirus genome were cycle-sequenced and compared to known NS5 sequences in Genbank. The sequences had less than 78% identity with other known flaviviruses. The present data report the circulation in mosquitoes of potentially novel flaviviruses in the Nhecolandia sub-region of Pantanal, Brazil.

HV1393 - SEROEPIDEMIOLOGICAL STUDY INFECTION OF HEPATITIS C VIRUS IN A PRISON IN MALE-ARAPI-RACA ALAGOAS-BRAZIL

Santos, E.O., Souza, A.R., Silva, E.E., Morais, V.M.S.

1. Universidade Federal de Pernambuco, UFPE, Av. Prof.
The infection caused by hepatitis C virus (HCV) is a serious public health problem in Brazil and worldwide. The prison population has a high risk for HCV infection due to the high prevalence of risk factors related to sexual practices, the use of tattoo / piercing and injecting drug use. The present study aimed to evaluate the seroepidemiological profile of HCV infection among inmates of Arapiraca-AL. A total of 100 peripheral blood samples (10mL), from the prison population in the medium-security male prison Judge Luis de Sousa Oliveira, were collected and sent to the Laboratory of General Biology, State University of Alagoas for serum separation and detection anti-HCV. To detect the anti-HCV was used a commercial kit from the Wiener® lab following the manufacturer’s instructions. Of the total of 187 inmates of the closed regime, 100 of them agreed to participate. Anti-HCV hasn't been identified in any of the processed samples. The ages of the prisoners was between 18 and 60 years, mostly mulatto (65%), low educational level (55%). All from Alagoas. With regard to sexual orientation, 3% said being homosexual. The most (58%) reported not using condoms. The use of inhaled drugs was referenced by 64%, since the use of injecting drug use and tattooing / piercing were reported by 1% and 54% respectively. Blood transfusion was performed in 22% of those investigated. The absence of HCV infection among inmates shows that this population does not constitute a risk group for this type of infection. Financial support: State University of Alagoas.

Brazil presents a HIV-1 epidemic characterized by the co-circulation of the subtypes B, F1 and C, as well as mosaic genomes involving the recombination of these subtypes. Currently, about 32 thousand children and adolescents are infected with this virus in the country. This study aimed to evaluate pol gene diversity and the prevalence of transmitted drug resistance mutations among vertically infected children and adolescents in Rio de Janeiro State, Brazil. HIV-1 from plasma samples of 94 patients with age <19 years collected between 2008 and 2012, at Laboratory of AIDS and Molecular Immunology of the Oswaldo Cruz Institute-Fiocruz, were
Hepatitis B virus (HBV) infection is a public health issue, one of main causes of death from infectious diseases worldwide. Brazil public health system (SUS) has provided antiviral drugs for chronic hepatitis B treatment for over 10 years, but a system for monitoring for drug-related resistance mutations is not available. This study aims to determine the presence of HBV primary mutations associated with nucleoside and nucleotides analogs in antiviral-naïve patients with chronic hepatitis B infection. HBV reverse transcription (rt) gene sequences from 37 isolates from antiviral-naïve patients from Cruzeiro do Sul Hospital were analyzed. These sequence data were obtained to validate molecular methods and HBV genotyping from previous collaborating studies with HUPES and FIOCRUZ-BA. Briefly, HBV-DNA was amplified with a nested-PCR with primers FHBS1-RHBS1 and FHBS2-RHBS2, and sequenced using ABI Prism 3100 (Applied Biosystems, USA). Sequences from forward (FHBS2) and reverse (RHBS2) primers were aligned to obtain a contig with length ranging from 310 to 369 bp, corresponding to the rt amino acid position from 51 to 172. Conflicting sites were edited by comparison with reference sequence X04615 after visual inspection. Consensus sequences were used for interrogating a local HBV drug resistance database (HBVrt DB, Stanford University, USA) to retrieve the prevalence of each mutation according to genotype and treatment. HBV genotype A (62.1%) was most prevalent followed by genotype F (31.0%) and D (6.9%). Despite the high rate of co-infection with Delta virus (58.6%), no primary drug-related resistance mutation was observed in this rt
region. Other regions will be evaluated in the future. After the initiation of drug therapy it is extremely important to monitor viral load and identify drug-related resistance mutations in order to support clinical decision about the patient management in addition to preventing the emergence of multidrug-resistant viruses. Financial support: FAPESB/CNPq No 020/2009 PRONEX/CNPq/FAPESB, Application number: 7201/2009.

HV1404 - MOLECULAR CHARACTERIZATION OF ROTAVIRUS, NOROVIRUS AND ASTROVIRUS FROM PATIENTS WITH ACUTE GASTROENTERITIS IN SALTO CITY, URUGUAY


Laboratorio de Virología Molecular, Regional Norte, LVMS, RN, UDELAR, Gral. Rivera 1350, Salto, Uruguay Hospital Departamental de Salto, HDS - ASSE, Cervantes esq. 18 de Julio, Salto, Uruguay Centro Medico Salto, CAM, Artigas 937, Salto, Uruguay Laboratorio de Virología Molecular, CIN, UdelaR, CIN, Mataojo 2055 E-mail: fernandolopezltort@gmail.com

Group A Rotavirus (RVA), Norovirus (NV) and Human Astrovirus (HAstV) are the major cause of acute gastroenteritis (AG) in children under five years old worldwide. These viruses are the leading cause of hospitalization and death due to AG among infants of this age group, mostly in developing countries. In this study, we analyzed clinical samples of young children with AG who were treated in two health institutions of Salto city: the public hospital of the city and a private medical health institution. 136 clinical samples were analyzed, 119 of them were fecal material and 17 vomit. The collection period was from February 2011 to June 2012. The samples were collected from pediatric patients (most of them between 0 and 5 years old). Viral RNA extraction was conducted from the clinical samples, and cDNA was generated from the RNA extracted through Retrotranscription (RT) using random hexamer primers. Worldwide standardized specific PCR protocol directed against capsid gene(s) were conducted for RVA, NV and HAstV molecular identification and genotyping. The RT-PCR analysis of the samples showed the following results: 39% were positive for RVA (n=48), 8% for NV (n=12) and 13% for HAstV (n=18). Thirty-eight DNA sequence were obtained from RVA RT-PCR positive samples (VP7 and/or VP4 genes), and according with the sequence information the genotypes distribution were as follow: P[4]G2 (n=9), P[8]G2 (n=4), P[8]G3 (n=1), P[8]G12 (n=1), P[2]G[ND*] (n=14), P[4]GND (n=4), P[8]GND (n=5). From the 13% RT-PCR positives samples for HAstV, 78% (n=14) were confirmed by DNA sequencing, and the genotype distribution were as follow: 42% Genotype 1 (n=6), 29% Genotype 2 (n=4), and 29% Genotype 3 (n=4). RT-PCR NV positive samples are actually being sequenced. These results represent the first report of the circulation of RVA, NV and HAstV in the country outside the Capital city (Montevideo), and the first report of the circulation of NV and HAstV in Uruguay. * (ND = Not-determined)

HV1405 - HEPATITIS B SUBGENOTYPES CHARACTERIZATION IN CHRONIC HEPATITIS B PATIENTS IN
BRAZIL: A MORE ACCURATE VIEW ON HBV VARIABILITY


1. Instituto de Medicina Tropical - FMUSP, IMT- FMUSP, Av. Dr. Enéas Carvalho de Aguiar, n° 500/Prédio II 2°andar Faculdade de Medicina da Universidade de São Paulo, FMUSP
2. Faculdade de Medicina do ABC, FMABC
3. Universidade Federal de Minas Gerais
4. Universidade Federal do Maranhão
5. Secretaria Municipal de Saúde de Ribeirão Preto
6. Serviço Municipal de Infectologia de Caxias do Sul
7. Santa Casa de Misericórdia do Pará
8. Instituto Evandro Chagas,, E-mail: gomesmic@yahoo.com.br

Hepatitis B virus (HBV) shows great variability, at least 10 genotypes (HBV-A through J) have been identified and subgenotypes have been classified within some these genotypes. Most genotypes and some subgenotypes show heterogeneity in their global distribution that may reflect the different patterns of human migration. Progression to chronic infection, the outcome of chronic hepatitis B (CHB) and the response to HBV treatment have been associated with this variability. Some studies suggest important pathogenic differences between HBV genotypes and subgenotypes. In Brazil, HBV genotype distribution was described, but the current available data is still incomplete, as few of them have characterized the HBV subgenotypes. In this study, we identified HBV subgenotypes isolated from 557 chronic hepatitis B carriers originating from five different Brazilian states (SP, MG, RS, PA and MA). A fragment of 1306 bp partially comprising HBsAg and the DNA polymerase coding regions (S/POL) was amplified and sequenced. HBV genotypes/subgenotype were determined by Bayesian phylogenetic analyses. HBV genotype A was the most prevalent (69.1%; 385/557) followed by genotype D (23.7%; 132/557) and F (4.8%; 27/557). Genotypes B, C, E and G were also found in few samples. HBV/A was more common in all regions, except in RS where HBV/D prevails. In MG, almost all patients were infected by HBV/A (91.7%). HBV/A1 and A2 were identified among HBV/A genotypes with higher prevalence of A1 (97%; 372/385). HBV/D showed high variability: subgenotypes D1, D2, D3 and D4 were found. HBV/D2, D3 and D4 were the most prevalent: 18% (24/131), 51% (66/131) and 28%, respectively. HBV/D1 only was found in three cases (2%): two from RS and one from SP. HBV/D subgenotypes showed a heterogeneous geographic distribution, but HBV/D3 was the most prevalent subgenotype in all regions excluding MA where D4 prevailed. HBV/F was found in samples from all studied regions, with almost all of them classified as subgenotype F2. Only two cases from SP were infected.
by F4. Among genotypes B cases, subgenotypes B1 and B2 were found and genotype C cases were classified as C2. In conclusion, in this study, we observed that HBV strains circulating in Brazil shows great diversity and that genotype identification did not reflect the accurate variability of the virus, especially in genotype D cases. Financial support: FAPESP (2010/50081-9 and 2010/51208-2).

HV1406 - ROTAVIRUS INFECTION AMONG COMMUNITY CHILDREN IN GUARAPUAVA, PARANA


Universidade Estadual do Centro-Oeste-PR, Unicentro-PR, Rua Simeão Camargo Varela de Sá, 03. Vila Carli, 85040-080, Guarapuava, PR. E-mail: veronika_ambrosini@hotmail.com

Rotavirus is the main etiological agent of diarrhea in childhood. Rotavirus immunization was introduced in Brazilian 6-month-old children in 2006. The present study was aimed to evaluate human Rotavirus occurrence in stool samples obtained from community children with gastroenteritis in a municipal laboratory of health public service. 83 stool samples collected in the 2011-2012 period were analyzed by RT-PCR for human Rotavirus detection. Rotavirus was detected in 4.1% of samples in a population with a coverage upper 80% previous immunization. These results indicate low incidence of human Rotavirus infection in community children with higher vaccine coverage. Financial Support: Fundacao Araucaria (Gestao compartilhada em saude - PPSUS).

HV1407 - STUDY OF TYPE I IFN LEVELS ON SERUM SAMPLES OF PATIENTS WITH DIFFERENT CLINICAL FORMS OF DENGUE


1. Universidade Federal de Pernambuco, UFPE, Av. Prof. Moraes Rego, 1235 - Cidade Universitária, Recife - PE

2. Centro de Pesquisas Aggeu Magalhães, CPqAM, Av. Professor Moraes Rego, s/n - UFPE - Cidade Universitária | Recife/PE

3. Centro de Pesquisas René Rachou, CPqRR, Avenida Augusto Lima, 1715, Barro Preto, Belo Horizonte - MG

4. Center for Vaccine Research, University of Pittsburgh, CVR, Pitt, 9014 Biomedical Science Tower 3, 3501 Fifth Avenue, Pittsburgh, Pennsylvania E-mail: mcs.mayara@gmail.com

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are increasingly important public health problems in the tropics and subtropics. Emphasis has been placed on the role of the adaptive immune system in dengue pathogenesis. However, there is increasing evidence about the role of innate immune system in regulating dengue infection and possibly influencing the disease outcome. The signaling system of type I interferon (IFN-I/IFN alpha-beta) is integral to the innate immune system’s ability to create an antiviral state. Counteracting mechanisms developed by viruses often hinders the antiviral IFN-mediated response, creating the mechanisms
necessary for the disease installation. In the present study, IFN alpha and beta levels on serum samples of 50 dengue virus infected patients (25 DF and 25 DHF), collected at the acute phase of the disease (within 3-5 days of fever), were quantified using Human IFN-alpha Matched Antibody Pairs kit (eBioscience) and ELISA Human IFN beta kit (Invitrogen), respectively, according to the manufacturers instructions. We observed that circulating levels of IFN-I were similar for both DF and DHF patients with no statistical significance. Therefore, we observed no correlation between mRNA and protein levels of type I IFN.

**HV1410** - **VIRAL MENINGITES: INVESTIGATION OF THE HUMAN PARECHOVIRUS AS ETIOLOGIC AGENT OF LINFOCITARY MENINGITES IN CEREBROSPINAL FLUID SAMPLES**

Vidal, L.R.R., Almeida, S.M., Nogueira, M.B., Raboni, S.M., Cavalli, B., Rossa, M.C.D., Cavalcanti, E.

1. Universidade Federal do Paraná, UFPR, Rua Pe. Camargo, 280, Setor de Ciências da Saúde, Alto da Glória, Cep; 80060240
2. Laboratório Central do Estado do Paraná, LACEN, Rua Sebastiana Santana Fraga 1001 - Guatupê, Curitiba - Paraná E-mail: lrrvidal@yahoo.com.br

Meningitis can be caused by several agents is attributed to enterovirus (EV) a rate of 90% of cases of viral meningitis, including the Human Parechovirus (HPeV) that has been described as the second most common pathogen in neurotropic diseases in humans. Global data related to HPeV are scarce, in Brazil there is a reporting of cases of HPeV type 8 in an outbreak of gastroenteritis. The study aims to define the epidemiological profile and characterize the molecular detection of HPeV, using CSF samples collected from patients with signs and symptoms of viral meningitis treated at Hospital de Clínicas, Federal University of Parana. The methodology used for the detection of HPeV genome 5’NCR was reverse transcription followed by real-time PCR. Positive samples were sequenced targeting the VP1 gene. Phylogenetic analysis of the positive samples showed a 96-100% similarity with the strain of the Netherlands (AMS721), confirming the circulation of HPeV in our region. Patients, with positive results for HPeV, presented ages ranging from 7 to 12 years, with prevalence of symptoms of fever, vomiting and headache, 60% of patients had neck stiffness and 20% showed signs of Kernig-Brudzinski. The median age was 8 years (p = 0.03), lactic acid 2.4 mmol / L (p = 0.03), 4.8 log10 viral load. There was a greater number of positive samples during the months with higher temperatures and coincide with the summer and spring, however, sporadic cases were observed in other months. In 2006, a greater number of positive samples also occurred when the rainfall was higher. Most samples were collected in March. Infection of the central nervous system (CNS) by HPeV was observed during the summer months and the use of the technique of real-time PCR was rapid and effective to define the etiological agent of infections of the CNS.

**HV1413** - **SEROLOGICAL EVIDENCES OF INFECTION BY FLAVIVIRUS ROCIO, SAINT LOUIS ENCEPHALITIS AND WEST NILE IN RIO GRANDE DO NORTE STATE, BRAZIL**

1. Faculdade de Medicina de Ribeirão Preto- USP, FMRP- USP, Av. Bandeirantes 3900 - Monte Alegre

2. Universidade Federal do Rio Grande do Norte, UFRN, Av. General Gustavo Cordeiro de Farias S/N. CEP 59012-570 E-mail: mafarignoli@hotmail.com

The Flavivirus genus includes the most important causatives of arboviral disease in tropical and subtropical countries. In Brazil, dengue viruses are responsible for several outbreaks every year. Other flaviviruses also reported in Brazil are yellow fever and those related to Japanese Encephalitis Complex (JECV) such as Saint Louis encephalitis virus (SLEV) and Rocio virus (ROCV). Although West Nile virus (WNV) has not been isolated in our country, serological evidence of WNV infection in horses has been reported. We show here results of a serologic survey including 85 participants from the County of Espirito Santo, Rio Grande do Norte State (RN). These participants, all healthy at the time of blood collection, were 55 females and 30 males, 2 to 59 years old (y.o.). Sera of the participants were tested by an in-house IgG-ELISA using specific recombinant peptides of domain III of E protein (rDIII) from ROCV, SLEV and WNV as antigen. It was observed that 8 samples (9.41%) from 5 women and 3 man, 4-45 y.o. presented monotypic IgG antibodies to WNV; one serum (1.17%) from a 20 y.o. woman presented monotypic IgG antibodies to ROCV; one serum from a 59 y.o. woman presented monotypic IgG antibodies to SLEV. Our results suggest that ROCV, SLEV and WNV could circulate in RN, being misdiagnosed with DENV or even remaining undiagnosed due to the limited health assistance sources to this population. Further studies including neutralization test are necessary in order to confirm these ROCV, SLEV and WNV infections. Financial Support: FAPESP and FAEPA

HV1414 - ANALYSIS OF RESISTANCE MUTATIONS TO ANTIVIRAL THERAPY USED IN THE TREATMENT OF HEPATITIS B IN PATIENTS FROM RONDONIA STATE


1. Instituto de Pesquisa em Patologia Tropical, IPEPATRO, Rua da Beira, 7175, Lagoa.


3. Fundação Oswaldo Cruz-Rondônia, FIOCRUZ, Rua da Beira, 7175, Lagoa.

4. Faculdade de Medicina da Universidade de São Paulo, USP, Av Dr Enéas de Carvalho Aguiar, 470 05403-000 E-mail: alcione.m@hotmail.com

Patients with chronic hepatitis B (CHB) can be successfully treated using nucleos(t)ide analogs (NA), but the occurrence of mutations in the HBV genome that confers resistance to these drugs is one of the most important factors in treatment failure.
Drug resistance may emerge during prolonged treatment, therefore monitoring of HBV variability, specifically at polymerase gene, is very important to more efficient treatment. The aim of this study was to identify the prevalence of HBV with resistance mutations to NA and characterize HBV genotypes in samples from patients that were followed up at the specialized clinic for Viral Hepatitis at Rondonia state. Twenty-two patients were included in this study and among these 18 were under NA therapy. A fragment of 741 bp comprising partially S and POL genes of HBV DNA was amplified by nested PCR and its sequence determined by direct sequencing. HBV genotypes were determined by phylogenetic analysis and the presence of mutations was determined by analysis of the amino acid changes associated with resistance at POL sequence. HBV DNA was detected in 22 samples and these 16 shows sequences with good quality. Among these samples genotype D was the most frequent (9/16; 56%) followed by genotype A (6/16; 38%). Genotype F was only detected in one sample. HBV harboring lamivudine resistance mutations (rtM204V or rtL180M + rtM204V) was detected in 38% (6/16) of the patients. One of these samples also carry a mutation rtS202G associated with rtL180M + rtM204V mutations, a pattern that additionally confers resistance to Entecavir. Another mutation (rt238T) that was potentially associated with Adefovir resistance was identified in only one sample. This study shows a higher prevalence of resistance mutation in therapy-experienced CHB patients from Rondonia. Considering that some mutations confers cross-resistance to different drugs its characterization is of great value to adequate treatment management and consequently to prevent disease progression. Support: IPEPATRO, CEPEM, FIOCRUZ.

HV1426 - EVALUATION OF TWO DIFFERENT RT-PCR PROTOCOLS FOR DETECTION OF DENGUE-1

Barboza, M.M.O., Araújo, F.M.C., Perdigão, A.C.B., Cruz, J.N.M., Lima, D.M., Pires Neto, R.J.

1. Universidade Federal do Ceará, UFC, Rua Alexandre Baraúna, 949 - Rodolfo Teófilo - CEP 60430-160 - Fortaleza - CE
2. Laboratório Central de Saúde Pública, LACEN-CE, Av. Barão de Studart, 2405 - Aldeota, Fortaleza-CE
3. Universidade de Fortaleza, UNIFOR, Av. Washington Soares, 1321, Edson Queiroz E-mail: morganabiologia@gmail.com

Dengue is the most important arboviral infection that affects humans, causing epidemics in more than 100 countries in tropical and subtropical regions of the world. The dengue virus belongs to the Flaviviridae family that comprises four serotypes (DENV1-4) involved in both dengue fever and dengue haemorrhagic fever. Early identification of dengue infection facilitates monitoring and treatment of patients while minimizing risks of complications. Laboratory confirmation is based upon direct or indirect methods which are applied according to infection period. During the acute phase, the virus or viral components are detectable. The aim of this study was to test the sensitivity of two different RT-PCR protocols.
for molecular diagnosis of DENV-1 infection. Serum samples from 20 patients in the acute phase of dengue fever were inoculated into C6/36 cells. DENV-1 strains were identified by indirect immunofluorescence assay using monoclonal antibodies. The protocol I was a semi-nested RT-PCR amplifying the C/prM region (Lanciotti et al, 1992). The protocol II was a One Step RT-PCR amplifying the E/NS1 region (Rico-Hesse, 1990). DENV-1 RNA was extracted by magnetic method. Amplification by protocol I was possible in only 2 samples (10%). Amplification by protocol II was possible in 9 samples (45%). Both protocols had low sensitivity. The small number of samples is a limiting factor in this work. The low sensitivity observed for DENV-1 in the protocol I had been previously described. Protocol II was originally validated for genotyping. In the state of Ceará DENV-1 began to run since 1985, and there may be inadequate homology between the sequences of the primers and the genome of the viral strain currently circulating in the state. In addition, other factors, including the RNA extraction method could explain the low sensitivity. Using a more significant number of samples is necessary for a more accurate assessment of both protocols.

HV1431 - HTLV-DNA DETECTION IN PREGNANT WOMEN BY IN HOUSE NESTED-PCR


1. Universidade Federal de Pernambuco, UFPE, Av. Prof. Moraes Rego, 1235 - Cidade Universitária, Recife - PE - CEP: 50670-901

2. Universidade Estadual de Alagoas, UNEAL, Rua Governador Luiz Cavalcante, S/N - Alto Cruzeiro, Arapiraca-AL CEP: 57312-000
E-mail: erlon.medtropical@hotmail.com

The human T cell lymphotropic virus (HTLV) is a retrovirus which is associated with some diseases, like adult T-cell leukemia/lymphoma and myelopathy/tropical spastic paraparesis. The HTLV-1 is present in all Brazilian regions, but its prevalence varies among the states, being highest in Bahia, Pernambuco and Pará. HTLV testing is very important in prenatal care of pregnant women to avoid the virus transmission during breast feeding of newborns. The purpose of this study was to investigate the presence of HTLV-DNA by in house nested polymerase chain reaction (PCR) in pregnant women followed in the Family Health Program in Penedo, Alagoas, Brasil. The pregnant women were screened for HTLV 1/2 antibodies by enzyme immunoassays kit (Ortho HTLV-I/HTLV-II ab-capture ELISA test system), according to the manufacturer’s instructions. For peripheral blood mononuclear cells (PBMC) isolation, EDTA-anticoagulated blood samples were treated with Ficoll-Hypaque (Sigma-Aldrich, USA). After isolation, the PBMC was stored in RPMI medium at -80°C until the DNA extraction. A commercial kit (QIAamp DNA Blood Mini Kit) was used for genetic material extraction, following the manufacturer’s instructions. An in house nested-PCR was applied for
detection the sequence env-tax of HTLV-1. The SK110 and SK44 primers were used in the first part of the reaction and SK248 and SK249 in the second part, generating a fragment of approximately 400 pairs of bases. Two hundred and nine pregnant women were analyzed and 8.2% (17/209) shown a positive serology for HTLV 1/2, of which 7.8% (9/17) were in the 18 to 28 years age group. However, 11.76% (02/17) refused to submit to nested-PCR. Thus, the HTLV-DNA was detected in 46.7% (7/15) of the other participants. Therefore, the HTLV infection was present in 3.4% (7/209) of evaluated pregnant women. The presented data show evidences to support the introduction of HTLV serological test in prenatal care by public health service to avoid the possible clinical consequences in newborns infected during breast feeding in the study area. Financial support: Universidade Estadual de Alagoas (UNEAL)

HV1437 - GENETIC CHARACTERIZATION OF HCV GENOTYPE 2 VARIANTS IN ISOLATES FROM BRAZIL


1. Laboratório de Hepatites Virais, Instituto Oswaldo Cruz, , IOC, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

2. Instituto de Patologia Tropical e Saúde Pública, Universi, UFG,

Hepatitis C is a single-strand, positive RNA virus classified within the Hepacivirus genus of the Flaviviridae family. The sequencing of HCV isolates is genetically identified into 6 major genotypes, each of which may have various subtypes. Phylogenetic analysis of NS5B region has been commonly used for identification of HCV subtypes and epidemiological applications. In Brazil, HCV subtypes 1a, 1b followed by 3a are the most prevalent strains, the 2 and 4 are less common strains and the genotype 5 uncommonly detected. Few Brazilian studies have analyzed genotype 2 and the molecular characterization is practically unknown. Moreover, its origin is not fully clear. The aim of this study was to characterize the HCV genotypes 2 circulating in Brazil. Between 2007 and 2012, a total of 8 serum samples were collected and tested for anti-HCV and HCV-RNA by RT-nest PCR of the NS5B region (nt 8279-8619) of HCV genome. For sequence analysis, the NS5B region was amplified and the PCR product obtained was purified and submitted to direct sequencing. The DNA alignments of obtained nucleotide sequences were generated with Clustal X program and Neighbor-joining and maximum-likelihood phylogenetic analysis were performed by MEGA3 program. A multiple sequence alignment of the examined region and the related sequences in the GenBank/EMBL database was performed. The phylogenetic tree showed that six isolates belonged to genotype 2 subtype b and another one was classified as subtype 2c. Besides, one isolate was found out of the group of isolates subtype 2b of Brazil’s cluster. This study suggests that the frequency of subtype 2b is higher than subtype 2c in Brazil. However, analyses of other regions are necessary to better characterize this genotype. Financial support: CAPES, CNPq and FDTIS/FIOCRUZ
HV1438 - DETECTION OF DENGUE VIRUS DIRECT IN VECTORS CAPTURED IN THE CITY OF MANAUS, AM, BRAZIL


1. Instituto Nacional de Pesquisas da Amazonia, INPA, Av. André Araújo, 2936, Aleixo, CEP 69060-001, Manaus – AM, Brazil.

2. Instituto Leonidas e Maria Deane / Fundação Oswaldo Cruz, ILMD / FIOCRUZ, Rua Terezina, 476, Adrianópolis, CEP: 69.057-070, Manaus – AM, Brazil. E-mail: antoniocardoso01@yahoo.com.br

Dengue virus (DENV) is an RNA genome arbovirus, family Flaviviridae, genus Flavivirus, caused by four distinct serotypes (DENV 1-4). Dengue is the most important arbovirus in Brazil, in number of cases and lethality. Differences in severity are associated with serotypes or particular genotypes. The city of Manaus, with 1.739.000 inhabitants has been infested by Aedes aegypti since 1996 and the first case of dengue fever (DF) episode appeared in 1998 and, in 2008 was the first case of DENV 4. This study was part of a dengue virus surveillance performed in the districts of Manaus. The mosquitoes were collected in the year 2011. In the Tropical Virology Laboratory of the National Institute of Amazon Researches (INPA), the specimens captured were identified and grouped in numbers up to 10 specimens per microtube (pool). A number of 165 Aedes aegypti were captured and pooled into 28 lots and five Aedes albopictus captured were pooled into four lots. Each pool of mosquitoes was macerated and diluted in a 1% solution of bovine albumin in phosphate buffered saline (PBS). Mosquito macerates had the RNA extracted and then were submitted to three different techniques, an RT-PCR (Reverse Transcriptase – Polymerase Chain Reaction) for detection of flavivirus genus followed by a Nested-PCR for identification of DENV species, both based on size of amplicons, a Multiplex-Nested-PCR and a Real Time PCR (qPCR) to detect the DENV direct in vectors. The three techniques confirmed the presence of the virus in different pools, being DENV 2, DENV 3 and DENV 4 serotypes. According to the molecular studies, which detected the presence from three of the four DENV serotypes, it demonstrates that the circulation and transmission of DENV is occurring at different locations in Manaus by Aedes aegypti mosquitoes during the last year as a part of a continuous transmission situation.

HV1439 - INCIDENCE OF CO-INFECTION BY HUMAN IMMUNODEFICIENCY VIRUS (HIV) IN INDIVIDUALS WITH PULMONARY TUBERCULOSIS TREATED IN THE CENTER OF TESTING AND COUNSELING IN STD/AIDS FROM ILHÉUS, SOUTHERN FROM BAHIA

Almeida, C.S., Santos, S.S., Costa, G.B., Oliveira, F.C.S.

1. Centro de Testagem e Aconselhamento em DST/ HIV, CTA/DST/HIV, Avenida Canavieiras, s/n, Centro, Ilhéus, Bahia

2. Universidade Federal de Minas
Gerais, UFMG, Avenida Antônio Carlos, 6627, Pampulha, Belo Horizonte, Minas Gerais E-mail: kris_almeida@hotmail.com

The infection by Human Immunodeficiency Virus (HIV) is one of the most important risk factors to contract tuberculosis due to immunological changes that it determines, creating favorable conditions for the activation of tuberculosis infection and disease development. The aim of this study was to assess the HIV co-infection in individuals infected with Mycobacterium tuberculosis who sought the services of the Counseling and Testing Center for STD/AIDS from Ilheus, southern Bahia, during the year 2011. The diagnosis of HIV infection was performed using the rapid test and by ELISA, and confirmed by immunofluorescence and/or western blot. The study included a total of 134 individuals, 81 men and 53 women, aged between 7 and 80 years. After serology, it was found that 10 patients (7.4%) were HIV positive. It is worth adding that all HIV-positive patients had pneumonia and/or liver disease. Most individuals (88%) belongs to the city of Ilheus, while the other 12% belong to other municipalities in southern Bahia, as Canavieiras, Itacaré, Ubaitaba, Una and Uruçucu. Understand the psychodynamics involved in the interaction process between the patient and the disease is of utmost importance for surveillance strategies are planned and enhanced. Still, it must be emphasized the need to return the individuals to confirm the diagnosis and therapeutic management.

HV1440 - EVALUATION OF SUSCEPTIBILITY OF CHILDREN TO HEPATITIS

Kury, C.M.H., Cruz, O.G., Teixeira, C.L., Riguetti, T.P., Conte, P.H., Pereira, R.C., Melgaço, J.G., Silva, J.P., Pinto, M.A., Vitral, C.L.

4. Laboratório De Desenvolvimento Tecnologico Em Virologia, Ioc / Fiocruz, Avenida Brasil, 4365, Manguinhos. Rio De Janeiro-Rj
5. Secretaria Municipal De Saude De Campos Dos Goytacazes-Rj, Sms/Campos-Rj, Rua Gil De Góis, 157, Altos, Centro, Campos Dos Goytacazes-Rj E-Mail: charbellkury@hotmail.com

The municipality of Campos dos Goytacazes is the only city in Brazil that implemented hepatitis A vaccination for all toddlers under the age of two in its public immunization program.
In order to evaluate the future impact that this immunization program will bring in the epidemiology of hepatitis A, a seroprevalence study is being conducted in individuals under the age of 19 years randomly selected at public and private schools from all 14 districts of this county. Sample size calculation based on 50% HAV estimated prevalence, 5% precision rate, and 80% confidence level yielded a total of 1028 subjects. Herein, anti-HAV results from the first 415 individuals included in the study are shown. After formal consent, blood spot samples were obtained for subsequent anti-HAV testing (Bioelisa HAV IgG, Symbiosys). Each participant or legal tutor was submitted to an interview using a standardized questionnaire. The overall prevalence of anti-HAV was 17.8%, being 96.2% of children under the age of five susceptible to HAV infection. Risk factors associated with seropositivity were: age (>5 years old) (RR=0.84 (95% CI:0.76-0.92) and skin color negro and mulatto (RR=0.85 (95% CI:0.78-0.96). No statistical difference was observed in gender (RR=0.94 95% CI: 0.86-1.03) and water source (filtered, mineral and untreated), with RR= 0.91 (95% CI: 0.78-1.05). An impressive increase in the anti-HAV prevalence was observed from the 1-4 year group (3.8%) to the 5-9 year group (15%), which may be related with the beginning of school attendance. Results obtained so far supports the municipality decision to introduce Hepatitis A vaccination in children before school admittance, and corroborate with data from other Brazilian seroprevalence studies that have been shown that a large proportion of children under the age of five are susceptible to HAV infection. The introduction of a hepatitis A vaccination program may be an important strategy for controlling HAV infection in Brazil.

HV1444 - ISOLATION AND TYPING OF DENGUE VIRUS FROM VECTORS Aedes Aegypti Captured In The Urban Area Of Manaus, Amazonas


1. Instituto Nacional de Pesquisas da Amazonia, INPA, Av. André Araújo, 2936, Aleixo, CEP 69060-001, Manaus – AM, Brazil. E-mail: stefane.reis@gmail.com

Dengue is the most important arbovirus in Brazil, in number of cases and lethality, it is an old zoonosis of the primates in the Southeast Asia that adapted to humans taking as vectors Aedes spp. mosquitoes and, thus, eliminating a sylvatic cycle. The city of Manaus, with 1.739.000 inhabitants has been infested by Aedes aegypti since 1996 and the first case of dengue fever (DF) episode appeared in 1998, caused by the DENV 1 and DENV 2 serotypes. Were collected mosquitoes of the genus Aedes aegypti in all areas of the city of Manaus. This work aims at the isolation and typing, from vectors A. aegypti, the four dengue serotypes (DENV-1, DENV-2, DENV-3 and DENV-4). The captured mosquitoes were separated into pools according to species and genus. The pools were soaked in phosphate buffer pH 7.4 with salt, containing bovine serum
albumin. An aliquot of the macerated inocula were prepared for isolation attempts and typing of dengue virus. To isolate viral cell culture were used mosquito Aedes albopictus, clone C6/36. The culture medium used was supplemented MEM medium at 20% Fetal Bovine Serum and 10,000U/ml penicillin and streptomycin 1.000μg/ml. The monolayers were inspected daily using an inverted microscope for detection of cytopathic effect (CPE). On the seventh day after infection, the tubes were removed and centrifuged two aliquots, one for the second pass of infection and another that was subjected to polymerase chain reaction reverse transcription RT-PCR for detection of the dengue virus. The samples of the second passage was also subjected to RT-PCR. Isolation attempts were made in 24 samples of vectors, of which eight samples were positive by RT-PCR for dengue virus. The DENV-2 was isolated in 50% of the eight positive samples, and DENV-4 corresponded to the other 50%. Of the isolates, some tubes showed cytopathic effect, so its no detection shall be conclusive for negativar a sample for dengue virus. In some isolates took two passages of infection for detection of dengue virus by RT-PCR, that because the viral load in the first pass may be very low and is not detected by RT-PCR.

HV1445 - GENOTIPING OF HEPATITIS B AND D VIRUS IN ISOLATED OF PATIENTS IN STATE OF AMAZON, BRAZIL

Oliveira, C.M.C., Braga, W.M., Castilho, M.C., Galvao, R.S., Vasconcelos, H.L., Gimaque, J.B.L., Filho, S.A.

1. Universidade do Estado do Amazonas, UEA, Av. Pedro Texeira

2. Universidade Federal do Amazonas, UFAM, General Rodrigo Otávio Jordão Ramos

Introduction: co-infection between hepatitis B (HBV) and D (HDV) virus is an important public health problem worldwide. Both are responsible for causing chronic disease with high evolutionary potential gravity. In the Amazon, the co-infection HDV and HBV causes severe forms of hepatitis, leveraging the rapid progression to liver cirrhosis and fulminant hepatitis. In addition to a worse prognosis, complicates the host immune response. In view of this situation, the work was Objectives: To detect and characterize the genotypes of the virus of hepatitis B and D in blood samples of patients treated in ambulatory viral hepatitis of Foundation of Tropical Medicine Heitor Vieira Dourado. In the period January to July 2012. Materials and methods: the extraction of viral nucleic acid was extracted from 200 uL serum using a High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Germany), according to the manufacturer’s instructions. The cDNA was obtained using the VHD RevertAid First Strand cDNA Synthesis (Fermentas-Life Sciences). After DNA amplification of HBV and HDV cDNA the PCR products were submitted to cycle sequencing with the ABI Prism BigDye Terminator cycle sequencing kit (AppliedBiosystems, Foster City,CA,USA), according to the manufacturer’s instructions.
Sequencing were run on an automated ABI Prism 3130 DNA Sequencer (Applied Biosystems). Results and conclusions: The study included a total of 29 blood samples from patients co-infected with HBV and HDV of patients. The characterization of HBV genotype was identified genotypes A and F each in 10/29 (34.5%) of the samples followed by genotype D in 9 (31.0%). As for the VHD all samples belong to the genotype 3. Demonstrating that these results are in accordance with the literature in relation genotyping of HDV on HBV virus and Highlighting the importance of genotyping VHD genotype 3 is associated with the most divergent and most aggressive, often causing fulminant disease through a cytopathic noninflammatory process of liver microsteatosis.

HV1451 - EVALUATION OF SINGLE-NUCLEOTIDE POLYMORPHISMS (SNPS) IN THE GENE OF THE INTERLEUKIN-10 -1082A/G (IL-10) AMONG BRAZILIAN WOMEN WITH HUMAN PAPILLOMAVIRUS RELATED CERVICAL LESIONS


1. Universidade Federal de Pernambuco, UFPE, Av. Prof. Moraes Rego, 1235 - Cidade Universitária, Recife - PE - CEP: 50670-901
2. Centro de Medicina Integrada de Sergipe, CEMISE, Hospital Universitário Oswaldo Cruz, Universidade de Pernambuco, UPE, E-mail: babisimas@gmail.com

Human papillomavirus (HPV) is the main etiologic agent of malignant lesions such as cervical cancer. The mechanism of HPV infection is a process dependent on basal keratinocytes differentiation. Thus, HPV infection promotes little or no alert to the human immune system, since the viral release does not promote cell lysis. Interleukin-10 (IL-10) has an important role in the immune response against viral infection, acting through the determination of a pattern of immune response and inhibition of viral replication. Polymorphisms in IL-10 have been related to the susceptibility of the development of cancers. In our study, we analyzed the single nucleotide polymorphisms -1082A/G present in the promoter of the gene IL-10 in a group of 77 patients infected with HPV and cervical lesions of low degree and high degree in order to evaluate their influence on the onset of infection and the cancer development. As a control group, we studied 77 healthy individuals not presenting HPV infection. The biological material was obtained from peripheral blood and cytobrush. The detection of HPV was performed by PCR (MY09/11). The SNP located in the promoter of the IL-10 were genotyped by real-time PCR using High Resolution Melt. The results allowed the identification of three possible genotypes AA, AG and GG. All populations analyzed were found in Hardy-Weinberg equilibrium. In relation to the allele frequencies no evidence of any association of IL-10 SNP (-1082A/G) between patients with HPV/cervical lesion and the control group was found (p = 1). For the genotypic frequencies, as observed before, no differences were identified between the groups (p = 0.72). Although...
interleukin is an important molecule in the immune system, our results did not show any direct association between the SNPs presence in the IL-10 gene and an increased susceptibility to cancer development when associated with HPV. However, this is a preliminary study and the results need to be confirmed in a larger cohort. Financial support: FACEPE

HV1452 - DENGUE VIRUS: ANALYSIS OF EPIDEMIOLOGICAL PROFILE IN PARÁ STATE, BRAZIL, IN THE FIRST HALF OF THE 2012


Arbovirology & Hemorrhagic F. Dept.-Evandro Chagas Institute, IEC, BR 316, KM 7, s/n E-mail: valeriacarvalho@iec.pa.gov.br

Introduction. Dengue fever is a mosquito-borne virus infection that in recent decades has become a major international public health concern. Dengue is found in tropical and subtropical regions around the world, predominantly in urban and semi-urban areas. The incidence of dengue has grown dramatically around the world in the recent decades. At least 2.5 billion people are now at risk for dengue. The World Health Organization currently estimates an annual occurrence of 50 million dengue infections worldwide, mainly in the Americas and Southeast Asia. The spread of dengue is attributed to the expanding of geographic distribution of the four dengue virus serotypes and their mosquito vectors. On the other hand, in the last decades, only the serotypes 1 (DENV-1), 2 (DENV-2) and 3 (DENV-3) circulated in Brazil. However, in July 2010, the serotype 4 (DENV-4) reemerged in Boa Vista, the capital of Roraima State, in Northern Brazil after an absence of 28 years in Brazil. The study aimed to perform a passive virologic surveillance of dengue in the Pará, Northern Region, Brazil, in the first half of the 2012 from samples received by the Department of Arbovirology and Hemorrhagic Fevers - Evandro Chagas Institute.

Material and Methods. A total of 1009 samples of suspect patients were inoculated into C6/36 cells culture for attempt of virus isolation. The indirect fluorescence assay using monoclonal antibodies was used to confirm viral infection. Results. A total of 280 (28%) Dengue Virus strains were isolated as follows: 12 (4.3 %) DENV-1; 24 (8.6 %) DENV-2; and 244 (87.1 %) DENV-4. Conclusion. These results showed an intense circulation of the DENV-4 in Pará State, Brazil and low circulation of DENV-1 and DENV-2 with an apparent absence of DENV-3. Financial support: Evandro Chagas Institute, CNPq.

HV1453 - INCIDENCE OF DENGUE VIRUS INFECTION IN A COHORT OF CHILDREN, GOIÂNIA, GOIÁS, CENTRAL BRAZIL, 2010-2012


Faculdade de Farmácia Universidade Federal de Goiás, CEP: 74605-220, Brasil acmessiasoliveira@gmail.com, vcrisrezende@gmail.com

Recent studies in Brazil reported a change in the prevalence of dengue cases in children with increasing cases hospitalized for DHF. In children below one year the potential risk to development of severe cases from
decay of maternal antibodies has been reported in the literature. This study aims to determine the incidence of infection by dengue virus in a cohort of children born in public hospital in the city of Goiania-GO (~1.2 million inhabitants), Central Brazil, 2010-2012. The baseline prevalence of dengue in Goiás refers to a study of prevalence (53.9%) of dengue virus infection in pregnant women conducted in 2010. The IgM antibodies anti-dengue (PanBio) were tested in samples from neonates to evaluate acute dengue infection. Monitoring the reporting of dengue cases was done by the national dengue surveillance system and active investigation of symptomatic dengue cases in children was conducted by telephone interviews with the mothers to identify clinical care for suspected dengue in the follow-up period of 2 years. Data collected during the study were analyzed in SPSS.17. The incidence of dengue virus infection in neonates was 8/235 (3.4%) (95%CI 1.3-6.0%). The kappa index evaluated the concordance of IgM positivity between mothers and neonates (87.4%). Monitoring of reported cases of dengue in the cohort was 3/235 (1.3%) (95%CI 0.0-3.0%). There was significant loss to follow-up of mothers to contact by phone. Of the 68 children studied 0.8% were reported for suspected dengue clinical care. These results reflect the accumulated incidence of the disease among children (5.5%) in Goiania-GO, an endemic area for viral circulation the four serotypes of dengue. Children in the cohort had dengue fever after one year of age, suggesting protection by maternal antibodies against severe infection. Epidemiological studies that assess the incidence of dengue in children may contribute to age-appropriate vaccinations and are in line with recommendations of the WHO. Financial support: Pronex Rede-dengue/FAPEG/CNPq/2009

HV1455 - AUTOIMMUNE HEMOLYTIC ANEMIA IN THERAPY-NAÏVE PATIENTS WITH CHRONIC HEPATITIS C VIRUS INFECTION: EVALUATION OF CLINICAL, BIOLOGICAL AND VIROLOGICAL PARAMETERS

de Almeida, A.J., Muniz, L.P., Campos de Magalhães, M., Brandão-Mello, C.E., Lampe, E.

1. Laboratório de Hepatites Virais (LAHEP)/IOC, FIOCRUZ, Av Brasil, nº4365, Manguinhos, Rio de Janeiro, RJ, Brasil

2. Setor de Hematologia-CMB/H.U. Gaffrée e Guinle (HUGG), EMC-UNIRIO, Rua Maria e Barros, nº 775, Tijuca, Rio de Janeiro, RJ, Brasil

3. Setor de Hepatologia-CMA/H.U. Gaffrée e Guinle (HUGG), EMC-UNIRIO, Rua Maria e Barros, nº 775, Tijuca, Rio de Janeiro, RJ, Brasil E-mail: adilsonjoal@ig.com.br

ChronichepatitisCvirus(HCV)infection is widely reported in association with an array of extrahepatic manifestations, including autoimmune disorders such as thrombocytopenia, thyroid autoimmunity, and hemolytic anemia. Uncommon reports of Coombs’positive autoimmune hemolytic anemia (AIHA) related to chronic HCV infection have been published in the literature, however, its clinical, biological, and virological features are not well known. The aims of this study were to evaluate the prevalence
and immunohematological profile of HCV-related AIHA, as well as its association with other immunological abnormalities and virological aspects in a cohort of antiviral therapy-naïve patients with chronic HCV infection. HBsAg and anti-HIV positive patients were excluded from the study. Between January 2011 and June 2012 a total of 190 patients, 84 (44.2%) males and 106 (65.8%) females with a mean age of 49.9 ± 11.0 (21-70) years were studied. Five (2.6%) patients were found to have AIHA, 3/5 females, with age ranging from 32–66 years. Regarding immunohematological features, all cases were due to warm-reacting autoantibodies (IgG isotype), with distinct specificities (anti-C, anti-D, anti-E, and anti-k) and variable intensity (1+ to 2+/4+). Other immunological abnormalities were detected in association with AIHA cases: antithyroperoxidase (n=1), antithyroglobulin (n=2), and anticardiolipin IgM antibodies (n=1). Infected HCV genotypes were 1a (n=2), 1b (n=2), and 3a (n=1), with only one patient presenting high plasma viral load (>600,000 IU/mL). In conclusion, the low prevalence of AIHA suggests that this alteration is an infrequent extrahepatic manifestation of chronic HCV infection. HCV-related AIHA cases were found to be caused by warm-active antibodies of IgG isotype with distinct specificities. HCV-related AIHA was also diagnosed in association with other immunological abnormalities and appears not to be an extrahepatic manifestation dependent on a particular HCV genotype. Financial support: PROEP/CNPq

HV1456 - EVALUATION OF IL28 POLYMORPHISM IN PATIENTS INFECTED WITH ACUTE HEPATITIS C


Fundação Oswaldo Cruz, Fiocruz, Av. Brasil, 4365 - Manguinhos, Rio de Janeiro - CEP: 21040-360 E-mail: nmdr@ioc.fiocruz.br

Hepatitis C virus (HCV) infects approximately 170 million people worldwide. Only 20-30% of individuals spontaneously clear acute HCV infection, most of infected will develop chronic hepatitis. Patients with acute HCV infection are difficult to identify due to the lack of symptoms and signs of acute phase. The therapy available based on interferon and ribavirin is effective in about half of the patients. Recently, several studies have shown that host genetic factors exert influence on the response to antiviral therapy and the spontaneous resolution of infection. A single nucleotide polymorphism (SNP) upstream of the IL28B gene (rs12979860) has been associated with chronic hepatitis C and also with spontaneous clearance of HCV. The homozygous CC genotype is rather associated with spontaneous resolution of infection than heterozygous CT or the homozygous TT genotype. Aim: To evaluate the SNP rs12979860 genotype and its relation to the outcome of the disease towards spontaneous viral clearance or chronicity. Methods: 14 well characterized patients acutely infected with HCV (clearance, n=6 vs chronic, n=8) were analyzed by restriction fragment length polymorphism (RFLP) using BstUI and real-time PCR with a commercial kit (Roche). Results: The rs12979860-CC and CT variants were detected, respectively, in 83%
(5/6) and 17% (1/6) of patients which spontaneous clearance of HCV. Concerning patients with chronic HCV, 50% (4/8) showed CC genotype, 25% (2/8) genotype CT and 25% (2/8) TT genotype. A complete concordance between the two methods was observed and both are suitable for routine testing. However, screening by real-time PCR detection had a reduced execution time. Conclusion: These preliminary results demonstrated that the protective C allele of rs12979860 was more common in patients with spontaneous clearance (83% vs 50%) suggesting that IL28B polymorphism has a marked impact on natural clearance of acute HCV infection in the cohort studied.

HV1457 - RECOMBINANT VESICULAR STOMATITIS VIRUS-BASED DENGUE VACCINES


1. Universidade de São Paulo, USP, Av Bandeirantes 3900
2. Yale University School of Medicine, Yale, New Haven, Connecticut 06510 E-mail: lauretti_2000@yahoo.com

Dengue is a viral disease with widespread distribution throughout tropical and subtropical areas of the world. It is transmitted by a mosquito bite and most infections are asymptomatic or present with mild symptoms, but some patients progress to a severe disease (dengue hemorrhagic fever/dengue shock syndrome). Although experimental vaccines are undergoing clinical trials, there is no licensed vaccine. In this study, vesicular stomatitis viruses recombinants (rVSV) were constructed to express envelope (E), membrane and its precursor (prM) proteins of dengue 1 and 2 to be tested as live vaccine candidates. rVSVs have successfully been used as vaccine vectors for several viruses to induce strong humoral and cellular immune responses. The DENV-1 recombinant was constructed using codon optimization for mammalian cell expression of prM-E sequence of a Brazilian strain (DENV-1/BR/BID-V2392/2005) cloned downstream a VSV-G signal peptide sequence. The DENV-2 construct was prepared by RT-PCR cloning of E protein domain III (EDIII) of the Thailand/16681/84 strain. Each construct was inserted in the in the full length VSV plasmid DNA for recombinant VSV-DENV recovery. Infectious recombinants were recovered and plaque purified in BHK-21 cells. The preliminary results shown that recombinants grew at high titers (3.5 x 10^7) and (2 x 10^8) for VSV-DENV-1 and -2, respectively. VSV proteins could be detected four hours post-infection by immunofluorescence (IF) and western blot (WB) analysis. A 22 kDa EDIII of DENV-2 was detected in WB analysis of VSV-DENV-2 but not by IF. Confirmation of prM and E proteins expression by VSV-DENV-1 recombinants are under way to test these viruses in mice in order to determine if they are able to elicit neutralizing antibodies and protect mice against lethal challenge with wild type DENVs. If this strategy proves successful, manufacturing VSV recombinants could be an useful strategy for production of tetravalent vaccine studies.

HV1461 - BLOOD CELL COUNT CHANGES IN DENGUE DIAGNOSIS: A
STUDY OF 1269 CASES IN UBERABA, MINAS GERAIS


1. Universidade Federal do Triângulo Mineiro, UFTM, R Frei Paulino, 30 - Nossa Senhora da Abadia -Uberaba-MG

2. Universidade de Uberaba, UNIUBE, Avenida Nenê Sabino, 1801- Uberaba-MG

3. Universidade Federal da Paraíba, UFPB, Cidade Universitária, S/N - Castelo Branco-João Pessoa-PB

Sabin laboratório clínico, sabin, E-mail: anasantanoli@yahoo.com

Dengue is an important infectious disease present in a large number of tropical countries around the world. It is an acute febrile disease caused by an arbovirus, a member of the family Flaviviridae. Dengue virus (DV) has four antigenically related serotypes known as DV-1, DV-2, DV-3 and DV-4 which has the vector mosquitoes of the genus Aedes (aegypti and albopictus). The diagnosis of dengue is based on clinical and laboratory findings. Among the laboratory tests blood cell count shows a good ally in the diagnosis and monitoring of disease progression. Therefore, the objective of this study was to evaluate changes in the blood cell count of patients affected by dengue in Uberaba (Minas Gerais) comparing the 2005-2006 and 2009-2010 biennia. The choice of the biennia was based on the fact that occurred a large number of dengue cases in these periods and also because different serotypes of the virus circulated in each biennium: serotype 2 and 3 in 2005-2006 and predominance of serotype 1 in the biennium 2009-2010. We analysed blood cell counts of patients with serology (IgM) positive for dengue, attended in a private clinical laboratory and in a public emergency unit of the city of Uberaba. Blood cell counts were performed using the automated method and differential counts in lamina and the detection of IgM antibodies of dengue virus was performed by capture elisa. The analysis was made using X2 (chi-square). In biennia 2005-2006 and 2009-2010 were selected, respectively, 1061 and 208 blood cell count for inclusion in the study. The most frequent findings were: leukopenia in 2005-2006 (66%) significantly higher than in the subsequent biennium (32%), lymphopenia in 2005-2006 (45.12%) that were also significantly higher than the 14.9% observed in 2009-2010 and thrombocytopenia 40% in 2009-2010, significantly higher than in 2005-2006 (16.12%). In the other parameters of white and red series there were no significant changes. Thus, it was possible to better understand the profile of changes that infection with this flavivirus causes in blood cell count of individuals from the region of Uberaba. Haematological abnormalities were similar in both periods studied, so the clinical symptoms combined with classical results found in the blood cell count, can anticipate the treatment of patients especially during epidemics, when the demand of serology extrapolates the stocks of public and private laboratories and seropositivity delay.

HV1465 - ANALYSIS OF HIV VIRAL LOAD AND GENOTYPIC RESISTANCE PROFILE IN VAGINAL SECRETION OF
PREGNANT WOMAN SUBMITTED TO CHEMOPROPHYLAXIS FOR MOTHER TO CHILDREN VERTICAL TRANSMISSION

Rodrigues-Pedro, A., Grinsztejn, B., Pilotto, J.H., Morgado, M.G.

1. Instituto Oswaldo Cruz-Fiocruz, IOC-Fiocruz, Av. Brasil, 4.365, Manguinhos, Rio de Janeiro Brasil

2. Instituto de Pesquisa Clínica Evandro Chagas-Fiocruz, IPEC-Fiocruz, Av. Brasil, 4.365, Manguinhos, Rio de Janeiro Brasil

3. Hospital Geral de Nova Iguaçu - Rio de Janeiro, HGNI-RJ, Avenida Henrique Duque Estrada Mayer, 953 - Posse- Nova Iguaçu - E-mail: arodrigues@ioc.fiocruz.br

The HIV-1 in the female genital tract has an important role in mother-to-child transmission (MTCT) of virus. Quantitation of HIV-1 levels in mucosal secretions is essential to study compartmentalization of HIV-1. The aim of this study was to analyze the profile of HIV-1 viral load in genital secretions in HIV-infected pregnant women submitted to chemoprophylaxis for the prevention of MTCT before treatment and after its discontinuation at delivery. The quantification of HIV-1 viral load was undertaken in the women’s genital tract to evaluate a possible correlation with plasma HIV viral load. A cohort of 274 HIV-1 infected pregnant women, antiretroviral-naïve, was followed up. Of that group, a total of 92 pregnant women agreed to participate in this study, all women collection of plasma samples and genital secretions at the inclusion in the study, before starting antiretrovirals. HIV-1 RNA has been quantified in these two compartments.

The methodology bDNA ® was used to determine viral load in plasma and EasyQ Nuclisens NASBA ® was used for the quantification of genital samples. The HIV-1 RNA in plasma was compared to that of vaginal secretions. The HIV-1 RNA was detected in 40 (43.5%) of 92 genital secretion samples and in 83 (91.3%) of 92 plasma samples. The comparative analysis of HIV-1 viral loads between these two compartment was performed for 74 samples. It was shown that the viral load in about 50% (36/74) of the plasma and genital secretion samples were found to be in the same range. Of these, 13 (36%) were in the detection range below 1.000 copies/ml, 9 (25%) in the intermediate range (1.000 to 10.000 copies/ml) and 14 (39%) were above 10.000 copies/ml. Thirty-eight samples (51%) were discordant in viral load analysis in both compartments, with a greater discrepancy observed in the intermediate range (1.000 to 10.000 copies/ml), confirming the differences of plasma and genital secretions viral loads before the introduction highly active antiretroviral therapy (HAART) (p=0.013). After HAART discontinuation, 43 women had samples of plasma and genital secretion available for HIV-1 viral load analyses. These results suggest that HIV-1 viral load detection in plasma is an important predictor of HIV-1 viral load in the genital secretions. However, there may be excretion of HIV-1 in genital secretions, even in the absence of detectable HIV-1 viral load in plasma, suggesting that the genital compartment may act as a reservoir for HIV-1 replication.

HV1467 - FIRST DETECTION OF NEW ASTROVIRUS MLB1 IN BRAZILIAN
The family Astroviridae comprises non-enveloped, positive-sense, single-stranded RNA viruses. The classical human astroviruses are genetically closely related and can be classified into eight serotypes (HAstV1–8). In addition, several genetically distinct human astroviruses (e.g. MLB1, MLB2, VA1, VA2 and VA3) have been recently identified in stool samples from patients suffering from gastroenteritis. Although astrovirus is one of the major causative agents for gastroenteritis, there is relatively little information on the prevalence and circling these viruses. The recent discoveries of genetically diverse astroviruses in human highlight the genetic diversity of astroviruses in nature and suggest that there might be many more novel astroviruses circulating in children and adults human. The currency study, described a case of AstV-MLB1 infection in a pediatric patient with diarrhea in Sao Luis, Northeastern Brazil. 200 stool samples from acute gastroenteritis in children under two years old were selected from a Laboratory of Comparative and Environmental Virology collection. A two-phases screening strategy to detect and identify astrovirus was used. The first phase, the OneStep RT-PCR Kit was used to screen 5 µL of extracted material from sample using consensus primers that target highly conserved regions in the ORF1b (RNA polymerase) of astrovirus. The second phase was used primers specific for classic human astrovirus [Mon 269/270], AstV-MLB1 [SF0053/SF061] and AstV-VA1 [SF0178/SF0179], using OneStep RT-PCR. Amplicons were sequenced by the Genomic Platform of DNA PDTIS/Fiocruz. The results confirm the MLB-1 for two different regions ORF1b and ORF2. This is the first description of AstV MLB-1 in Brazilian sample and corroborate with the possibility that Astv-MLB-1 is associated whit acute diarrhea disease in children. Financial support: Financial support: CNPq; IOC-Fiocruz.
eight genotypes (HAstV1–8). In order to investigate the circling of different genotypes of HAstV in Rio Grande do Sul, Brazil, we analyzed 65 positive stool samples from acute gastroenteritis in children under five years old from a Laboratory of Comparative and Environmental Virology collection, during January 2005 through December 2010. All samples had prior negative results for other enteric viruses (Rotavirus and Norovirus). The OneStep RT-PCR was used to screen 5 µL of extracted material from sample using primers specific for classic human astrovirus [Mon 269/270]. For molecular characterization, the products generated by One Step RT-PCR were sequenced in both directions. The sequences were aligned and edited using the program BioEdit and compared to eight prototypes of HAstV available in GenBank. The analysis of a 449 bp ORF2 fragment revealed that HAstV-1 was the predominant genotype detected (65%), followed by HAstV-2 (12%), HAstV-4 (3%) and HAstV-8 (3%). This study is the first of HAstV in the State of Rio Grande do Sul, Brazil. Our findings pointed out the importance of epidemiology molecular of HAstV in cases of infantile acute gastroenteritis showing the circulation of different strain contributing for further designing to prevention and control strategies of gastroenteritis transmitted by this important etiologic agent. Financial support: Financial support: CNPq; IOC-Fiocruz.


Laboratório de Virologia Comparada e Ambiental-Fiocruz, LVCA-FIOCRUZ-IoC, Avenida Brasil, 4365 E-mail: juliana@ioc.fiocruz.br

Norovirus (NoV) is recognized as the leading etiological agent in sporadic cases and outbreaks of nonbacterial gastroenteritis worldwide in individuals of all ages. They are divided into five genogroups (G): GI-GV, within which 35 genotypes were identified, classified according to the molecular analysis of the amino acid sequence of the viral capsid protein VP1. This study of NoV in the state of Rio Grande do Sul aims to perform a molecular characterization of NoV circulating in the state, contributing to the establishment of molecular surveillance of NoV from outbreaks of acute gastroenteritis occurred between 2004 and 2010. These samples were received at the Laboratory of Comparative and Environmental Virology (LVCA) for elucidation of acute gastroenteritis cases attended at the Central Laboratory (LACEN) of Rio Grande do Sul. One hundred and twenty two samples previously positive for Nov by RT-PCR (region B) were tested by RT-PCR for region D to determine the GI (Cap A, B1 e B2) and GII (Cap C, D1 e D2) genotypes. A total of 88 samples were amplified for GII and 2 to GI. It was not possible to characterize 32 samples. Analysis of partial nucleotide sequences showed that genotype GII.4 was prevalent and was found in all years studied. The second prevalent was the genotype GII.6 and, the remaining samples were characterized as GII.2, GII.3, GII.12,
GII.13, GII.14, GII.17, GII.21, and GI.1 GI.3. These results show the great diversity of NoV circulating in the state of Rio Grande do Sul, also observed in other Brazilian states. The relevance of the molecular characterization of these viruses show their impact on health of the population and provide important information for developing an effective vaccine against these viruses. Financial support: CNPq/FIOCRUZ-IOC/ Plataforma Genomica de Sequenciamento PDTIS-FIOCRUZ Acknowledgments to staff of LACEN of Rio Grande do Sul.

HV1480 - ENTEROVIRUS DETECTION IN CASES OF ASEPTIC MENINGITIS IN MOZAMBIQUE

Pinto, G.C., Costa, E.V., Gudo, E.S., Jani, I.V., da Silva, E.E.
1. Instituto Nacional de Saúde - Moçambique, INS - MISAU, 264 Av. Eduardo Mondlane/Salvador Allende MAPUTO – Republica de Moçambique
2. Fundação Oswaldo Cruz (Laboratório de Enterovirus), FIOCRUZ, Av. Brasil. 4365. Pav. Helio e Peggy Pereira. Sala B217 E-mail: gabbydocarmo@gmail.com

Aseptic meningitis refers to a clinical syndrome of meningeval inflammation in which common bacterial agents cannot be identified in the cerebrospinal fluid (CFS). Rapid diagnosis of viral meningitis using PCR testing of CSF can help shorten hospitalization, and avoid the unnecessary use of antibiotics. Non-polio human enterovirus is the leading recognizable cause of aseptic meningitis, accounting for 80–92% of all the cases in which a pathogen is identified. Most of the cases occur during the summer and autumn although sporadic cases can occur throughout the year. In our study 163 CSF samples were collected in three Hospitals in Southern (Maputo), Northern (Nampula) and Center (Beira) of Mozambique, from July 2011 to February 2012. All 163 CSF samples from suspected aseptic meningitis were negative when submitted to culture for bacterial and fungi and Indian ink stain negative for fungi. Seventy CSF samples have been so far tested using two methods; TaqMan real-time RT-PCR (rRT-PCR) and virus culture using RD cells. Twenty-six specimens were enterovirus positive by rRT-PCR. From these, 13 were positive regarding enterovirus isolation. This is the first set of data associated to a broader study concerning the etiology of aseptic meningitis in Mozambique. Financial support: CNPQ, FIOCRUZ, INS