A BRAZILIAN MARSEILLEVIRUS IS THE FOUNDING MEMBER OF A LINEAGE IN FAMILY MARSEILLEVIRIDAE

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Acanthamoeba polyphaga mimivirus (APMV) was discovered as parasitizing Acanthamoeba. It was revealed to exhibit remarkable features, especially odd genomic characteristics, and founded viral family Mimiviridae. Subsequently, a second family of giant amoebal viruses was described, Marseilleviridae, whose prototype member is Marseillevirus, discovered in 2009. Currently, the genomes of seven different members of this family have been fully sequenced. Previous phylogenetic analysis suggested the existence of three Marseilleviridae lineages: A, B and C. Here, we describe a new member of this family, Brazilian Marseillevirus (BrMV), which was isolated from a Brazilian sample and whose genome was fully sequenced and analyzed. Surprisingly, data from phylogenetic analyses and comparative genomics, including mean amino acid identity between BrMV and other Marseilleviridae members and the analyses of the core genome and pan-genome of marseilleviruses, indicated that this virus can be assigned to a new Marseilleviridae lineage. Even if the BrMV genome is one of the smallest this family. In addition, the BrMV genome encodes 29 ORFans. Here, we describe the isolation and genome analyses of the BrMV strain, and propose its classification as the prototype virus of a new lineage D within the family Marseilleviridae.

Palavras-chave: Marseilleviridae, Marseillevirus, Giant virus, Lineage D, Brazilian Marseillevirus
Return of treated sludge to the environment poses concerns and has stimulated the development of studies on viral monitoring in this matrix, in order to assess its potential risks for public health. Human adenovirus (HAdV) has been identified as a putative viral marker of faecal contamination due to its stability and resistance to the sewage treatment process. The objective of this study was to establish a more efficient viral concentration protocol for the recovery of HAdV in sewage sludge samples with optimized skimmed-milk flocculation technique. A single sludge sample was collected at a Wastewater Treatment Plant (WWTP) and submitted to four protocols including modifications in the initial sample dilution in glycine buffer pH 9.5 (1:9, v/v), in the stirring time of sample in glycine buffer (30 min or 60 min) and in the final concentration of skimmed-milk (0.01% or 0.02%, pH 3.5). In each protocol, three aliquots were inoculated with HAdV and bacteriophage PP7 and a non-inoculated one was used as negative control. The viral load and recovery rate were determined by quantitative PCR. The highest recovery rates of HAdV and PP7 were obtained when a lower stirring time and higher concentration of skimmed-milk were performed. This protocol was assessed in a field study conducted with 14 samples of activated, thickened and digested sludge obtained in two WWTPs. HAdV was detected in all samples, with a similar or higher viral load than those obtained with other concentration techniques already applied to sludge. This study corroborates the wide use of the organic flocculation method for virus recovery in environmental samples with adaptation of the protocols to different aquatic matrices. Financial support: FAPEMIG, CAPES, UFJF

**Palavras-chave:** adenoviruses, sludge sewage, optimization, concentration method, organic flocculation
003 - ÁREA: AMBIENTAL

COMPARISON OF METHODOLOGIES FOR VIRUS RECOVERING FROM CHEESE SAMPLES AND MICROBIOLOGICAL EVALUATION OF COMMERCIALLY OBTAINED PRODUCTS, RIO DE JANEIRO, BRAZIL

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Resumo

Norovirus (NoV) is an important agent of foodborne outbreaks of acute gastroenteritis and its recovering from dairy products has been a challenge mainly due to the low levels of virus particles and the presence of inhibiting substances in this food matrix. This study evaluated the efficiency of human NoVGII.4 (NoV GII.4) and murine norovirus (MNV-1) recovery from a typical Brazilian fresh white ("Minas" cheese) and yellow ("Prato" cheese) cheeses by using two variations of skimmed milk flocculation method, using chloroform/butanol (1:1) or TRIzol® reagent treatment, and direct extraction by TRIzol®. The organic flocculation showed results ranging from 0.2 % to 25.1 according to treatment associated, while the direct extraction by TRIzol® reagent showed 51.0% and 79.9% of Norovirus for “Minas” and “Prato” cheese, respectively. For field study, we evaluated bacteriological and virological naturally contamination in a total of 90 cheese samples. NoV GI and GII were detected in one sample (1.1%) each and human adenovirus (HAdV) in nine samples (10.0%). Bacteriological analysis revealed five samples (5.5%) contaminated with Listeria monocytogenes, 27 (30.0%) with fecal coliforms and 10 (11.1%) with Staphylococcus aureus. The establishment of methods for viral concentration from food matrices, especially dairy foods, will assist epidemiologic investigations expanding the investigation of agents in these matrices.

Palavras-chave: “Minas” cheese, “Prato” cheese, norovirus, food
DEVELOPMENT OF MOLECULAR TOOL FOR LYSSAVIRUS DETECTION IN SAMPLES OF SMALL MAMMALS OF URBAN, RURAL AND WILD AREAS OF MINAS GERAIS

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Resumo

The genus Lyssavirus comprises viruses enveloped with an RNA genome that has as prototype Rabies virus, one of the etiological agents of rabies. This virus is transmitted by the bite or counted from the saliva of an infected animal being able to attack mainly the nervous system of mammals, including humans, in which they cause encephalitis and encephalomyelitis, leading to death 99% of those who contract it besides irreversible sequelae. To those who can survive. Techniques for laboratory diagnosis of rabies virus are traditionally serological, which does not allow the distinction between infected and vaccinated animals, and most of the time, post mortem are carried out only for confirmation and notification of the cases. Thus, the use of molecular tools becomes a more adequate and rapid alternative for viral detection. This virus has as hosts reservoirs bats, hematophagous or herbivores, domestic animals such as horses, cattle, goats, swine and wild animals such as possum, fox, wolf, raccoon, monkey and coati. In Brazil the urban, rural and wild transmission cycles of rabies are observed, although there are still no reports of the participation of rodents and marsupials in this cycle, although there are already serological and molecular evidences of the virus circulation in these animals in other countries. Since rodent-associated zoonotic agents are of great public health relevance, associated with great diversity, remarkable reproductive potential and adaptability to different niches, the objective of this work is the construction of primers for the detection of Lyssavirus in samples of Serum, viscera and marrow of rodents and marsupials collected between the years 2011 to 2013 in urban, rural and wild regions of Sabarã, Serro and Rio Pomba. For this, alignments of several Lyssavirus genes, available on Genbank, from Brazilian canines and cattle and rodents from several countries were made to define the best regions for primer construction. In view of the great genetic variability of these viruses, only three regions of the nucleoprotein gene were selected for the construction of degenerate primers capable of detecting several Lyssavirus species and lineages in conventional or semi-nested PCR amplifying regions of approximately 227bp and 318bp, which also allow phylogenetic inferences. Financial Support: CNPq, FAPEMIG, CAPES

Palavras-chave: Lyssavirus, Rodent, Marsupials, Molecular detection
Resumo

Nowadays, wastewater treatment is amongst the major challenges in developing countries, specially in rural areas where often there is no sanitation infrastructure. Viruses are commonly detected and utilized as biomarkers of fecal contamination in water. Resistant viruses, such as adenovirus (AdV), rotavirus (RV) and enterovirus (EV) are often assayed in water, providing important information such as presence of fecal contamination and also host species responsible for this contamination, since most of these viruses infect hosts from a single species. In cattle husbandry and in public health, diarrhea is one of the major causes economic losses worldwide. Infectious agents are frequent causes of diarrheic outbreaks in cattle, and RV is one of the major infectious agents related to diarrhea outbreaks in calves. Poor sanitation and high population density is among the factors that contributes with diarrhea prevalence in cattle herds. In order to investigate virus shedding and sources of viral contamination in water of rural areas, we visited farms with diarrheic bovine and collected diarrheic feces from cattle and water samples from drinking source and effluents in these farms to detect AdV, EV and RV, and estimate the effect of cattle diarrheic outbreaks in virus shedding through water. AdV, RV and EV detection through polymerase chain reaction (PCR) assays were carried out in 8 diarrheic samples, in 8 water source samples and in 4 effluent samples from small farms with cases of bovine diarrhea. AdV and RV positive samples were sequenced and analyzed. Additionally, fecal coliforms quantification was performed in water samples. Reverse transcriptase PCR (RT-PCR) was positive for RV in 3 fecal samples. EV was detected in 1 fecal sample and AdV was negative in all fecal samples. Water source samples were positive for AdV in 6 farms, EV in 2 farms and negative for RV in all farms. AdV was detected in 2 of 4 effluents, EV was detected in 1 of 4 effluents, and RV was negative in all 4 effluents analyzed. Fecal coliforms were detected in 10 of 12 water samples. AdV was detected in 6 water sources, of these 6 were closely related to human AdV, revealing that even in bovine diarrheic cases, the main source of water contamination is human dung. None of the farms with RV diarrheic cases presented RV in water sources and effluents. RV is an important cause of diarrhea in calves, maximum age of positive fecal samples in this study was 6 months.

Palavras-chave: Diarrhea, Adenovirus, Rotavirus, Biomarker, Water
Aquatic ecosystems, especially river basins, are being heavily affected by various anthropogenic actions. There is a constant process of deterioration/contamination that has harmful effects on the environment and the population. The discharge of human and animal waste directly into the rivers due to lack of basic sanitation and inadequate sewage treatment stand out as the main contributors to this process of dissemination of microorganisms as viruses and bacteria from faecal origin in the environment. Human Adenovirus type F 40/41 (HAdVF-40/41) and animal Adenoviruses (Bovine adenovirus, BAdV; Porcine adenovirus, PoAdV; Canine adenovirus, CAV), are excellent markers of anthropic and animal pollution and transmitted fecal-oral. Paranhana river is located in Rio dos Sinos Watershed (RSW) in the state of Rio Grande do Sul, Brazil, and is considered one of the most polluted water systems in the country, also used as a source of water abstraction for public supply. Paranhana river, one of the main contributors to RSW, it is a receiver of domestic and animal waste through rural and urban areas with its mouth in the Rio dos Sinos.

The present study aimed to research the presence of HAdVF-40/41 and animal (BAdV, PoAdV, CAV) in water samples from Paranhana river. Six bimonthly water collections were carried out in May/2015 until March/2016, totaling 72 samples, distributed in 10 points of the rural and urban areas from the spring to the mouth. The method used for water concentration was by adsorption / elution negative membrane was took after extraction of the viral DNA samples. Viral detection was obtained by quantitative polymerase chain reaction (qPCR). The viral genomes researched showed the presence of HAdVF 40-41 in 29.2% (21/72) of the samples, with variation in the viral quantification from 5.68x10³ to 2.65x10⁶ gc/L; 40.3% (29/72) of BAdV, with quantifications of 1.83x10⁴ to 1.21x10⁸ gc/L; 27.8% (20/72) PoAdV between 3.01x10⁵ and 1.21x10⁹ gc/L; 38.90% (28/72) of CAV showing 1.07x10⁵ to 7.74x10⁷ gc/L. It was evidenced a process of continuous contamination in the Paranhana river identified by the presence of animal AdV due possibility at rural residue and HAdVF with higher viral load and presence in the month of July demarcated with greater rainfall in the region.

Palavras-chave: Adenovirus, fecal contamination, Paranhana River, water
FIRST HUMAN ADENOVIRUS STUDY AND THEIR SPATIAL MAPPING IN THE IMPORTANT TRIBUTARY OF THE RIO DOCE BASIN /MINAS GERAIS AFTER THE WORST ENVIRONMENTAL DISASTER IN BRAZILIAN HISTORY

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Resumo

Adenovirus (HAdV) can be considered a safe biomarker of fecal contamination, being more resistant and stable in the environment than bacterial and parasitic indicators, for example. In this context, the objective of this work was to analyze the spatial distribution of HAdV in the Rio Gualaxo do Norte and their basin, principal tributary of the Rio Doce, which was affected in 2015 by 50-60 million m3 of mud the iron from the dam of Fundão / Mariana-MG. For this, 27 collection sites were monitored for HAdV presence and quantification during dry and rainy periods / 2016-2017. Two liters of water samples were collected monthly during one year and the viruses concentrations was performed by organic flocculation (using skin milk), followed by DNase treatment for further molecular detection and quantification of integral HAdV particles, by qPCR method. The geo mapping of HAdV was generated using the ArcGIS 10.2® software. During dry and rainy periods high HAdV concentrations were detected (ranging from 103 to 107 genomes copies/L – GC/L). During rainy season 66.66% of the samples were positives for HAdV and during dry season the presence of HAdV was detected in 85.18% of the water samples. Main source of pollution can be the discharge of domestic effluents into water bodies, since there is no treatment of domestic sewage in the river basin. In addition, due to the rupture of the dam extrapolated mud and this fact may have contributed to the fecal material flow, intensifying its contamination. The geo mapping generated showed the contamination mainly in the tributaries of the Rio Gualaxo of the North affected by the mud from Fundão dam. Results can be used in management of disease risks in strategic locations and minimize the impacts caused by viral, bacterial and parasitic contamination, with a view to improving human, animal and environmental health.

Palavras-chave: Environmental disaster, Rio Doce basin, Human Adenovirus
HEPATITIS A DETECTION IN FOOD SAMPLES COLLECTED OR SEIZED FROM ARGENTINA AND URUGUAY

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Resumo

Hepatitis A virus (HAV), a member of the family Picornaviridae, is a nonenveloped virus with a linear, single-stranded, positive-sense RNA genome of approximately 7.5 kb in length. In children infections are usually asymptomatic, but in non-immunized adults the infections could be more severe. HAV is often found in vegetables, fish or edible mollusks grown in contaminated water, but the virus may also reach meat due to improper manipulation, packaging and hygienic conditions. They are transmitted through fecal-oral route, and can cause asymptomatic infections, gastroenteritis and hepatitis. The purpose of this work was to analyze the presence of HAV genomes in meat samples (chicken, bovine, swine) from illegally transported and collected by the authorities on the border between Rio Grande do Sul, Argentina and Uruguay. In total, 159 food samples were collected 1g was macerated with 1mL of E-MEM and after extracted with TRIZOL. cDNA synthesis was performed using High-Capacity cDNA Reverse Transcription Kit following the manufacturer's protocol and after real-time polymerase chain reaction using Hav-specific primers and a TaqMan® probe. In 15,7 % of the samples showed positive for the presence of HAV genome. The findings are more likely related to poor hygienic conditions of places in which the food was prepared, pointing to health-risk related to the consumption of these meats. Financial support: CNPq, CAPES,FAPERGS

Palavras-chave: hav, q-PCR, Environment, food
These results demonstrate anthropic contamination of faecal origin in water resources, demonstrating precarious water management systems and lack or ineffective sanitation. In addition, the presence of these pathogens poses a risk to public health.

Concentrations were carried out by ultracentrifugation method and viral DNA extraction. Viral molecular detection occurred via qPCR, primers specific hybridization to the viral genome. The results show that 52.5% (21/40) of the samples confirm the presence of HAdV type C and 50% (20/40) HAdV type F. The viral load ranged from 6.17 x 10^5 genome copies/Liter (gc/L) to 7.82 x 10^6 gc/L for HAdV-C and 1.10 x 10^5 gc/L to 1.14 x 10^7 gc/L for HAdV-F. These results demonstrate anthropic contamination of faecal origin in water resources, demonstrating precarious water management systems and lack or ineffectiveness of basic sanitation. In addition, the presence of these pathogens poses a risk to public health.

Palavras-chave: fecal contamination, Human adenovirus, Rio Caí, water
010 - ÁREA: AMBIENTAL

HUMAN AND ANIMAL FECES CONTAMINATION IN PUBLIC RECREATIONAL WATERS

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Resumo

Waters intended for primary contact recreation can expose users to contamination by microbiological agents. Several are the factors capable of changing the quality of the water, mainly the domestic sewage, which is generally contaminated with pathogens of fecal origin. Other natural elements can also contribute to the deterioration of the water, such as precipitation, which carry the impurities contained in the soil into the water bodies. In Brazil the recreational waters are regulated by CONAMA resolution 274 from 2000, which defines the criteria for bathing, classifying the waters on the basis of indicators such as Escherichia coli. Studies show that only the use of this bacteria is not sufficient, in particular by not allowing to trace the origin of fecal contamination and not excluding the presence of other pathogens. The adenovirus is an enteric virus commonly used for environmental monitoring, since it remains viable for several months. From molecular analysis it is possible to differentiate the human, (HAdV), bovine (BAdV), porcine (PoAdV) and canine (CAV) adenovirus. The aim of this study was to differentiate the source of fecal contamination by the detection of human and animal adenovirus in waters intended for recreation. Water samples were collected from Parque das Laranjeiras, located in the city of Três Coroas, Rio Grande do Sul, from 2015 to 2017. Ultracentrifugation methods were used for viral concentration, followed by nucleic acid extraction and real-time PCR. It was verified the presence of HAdV in 95% of the samples (mean: 2,55x10⁷ cg/L). When it comes to animal adenovirus, the presence of BAdV was observed in 55% of the samples (mean: 1,36x10¹⁰ cg/L), followed by 30% of CAV (mean: 7,21x10⁹ cg/L) and 20% of PoAdV (mean: 2,34x10⁹ cg/L). The results indicated a fecal contamination of diffuse source. Although the HAdV was the virus more frequently detected, the animal adenovirus presented a higher viral load. Therefore, even if it is not always present, the animal contamination appears relevant. This contamination can be related to rural activities in the area of study due to an inappropriate management of animal wastes, which end up being loaded into the water bodies. The present study identified different sources of contamination in the waters. These findings allow the proposal of measures aimed at adaptation of waste management to minimize the impact on water quality.

Palavras-chave: Human Adenovirus, water quality, PCR real time, recreational water, Animal Adenovirus
IDENTIFICATION OF PROPHAGE-LIKE ELEMENTS IN DESULFOVIBRIO AND ITS RELATION WITH CRISPR-CAS SYSTEM

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Resumo

Desulfovibrio is the second bacterial genus that has the largest number of species of the group of Sulphate Reducing Bacteria (SRB). The SRB cause great damage in the oil industry by the ability to produce H2S, biofilm formation and iron structures corrosion. Bacteriophages are an alternative form control, whose information about these viruses is almost non-existent for SRB. This work aims to identify and analyze sequences of prophage-like elements of 47 Desulfovibrio genotypes from GenBank, as well as to identify and analyze the relationship of the CRISPR-Cas system with the acquisition of these elements. The elements were identified through the PHASTER program, the sequences aligned by ClustalW and the dendrogram obtain by MrBayers. To identify shared proteins among phylogenetic groups and to perform the sequence comparison were used the OrthoVenn and Mauve programs, respectively. The CRISPR-Cas system was identified by CRISPRFinder. 128 elements were found in 97,8% (46) of the analyzed genomes, being 41,40% (53) of the elements classified as complete, 29,68% (38) as incomplete and 28,90% (37) as hypothetical. All the complete elements were classified within the order Caudovirales. Poly-lysogenesis was common among strains, which have a range of 1-8 elements. Among the poly-lysogenic strains were found elements belonging to the family Myoviridae, Siphoviridae and Podoviridae composing the same strain. Despite the great diversity of complete elements found, they form very related phylogenetic groups and share proteins. Four genomes presented two complete elements with identity above 50% into same strain, whose comparative analysis demonstrated the collinearity between the sequences of prophages from the same strain. Three of these genomes with similar elements had the CRISPR-Cas system classified as inactive, and the other genome, although classified as active, has no similarity between the spacers of CRISPR and the prophage-like elements, which would justify the acquisition of very related elements. Of the 17 closed genomes, only 8 had the system classified putatively as active. Most of strains with CRISPR-Cas active have lesser numbers of elements than those with CRISPR-Cas inactive, indicating that the system may be related to protection against bacteriophage in Desulfovibrio. This work was the first step to understand the relationship between prophage-like elements present in Desulfovibrio. Financial support: PETROBRAS, CNPq, FAPEMIG and CAPES.

Palavras-chave: Bacteriophage, CRISPR-Cas, Desulfovibrio, Prophage, Sulphate Reducing Bacter
IRON AS PUTATIVE ENTERIC VIRUS PRECIPITATOR IN WATER FROM AFFECTED AREAS BY MINE DAM BURST IN MINAS GERAIS STATE, BRAZIL

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Resumo

A big catastrophe had occur in November 2015 in the city of Mariana, Minas Gerais: the iron mine dam spilled 60 million m3 of mud at Rio Doce Basin (RDB), contaminating its water with iron residues, as well as transporting human and animal sewage to this river. Waters from RDB are widely used to direct human consumption, irrigation, fishing and bathing. As a pioneer group in Minas Gerais state-Brazil, the Laboratório de Microbiologia e Bioprospecção Tecnológica (Universidade Federal de Ouro Preto-MG), in partnership with the Laboratório de Virologia Aplicada (Universidade Federal de Santa Catarina-SC), started a collaborative project aiming to investigate endogenous hepatitis A virus (HAV), hepatitis E virus (HEV) and rotavirus A (RVA) in RDB. Eight water samples were collected, and a high turbidity was observed (total solids average of 120±80 mg/L). The water samples were artificially inoculated with 6Log10/L of human adenovirus 2 (HAdV-2) as internal control. Due to the nature of these samples, we observed that conventional methods used for virus concentration, as filtration and organic flocculation, were not efficient to recover the seeded adenoviral particles (<1Log10 recovery). In order to study the iron (Fe++) content influence for the viral recovery, the water samples were placed in cone Imhoff during 4 h. After sedimentation, 100µL of the precipitated samples were eluted in phosphate buffer (pH 9.0) and the eluate was used for nucleic acids extraction by commercial kit. Viral quantification was performed by qPCR (genomic copies detected (GC) by TaqMan method using specific probes). The results showed that the increasing of Fe++ concentration was directly related to the seeded HAdV-2 recovery in samples as observed for the following results: 10 µg/L of Fe++ (0.6 Log10 of HAdV-2); 100 µg/L (1Log10), 500 µg/L (2.0 Log10), 1,000 µg/L (2.4 Log10) and 6,000 µg/L (3.7 Log10). In samples containing Fe++>500 µg/L, endogenous HAV, HEV and RVA were detected, in average concentrations of 104, 102 and 106 GC/L, respectively. In conclusion, we observed that the Fe++ in samples can act as putative viral adsorbate, allowing segregation of the viral particles from the water to the sediment generated. The methodology can be used to evaluate the enteric virus contamination in the area affected by mining, as well as in water sources with high concentration of Fe++ due the geological formation.

Palavras-chave: Iron precipitation, Viruses detection, Mining
TRABALHOS APROVADOS PARA APRESENTAÇÃO EM FORMA DE PÔSTER

013 - ÁREA: AMBIENTAL

MOLECULAR DETECTION OF HEPATITIS A VIRUS IN OYSTERS AND CULTIVATION WATER IN NORTHEAST OF PARÁ

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Resumo

In Pará, oyster farming is a growing activity in the Northeast of the State and helps to improve the family economy of many riverside communities, where this activity is the main source of income. Because oysters are bivalve filtering molluscs, they are commonly used as environmental quality bioindicators. These organisms may serve as a vehicle for the transmission of various water-borne diseases, such as hepatitis A, an endemic disease of Northern Brazil, caused by hepatitis A virus (HAV), which has the fecal-oral route as the major means of transmission. In this context, the aim of this study was to evaluate the occurrence of hepatitis A viruses in samples of oyster and cultivation water from five communities located in the northeast of Pará. From January to June 2017 five communities of oyster farmers were sampled twice. These communities were located in the municipalities of Curuçá, Maracanã, São Caetano de Odivelas, Salinópolis and Augusto Correa. For each community, three points of collection were established. In two of them, samples of water were collected, while in the third one, we collected samples of water and oysters, totaling 30 samples of water and 10 of oyster. The water samples were concentrated by the organic flocculation method with skimmed milk (Sigma) and the intestine of the oysters were dissected and subsequently eluted, clarified and concentrated with Polyethylene glycol 6000 (PEG 6000). The extraction of viral RNA was completed with the QIAamp Viral RNA kit (QIAGEN), followed by reverse transcription with SSIII-RT (Invitrogen). Detection of HAV was performed using the nested-PCR technique with amplification of the VP1/2A region of the genome using Taq platinum DNA polymerase (Invitrogen). Of the 30 water samples, 53.33% (16/30) showed a positive result for HAV: 3 from the municipality of Augusto Correa, 3 from São Caetano de Odivelas, 3 from Salinópolis, 4 from Curuçá and 3 from Maracanã. In the oyster samples, the HAV detection was positive in 70% (7/10) of the analyzed samples: Maracanã (1/2), Augusto Correa (1/2) Salinas (1/2), São Caetano de Odivelas (2/2) and Curuçá (2/2). The results demonstrated that the hepatitis A virus is circulating in the coastal area of Pará, indicating the occurrence of fecal contamination in the water bodies used for the cultivation of these molluscs, since the HAV is eliminated in the feces of infected individuals, representing a potential health risk for the population that consume this aliment.

Palavras-chave: hepatitis A virus, cultivation water , OYSTERS
014 - ÁREA: AMBIENTAL

MONITORING OF HUMAN ADENOVIRUS IN THE NORTHERN COAST OF RIO GRANDE DO SUL, BRAZIL

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Resumo

Coastal environments suffer significantly from anthropogenic activities. Through the disposal of domestic effluents, a significant amount of contaminants is dumped in these places. Such contaminants can accumulate in aquatic organisms and infect humans who make recreational use of these environments. Among these contaminants, the human adenoviruses (HAdV) are highlighted, being the most often used viral markers of human fecal contamination. The sea surface microlayer (SML) is the water atmosphere interface that can act in the storage of these viruses. The sediment can serve as a viral reservoir, aiding in water analysis. Bivalves are organisms that can bio accumulate viruses, since they filter and accumulate the particles present in the water, often at higher levels than the water samples. The objective of this study was to evaluate HAdV in beaches located in the north coast of Rio Grande do Sul, Brazil, through different samples. Sampling occurred in eight beaches during three months (October, January and April), totaling 32 samples for each sampling. For the detection of HAdV the molecular method of real-time polymerase chain reaction (qPCR) was performed, using the primers VTB2 (HAdV-C) and VTB1 (HAdV-F). In October, HAdV-C was positive in 59% (19/32) of the samples, with the SML and bivalves being the most representative, both presented 37% (7/19) of positivity, being the highest value for genomics copies (GC) in 2.08x10^7 GC/mL and 3.91x10^4 GC/g, respectively. Also were detected in water and sediment samples in 16 (3/19) (5.31x10^5 GC/L) and 10% (2/19) (8.91x10^5 GC/g), respectively. HAdV-F was positive in 6.25% (2/32) of the samples, both SML samples (2.92x10^6 GC/mL). In January, HAdV-C was positive in 22% (7/32), being detected at 43% (3/7) in the sediment (3.82x10^6 GC/g), 29% (2/7) in water (2.42x10^6 GC/L) and 14% (1/7) in SML and bivalves with 1.78x10^6 GC/mL and 3.47x10^5 GC/g, respectively. HAdV-F wasn’t detected in this month. In the sampling of April, HAdV-C and F were negative. This is the first study of viral contamination in this area and all the beaches presented human viral contamination, reflecting the anthropic interference in the proximities with the coast. We highlight the relevance of evaluating different samples in the environment since all samples present capacity for viral retention. We also emphasized the importance of using viral indicators for safer monitoring of water quality. Financial Support: CAPES, CNPq, FAPERGS, Feevale.

Palavras-chave: HAdV, Water, Sediment, Bivalves, Sea Surface Microlayer
THE CONTRIBUTIONS OF VECTOR MONITORING AND SOCIAL MOBILIZATION, BY OVITRAMPAS, IN MICROTERRITORIES, WHILE STRATEGIES FOR THE PROMOTION OF HEALTH.

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Resumo

The present work results from the monitoring, by means of ovitraps, of vectors Aedes and Culex, in the rural zone of Uberlândia - MG. Expansions of human activities cause the environmental degradation of natural habitat vectors, which transmit pathogens to humans and other animals. These pathogens can undergo transition from a health problem from a restricted area to a global dimension. These viruses are kept in the wild by epidemiological cycles involving vertebrate hosts and hematophagous arthropod vectors. Species of the genus Aedes, Culex, Haemagogus, and Sabethes make up the main vectors of Brazilian arboviruses. It is known that mosquitoes of the genus Aedes inhabit environments of tropical and temperate climates, and have anthropophilic and zoophilic behavior. The diseases related to vectors are not only due to the climatic nature and their vectors, they are also related to the habits and behaviors of the population. In Brazil, in recent years, the discomforts with Dengue, chikungunya and Zika arboviruses have increased. These, because of their symptomatology, incapacitate workers, take students out of classrooms, and reap lives. In addition to these factors, the treatment and prevention of these require large expenditures of public money. Campaigns to eliminate breeding sites and control the vectors need the effective participation of all. The University has a function of indissociable articulation of teaching, research and extension, in an educational process of transformation of society in relation to certain diseases. In this scenario, we intend to present results of social mobilization in the monitoring of vectors as health promotion strategies. We install and monitor weekly ovitraps in the residences of residents of the Federal Institute of Education, Science and Technology (IFTM). We performed collections, 2013/2016, detecting 25,981 eggs in stereomioscopy, 20,591 of which were viable, 3,186 were hatchlings and 2,209 were damaged. The vases with viable eggs were placed in plastic cups with water, within a mosquito net, to monitor the biological stages of the vectors, being 70% Aedes albopictus, 25% Culex quinquefasciatus and 5% Aedes aegypti. We mobilize people with the purpose of achieving meaningful learning related to the knowledge of vector biology and disease, also in understanding environmental conditions and subjects' lifestyles, in the elimination of breeding sites. FINANCIAL SUPPORT: Universidade Federal de Uberlândia e FAPEMIG.

Palavras-chave: ARBOVIROSES, MICROTERRITORIOS, VETORES
Noroviruses (NoVs) are considered the main cause of viral acute gastroenteritis outbreaks in adults and as the second most common viral agent in children after group A rotaviruses. NoV genotype GII.4 and their variants are generally associated with community outbreaks. In this study NoV genogroup II (NoV GII) were monitored during one year (April 2015 to March 2016) in urban sewage, treated effluents and reclaimed water samples collected in four wastewater treatment plants (WWTPs) from metropolitan region of São Paulo city. WWTPs 1-3 have a treatment process composed by activated sludge followed by sand-anthracite filtration and chlorination, while WWTP-4 operates with activated sludge followed by membrane bioreactor (MBR), reverse osmosis and chlorination. One hundred and forty one samples collected from raw sewage (48), treated effluents (48) and reclaimed water samples (45) were concentrated using celite and hollow-fiber ultrafiltration methodologies. Nucleic acids were extracted and partial amplification of the noroviruses genome were detected by semi-nested RT-PCR with primers towards ORF1/ORF2 junction and 5' region of VP1 gene (region C). Amplicons were purified and sequenced through of Sanger method. Sequences were edited and aligned with BioEdit® program and compared with norovirus typing tool database. Results demonstrated a high circulation of NoV GII in raw sewage samples (91.6%) and lower detection rates in treated effluents and reclaimed water samples (39.5 and 11.1%, respectively). No positive sample was obtained in reclaimed water samples produced by MBR/reverse osmosis systems. Out of the 68 positive samples, 63.2% (43) could be genotyped, resulting in the following distribution: GII.17 (n = 24), GII.4 (n = 9), GII.6 (n = 4), GII.1 (n = 4) and GII.5 (n = 1). NoV GII.4 Sydney 2012 variants were detected in 77.7% (7/9) of the samples, but the main found was the prevalence of genotype GII.17. It is important to note that this genotype began to be detected more often in August / September 2015 through 2016, coinciding with recent data about the entry and dissemination of a new emerging genotype (GII.P17-GII.17 Kawasaki 2014) in the country. This is the first large-scale study to evaluate genotypes of noroviruses in environmental samples in the city of São Paulo, providing a rapid screening of viruses circulating in the population. Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) Processo No 2013/26586-1

Palavras-chave: epidemiology, norovirus, public health, reclaimed water, urban sewage
UBIQUITOUS GIANTS: A PLETHORA OF GIANT VIRUSES FOUND IN BRAZIL AND ANTARCTICA

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Resumo

Since the discovery of giant viruses infecting amoebae in 2003, many dogmas of virology have been revised and the search for these viruses has been intensified. Over the last years several new groups of these viruses have been discovered in various types of samples and environments. In this work, we describe the isolation of 68 giant viruses of amoeba obtained from 776 environmental samples from Brazil and Antarctica. It was analyzed was analyzed: 495 soil samples, 124 water samples, 140 sewage samples and 17 capybara samples. Isolated viruses were identified by hemacolor staining, PCR assays and electron microscopy (scanning and/or transmission). A total of 64 viruses belonging to the Mimiviridae family were isolated (26 from lineage A, 13 from lineage B, 2 from lineage C and 23 from unidentified lineages), from different types of samples, including marine water from Antarctica, thus being the first mimiviruses isolated in this extreme environment to date. Furthermore, a new marseillevirus was isolated from sewage sample along with two pandoraviruses and a new cedratvirus (the third to be isolated in the world so far), adding new members to the putative Megavirales order. Although water samples have shown the highest number of isolated virus (34 isolates), we found a higher diversity of viral groups in sewage samples (26 isolates). In the water samples only Mimiviridae family viruses (12 of lineage A, 13 of lineage B and 9 unidentified) were identified, while in the sewage samples were found besides Mimiviridae(9 of lineage A, 2 of lineage C and 11 unidentified), 1 marseillevirus, 1 cedratvirus and 2 pandoraviruses. Our results reinforce the importance of prospective studies in different environmental samples, therefore improving our comprehension about the circulation and diversity of these viruses in nature. Financial support: CNPq, Capes, FAPEMIG, Pós-Graduação em Microbiologia da UFMG.

Palavras-chave: Giant viruses, Isolation, Antarctica, Brazil
018 - ÁREA: BÁSICA

ACTIVITY OF SYNTHETIC COMPOUNDS IN PSEUDO HUMAN PAPILLOMAVIRUS INFECTION IN VITRO

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Resumo

Human Papillomavirus (HPV) belongs to the Papillomaviridae family. The prevalence of HPV is high, reaching up to 10% of human world population and is directly related to cervix cancer. Thus, it is evident the need to develop new and more efficient therapies against the virus. Substances derived from natural compounds have attracted interest exhibiting bacterial, fungal, and viral activity. However, natural compounds have the difficulty of extraction, purification and are not patentable. In this context, synthetic compounds show an interesting source for the development of new antiviral treatments. They are described as having antiviral, anticancer, antioxidant and anti-inflammatory properties. Such effects added to the large-scale production capacity of the synthetic compounds, present a promising cost-benefit relation for the development of new antivirals. This work aim to produce HPV pseudovirus and evaluate the effect of synthetic compounds in infection of HPV in vitro. Human embryonic kidney 293T cells were transfected with p16SHELL e pClneoEGFP plasmids. New 293T cells were infected with the pseudovirus to evaluate the infection capacity. Firstly, the cell viability (MTT) was performed to evaluate the synthetic compounds cytotoxicity. Forty-two compounds from 10µM to 50µM concentrations were tested to identify which concentration was toxic. Compounds with cell viability rate greater than or equal to 80% were selected for pseudovirus infection assay. The pseudovirus was able to infect the 293T cells and to express fluorescence. After the cell viability assays, twenty-one synthetic compounds were selected in non-toxic concentrations, which will be tested for HPV infection in vitro. Financial Support: ROYAL SOCIETY (NA150195); FAPEMIG (APQ-00587-14; SICONV 793988/2013), CNPq (445021/2014-4), FAPESP (2014/22198-0).

Palavras-chave: Antiviral , HPV, Synthetic Compounds
AN IRES ELEMENT WITHIN THE HIV-1 GENOME INDUCES MICRO RNA 178 AND REDUCES VIRAL INFECTIVITY

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Resumo

HIV-1 is responsible for 36 millions of infections worldwide. HIV codes for the viral protein Nef, which is associated with the increase in viral infectivity. Although, Nef is considered non-essential for viral replication in vitro. Several infectious clones of HIV-1 exist and are used in studies from characterization of aspects of viral biology to the screening of anti-virals and assays for sensitivity of HIV to anti-retroviral compounds. To facilitate quantification of viral replication reporter genes are cloned within the coding region of HIV-1 genome. However, a viral non-essential gene, such as nef, is replaced by the reporter gene. In an attempt to obtain an infectious clone preserving all viral ORFs and harbouring a reporter gene, the clone pNL4-3_Nef_IRES_GFP was previously obtained. This infectious clone is derived from the HIV-1 infections clone pNL4-3, in which, an IRES element followed by the GFP ORF was cloned at the 3’ end of the nef gene, generating a bicistronic transcript for Nef and GFP. No study was carried out to access whether this change can impact on the viral replication capacity. Thus, our objective was to study the replicative capacity of pNL4-3_Nef_IRES_GFP. A 3-4-fold reduction in the infectivity of viral progeny produced from Hek-293T was noticed in pNL4-3_Nef_IRES_GFP when compared to pNL4-3. Expression levels of Gag, Nef and Vpu proteins was accessed and a 2-fold increase in protein synthesis was observed in pNL4-3_Nef_IRES_GFP when compared to pNL4-3 transfected cells. Semi-quantitative PCR was employed to amplify HIV full-length and multi-spliced transcripts. Surprisingly, levels of viral transcripts were 4-fold lower in pNL4-3_Nef_IRES_GFP than in pNL4-3. However, viral progeny incorporated similar levels of genomic RNA. We have analysed the expression of Micro RNA178 (miR-178) in pNL4-3 and pNL4-3_Nef_IRES_GFP transfected cells and found that while in the former, there was a reduction in miR-178 expression when compared to mock-transfected cells, in the later, levels of miR-178 were increased by 4-fold. Expression of miR-178 is induced by Toll-like-receptors (TLR) and miR-178 is involved in inducing PI3K/AKT/MTOR pathway by reducing levels of PTEN. Thereby we propose a model in which, the IRES element within pNL4-3_Nef_IRES_GFP induces TLR leading to miR-178 expression and mTOR activation, increasing protein synthesis but inducing an antiviral response in infected cells. This work was supported by CAPES, CNPq and FAPERJ.

Palavras-chave: HIV-1 infectivity, Ires element , HIV-1 genetics, mTor pathway in HIV-1, mRNA178/TLR
ANTIVIRAL ACTIVITY OF MAPK INHIBITORS: IMPROVEMENTS FOR DENV, ZIKV AND YFV

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Resumo

As part of an ongoing investigation in the Signal Transduction Group from the ‘Laboratório de Vírus’ at ICB-UFMG, we are testing cancer-developed kinase inhibitors to reduce Flavivirus in vitro multiplication. Actually, viruses need to activated and modulate Cell Signaling Pathways in order to complete a successful multiplication cycle. That is why using inhibitors design to counteract constitutive kinase activation in cancer have shown good results as antivirals. Our group have already shown the effect of MAPK pathway inhibitors in DENV; reducing viral titers in more than 10-fold in vitro, and protecting infected mouse from lethal viral doses. Now, due to the urgent public health priority, we are including ZIKV and YFV for testing. Results indicate that a specific MAPK inhibitor reduces ZIKV titter 1000-fold and YFV titter 100-fold with 24h treatment, in vitro. Furthermore, the team last breakthrough exhibits a higher effect of inhibitors when functionalized with β-cyclodextrin, which forms a reversible complex with the inhibitor, enhancing solubility and bioavailability. In the long run, these results bring us closer to our goal of finally find a treatment to Flavivirus infections. Highlighting that, all side effects found in long-term cancer therapy are undermined when endorsing treatment for a viral acute infection.

Palavras-chave: DENV, ZIKV, YFV, MAPK inhibitor, β-cyclodextrin
Chikungunya fever, caused by the infection with chikungunya virus (CHIKV), is characterized by symptoms as fever, muscle aches and joint pains. Currently, there is no vaccine or antiviral treatment against CHIKV infection. Natural compounds extracted from plants present as an alternative approach to new therapies, being a rich source for the development of antivirals. This work aimed to evaluate the antiviral activity of 30 compounds isolated from Brazilian plants on the replicative cycle of CHIKV. To access the therapeutic potential of these compounds, BHK-21 cells were infected with CHIKV-ECSA strain containing the Gaussia Luciferase reporter (MOI of 0.01) in the presence or absence of compounds. After 16 hours, the supernatant was collected and Gaussia luciferase levels were measured. The results demonstrated that compounds Ac.O, K1, S6 and S8 inhibited up to 76% of the CHIKV infection. We further investigated the effect of 6 compounds on the replication stage of virus lifecycle by using Huh-7.5 cells stable line expressing NCT. Cells were treated with compounds at maximum nontoxic concentrations for 72 hours and cell viability and replication levels were accessed. The compounds APS and 3*20 demonstrated to reduce CHIKV replication up to 48.4% and 39.2%, respectively. Additional analyzes have been carried out to a better understand of the mode of action of these compounds. Financial Support: CNPq, Royal Society

Palavras-chave: Antiviral, Chikungunya, Natural compounds
ASSOCIATION OF CD209 -336 A/G (RS4804803) GENE POLYMORPHISM AND DENGUE: A META-ANALYSIS.

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Resumo

Dengue is the most prevalent arboviral disease that affects humans in the world. Research has demonstrated that the pathogenesis mechanisms of this disease are complex. Interaction among factors inherent to Dengue virus (DENV), environment and the human host are determinant in individual susceptibility to dengue, as well as, in variations of clinical manifestations and risk of progression to severe forms. In recent years, Single Nucleotide Polymorphisms (SNPs) in genes related to the pathogenesis of dengue has been studied and associated with susceptibility and/or protection. Among these, -336 A/G SNP in the CD209 gene (DC-SIGN) showed controversy role in pathogenesis of dengue in variety populations. The aim of the study was to realize a meta-analysis to evaluate the role of the SNP -336 A/G in DC-SIGN gene as a protective factor and/or susceptibility to dengue progression from published studies that relate this SNP between groups of patients (control group, Dengue Fever patients and Dengue Hemorrhagic Fever patients). A systematic search was conducted using databases for publications and nineteen articles were selected. After analysis and detailed evaluation of two examiners, four articles and internal data from the research group realized in a population from the Northeast of Brazil were included, with a total of five studies included in this meta-analysis. The Review Manager version 5.3 program was used for statistical analysis. The results indicate that the AA genotype is related to protection in the control group (OR= 0.59, p= 0.03) and AG genotype is related to the risk of developing DHF (OR= 1.34, p= 0.02). Comparing patients with DHF and DF, genotype AG (OR= 2.59, p = <0.00001) and G allele (OR= 2.73, p= <0.00001) are associated with the risk of Dengue Hemorrhagic Fever, as well as AA genotype (OR= 0.34, p= <0.00001) and A allele (OR= 0.37, p= <0.00001) are related to protection in the DF group. In subgroup analysis, AG genotype (OR= 3.12, p= <0.00001) and G allele (OR= 2.71, p= <0.00001) are associated with susceptibility to DHF in Asians, but not in the Brazilian population. Therefore, literature-based meta-analysis suggests that AG genotype and G allele of the -336 A/G SNP is associated with Dengue Hemorrhagic Fever. Financial Support: CNPq, FAPEPI, Prefeitura de Parnaíba.

Palavras-chave: DC-SIGN, Dengue, polymorphism, host
BIOLOGICAL CHARACTERIZATION OF TWO BRAZILIAN ISOLATES OF ZIKA VIRUS.

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Zika virus (ZIKV) is an arbovirus belonging to the family Flaviviridae. Currently this virus is widespread in the Americas. In Brazil, ZIKV infections have been associated since 2015 with microcephaly, ocular lesions, among other serious diseases in newborns, as well as nervous system disorders in adults. Currently, the biologic differences among the different Brazilian ZIKV isolates are poorly understood. Therefore, we studied two Brazilian ZIKV clinical specimens in two cell lineages. They were isolated in Recife (PE243/2015; herein named PE-ZIKV) and Sao Paulo (SPH/2015; herein named SP-ZIKV). Viral stocks were respectively prepared on C6/36 and Vero cells. Both isolates were initially characterized by plaque size on VERO monolayers under carboxymethyl cellulose overlay. Remarkably, SP-ZIKV displayed larger lysis plaques. Further, the kinetics of multiplication of both isolates was assessed in Vero and C6/36 cells infected with M.O.I. 0.1 or 0.01. Irrespective the M.O.I which was used, PE-ZIKV attained higher titers in VERO cells when compared to SP-ZIKV. In addition, although SP-ZIKV displayed a delayed kinetics in C6/36 cells infected with 0.1 M.O.I, peaking at day 7 (whereas PE-ZIKV peaked at day 4), the viral titers attained in C6/36 cells were higher than those found in cells infected with PE-ZIKV. In contrast, we found no differences when C6/36 cells were infected with 0.01 M.O.I of either isolate. Moreover, higher infectious titers were observed in VERO cells infected with PE-ZIKV (irrespective the M.O.I used) when compared to the titers found in C6/36. In contrast, the infectious titers detected in C6/36 cells infected with SP-ZIKV were higher (M.O.I=0.1) or similar (M.O.I=0.01) to those found in Vero cells. In conclusion, these results suggest that there are biological differences among Brazilian ZIKV isolates. Their analysis by sequencing and further experiments in other cell lineages and animal models will be necessary to their better characterization. **Financial support:** FIOCRUZ/PDTIS-Vacinas, Universal FAPEMIG and Universal CNPq. CNPq provided fellowships to AMVM, MASC, EGK, LAM. CAPES, the Brazilian Ministry of Health (DECIT/SVS), and a grant to Monash University from the Foundation for the National Institutes of Health through the Vector-Based Transmission of Control: Discovery Research (VCTR) program of the Grand Challenges in Global Health Initiatives of the Bill and Melinda Gates Foundation provided fellowships to LAM.

Palavras-chave: BRAZILIAN ZIKV ISOLATES, FLAVIVIRIDAE, FLAVIVIRUS, ZIKA VIRUS
024 - ÁREA: BÁSICA

CS7BL/6 MICE AS A MODEL FOR BOVINE ALPHAHERPESVIRUS 5 STUDIES

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Resumo

Bovine alphaherpesvirus 5 (BoHV-5) is a Varicellovirus that is associated to non-suppurative meningoencephalitis causing sanitarian and economic losses in cattle herds. Frequently rabbits are used as models to study BoHV-5 biology and pathogenesis, but the use of murine model still incipient, although presents important advantages as the short life cycle and well-established genomics and proteomics approaches. This work aimed to evaluate the susceptibility of CS7BL/6 wild-type (WT) mice to BoHV-5 infection by intracranial (IC) inoculation and the use of glial cells derived from neonatal CS7BL/6 mice to analyze viral growth curves and innate immune response. In contrast to mock, BoHV-5 infected animals showed clinical signs (e.g. circling and hunched posture) and histopathological changes in CNS (meningoencephalitis, reactive blood vessels and microgliosis). CS7BL/6 neonatal glial cells supported the BoHV-5 infection in one-step (m.o.i=10) and multiple step growth curves (m.o.i=0.1) showing production of viral titers of 2x10^5,57 and 2x10^5,87 TCID50/mL, respectively. Optical microscopy evidenced vacuolization, rounding and cell lysis and ultrastructural analysis of the infected cells indicated large electron-dense clusters in the cytoplasm, cell vacuolization, as well as distension of the carioteca and clusters of viral particles visualized inside vesicles. Analyzing the antiviral immune response by qPCR, infected cells had significantly higher expression of TLR7, MyD88, IRF-3, IRF-7, IL1-β and IFN-β mRNA in comparison to mock infected cells. IRF-3, IL1-β and IFN-β were triggered early (6 h.p.i) in comparison to the TLR7 mRNA (peaks only at 24 h.p.i.) pointing out that other PRRs molecules can be involved in antiviral response during BoHV-5 infection in murine model. To further investigate the participation specifically of the endosomal TLRs 3, 7 and 9 during BoHV-5 infection, CS7BL/6 TLR3/7/9-/- mice were inoculated by IC route. Both BoHV-5 infected WT and TLR3/7/9 deficient groups showed similar degrees of meningitis, loss of weight and other clinical signs, suggesting that other compensatory multiple innate sensing pathways could be implicated during viral infection as already described to other hesperviruses. Together these data indicate the potential use of CS7BL/6 mice and of a primary cell culture obtained from their CNS as a tool to study aspects of BoHV-5 basic biology, immunology and pathogenesis of this cattle pathogen.

Palavras-chave: Animal model, Bovine alphaherpesvirus 5, CS7BL/6 mice, Glial cells
025 - ÁREA: BÁSICA

CELLULAR LOCALIZATION ANALYSIS OF THE HEPATITIS C VIRUS CORE PROTEIN DURING NUCLEOCAPSID-LIKE PARTICLES ASSEMBLY

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Resumo

Hepatitis C virus (HCV) infection is the major cause of chronic liver diseases, infecting around 210 million people worldwide. HCV capsid protein (HCVCP) is involved in several viral process, including the capsid assembly process, and cellular processes, as the interaction with a p53 tumor suppressor protein, increasing apoptosis and cell proliferation. This work aims to gain more information about the cellular localization and the assembly process of HCV in different cell models. We constructed vectors to express the full-length HCVCP, composed by 191 amino acids and fused to Green Fluorescent Protein (GFP) (HCVCP191GFP and GFPHCV191), in Huh7 cells, human hepatocytes. Also, we constructed, by deletions of HCVCP191GFP, two other forms of the HCV core protein, composed by 124 and 179 amino acids, HCVCP124GFP and HCVCP179GFP, respectively. The same was done for GFPHCVP191, generating GFPHCVP124 and GFPHCVP179 truncated forms. Transfected cells with HCVCP at its C-terminal (HCVC1GFP) and N-terminal (GFPHCVP1) were analyzed by confocal microscopy and number and brightness analysis (N&B). Confocal microscopy of HCVCP191GFP transfected Huh7 cells show that 36 hours post transfection, the HCVCP191GFP is mainly placed in the nucleus and, interestingly, this protein seems to be sited on lipid droplets surface, while the GFPHCVP191 form shows a diffuse cell distribution. The N&B data show that GFPHCVP191 is dimeric and monomeric in Huh7 cells. The deletions were confirmed by sequencing and the cellular distribution analyses are in progress in Huh7 cells. In order to understand the interaction between HCVCP and p53 tumor suppressor protein, analyzes of the cellular distribution of these proteins are in progress in Hep3B cells, a human hepatoma cell line. Our data reveal a new approach to understand the assembly of Hepatitis C virus capsid, which is an important target for drugs that may impair the Hepatitis C virus replication.

Palavras-chave: Hepatitis C Virus, Core Protein, Assembly
Characterization of Helicoverpa Armigera Stunt Virus (Hasv) Maturation Process.

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Resumo

HasV is a positive ssRNA virus that belongs to the alphatetraviridae family and infects Helicoverpa armigera caterpillars, an agricultural pest of great importance worldwide. HasV is a simple, non-enveloped virus, with a proteic capsid formed by 240 copies of the same protein. As other tetraviruses, HasV undergoes a maturation process involving a large conformational change followed by an autocatalytic reaction resulting in a covalently independent lytic peptide that remains associated with the particle. The detailed knowledge of tetravirus tridimensional structure and the exquisite control of their maturation in vitro, make these viruses excellent models for the study of viral maturation. In this work, we compare the maturation kinetics of HasV with a related well characterized virus, NwV. These viruses share 66% aminoacid identity and in both cases, the autocatalytic activity is controlled by pH. In order to induce maturation, uncleaved procapsids were transferred from pH 7.6 to pH 5.0, aliquots were flash-frozen at time intervals and analyzed by SDS-PAGE. HasV cleavage presented a typical curve, with a fast phase in which about 50% of the subunits were cleaved within 30 minutes and a slower phase in which the remaining subunits took hours to complete cleavage. A site-specific substitution in which a proline that is present in HasV cleavage site was exchanged by a glycine that in in the same position in NwV structure, caused a slow cleaving phenotype but rescued the reversibility property that was previously described with NwV but not observed with HasV WT particles. This result suggests that the cleavage dynamics is fine tuned by amino-acids that are not directly involved in cleavage reaction. The comparison between these two models can help to understand the molecular basis of the regulation of maturation and autocatalytic reactions in viral capsids. Support: FAPERJ, CNPq.

Palavras-chave: Maturation, auto-catalysis, Helicoverpa armigera, insect virus, biotechnology
COMPREHENSIVE INTEGRATION AND SPREAD OF ENDOGENOUS VIRAL ELEMENTS BASED ON SMALL RNA SEQUENCING

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Resumo

Mobile elements drive genome evolution in most organisms. Among these elements are Transposable Elements (TEs), Retroviruses and Endogenous Viral Elements (EVEs). Although progress is seen in the understanding of integration mechanisms of TEs and retroviruses on host genome, endogenization of viral sequences remain poorly described and mostly restricted to mammals. RNAi pathways play major role in the control of mobile elements through production of complementary small RNAs. Here, we took advantage of long and small RNA sequenced libraries from Aedes aegypti mosquitoes and Aag2 cell line to investigate mechanisms involved on endogenization and control of EVEs. Here we applied small RNA deep sequencing and Hidden Markov models to characterize EVEs in A. aegypti mosquitoes and investigate mechanisms involved on invasion and spread along host genome. We identified and characterized 248 putative EVEs with sequence similarity to at least 18 viral families. Some of them are not present on current version of host reference genome. We noticed vast majority of EVEs are flanked by TEs and repeats, which suggests they could orchestrate the integration of EVEs on host genome in a mechanism similar to observed in mammals. The expression of EVEs and flanking TEs were positively correlated closer the distance between them. Analyzing EVE-derived small RNAs we observed primary piRNAs but not siRNAs in mosquitoes and cell lines. The negative correlation observed between long and small RNAs also corroborate the action of piRNA pathway in control of EVEs. Financial support: FAPEMIG – Fundação de Amparo à Pesquisa do Estado de Minas Gerais CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico ANR – Agence Nationale de la Recherche

Palavras-chave: endogenous viral elements, mobile elements, small RNA sequencing, transposable elements, virus integration
028 - ÁREA: BÁSICA

DENGUE INFECTION OF ENDOTHELIAL CELLS PROMOTES MITOCONDRIAL DYSFUNCTION AND ROS PRODUCTION, WHICH AFFECTS CELL VIABILITY AND VIRAL REPLICATION

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Resumo

Dengue (DENV) infection induces a spectrum of clinical manifestations, including increased vascular permeability and plasma leakage, as a result of systemic inflammation and endothelial lesion. We have previously demonstrated that endothelial cells were permissive to DENV, which triggered activation of cytoplasmic RNA sensors, induced cellular activation, and death. Preliminary data also indicated that DENV infection lead to altered mitochondrial membrane potential, suggesting that endothelial cell death might be associated to activation-induced stress. Given that mitochondria is a major source of reactive oxygen species (ROS), we investigated whether virus replication promoted ROS production, and if these mediators would affect viral replication, endothelial activation and cell death. We also further analyzed mitochondrial function, by carefully investigating cell respiration. We used human brain microvascular endothelial cells (HBMECs) as an endothelial cell model. The cells were infected with DENV2 (16681 strain) and ROS production was analyzed flow cytometry. Virus replication was evaluated by qRT-PCR, flow cytometry and plaque assay. Cell death was evaluated by flow cytometry and XTT assay. Cytokine production was analyzed by Bioplex and ELISA. Cellular respiration was analyzed by respirometry. Dengue infection lead to mitochondrial dysfunction, as evidenced by altered cellular respiration, with decreased basal and ATP-dependent respiration, and diminished reserve capacity. HBMECs infection also resulted in increased ROS production, including mitochondrial ROS, and this was dependent on viral replication. ROS inhibition resulted in decreased viral load, prolonged cell survival, and diminished apoptosis of bystander cells. Interestingly, inhibition of ROS resulted in diminished cytokine secretion by HBMECs. These data suggest that ROS production might by associated to mitochondrial changes induced by virus replication. DENV-induced mitochondrial stress and ROS might then enhance the activation mediated by cytoplasmic sensing leading to endothelial cell activation and death.

Palavras-chave: DENV, HBMECs, ROS
DENGUE VIRUS ANTIBODY IS CROSS-REACTIVE TO ZIKV AND OTHER FLAVIVIRUS, DRIVING TO INFECTION ENHANCEMENT OR NEUTRALIZATION

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Resumo

DENV circulates in Brazil for more than 30 years. Along this time, one serotype has cyclically replaced the other, until recently, when all four distinct serotypes have begun to circulate together. Persistent circulation of DENV for so long makes possible sequential infections throughout a person’s life. After primary DENV infection, a long-life immunity specifically to the infecting serotype is developed. Severe dengue, characterized by hemorrhagic manifestations, is more frequent in secondary than in primary infections. Once the four DENV serotypes are antigenically related, Antibody-Dependent Enhancement (ADE) could be associated with severity. It suggests that antibodies generated by the primary infection would not be at a sufficient titer to neutralize secondary infection, instead it would opsonize and increase the virus entry in circulating monocytes leading to higher virus loads. Since ZIKV and DENV are antigenically similar, the possibility of cross-reaction has attracted attention, and in fact it has been demonstrated in vitro, raising concerns about the consequences for the pathogenesis of ZIKV infection. The aim of my work is determine whether immune-sera from DENV and ZIKV infected patients would cross-react in vitro with other flavivirus such as Yellow fever (YFV), West Nile Virus (WNV), Rocio Virus (ROCV), Ilhéus Virus (ILHV), and Saint Louis Virus (SLEV). For this we resorted of flow cytometry to study ADE and the plaque reduction neutralization test (PRNT) to characterize the samples regarding the presence of neutralizing antibodies. Using sera from DENV and ZIKV infected patients we demonstrated cross-reactivity of these samples with other flavivirus. We observed that some samples promoted ADE, enhancing virus entry in K569 cells, while others were able to neutralize infection. The protection generated by the cross-neutralization of flavivirus may be an indication that conserved epitopes exist in this group, which could be a potential target for the development of safe vaccines. Financial support: FACEPE.

Palavras-chave: ADE, DENV, Flavivirus, ZIKV
The *Anelloviridae* is a family of non-enveloped, circular single-stranded DNA viruses with genome size of 2.1 to 3.9 kba in length, which are currently described into 68 species and classified into twelve genera. Anelloviruses have been found in vertebrate and invertebrate hosts including primates, dogs, cats, pigs, rodents, bats, seals and mosquitoes. Also, they are highly diverse and prevalent in humans, they have been associated to a broad spectrum of human diseases, but they have not yet been directly related to cause it. In this study, we describe the application of the high-throughput sequencing to examine the frequency and diversity of anelloviruses in small mammal samples, which includes rodents (Cricetidae), bats (Molossidae and Phyllostomidae) and opossum (Didelphidae) species captured in São Paulo State, Brazil. We report a total of twenty-six anellovirus sequences with sixteen nearly complete genomes and ten partial genomes, which include eleven novel species identified. We describe their genome organization, which range in in length between 2.3 to 2.7 kba, and most of them have a predicted untranslated region (UTR) with a GC-rich content, putative TATA box regions and polyadenilation tail. The anellovirus sequences varied in position and numbers of putative open reading frames (ORFs), but all of them included at least ORF1, which encodes the putative ORF1 protein. We also have described the phylogenetic analysis of the novel viruses, and we have proposed the inclusion of two new genera within the *Anelloviridae* family, named *Omegatorquevirus* and *Sigmatorquevirus*, including six and three novel species of anelloviruses, respectively. In addition, the most prevalent host species positive for anelloviruses were *Necromys lasiurus* and *Mus musculus* with 100% of detection in the sample pools, followed by *Calomys tener* with 70%, all of which were detected in blood samples. On the other hand, the greatest diversity of anelloviruses species were observed in *Oligoryzomys nigripes* and *Akodon montensis* with 36% and 27% of the total eleven novel anellovirus species, respectively. In sum, this is the first epidemiological study of anelloviruses in wild rodents and marsupials in the Americas, and it provides new information on the diversity and host range of anelloviruses in nature. Financial Support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)

**Keywords:** Anellovirus, rodent-borne virus, bat-borne virus, ssDNA virus, zoonotic virus
EFFECT OF A TBK1 INHIBITOR, BX-795, ON POXVIRUS REPLICATION

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Resumo

Poxviruses are complex dsDNA viruses with an impressive capacity of modulating different cellular pathways. Cotia virus SPAn232 (COTV) has been poorly characterized and belongs to a new genus within the Poxviridae, whereas vaccinia virus (VACV) is the well-studied prototype of the Orthopoxvirus genus. In previous studies, our group screened several signal transduction pathways that would be preferentially activated in cells infected with COTV but not with VACV. Preliminary results suggested that TBK1 was a potential target for modulation during COTV infection. Therefore, the aim of this work is to investigate the involvement of TBK1 activation during infection of cells with COTV and VACV. Cells were infected with COTV at an MOI of 13 and after different times post-infection cell extracts were analyzed by Western Blot. Our results show that phosphorylated TBK1 was detected at 1h post-infection and progressively decreased as infection progressed until 24 h. Total TBK1 and β-actin remained constant at all time points. To evaluate the effect of TBK1 phosphorylation on the replication of COTV, we used the commercial inhibitor BX-795 that was added to cells after virus adsorption. Our results show a complete inhibition of TBK1 phosphorylation at all time points. To evaluate the effect of BX-795 on the production of virus infectious particles, cells were infected with COTV in the absence or presence of 10 μM BX7-795 for 24 hours and virus titer was determined by plaque assay. BX-795 inhibited the production of infectious progeny by 90.6%. We then asked whether BX-795 had an effect on the production of VACV infectious particles. For that, we screened several concentrations of BX-795 a 20-h infection with two VACV strains: VACV-IOC and VACV-WR. IOC is a low virulent strain of VACV and WR is a highly pathogenic strain. To our surprise, we observed that the yield of VACV strain IOC was inhibited by 90.9% and 93.5% at 5 μM and 10 μM BX-795, respectively, whereas the yield of VACV strain WR was inhibited by 90.7% and 98.8% at 5 μM and 10 μM of the inhibitor, respectively. Because previous results had shown that TBK1 was not activated during VACV infection, we will confirm these findings with both VACV. In addition, we will investigate the inhibitory effect of BX-795 on other potential targets such as the kinases IKKe and PDK1.

Palavras-chave: virus vaccinia, poxvirus, virus cotia
032 - ÁREA: BÁSICA

EVALUATION OF A CANDIDATE FOR MAYARO FEVER VACCINE IN AN IMMUNOCOMPETENT ANIMAL MODEL

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Resumo

Global warming and the increasing rainforest use facilitates interactions of antropophillic mosquitoes with humans leading to a high number of people at risk of arboviral infections. Despite the increasing number of cases, some arbovirus remains neglected. Mayaro virus (MAYV) is a neglected arbovirus that causes a Chikungunya-like illness usually presented with incapacitating arthritis/arthralgia. In nature, mosquitoes transmitted MAYV to vertebrates; human cases are accidental spillovers of this sylvatic cycle. The virus was found causing only sporadic outbreaks in Central and South America, but this can be an underestimation of the real circulation of this virus since there are few epidemiological inquires about MAYV. Besides, MAYV have the potential to become urbanized, with a broader area of circulation since Aedes mosquitoes can transmitted it, following the steps of chikungunya. There are no specific treatment or approved vaccine for MAYV. Most of our knowledge about Mayaro fever relies on chikungunya studies, since both are close genetic-related alphaviruses causing a very similar disease. Studies on the MAYV are based on immunologically impaired mice strains that are expensive and difficult to manage. Immunocompetent animal models are more suitable for development of a vaccine. In this work we develop a immunocompetent animal model for survey the immune response of a live-attenuated vaccine candidate for MAYV. Immunocompetent Balb/c mice were used to compare vaccinal or wild type (wt) strains of MAYV. Clinical, histological, and immunological parameters were analyzed. The vaccinal strain had initially higher counts of lymphocytes and neutrophils; elicit more IFN-γ, IL-1β, MCP-1, and specific antibodies against the wt MAYV strain. The wt strain had higher mononuclear count in first days post-infection and also causes an acute inflammation, absence in the mice inoculated with vaccinal strain. In this immunocompetent model, the vaccine candidate elicit a strong and protective immune response without inflammation. Besides, the immunocompetent animal model have a lower cost and is easier to manage in comparison to other models for alphavirus as CD1 mice. Despite the absence of chronic inflammation, this model proved to be useful for immunological survey. More studies are necessary to verify the response for longer times. Financial Support: FAPESP.

Palavras-chave: Arboviroses, Alphavirus, Mayarovirus, Vaccine, Animal model
EVALUATION OF ANTIVIRAL ACTIVITY OF EXTRACT AND ISOLATED OF PSYCHOTRIA SP. AGAINST THE MAYARO VIRUS

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Resumo

The fever caused by Mayaro virus (MAYV) is an arbovirose endemic in several countries in South America. It is related to incapacitating arthritis, involving myalgias and arthralgia, which can remain for various months. In recent years, an expansion into urban communities has been reported, and MAYV was noticed as an emerging. To date, there are no antiviral drugs or vaccines against the MAYV, and antiviral research has gained relevance. Bioactive extracts from about 1000 species of plants belonging to the genus Psychotria have been reported with antibacterial, antiviral, antifungal and anti-inflammatory activity. Therefore, the objective this work was evaluate the antiviral activity and its possible mechanisms of actions in methanolic extract D4 of Psychotria sp and its DX isolated. Initially, using Vero mammalian cells and methylthiazol-tetrazolium (MTT) technique, cytotoxicity tests were performed, giving the cytotoxic concentration to 50% of the cells (CC50). Then, the protective effective concentration was obtained for 50% of the infected cells (CE50). For the D4, no cytotoxic activity was detected until the highest concentration evaluated (500μg/mL). For the DX, CC50 was 119.3μg/mL. From numbers, antiviral tests were directed, and the same did not occur with the isolated DX. When evaluating the effect on virus adsorption, both the extract and the isolated showed no selectivity index (IS) was calculated, all being above 3. The following tests were made in order to perform the characterization of possible mechanisms of action, adding the antivirals before, during and at different times after infection, including adsorption, penetration, early and late stages of the viral cycle or virucidal effect. The virucidal effect was observed for the D4 from 31.25μg/mL, and the same did not occur with the isolated DX. When evaluating the effect on virus adsorption, both the extract and the isolated showed no effective protection. In the viral penetration stage, activity was observed for the D4 from 125μg/mL. Evaluating the effect in early and late stages of the viral cycle, the results showed that, when added 1h and 3h after infection, only the DX was able to partially inhibit the infection. These data show that the extract of this plant has antiviral action, and the DX may be one of active principles, possibly acting in synergy with other compound(s).

Palavras-chave: Psychotria sp, Mayaro virus, antivirals
The World Health Organization and the Brazilian Ministry of Health estimates that a new outbreak of Zika can occur in 2017/2018, specially on Northeast of Brazil. Similar to other flaviviral arboviruses, there is still no specific treatment for Zika to date. Considering the high number of possible infections, the risks it has to the brain and the fact that there is no vaccine or specific prophylaxis available to date, an effective treatment able to prevent the infection would be extremely desirable. Xanthene derivatives have diverse properties and biological activities such as antiviral, antibacterial and anti-inflammatory. Cytotoxicity of xanthenedione derivatives were evaluated in VERO cells. Cell viability was measured by MTT method, and the CC50 value was determined. Considering the 31 xanthenedione derivatives, only 5 presented high cytotoxicity in the highest concentrations. Concentrations used in subsequent neutralization assays do not present a reduction in cell viability. The screening assay was made using the highest non-toxic dilution in VERO cells, and the cell viability was measured by MTT method. From the 31 compounds tested, 9 presented antiviral activity higher than 50%, and the compounds #06 and #23 were selected for the next steps. To evaluate if the xanthenedione derivatives acts under the viral infection, PRNTs were performed. A viral activity reduction of 99,9% in the number of lysis plaques was observed for the compound #06 (CC50 = 27,64 mM) on the highest non-toxic dilution and a reduction of 99,9% for the compound #27 (CC50 = 224,4 mM) even under a dilution of 1:8. Based on dose-response viral activity reduction the IC50 value was determined 9,715 mM and 7,466 mM for the compounds #06 and #27 respectively. With CC50 and IC50 value it is possible to calculate the selectivity index (SI) which determine if is possible to neutralize the virus before killing the host. Values of 3.61 and 31.60 were calculated respectively for the compounds #06 and #27. Four inhibition mechanisms assays were performed for the compound #27: post-treatment, pretreatment, inhibition of internalization and inhibition of attachment. According with number of lysis plaques, attachment was inhibited in 99%. In silico analyses show a strong bond between the compound and Zika E protein. Altogether, is possible to conclude that the compound #27 can be a promising candidate against ZIKV infection and it may act blocking the viral E protein. Financial Support: FAPEMIG.

Palavras-chave: Zika Virus, Antiviral, Xanthenedione
Fungi and plant extracts are potential sources for the development of antiviral drugs for the treatment of diseases caused by arboviruses such as Dengue virus (DENV), Zika virus (ZIKV) and Chikungunya virus (CHIKV). To date, our group screened more than 6,000 extracts from plants and fungi for their antiviral activity against DENV-2 using a simultaneous treatment in BHK-21 cells. The antiviral effect was analyzed by observing the degree of inhibition of the viral cytopathic effect (CPE) and by MTT colorimetric assay. To date we have identified 115 extracts active against DENV-2. Among these 115 active extracts against DENV-2, we chose the 10 most promising extracts to be tested against ZIKV, CHIKV and the other serotypes of DENV (DENV-1, -3 and -4). Some fungi extracts showed reproducible EC50 values ranging from 3.1 to 12.5 µg/mL against DENV-2, even after recultivation of the original fungal isolate, and with no apparent cytotoxicity at the concentration of 100 µg/mL. Selected extracts will be fractionated by HPLC and the fractions will be bioassayed against those viruses. This process will be repeated and the analysis of the active fractions by HPLC-high resolution mass spectrometry will allow us to identify compounds responsible for the antiviral activity. In addition, by gene expression/proteomic tools, the gene/protein expression profile of ZIKV/CHIKV/DENV-infected cells treated with the selected purified compounds/fractions will be performed to prospect potential antiviral therapeutic substances against DENV, ZIKV and CHIKV, which hopefully could be useful to treat other viruses. Financial support: IRR - Fiocruz, CNPq, Capes, Fapemig, PROEP/P3D

Palavras-chave: antiviral, dengue, zika, chikungunya, natural products
GENOMIC AND MICROSCOPICAL CHARACTERIZATION OF BORELYVIRUS: A NEW GIANT VIRUS ISOLATED FROM SERRA DO CIPÓ NATIONAL PARK, MINAS GERAIS

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Resumo

Over the last decade, new members of the family Mimiviridae were isolated from a large variety of samples and regions. The identification and characterization of this increasing number of giant viruses have raised new questions concerning diversity, phylogeny and viral evolution. In order to clarify some of these issues, our research group has been devoting efforts to discover and describe novel giant viruses from different sources. Recently, a new-found member of the genus Mimivirus, named Borelyvirus, was prospected by our group using amoeba-associated virus co-culture from a sample collected in a small river located in the Serra do Cipó National Park, Minas Gerais. After this discovery, Borelyvirus was submitted to transmission electron microscopy (TEM) analyses, biological assays and complete genome sequencing using the Illumina MiSeq instrument (Illumina Inc., San Diego, CA, USA) with the paired end application. The DNA polymerase, RNA helicase and major capsid protein genes were used for phylogenetic analysis. The morphologic observation by TEM revealed icosahedral particle viruses with approximately 400 nm covered by an unusual less dense fibril layer when compared to other mimiviruses. The sequencing analysis showed that the genome of Borelyvirus has 1.038.187 bp of size. Its genome presented a low C+G% content (25.2%), similar to other mimiviruses. Furthermore, the genome of Borelyvirus encodes five tRNA molecules: three tRNA-Leu, one tRNA-His and one tRNA-Cys. The gene prediction showed that the genome encodes a total of 1005 ORFs, and among this 7 were considered ORFans, once were predicted without similarity with previously reported sequences. Moreover, 45 sequences, also without any predicted function, presented less than 100 aa and were characterized as pseudo-ORFs. The functional annotation showed putative proteins related to several metabolic processes, nucleic acid binding and manipulation. The analysis of paralogous proteins revealed the presence of 226 proteins divided in 47 clusters, of which is possible to highlight an ankyrin-like protein and an f-box/fnip (repeat containing) protein families, comprised of 56 and 43 sequences, respectively. Finally, for the ORFs predicted, the best hits were most frequently proteins from mimivirus lineage B (87.86%). Corroborating these results, the phylogenetic analysis of different genes also clustered the Borelyvirus with other members of lineage B, including moumouvoir-like strains.

Palavras-chave: Giant virus, mimivirus, genomic characterization, microscopical characterization, prospection
Human Adenovirus (HAdV) in allogeneic stem cell transplant (ASCT) recipients may cause prolonged and disseminated disease that often results in fatality. A 57-year-old patient diagnosed with chronic myeloid leukemia was submitted to an HLA-identical stem cell transplant. The patient’s fecal and sera samples were monitored for HAdV and norovirus. First sample was collected at day after transplant (D+) 1, and after that, samples were obtained weekly until patient’s discharge. Samples were then obtained in outpatient clinic visits and were extracted using commercial kits. For HAdV screening, samples were subjected to qPCR (Taqman), for norovirus screening conventional RT-PCR was used. HAdV and norovirus positive samples were also sequenced. Patient remained asymptomatic until D+9, when he presented nausea, vomit, abdominal pain, fever and diarrhea. Species F HAdV was detected in serum. This sample was also positive for Norovirus GI.3 by RT-PCR. At D+17, patient still presented diarrhea, and his fecal sample tested positive for species D HAdV with a viral load of 1.97x10^8 genomic copies/mL (gc/mL). Sample was also positive for Norovirus GI.3. At D+21, patient was discharged. A fecal sample, at D+27, was positive for HAdV at a higher load and also for NoV (GI.3), at the time patient presented diarrhea, abdominal pain and vomit. At D+41, samples were negative for HAdV, but positive for Norovirus GI.3. At D+76, serum sample was positive for species F HAdV (3.78 x 104 gc/mL). Phylogenetic analysis revealed that the HAdV sequence was identical to the sequence found in the sample obtained at D+9. At D+153, patient had diarrhea and was positive for species C HAdV in serum. The following samples were negative for HAdV and norovirus, and patient remained asymptomatic. More than 3 years after transplant, patient remains alive without immunossupression. In the studied case, three different species of HAdV and norovirus GI.3 were detected in samples from the same patient, up to D+153. After a sequence of negative sera samples, at D+76 one sample was positive for species F HAdV that had identical sequence to that sample detected on D+9, this time with lower viral load suggesting that viremia was intermittent. In Brazil, patients undergoing ASCT are not monitored for HAdV infection. The data demonstrate the importance of monitoring clinical samples of these patients in order to provide an appropriate treatment when the infection and clinical symptoms are present.

Palavras-chave: adenovirus, norovirus, allogeneic stem cell transplant, immunossupressed, viral infections
The Mayaro virus (MAYV, Alphavirus genus, Togaviridae family) is an emerging arbovirus, responsible for sporadic outbreaks of febrile illness in countries of South and Central America. The clinical manifestations of MAYV are characterized by fever, headache, rash, myalgia, and severe and long-lasting polyarthralgia. Studies with other alphaviruses in animal models and patients have elucidated the role of immune factors in the development of arthralgia. Thus, the aim of this work was to evaluate the immune response pattern elicited by the MAYV using animal model. In this experiment were used BALB/c mice with 15 days old, divided into two groups according to the infection route, below forelimb (G1) and in the rear footpad (G2).

On days 3, 10, 15 and 30 post-infection serum was collected for cytokine levels analysis, which were determined using the kit CBA (Cytometric Bead Array) mouse Th1/Th2/Th17 (IL-2, IL-4, IL-6, IL-10, IL-17A, IFN-γ and TNF), CBA flex IL-9, IL-13, IL12-p70 and chemokine MCP-1. Both groups showed a similar cytokine profile. On day 3 post-infection high levels of MCP-1, TNF, INF-γ and IL-6 were detected. On day 10 PI, TNF and IFN-γ levels remained high, with high concentrations of IL-10. At day 30 PI a second TNF increase was observed in G2. The presence of IL-4 was observed at day 30 PI in G2 and G1 day 10 PI. Therefore, these results show that the MAYV induces a strong pro-inflammatory response in the early post-infection days, which is rapidly controlled by the anti-inflammatory action of IL-10. In addition, the presence of INF-γ and IL-4 shows the infection induces both Th1 and Th2 patterns, highlighting the role of acquired immunity in infection control. Financial support: FAPEMIG

Palavras-chave: alphavirus, animal model, Cytokine, Mayaro virus, pro-inflammatory response
IDENTIFICATION OF A MAMMALIAN CELL LINE THAT DISTINGUISHES VIRULENT FROM ATTENUATED ISOLATES OF THE MORBILLIVIRUS PESTE DES PETITS RUMINANTS

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Resumo

Comprehensive pathogenesis studies on Peste des Petits Ruminants virus (PPRV) have been delayed so far by the absence of a small animal model reproducing the disease or an in vitro biological system revealing virulence differences. In this study, a mammalian cell line has been identified as presenting different susceptibility to virulent and attenuated PPRV strains. These cells, transfected or not with the PPRV cell receptor SLAM goat, were infected with either virulent or attenuated PPRV strains: Nigeria 75/1 wild-type and vaccine strains, wild-type India 94/1 and vaccine Sungri 96/1 strains. All PPRV strains penetrated and initiated the replication cycle in this cell, independently of the presence of the SLAM goat receptor as evidenced by an immunofluorescence test detecting PPRV nucleoprotein and by RT-PCR to PPRV mRNAs. However, while all the vaccine strains did not replicate, the wild-type strains successfully completed their replication cycle. Since this mammalian cell are interferon-producing, this could differentially modulate replication of virulent and attenuated strains. Therefore, we stimulated or repressed the expression of type I interferon in the study cell using respectively poly I:C and RNA interference methods, and subsequently infected them with PPRVs. Modulation of type I interferon response did not improve the replication of the vaccine strains, indicating that cell factor(s) other than type I IFN may hinder the replication of attenuated PPRV in this cell line. We propose this mammalian cell line as a new in vitro tool for PPRV-host interaction and virulence studies. The cell line name was not mentioned due the application of a patent for its use in the morbillivirus studies field. Financial support: CIRAD, AIRD

Palavras-chave: PPRV, cell permissivity, SLAM receptor, type I IFN, virus-host interactions
IDENTIFICATION OF CIRCULATING DENGUE SEROTYPES AND GENOTYPES FROM DIFFERENT MUNICIPALITIES OF THE AMAZONAS STATE, 2011 TO 2017

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Resumo

Dengue is considered one of the leading public health problems of the humanity, and it is estimated that almost half the world population is at risk. Dengue virus (DENV) belongs to the genus Flavivirus, family Flaviviridae, and is composed of four antigenically distinct serotypes named DENV-1 to DENV-4. The Amazonas state, as well as other Brazilian states, is considered endemic for dengue, with outbreaks records almost every year. However, there is little information on which viral serotypes circulate in the municipalities of the interior of the State. Thus, this study aimed to identify DENV serotypes in the Amazonas State between 2011 and 2017. We analyzed 155 serum samples from patients with clinical suspicion of dengue collected in public laboratories of the municipalities of Anorí, Boa Vista do Ramos, Borba, Coari, Humaitá, Itacoatiara, Manacapuru, Manaus, Maúés, Novo Airão, Parintins, São Gabriel da Cachoeira, and Tefé. Viral RNA extraction was performed using a commercial kit according to the manufacturer's instructions. Subsequently, samples were submitted to reverse-transcription real-time PCR (RT-qPCR) and semi-nested PCR to confirm the presence of DENV genetic material and serotyping, respectively. Until now, 98 samples were tested by RT-qPCR, of which 47 samples (48%) were positive. A total of 104 samples were tested by the semi-nested PCR protocol, including the RT-qPCR positive samples. Serotyping was accomplished to 67 (43.2%) samples, and the results showed two (3%) DENV-1 (2017); five (7.5%) DENV-2 (2013 and 2017); 13 (19.4%) of DENV-3 (2013 and 2016) and 47 (70.1%) DENV-4 (2012, 2013, 2014 and 2017). To date, eight samples were sequenced, of which seven samples are DENV-4 genotype 2, collected in the municipalities of Manaus (2012 and 2013), Maúes (2013) and Itacoatiara (2014) and one sample DENV-2, Asian-American genotype, from Novo Airão (2013). The data obtained in this study contribute to the increase of epidemiological information of dengue in the Amazonas state, mainly from the municipalities of the interior where there is no access to the molecular diagnosis of dengue. Further experiments are being conducted to characterize all the remaining DENV samples.

Palavras-chave: Dengue virus, Amazonas State, Serotypes, RT-qPCR, Semi-nested PCR
IMMUNOGENICITY AND STRUCTURAL STABILITY OF VIRUS-LIKE PARTICLES BASED ON BACTERIOPHAGE MS2 AS POTENTIAL VACCINE PLATFORMS

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Resumo

Virus-like particles (VLPs) are considered to be a safe and effective approach in vaccine development. Their particulate nature and dense repetitive subunit organization makes them ideal platforms for surface display of vaccine antigens. The stability and immunogenicity of a VLP is an important consideration for its use. Here, we study a platform for vaccine development based on the VLPs of bacteriophage MS2 displaying peptides representing the V3 loop of HIV gp120 and the ECL2 loop of the HIV coreceptor, CCR5. We aim to evaluate the stability of these VLPs and to investigate the immunological response of peptides presented in VLP when compared to peptides alone. The effects of the insertion of these peptides on VLP structure were assessed mainly by dynamic light scattering and small-angle x-ray scattering (SAXS). To evaluate the immunogenic capacity of VLPs V3 and ECL2 we used female Balb/c mice. The animals were immunized with 2 doses of 100 µg/mL VLPs ECL2, VLP V3, peptide ECL2 and peptide V3. After vaccination, IgG, IgG2a, IgG1 and IgA were measured in sera using ELISA. To further investigate the ability of the VLPs in stimulating the immune system we evaluated the production of cytokines (IL-17, IFN-γ and TNF-α) in the supernatants of splenocytes. The VLPs displaying peptides on their surfaces showed a similar structure and slightly lower stability than VLPs formed by the native coat protein. The VLPs do not appear to modify the cellular response profile. However the antibody response was increased when the animals were vaccinated with the antigen presenting VLPs. The construction of the VLP MS2 V3 and ECL2 promoted small changes in particle stability without significantly altering VLP size. Our results showed that the animals present a satisfactory immune response after vaccination with VLPs when compared to vaccination with free peptides.

Palavras-chave: Virus-like particle, Structural stability, Vaccine platform, Immunogenicity
IN VIVO AND IN VITRO CHARACTERIZATION OF TWO ZOONOTIC ISOLATES OF VACCINIA VIRUS - GUARANI (GP1V) AND PASSATEMPO (PSTV) - AND EVALUATION OF THEIR CAPACITY TO MODULATE HOST RESPONSES TO INFECTION

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Resumo

Vaccinia virus (VACV) is a member of Poxviridae family and Orthopoxvirus genus. The Poxvirus replicative success is dependent on its ability to block, evade or subvert essential elements of the immune response. However, the host immune responses to different virus strains are variable, and responses against recently isolated strains are poorly understood. This project aims to investigate the immunomodulatory and virulence profiles of the Brazilian VACV strains (VACV-Br), Guarani P1 virus (GP1V) and Passatempo virus (PSTV), in vitro and in vivo, comparing to VACV Western Reserve (WR). BSC-40 cells were used to evaluate One-step and Multi-step growth curves. Plaque phenotype assays were also performed. Infected cells were incubated for 2, 6, 12, 24 and 48 hours for one-step (M.O.I 10) and 6, 12, 24, 48 e 96 hours for multi-step growth (M.O.I 0,01). In parallel, Balb/C mice were inoculated with 103 or 106 PFU of purified virus. The animals were monitored for weight loss and the appearance of clinical signs. They were euthanized on 7 d.p.i and their organs were collected for viral quantification by means of real time PCR. MEFs cells were also infected to evaluate the splicing of the XBP1 transcription factor (TF) mRNA by RFLP; this TF is essential for a myriad of immune process. The one-step growth curve of GP1V and PSTV strains are similar to WR. However, PSTV seemed less productive at the 6 hours’ time-point. The multi-step growth curves also showed great similarity between GP1V and WR. The PSTV isolate, however, was again less productive than GP1V. Concerning plaque phenotypes GP1V with WR produced similar plaques whereas PSTV presented small plaques. The qPCR assay indicated that the amount of viral DNA found in the lungs of inoculated animals with 103 PFU of VACV isolates was higher in the group infected with PSTV and lower with GP1V, when both are compared to WR. Animals inoculated with 106 PFU of VACV isolates showed distinctive patterns: those inoculated with GP1V or WR became similarly sick, exhibiting typical symptoms of VACV infection, whereas those infected with PSTV showed only moderate symptoms. In summary, GP1V presents a virulence profile that is similar to WR, while PSTV displays a more moderate profile. Financial Support: CAPES, FAPEMIG, CNPq

Palavras-chave: GP1V, PSTV, VACCINIA VIRUS, Virulence profile, WR
INVESTIGATION OF CORONAVIRUS IN BATS

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Resumo

Viruses are important agents of emerging zoonosis, being a great public health issue. Several emerging and reemerging viral diseases have been described in bats. The coronavirus (CoV), a group initially known to cause mild respiratory illness, gained special attention in the last years due to the emergence of two new viruses, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV). These new viruses cause severe respiratory syndrome with high mortality rate and studies indicate that both are zoonosis originated in bats. Recently, bats were considered as the major evolutionary reservoirs and ecological drivers of CoV diversity. These vertebrates have abundant geographical distribution and are frequently in direct or indirect contact with the human population. Lately, such mammals, have received increasing attention as an important source for the emergence of zoonosis and possibly as viral reservoirs. Based on the importance of the analysis of potential viral reservoirs for zoonosis control and expand our knowledge on bat viruses, this study aims to investigate the presence of viruses of the Coronaviridae family, in intestinal samples, of bats. The animals were collected from São José do Rio Preto (São Paulo) and Barreiras (Bahia), were euthanized, followed by removal of the intestines of each specimen. The RNA was extracted and after quantification and quality analysis, cDNA was synthesized. PCR for the endogenous gene β-actin was performed for all samples in order to evaluate the cDNA quality. Samples were tested for the by Nested-PCR using specific primers targeting the RNA dependent RNA polymerase region. Positive samples were sequenced and phylogenetic analysis were performed. Ten samples were positive for bat specific coronaviruses. Nine (14,1%) were from São José do Rio Preto – SP and one (3.6%) from Barreiras – BA. Phylogenetic analyzes indicated that all identified coronaviruses belong to the genus Alphacoronavirus and that they form clusters according to the bat genus. This is one of the few studies to investigate these virus in both regions. Results revealed a high prevalence of bat coronavirus in São José do Rio Preto when compared to other studies. Further studies are needed to clarify the role of bats as reservoirs and source of infection of this viral zoonosis. Financial support: CNPq (165802/2015-4) and FAPESP (2015/09704-6).

Palavras-chave: Bats, Coronaviruses, Emerging viruses, Zoonosis
MAYARO AND CHIKUNGUNYA VIRUS REPLICATION IN MUSCULAR CELLS RESULTS IN REACTIVE OXIGEN SPECIES GENERATION, MITOCHONDRIAL DYSFUNCTION AND CELL DEATH.

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Resumo

The alphaviruses Chikungunya (CHIKV) and Mayaro (MAYV) are enveloped viruses members of the Togaviridae family. These viruses are usually related to an acute febrile illness followed by a debilitating symptom of arthralgia and myalgia that can last days and in some cases, for months or years. Clinical evidences highlights the importance of muscle in the progression of these alphaviruses diseases, but the molecular mechanisms associated with muscle damage, extent of injury and persistence of muscle symptoms are poorly understood. Previous studies have shown that reactive oxygen species (ROS) are involved in the progression of inflammatory myopathies. The aim of these work was assessed the involvement of ROS on muscular damage induced by MAYV and CHIKV infection. For this, we used C2C12 myoblasts and myotubes cells to evaluate the susceptibility, viability and oxidative stress during MAYV and CHIKV infection. Myoblasts were infected with MAYV and CHIKV in a multiplicity of infection (MOI) of 5 and differentiated myotubes were infected with a MOI of 1. After 24 hours of infection, we observed an increase of more than 3 orders of magnitude in viral titers in both cell populations. Despite similarities on viral replication, infected myoblasts exhibited 18% and 11% of cell death, while myotubes showed 47% and 59% of cell death induced by MAYV and CHIKV infection, respectively. The content of total cellular and mitochondrial ROS was determined by fluorescent microscopy using 2′,7′- dichlorofluorescein (DCF) and MitoSOX™ dies, respectively. Total ROS image analyses showed an increase of over 5.5- (MAYV) and 4.7-fold (CHIKV) against non-infected myoblasts. The contents of mitochondrial ROS (mtROS) showed an increase of over 1.4- (MAYV) and 1.9-fold (CHIKV). In infected myotubes, the total ROS content increased 3.3- and 4.2-fold for MAYV and CHIKV, respectively, and the content of mtROS also increased 2.4- and 1.7-fold. Mitochondrial integrity of infected myoblasts was also analyzed by High-resolution Respirometry and shows prejudice in mitochondrial activity after infection by MAYV and CHIKV. These results indicate that both, MAYV and CHIKV, may induces muscle cell damage associated with mitochondrial dysfunction and ROS generation. Thus, it is possible that the oxidative stress in the muscle induced by the infection may contribute to the progression of the lesions and inflammation in this tissue.

Palavras-chave: Mayaro, Chikungunya, ROS
Mayaro virus (MAYV) is an arthropode-borne viruses endemic in American continent, which together with other members of the genus alphavirus, such as the Chikungunya virus, is classified as arthritogenic alphavirus. MAYV infection induces a febrile condition with severe arthralgia and myalgia, which may persist for months characterizing a chronic condition of the disease. Despite the similarities of the clinical aspects among other viruses of this same group, the molecular mechanisms associated to the pathogenesis of the MAYV are completely unknown. Therefore, the aim of this work was feature an in vivo model for the study of MAYV-induced muscular and articular disorders. For this, adult mice deficient for IFN-α/β receptor (IFNR-/-), RAG-/- deficient mice and wide type (WT) with 6, 11, 21 days-old or adults (eight weeks), were infected subcutaneously in the left hind footpad with 20 μL of inoculum containing 10⁶ P.F.U. of MAYV. Clinical signs such as weight loss, weakness of hind limbs and difficulties in locomotion were observed in WT infants up to 21 days and adult IFNR-/- mice. The infection promoted a 100% lethality in 6 day WT and IFNR-/-; 80% in WT of 11 days. All 21-day animals recover after 5-6 days, coinciding with the decrease in viremia. The RAG-/- animals did not present any alteration in the clinical score, even though they presented high viremia. At different days post-infection (dpi) tissues such as footpad, knee, brain, liver and muscle from 11 day-old WT and adult IFNR-/- and RAG-/- mice were collected for viral quantification by plaque assay on BHK-21. A high level of MAYV was detected in serum on the first day post-infection, reaching a title of 10e7 P.F.U/ml. The virus was able to spread to all tissues tested, sustaining high viral loads, including muscle. Interestingly, in RAG-/- animals, even without clinical signs, MAYV was detected in tissues even 40 dpi. Additionally we quantify cytokine expression in footpad and muscle by qPCR of 11 day-old WT and adult IFNR-/- The MAYV infection promoted an increase of TNF, IL-1β, KC, IL-10 and MCP-1 expression in mouse muscle and footpad WT and IFNR-/-, four dpi. This study provides evidence that MAYV infection in young and IFNR-/- mice could replicates and promotes muscular and articular inflammation. The establishment of this animal model constitutes an important tool to study pathogenesis of MAYV and to develop control strategies for this virus.

**Palavras-chave:** Mayaro Virus, Muscle, Articulation, Murine model
MUTATIONAL STUDY ON E, NS3, AND NS4B VIRAL PROTEINS OF YELLOW FEVER VACCINE VIRUS 17D.

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Resumo

Yellow Fever (YF) is an infectious disease, caused by a flavivirus and is the subject of worldwide health concern. The main measure to control the disease is vaccination with the attenuated Yellow Fever virus (YFV), strain 17D, which can induce lifelong protective immunity with a single dose administration. Because of its viral properties, the YFV 17D is considered a potent expression vector in the development of recombinant human vaccine prototypes. In this work, the recombinant YFV 17D expressing the reporter Enhanced Green Fluorescent Protein (EGFP) was used to evaluate the impact of specific mutations that can modulate viral replication. The mutations in residues E$_{400}$ (F$\rightarrow$L), E$_{403}$ (T$\rightarrow$I), NS3$_{439}$ (V$\rightarrow$S) and NS4B$_{54}$ (L$\rightarrow$F) were inserted into the YF/EGFP virus genome to characterize its effect on viral proliferation and induction of humoral immune response. The cDNA of the viral genome YF/EGFP was used to generate recombinant viruses carrying one, two or three mutations. The viral proliferation was tested through infection of Vero, C6/36, and Huh7 cell lines. The results show that the mutations did not strongly influence the proliferation. However, viruses carrying a mutation in NS4B$_{54}$ presented a slightly improved fitness and proliferated more than the non-mutated recombinant virus in C6/36 cell line. On the other hand, the double mutation E$_{400}$/NS3$_{439}$ significantly decreased viral proliferation. Infectious viral particles could not be recovered from the cell culture transfected with the viral RNA bearing the mutation E$_{400}$, suggesting that it prevents the proper viral replication cycle. Comparative modeling of the YFV E protein has shown that this mutation influences the conformation of nearby residues, probably interfering with the protein’s interactions. To verify the induction capacity for YFV neutralizing antibodies, BALB/c mice were immunized with the viruses carrying single mutations and the sera were analyzed by Plaque Reduction Neutralization Test. The recombinant viruses YF/EGFP/NS3$_{439}$ and YF/EGFP/NS4B$_{54}$ induced lower titers of neutralizing antibodies compared to the group immunized with the recombinant virus without any mutation. We conclude that although the mutant E$_{400}$ did not alter the proliferation rate, it has a better capacity of inducing humoral immune response than the mutants NS3$_{439}$ and NS4B$_{54}$. Also, the E$_{400}$ residue is essential for the formation of infectious viral particles. Financial support: CAPES, CNPq, Fiocruz.

Palavras-chave: Yellow Fever, Viral vector, Mutations, Recombinant vaccine prototype
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NOVEL PARAMYXOVIRUSES IN BATS FROM BRAZIL

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Resumo

Paramyxovirinae family is composed by enveloped negative-sense RNA viruses divided into Pneumovirinae and Paramyxovirinae subfamily. Paramyxoviruses have been increasingly associated with bat species worldwide and some of them have been involved in cross-species transmissions with fatal consequences. The objective of this study was to investigate the paramyxoviruses in bats captured in Araçatuba, São Paulo State, Brazil. To this end, we sampled 42 individuals that represented five different species. Samples were distributed in pools based on species (Molossus rufus, Artibeus lituratus, Carollia perspicillata, Glossophaga soricina and Desmodus rotundus), tissue (kidney and liver), and date of collection. Viral RNA was extracted, followed by synthesis of double-stranded cDNA. Double-stranded RNA was sequenced using the Illumina platform. Sequence reads were quality-filtered, removed the adapter sequences and remaining reads were assembled by the de novo strategy using the MetaViC pipeline. We identified contigs from three novel paramyxoviruses, which showed similarity to proteins of paramyxoviruses previously described. Interestingly, paramyxovirus contigs were only identified in samples from Carollia perspicillata and Desmodus rotundus. These contigs shared less than 75% identity in amino acid level with homologous fragments of paramyxoviruses sequences available in GenBank. Phylogenetic analysis of complete sequences of polymerase gene of the polymerase gene revealed that all virus sequences formed a monophyletic clade and shared a common ancestor with Tailan, Beilong, and J virus, which has been proposed as Jeilongvirus genus. Also, a total of 21% (9/42) individual were positive when tested by RT-PCR targeting the conserved polymerase gene of Respirovirus, Morbillivirus, and Henipavirus genus. Interestingly, positive samples were derived exclusively from kidneys, indicating a potential excretion of the viruses in the environment. In addition, we have propagated a novel paramyxovirus from Desmodus rotundus in on Vero cell monolayers, and observed the cytopathic effect seven days post-infection on the third passage. In sum, we have identified three potentially novel paramyxoviruses in bats from Araçatuba, São Paulo State, Brazil.

Palavras-chave: Bat borne virus, Emerging viruses, Paramyxoviridae, Paramyxovirus, Zoonotic viruses

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Resumo

Bovine herpesvirus 1 and 5 (Bovine alphaherpesvirus 1 - BoHV-1 and Bovine alphaherpesvirus 5 - BoHV-5) are responsible for a variety of clinical syndromes infectious as bovine rhinotracheitis, infectious pustular vulvovaginitis, balanoposthitis, conjunctivitis, abortions and meningoencephalitis. These viruses are widely disseminated in beef and dairy cattle, presenting high prevalence in Brazil, representing an important economic problem. The vaccination has been the most used strategy for these viral infections being able to reduce viral transmission and clinical signs. The use of viral vectors as a vaccine has been widely used. The favorable properties of the Modified Virus Ankara (MVA) platform include the good stability of the virus, allowing the storage of the vaccine, and their great immunogenicity, already demonstrated for a series of antigens. We recently produced a recombinant virus expressing BoHV-1 and BoHV-5 multiepitope protein (MVA-recBoHV), a vaccine candidate for the control of bovine herpesviruses. Aiming transferring it to the industries which presents difficulties to adapt to the use of cellular systems for vaccine production, this work aimed to compare the expansion of MVA-recBoHV in different biological systems: primary and immortalized cell lines and chicken embryo egg chorioallantoic membrane (CAM). The shelf test was also carried out to analyze the maintenance of virus titre after lyophilization and storage at different temperatures. MVA-recBoHV was expanded in BHK-21 (Baby hamster kidney cell – ATCC CCL-10), DF-1 (Chicken embryonic fibroblasts – ATCC CRL-12203), CEF (primary chicken embryonic fibroblast) and CAM. The titration in BHK-21 of the viral stocks produced in the different biological systems showed equivalent results for MVA-recBoHV production in BHK-21, DF-1 and CEF. In MCA system, however, the viral production was lower than the cell cultures in 4 logs. It is important to emphasize that there is no correspondence between the cellular inoculum used for the continuous and primary cells and the number of cells contained in CAM. Lyophilization of MVA-RecBoHV followed by 60 days storage at 4 °C, -20 °C or at room temperature did not affect viral titre, confirming its stability and potential use as a vaccine candidate.

Palavras-chave: BOHV, EMBRYONATED EGG, MVA
PROLONGED AND INTERMITTENT DETECTION OF ZIKV IN THE URINE OF PREGNANT WOMEN AND NEWBORNS OUTCOME

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Resumo

Zika virus (ZIKV) is a re-emerging flavivirus that causes a mild febrile illness without severe outcomes. However, the cases of congenital malformations or neurological alterations reported in Brazil reshaped knowledge on the course of infection caused by a flavivirus and demanded a rapid approach to diagnosing the virus. The molecular diagnosis of flavivirus is usually performed using blood/serum samples; however, urine, saliva, and semen have been identified as additional sources since detection can be prolonged in these fluids even after viremia clearance. This study describes the long-term detection of ZIKV RNA in the urine from a group of ZIKV-positive pregnant women. From February to October of the 2016 ZIKV outbreak, 13 pregnant patients with Zika-like symptoms, with 4 to 38 weeks into their pregnancies, were treated at the Public Health Authority in the Brazilian city of São José do Rio Preto. Serum and/or urine samples were collected from the mothers during their first visits to facility after the onset of symptoms, and the patients were tested for ZIKV. The viral RNA was extracted and TaqMan® RT-qPCR assay was performed using primers targeting the ZIKV envelope (E) gene. After their first ZIKV-positive RT-qPCR (ct ≤ 38.5), these pregnant women were monitored, and viruria was measured until delivery. The viral RNA was detected more than four weeks (and up to seven months) after the first detection, even with intervals of negative detection. Among the newborns, only three presented outcomes, being unilateral abnormal OAE and subependymal cysts. None of them presented microcephaly. The use of urine for diagnosis represents an additional tool for virus detection. It is easy to obtain (as it does not put physiological stress upon pregnant women), and it can be obtained more than five days after the onset of clinical symptomatology. The outcome may or may not be related to the viruria, since mothers with a positive ZIKV RNA detection just before the delivery had newborns with and without outcomes. Because microcephaly and fetal abnormalities have been attributed to ZIKV, the monitoring of ZIKV viruria in pregnant women through the regular collection of urine samples proved to be an important approach over the course of the pregnancy; however, the meaning and the consequences for newborns will need to be evaluated in the future.

Financial Support: FAPESP

Palavras-chave: ZIKA, PREGNANT, URINE, VIRURIA
ROLE OF INTERFERON TYPE I AND III DURING ZIKA VIRUS INFECTION IN HUMAN BRAIN MICROVASCULAR ENDO The role of interferon type I (IFNα/β) and III (IFNλ) in the control of ZIKV replication in microvascular endothelial cells (HBMEC) is evaluated. HBMECs infected with African or Asian ZIKV strains showed decreased viral replication and IFNα expression when treated with IFNα or IFNλ. No luciferase activity was detected upon ZIKV infection, suggesting that IFN expression, secretion, or IFN-mediated response were hampered. Furthermore, luc activity induced by exogenous IFN was severely diminished in ZIKV-infected cells, indicating that virus replication blocked IFN-mediated signaling in this cell type. Accordingly, IFN-mediated STAT1 phosphorylation and ISG expression were diminished. Addition of IFNα to the cultures did not affect virus replication, corroborating that ZIKV escape from this response. However, when ZIKV-infected HBMECs were treated with anti-IFN neutralizing antibody, an increase of virus released was observed suggesting that, although the virus is capable of countering the IFN response, cytokine produced during infection modulates viral replication. In addition, pre-treatment of HBMECs with IFNβ 6h or 2h prior to ZIKV infection, led to a decreased viral replication, indicating uninfected cells may become resistant to infection upon IFNβ treatment. These data suggest that, whereas ZIKV-infected HBMECs block antiviral IFN responses, allowing some degree of virus replication and extravasation, IFNs produced during HBMEC infection may restrict virus replication in bystander cells. Financial support: Capes, CNPq, FAPERJ

Palavras-chave: HBMECs, Interferon, ZIKV
SCREENING OF ANTIVIRAL ACTIVITIES IN AMAZONIAN MEDICINAL PLANTS AGAINST DENGUE VIRUS AND ROTAVIRUS

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Introduction: In the last 20 years, ~40% of new drugs were obtained based on studies with prototypes of natural origin. Considering the rich biodiversity of our flora and that there are no specific therapies licensed for treatment of infections caused by Dengue virus and Rotavirus, this work proposes to carry out screening of medicinal plants from Amazon biome in order to identify potential bioactive agents against such viral infections. Material and Methods: The plant species were selected using integrated strategies, involving chemotaxonomic and ethnopharmacobotanical criteria. Cytotoxicity assays of crude extract were performed incubating, for 48h at 37°C and 5% CO2, 96-well plates with different concentrations of extracts in LLC-MK2 and MA-104 cells, so that cytotoxicity was determined by MTT assay and morphological analysis under optical microscope. Then, antiviral assays were performed against Dengue virus and Rotavirus in 24-well plates, using the maximum non-cytotoxic concentration (MNCT) of the extracts for LLC-MK2 and MA-104 cells susceptible to infection by those viruses respectively. After 48 h incubation, under the same conditions as in the previous assay, RNA was extracted from 140 μL of each individual well using QiAamp viral RNA Minikit. An exogenous control was added to each sample prior to RNA extraction with the aim of correcting pipetting errors and normalizing the amount of RNA recovered. Viral RNA load of treated cells compared to untreated controls was performed by one-step qRT-PCR in a multiplex one-step reaction for viral target and internal control in both antiviral trials. Results: Once the cytotoxicity of the extracts was determined, we performed antiviral assays using the MNCT of each extract for both cell lines. Our data so far, showed potential activity of ethanolic extract from Montrichardia linífera leaf against Dengue virus, as well as potential anti-Rotavirus activity of the lapachol fraction from Handroanthus serratifolius, both inducing decrease of viral load higher than 90% when compared to untreated cells. Our next step will be to determine the selectivity index of those active extracts against the mammalian cells and viruses. Conclusion: Our data showed that M. linífera and H. serratifolius have antiviral activity against Dengue virus and Rotavirus respectively. Additional studies will be conducted to investigate the mechanisms of action involved in the antiviral effects. Financial support: Fapemig, FUNED.

Palavras-chave: antiviral, medicinal, plants, Dengue, Rotavirus
Resumo

Viral hepatitis is a major public health problem in the world, being the types B and C of major epidemiological importance, due to the clinical characteristics and high morbimortality. Hepatitis B is caused by the hepatitis B virus (HBV), which because of its high specificity infects the man who becomes the natural reservoir. Hepatitis C is caused by the hepatitis C virus (HCV) and is recognized as one of the leading causes of chronic liver disease worldwide. The objective this work was to investigate the presence of HBV and HCV infections in the manicure and pedicure professionals of the municipalities of Jataí-GO and Caiapônia-GO, through serological and molecular analyses, associating the occupational conditions of these professionals. This is a cross-sectional and quantitative study to determine the prevalence of HBsAg, anti-HBc, anti-HBs and anti-HCV serological markers through immunochromatographic, Immuno-enzymatic and molecular methods. Primarily, A standardized questionnaire was used to interview 163 professional manicures and pedicures. As the same time, 142 serum samples were obtained. The sample population was characterized predominantly by female individuals (99.4%), with a median age of 32 years (IIQ: 26-42), married or in a consensual union (52.1%), with children (74, 2%) and with study time greater than or equal to 12 years (59.5%). Aspects of working time, use of personal protective equipment and knowledge about biosafety and sterilization, vaccine protection, accident working was verified. As for the presence of HBV and HCV serological markers, the HBsAg, anti-HBs and anti-HBs markers were observed in the ELISA technique 5.6% (n = 8); 7% (n = 10) and 33.1% (n = 47) respectively and anti-HCV in 2.8% (n = 4). There was no confirmation of positivity by the molecular method tested. This research, through the use of serological and molecular methods for hepatitis B and C, has made it possible to better establish the diagnosis of these diseases with social impact and to reinforce the need for adherence to good biosafety and prevention practices during the workday of professional manicures and pedicures

Palavras-chave: Hepatitis B, Hepatitis C, ELISA, Manicures, Pedicures
The Zika virus (ZIKV) is an arthropod-borne Flavivirus, Flaviviridae family, transmitted mainly by the bite of female Aedes mosquitoes. Infection with Zika virus (ZIKV) manifests in a broad spectrum of disease ranging from mild illness to severe neurological complications. Host immune response plays an important role in the clinical course of patients with viral infection. Particularly, cellular immunity and key components of the innate immune response, such as interferons and other cytokines/chemokines, play an essential role in limiting the viral spread. In this study, we characterized the levels of circulating cytokines, chemokines and growth factors in 54 infected patients, 29 non-pregnant females and 25 males, at five different time-points after symptoms onset. All patients attended at the Hospital Adventista de Manaus, Amazonas state, Brazil, in the first semester of 2016. We used microbeads multiplex immunoassay; statistical analysis and data mining to analyze clinical and laboratory data. ZIKV-infected patients present a striking systemic inflammatory response with high levels of pro-inflammatory mediators. Despite the strong inflammatory pattern, IL-1Ra and IL-4 are also induced during acute infection. Interestingly, the inflammatory cytokines, IL-1β, IL-13, IL-17, TNF-α, interferon-gamma; chemokines, CXCL8, CCL2, CCL5; and the growth factor G-CSF display a bimodal distribution accompanying viremia. Moreover, biomarker network analysis demonstrated distinct dynamics in consonance with the bimodal viremia profiles at different time-points during ZIKV infection. Such robust cytokine and chemokine response has been associated with blood-brain barrier permeability and neuroinvasiveness in other flaviviral infections. High-dimensional data analysis further established CXCL10, a chemokine previously associated to neuronal apoptosis, as the most promising biomarker of acute ZIKV infection for a putative clinical application.

Palavras-chave: Zika virus, Biomarkers, Cytokines, Chemokine CXCL10
Viruses display a wide range of genomic profiles and, consequently, a variety of gene expression strategies. Specific sequences associated with transcriptional processes have been described in viruses, and putative promoter motifs have been elucidated for some nucleocyttoplasmic large DNA viruses (NCLDV). Among NCLDV, the Marseilleviridae is a well-recognized family because of its genomic mosaicism. The marseilleviruses have an ability to incorporate foreign genes, especially from sympatric organisms inhabiting Acanthamoeba, its main known host. Here we identified for the first time an eight-nucleotide A/T-rich promoter sequence (AAATATTT) that regulates 55% of marseillevirus genes and is conserved in all marseilleviruses lineages, more than any giant virus described to date. We instigated our prediction about the promoter motif by biological assays and by evaluating how single mutations in this octamer can impact gene expression. The investigation of sequences that regulate the expression of genes relative to lateral transfer revealed that the promoter motifs do not appear to be incorporated by marseilleviruses from donor organisms. Indeed, analyses of the intergenic regions that regulate lateral gene transfer-related genes have revealed an independent origin of the marseilleviruses intergenic regions that does not match gene-donor organisms. About 50% of AAATATTT motifs spread throughout intergenic regions of the marseilleviruses seems to be available in all marseilleviruses lineages and do not appear to regulate a downstream gene. Multiple copies of available AAATATTT motifs seem to be fundamental for the incorporation and expression of foreign genes, which increases the plasticity and viral fitness of the marseilleviruses genomes.

Palavras-chave: Marseilleviridae, promoter, gene expression, lateral gene transfer
**Introduction:** DENV capsid protein (DENVC) is a structural protein that binds to the viral genome forming the core of the virus particle. Recent data suggest the participation of DENVC in the viral RNA release into the cytoplasm during virus entry into the cell, both processes involving its interaction with nucleic acids. DENVC structure was solved by nuclear magnetic resonance revealing that each monomer folds as four α-helices connected by short loops. DENVC structure is unique in two aspects: (i) the dimer is composed by 8 intertwined α-helices and (ii) the quaternary contacts are much more prevalent than the tertiary. One face of the protein is basic and binds nucleic acid, while the opposite face is mainly hydrophobic and binds to lipid droplets. The main goal of this work was to measure the binding the nucleic acid, the protein stability, to determine the folding/refolding mechanism of DENVC as a way to get insights into capsid assembly. We believe that the unique characteristics of the dimer is directly linked to its ability to assembly as a capsid.

**Material and Methods:** Recombinant DENVC from serotypes 1 and 2 were expressed and purified. We used intrinsic fluorescence of Trp69 to measure binding to nucleic acid and thermal stability. The thermal stability of DENVC was evaluated by following the changes in fluorescence parameters (intensity, maximum emission wavelength and anisotropy) as a function of temperature. We also used circular dichroism to evaluate the changes secondary structure. **Results:** Thermal denaturation of DENVC from viruses of the serotypes 1 and 2 were compared. DENVC2 appears to be more stable than that of DENVC1, as its denaturation begins at higher temperatures. Circular dichroism experiments suggest that the denaturation of DENVC2 is reversible while DENVC1 is not. This is probably related to the kinetics of refolding, which should be slower for DENVC1. The kinetic of refolding will be measured by fluorescence. These experiments, along with the measurements of binding to oligonucleotides helps the understanding. In this way, we have insights of how the binding of the capsid protein with the nucleic acid stabilizes the dimer leading to the formation of the capsid. **Conclusion:** The results suggest that there are subtle differences in the folding and refolding processes of DENVC 1 and 2. We need further studies to understand how the binding to nucleic acid triggers the capsid assembly.

**Palavras-chave:** capsid, dengue, protein, thermal stability
The antiretroviral therapy provides an improvement in the morbidity and mortality of HIV infected patients however; drug resistance is the mainly limiting factor to the therapy. The treatment failure of regiments with protease inhibitors (PI) with medication adherence but no protease drug resistance mutations (DRM) is not well understood. Understanding mechanisms leading to 2nd-line, PI-based failure, especially in resource limited settings (RLS) is important to sustain treatment efficacy and maintain future therapy options; particularly in HIV-1 non-B subtypes. In order to understand the mechanisms related to DRM, we conducted phenotype drug resistance testing to lopinavir (LPV) in single genome clones obtained from plasma samples of Kenya patients who are infected with HIV-1 subtype A and were failing LPV/ritonavir based second line of therapy but presented LPV detectable plasma levels. The selected clones had the protease amplicons co-electroporated with a molecular infectious clone (HXBII with deleted protease) into MT4 cells and recombinant viruses were generated. We analyzed 5 samples and a total of 12 clones and for 10 clones we observe sensitivity to LPV which agree with the absence of PI DRM however, this clones seems to be more sensitive to LPV when compared to the wild type virus NL43. For the other 2 clones, obtained from different patients, we observed different levels of resistance: one clone presented high LPV resistance (> 250 fold compared to the wild type subtype A virus) and the other presented moderate resistance (2 fold). In the highly resistance clone, protease mutation C67Y was the only genomic difference detected compared with the other clones and with the subtype A consensus. The sample with moderate resistance to LPV also present a 2-fold resistance to the PI darunavir and the only genomic difference observed in this sample was the I64S mutation. These results suggest that unrecognized mutations can confer resistance to LPV and for the samples who presented sensitivity to LPV, other genomic regions of HIV could be related to therapy failure observed in patients. Importantly, the higher sensitivity of subtype A proteases to PIs when compared to subtype B highlights the differential responses of non-subtype B viruses to therapy.

Palavras-chave: HIV, Protease, Drug Resistance
Upon sensing of pathogen-derived danger signals (such as dsRNA or cytoplasmic DNA), pathogen recognition receptors (PRR) are triggered to activate transcription factors that translocate to the nucleus to induce the expression of cytokines, chemokines and interferons. After release from producing cells, these molecules engage their cognate receptors on the cell surface amplifying the immune response and attracting other immune cells to site of infection. The ultimate goal of these processes is to restrain and eliminate the infection whilst generating a long-lasting immunological memory against the infecting pathogen. The transcription factor NF-κB underlines all the processes described above, being activated upon engagement of PRRs as well as by cytokines receptors. Given its central role in the immune response, it is not surprising that NF-κB immune signalling is targeted by many viruses to evade recognition by the immune system. This is well illustrated with vaccinia virus (VACV), whose 191-kb dsDNA genome encodes ten known proteins that inhibit NF-κB activation intracellularly (A46, A49, A52, B14, C4, E3, K1, K7, M2, and N1). Nevertheless, a VACV lacking all known NF-κB inhibitors still blocks NF-κB after its translocation into the nucleus. A screen for potential NF-κB inhibitors by reporter gene assay identified additional VACV proteins that block this pathway. An initial characterisation of one of these proteins is presented. F14 is a 73-amino acid protein, predicted to be acidic and conserved in the genus Orthopoxvirus. F14 is expressed early during infection, and its stability might be regulated in a proteasome-dependent manner both during infection and when expressed ectopically. Deletion of F14 from VACV (vΔF14) does not affect viral replication in cell culture. Future work will determine if F14 is a specific inhibitor of NF-κB or affects other immune signalling pathways, and if the outcome of infection with vΔF14 is altered in mouse models. Deletion of virally-encoded immunomodulators usually leads to altered phenotype of infection in mouse models, such as attenuation, due to altered immune response. Study of VACV immunomodulators proteins may help us to gain insight into the interplay between innate and adaptive immune responses against pathogen infection, thereby assisting to develop safer and better therapeutics to fight infectious diseases. Funding: Wellcome Trust and CNPq-Brazil

Palavras-chave: immune evasion, NF-kB, vaccinia virus
VIRAL IMMUNOGENICITY DETERMINES EPIDEMIOLOGICAL FITNESS IN BRAZILIAN DENV-1 LINEAGES

The dynamics of dengue virus (DENV) depends on a series of changes in serotype, genotype and lineage turnover. In São José do Rio Preto, we observed that clade replacement did not occur and the L6 lineage of DENV-1 (genotype V) remained the dominant circulating lineage even after the introduction of the L1 lineage. This finding raised questions about differences in the fitness. Thus, we investigated the viral fitness of both lineages as well as their interaction with the host immune system during DENV-1 infection to determine which factors could interfere in increased epidemiological fitness. To explain L6 lineage dominance, we assessed the viral replication of L1 or L6 isolates in cell lines and in Aedes aegypti. Also, Aag-2 cells were coinfected with L1 and L6 isolates in a viral competition assay. We tested sera from symptomatic DENV-1 infected patients for anti-dengue IgG antibodies. We also investigated the produced levels of sFRNA and expression of type I interferon antiviral responses of DENV-1 lineages. Quantification of cytokines production was performed in DENV-1 human sera using Luminex assay. In addition, each sequence of DENV-1 L1 and L6 lineages was classified and plotted according to its potential immunogenicity after in silico analyses of the putative antigenic potential. C57BL/6 mice were intraperitoneally immunized with L1 or L6 isolates and the PBMCs were cultured with these isolates for then measuring the B and T cells activation by flow cytometry. The results showed a more efficient replicative fitness of L1 over L6 in cell lines and in mosquitoes. However, infections by the L6 lineage were associated with reduced antigenicity, lower B and T cell stimulation and weaker host immune system interactions, which were associated with higher viremia. Our data, therefore, demonstrate that reduced viral immunogenicity and consequent greater viremia determine the increased epidemiological fitness of L6 lineage in São José do Rio Preto. Financial support: FAPESP.

Palavras-chave: Dengue virus, Immune response, Molecular epidemiology, Phylogeny, Viral and epidemiological fitness
059 - ÁREA: BÁSICA

VIRUCIDAL ACTIVITY OF AN ISOLATE OF MAYTENUS SP. AGAINST MAYARO VIRUS

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Resumo

The Mayaro virus (MAYV), a sublethal arbovirus transmitted by Haemagogus janthinomys of wild habit, was considered endemic only to Amazonian riverine regions. However, lately it has been considered as emerging after the confirmation of possible transmission by Aedes aegypti and its isolation in metropolises, which reveals a latent threat to the public health system. With symptoms that are confused with those of Dengue, Mayaro fever is a highly debilitating disease, in which severe arthralgias can persist for months after infection, causing discomfort to the patient and loss of productivity. How besides the inexistence of an efficient diagnostic system, there is no therapy or vaccine available to this virus, the aim of work was to evaluate the antiviral activity of a flavonoid (FMI) isolated from Maytenus sp. Bioassays were performed using the Mayaro virus strain BeAr 20290 and Vero cells (ATCC CCL-81). As a preliminary test, was determined the cytotoxic concentration of FMI for 50% of the cells (CC50), and neither the highest concentration (1000 μg/mL) of FMI tested was toxic to Vero cells, presenting 100% viability. In the assay of global antiviral activity, cell and virus (MOI 0.1) were pretreated 30 minutes prior to infection with different concentrations of FMI, to determine the effective concentration for 50% of the infected cells (EC50) by the MAYV, which was equal to 23.15 ± 2.11 μg/mL. As recommended by the literature, the selectivity index (SI) was greater than 10 and FMI can be considered safe, once there is a significant window between the toxic and effective dose. In the evaluation of the mechanisms of action, it was verified that FMI has a potent virucidal activity as from 25 μg/mL and a moderate activity in the penetration step as from 100 μg/mL. However, this flavonoid was not able to block the viral adsorption step not even when used at the concentration of 200 μg/mL. When FMI was added after MAYV infection (1,3,6,12,24 and 36h) at a concentration of 31.25 μg/mL, antiviral activity was not as pronounced when compared to virucidal activity, that was able to of reducing the infection in 7 logs. However, a significant reduction was still observed in this condition, which in 15 times inhibited the number of viral plaque forming units (PFU), possibly inactivating the virus when it becomes extracellular from the 2nd cycle of infection, that could block your cell-cell advancement. FINANCIAL SUPPORT: FAPEMIG and CAPES

Palavras-chave: Mayaro virus, Alphavirus, Antiviral, Celastraceae, Maytenus sp.
Introduction: Dengue is the most important arbovirosis in the world with regard to morbidity, mortality and clinical implications. Objective: To analyze the acute and late clinical profile of dengue-suspected patients in a prospective cohort. Methodology: participants, over 10 years old, residents in São José do Rio Preto, São Paulo, Brazil, were attended due to acute dengue symptoms, and had a serum sample tested to Dengue, Chikungunya and Zika virus. These participants were evaluated after 60 days about late symptoms. Results: From November/15 to June/17, 64 participants of prospective cohort were attended in the health service due to dengue-suggestive signs/symptoms. Among them, 8 presented dengue-confirmed diagnostic (1 NS1 and 7 DENV RT-PCR positives), 11 had presumptive diagnosis with IgM anti-DENV reagent. DENV2 was the main serotype (n=4); DENV1 (n=1); DENV4 (n=1) and one case was Dengue co-infection (DENV1 and 4). Also, 4 RT-PCR identified ZIKV and none CHIKV. The main dengue-suggestive signs/symptoms were headache (9), ocular pain and myalgia (8), fever (7), rash (6), arthralgia and vomiting (5), while in the Zika cases were fever (3), headache and myalgia (2), ocular pain, arthralgia and rash (1). From 17 tourniquet test performed, 4 were positive (3 DENV/1 ZIKV confirmed diagnosis). No hospitalizations, dengue cases with alarm signs or severe dengue among participants with symptomatology were reported. For cases with late symptoms, 33 (51.5%) patients were evaluated, 7 of 9 presumptive/confirmed diagnostic to dengue maintained symptoms after 60 days of the acute phase, 2 presented an anxiety score as probable, and 1 depression score as probable. Among 2 patients confirmed for ZIKV, none maintained symptoms or established a probable relationship with anxiety and depression. Conclusion: In the face of a dengue outbreak, we must be alert for other simultaneous diseases, due to the symptoms overlapping that lead to error or underdiagnoses. The clinical knowledge of the most prevalent infections in each population makes it possible to minimize diagnostic confusions and to start earlier effective therapeutic practices. Virological tests are important tools to contribute in differential diagnosis. FINANCIAL SUPPORT: FAPESP

Palavras-chave: DENGUE, ZIKA, CHIKUNGUNYA, ACUTE CLINICAL PROFILE, LATE CLINICAL PROFILE
ADEQUATION OF PLAQUE REDUCTION NEUTRALIZATION TEST (PRNT) TO DETECT NEUTRALIZING ANTIBODIES AGAINST SIX FLAVIVIRUSES IN A LARGE AMOUNT OF SAMPLES

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Resumo

Currently in Brazil, three mosquito-borne flavivirus species presents great importance for public health: the four serotypes of Dengue virus (DENV 1 to 4), Zika virus (ZIKV) and Yellow Fever virus (YFV). Although very similar to one another (specially DENV 1 – 4 and ZIKV), all viruses are serologically distinct and the infection with one virus will not protect against infection with another. Among the serologic tests used to measure anti-flavirus antibodies, the Plaque Reduction Neutralization Test (PRNT) is the most accepted to measure neutralizing antibodies and is the most virus-specific assay. The aim of this study was to adequate the PRNT for the six viruses – DENV 1, 2, 3 and 4, ZIKV and YFV – in a relatively large amount of samples. The DENV and ZIKV used were isolated from clinical samples and the YFV was the 17d vaccine strain. All viruses were submitted to qPCR and genetic sequencing to quantify and ensure virus identity and sterility. Virus titers were calculated in plaque forming units (PFUs) considering the presence of 20 to 40 PFUs per well. Sera to be tested were inactivated and 4-fold diluted (1:5-1:5120) in a 96 well microplate, eight samples per plate, using DMEM. Virus was added – 120μL (DENV 1, 3, 4: 500 PFUs; DENV2, ZIKV, YFV: 600 PFU) to antigen-antibody reaction for 01 hour at 370C in 5% CO2. A control microplate was used with positive (virus) and negative (DMEM) controls, serum with known titer and virus titration. Sera/virus was then transferred (100μL in duplicate) to a 24-well plate (two samples per plate) with a monolayer of vero cells (1x105 cells/mL) with at least 80% of confluence and adsorbed for 01 hour, 370C in 5% CO2. An overlay of carboxymethyl cellulose (CMC) with DMEM, 2% of fetal bovine serum and antibiotics was used and plates incubated at 370C 5% CO2 for 96 hours (DENV 4, YFV), 144 hours (ZIKV) or 168 hours (DENV 1, 2, 3). Plates were washed with PBS pH7,2 and stained with Naphtol Blue Black (Sigma-Aldrich). The PFUs (plaques) were counted and the titers calculated using the NIH statistic tool (https://exon.niaid.nih.gov/plaquereduction/). The PRNT was used in human (100 samples for all viruses) and neotropical primates (120 samples for ZIKV) samples showing sensitivity and specificity (no cross-reactivity was observed). The test here described proved to be reliable, reproducible and can be used in 60 to 80 samples per test.

Palavras-chave: Zika virus, Dengue virus, Yellow fever virus, PRNT, Serology
RESUMO

Aperfeiçoamento de Pessoal de Nível Superior (CAPES), CNPq and FAPEMIG.

Antiviral studies to candidates to counteract CHIKV infection candidates to counteract CHIKV infection plaque assay in Vero cells. Our results show that MEK inhibitors are interesting, potential antivirals in Vero cells lines. Finally, four different MEK pharmacological inhibitors (PI) were tested as antivirals in Vero cells lines. Incubation at 37°C for up to 2 hours. Next, we tested CHIKV optimal growth conditions in both C6/36 and Drosophila. We demonstrated that CHIKV stock preparations are stable with very low decay in virus titer even after incubation at 37°C for up to 2 hours. Next, we tested CHIKV optimal growth conditions in both C6/36 and Vero cells lines. Finally, four different MEK pharmacological inhibitors (PI) were tested as antivirals in Vero cells infected with CHIKV. CHIKV yield in the absence or presence of MEK PI was determined through plaque assay in Vero cells. Our results show that MEK inhibitors are interesting, potential antiviral candidates to counteract CHIKV infection in vitro. The data gathered here will be supportive to expand our antiviral studies to in vivo models of CHIKV infection.

Palavras-chave: antiviral agents, chikungunya virus, mitogen-activated protein kinase

062 - ÁREA: HUMANA E SAÚDE PÚBLICA

ANALYSIS OF MAPK MEK/ERK SIGNALING PATHWAY INHIBITORS AS ANTIVIRAL CANDIDATES AGAINST CHIKUNGUNYA VIRUS

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Resumo

Chikungunya virus (CHIKV) is an Alphavirus belonging to the Togaviridae family. This reemergent arbovirus causes chikungunya fever and infects more than 1.5 million people only in Americas. Chikungunya fever is associated with severe outcomes such as long lasting joint pain that can decrease the productivity of infected individuals, and ultimately death. So far, anti-CHIKV vaccines and antivirals do not exist to counter this disease. Development of drugs and therapy against CHIKV is urgent. In this regard, the MAPK signaling pathway seems to be an interesting target in antiviral therapy, since it can control survival, proliferation, and many other biological responses that are key for viral replication success. The MAPK pathway is a promising participant of the CHIKV infective cycle, once it has been demonstrated that compounds affecting CHIKV replication also inhibit the phosphorylation of MAPK signaling kinases such as ERK1/2, JNK1/2 and p38. To test this hypothesis, we used CHIKV of ECSA strain in biological assays. First, was assayed the thermolability of virus stocks after exposure of viral preparations to higher temperatures. We demonstrate that CHIKV stock preparations are still stable with very low decay in virus titer even after incubation at 37°C for up to 2 hours. Next, we tested CHIKV optimal growth conditions in both C6/36 and Vero cells lines. Finally, four different MEK pharmacological inhibitors (PI) were tested as antivirals in Vero cells infected with CHIKV. CHIKV yield in the absence or presence of MEK PI was determined through plaque assay in Vero cells. Our results show that MEK inhibitors are interesting, potential antiviral candidates to counteract CHIKV infection in vitro. The data gathered here will be supportive to expand our antiviral studies to in vivo models of CHIKV infection. FINANCIAL SUPPORT: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), CNPq and FAPEMIG.

Palavras-chave: antiviral agents, chikungunya virus, mitogen-activated protein kinase
ANTI-TNF-A TREATMENT PREVENTS NECROPTOSIS IN MODEL INFLUENZA IN VIVO

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Resumo

Influenza A virus is a major concern in public health. WHO's data show that 20% of the world population is infected every year. Literature data demonstrate that the infection generates an exacerbated activation of the immune cells, which generates an uncontrolled production of several cytokines, that can contribute to several phenomena deleterious to the host. Several studies show that an intense cellular death in the infection by the influenza virus contributes to a worsening of the clinical picture in the infection, as for example the massive one. For many viruses infection, a singular cell death phenomenon has described, called necroptosis. The necroptosis is a cell death pathway-dependent on activation of RPK1 / RPK3, leading to a harms signal to the immune system. Since TNF-α production is associated with necroptosis, studies have focused on possible anti-TNF treatments in order to prevent this phenomenon of death. Among these treatments, Etanercept, a drug used in the treatment of rheumatoid arthritis, has been used in some studies in murine models of influenza infection. In this work, mice were infected with 1X103 PFU of H1N1 virus, and treated daily with 5mg / kg etenercept. The survival and weight of the animals was monitored for 15 days. In addition, animals were sacrificed on days 3 and 5 post-infection to collect bronchoalveolar lavage and lungs for quantification of infiltrate and cell death, dosage of proteins, LDH and cytokines, as well as evaluation of the expression of necrotic pathway molecules. Our data demonstrate that anti-TNF-α treatment was able to prevent mortality and weight loss of animals. In addition, bronchoalveolar lavage analyzes demonstrate that the treatment contributed to a lower concentration of proteins in the fluid. The treatment was also able to diminish the inflammatory infiltrate, as well as to prevent the death of the cells present in the washed, in addition to diminish the amount of pro-inflammatory cytokines produced. Analysis of the collected tissues demonstrates that the anti-TNF-α treatment also decreased the expression of key proteins of the activation of the necroptosis pathway, thus preventing the cellular death in the tissue. Our data, demonstrate that treatment with Etanercept is a potential therapeutic treatment in the control of influenza virus infection, since its action has been shown to be effective in controlling cell death in lung tissue. Financial support: CNPq and FAPERJ.

Palavras-chave: ETANERCEPT, INFLUENZA, NECROPTOSE, TNF-a
Emerging and re-emergent viral diseases represent a public health problem. Dengue, which can be caused by one of the four serotypes of Dengue virus (DENV-1, 2, 3 and 4), is one of the most important re-emergent arboviruses. It is estimated that approximately 400 million cases of this infection occur annually in the world. This alarming number is due to, among other causes, the difficulty of obtaining multivalent and safe vaccines for the different serotypes and the lack of specific treatment against DENV. Thus, the discovery of anti-dengue drugs becomes important, justifying investments in research and prospection of antivirals. In this context, synthetic compounds represent a widely source of substances with potential biological activity, and the classes of chalcones have shown activity against different pathogens such as bacteria, fungi and viruses. Thus, the aim of this study was to evaluate the antiviral activity of novel ten chalcones analogues in vitro and in silico against Dengue virus. Bioassays were performed using DENV-2 strain and neonatal hamster kidney cells (BHK-21). First, cytotoxicity concentration for 50% of cells (CC50) of chalconas was performed by MTT method at concentrations ranging from 3.12 - 200 μg / mL. In the global activity antiviral assay, cells and virus (MOI 0.1) were treated 30 minutes prior to infection at non-toxic concentrations to determine the effective concentration for 50% of the infected cells (EC50) by the DENV-2 also by the MTT method. Selectivity index (SI) was calculated by the ratio of CC50 to EC50. The CC50 results ranged from 47.21-192.51μg / mL. It was not possible to determine the EC50 for nine of chalcones evaluated, since they did not show activity at the concentration tested. However, the compound named B8, showed EC50 of 36.18 μg / mL and SI was 3.7. From this promising results, in silico molecular docking studies were conducted against NS3 protease and NS5 metiltransferase, most promising target to rational antiviral drug development. The contact points culminating in binding energies of -8.9 Kcal/mol. and -5.4 Kcal/mol., respectively to NS3 and NS5, indicating important interactions between compound B8 and the amino acids present in the proteins such as Tyr161, Gli151, Asp75 and Lys61, among others. The results obtained from in vitro and in silico tests for compound B8 showed that this molecule has a potential antiviral action, which could lead to the development of anti-dengue drugs.

Palavras-chave: Dengue virus, Chalcones, in silico, Antiviral
ANTIVIRAL ACTIVITY OF A HYDROALCOHOLIC EXTRACT OF BAUHINIA HOLOPHYLLA AGAINST ZIKA VIRUS

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Resumo

Zika virus (ZIKV) infection is significant global public health associated with severe neurological complications and congenital diseases such as microcephaly. ZIKV is a positive-sense single-stranded RNA genome belonging to Flaviviridae family and Flavivirus genus, which is transmitted to humans by infected mosquitoes of Aedes genus. There is no vaccine available and specific antiviral drug able to inhibit ZIKV replication are necessary. Currently, the treatment of infections is directed to symptom relief by the administration of analgesics and antipyretics. Several species of Bauhinia have been widely related to the treatment of gastrointestinal diseases, diabetes, and inflammation, due to their high composition of flavonoids such as quercentin and rutine. However, there are no studies in the literature to evaluate the antiviral activity of the hydroalcoholic extract of B. holophylla against ZIKV. So, the present work aimed to investigate the antiviral activity of the extract and flavonoids against ZIKV. Bioassays were performed using ZIKV and Vero mammalian cells. First, cytotoxicity concentration for 50% of cells (CC₅₀) was performed by the MTT method. The extract and flavonoids were tested at different concentrations (3.91 to 1000 μg/mL). In the global activity antiviral assay, cells and virus (MOI 0.01) were treated 30 minutes prior to infection at non-toxic concentrations to determine the effective concentration for 50% of the infected cells (EC₅₀) by the ZIKV also by the MTT method. Selectivity index (IS) was calculated by the ratio of CC₅₀ to EC₅₀. The CC₅₀ of hydroalcoholic extract were 90.2 ± 5.7 μg/mL, and quercentin and rutine showed CC₅₀ values higher than 125 μg/mL and 1000 μg/mL. No antiviral activity was detected to rutine. However, the EC₅₀ for quercentin and extract was 16.47 ± 5.7 μg/mL (SI ≥ 7.5) and 4.06 ± 1.25 μg/mL (SI = 22), respectively. The results of SI suggests a safe and significant window to the toxic and effective dose to inhibit ZIKV infection. Virucidal and mechanism of action assay for quercentin and hydroalcoholic extract of B. holophylla will be performed to further studies to identify new leads to anti-ZIKV drug discovery. Financial Support: CAPES, UFSJ, FAPEMIG, CNPq

Palavras-chave: Antiviral, ZIKV, Bauhinia, Cytotoxicity, Quercentin
ANTIVIRAL ACTIVITY OF BRAZILIAN NATURAL COMPOUNDS AND DERIVATIVE SYNTHETIC ANALOGUES ON ZIKV INFECTION.

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Resumo

Diseases caused by arboviruses involve complex cycles between vertebrate and hematophagous arthropod vectors and represent important causes of outbreaks and epidemics. Among these diseases, Zika fever has been receiving attention by the Brazilian government and public health authorities worldwide. There is no specific antiviral against Zika virus (ZIKV) and current treatment is palliative. The efforts to develop innovative and specific drugs against this virus are challenged by the high viral mutation rate and side effects to the host cell. In this context, natural and synthetic compounds are attractive candidates in the search for new therapeutic approaches, as numerous modern drugs have been developed from natural prototypes. Therefore, the aim of this study was to investigate the antiviral effects of a panel of natural compounds isolated from Brazilian natural sources and synthetic analogues based on natural scaffolds. Cells were infected with ZIKV in vitro and immediately treated with compounds at maximum nontoxic concentration for 72 hours. Initial screening of forty-four compounds was performed and 12 compounds demonstrated anti-ZIKV activity. Of these, eleven synthetic compounds reduced a minimum of 50% the replication stage and one natural compound inhibited up to 84% the infectivity of ZIKV. These results represent a preliminary analysis on the antiviral activity of Brazilian natural and synthetic compounds on ZIKV infectivity and further analyses are being performed in order to investigate the mode of action of those compounds.

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Palavras-chave: Antiviral, Zika virus, natural and synthetic compounds
067 - ÁREA: HUMANAS E SAÚDE PÚBLICA

ANTIVIRAL ACTIVITY OF CHONDRCANTHUS CHAMISSOI AND CHLORELLA PERUVIANA EXTRACTS AGAINST DENGUE VIRUS SEROTYPE -2 IN VERO-76 CELLS

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TRABALHOS APROVADOS PARA APRESENTAÇÃO EM FORMA DE PÔSTER

In Peru, dengue is hyperendemic to 18 departments, which the serotype 2 (DENV-2) is associated with large outbreaks and severe cases. There is no treatment for dengue, and it is necessary to develop new alternatives for the prevention and treatment of this disease. Chondracanthus chamissoi and Chlorella peruviana could have antiviral properties due to their high content of carrageenans and proteins against dengue virus. Therefore, the objective of this study was to evaluate the antiviral activity of C. chamissoi and C. peruviana extracts against DENV-2 in Vero-76 cells. In this study, the following extracts were used: C. chamissoi carrageenan in gametophyte phase (20 mg/ml), C. chamissoi carrageenan in sporophyte phase (2 mg/ml), C. chamissoi carbohydrates in sporophyte phase (825 μg/ml), C. peruviana carbohydrates (4 μg/ml) and C. peruviana crude extract. The cytotoxicity test was performed inoculating 1:5 and 1:10 dilutions of each extract in 24-well plates with complete monolayer of Vero-76 cells and evaluating morphological changes on the cellular monolayer during 9 days. Subsequently, the antiviral activity test was performed by plaque reduction neutralization test against a wild type strain of DENV-2 using Vero-76 cells in suspension and semi-solid method. Algae extracts and carrageenan controls were evaluated at 1:5 and 1:10 dilutions. As results C. chamissoi extracts and C. peruviana carbohydrates extract did not show cytotoxicity. The C. peruviana crude extract showed cytotoxicity at 1:5 and 1:10 dilutions. C. chamissoi extracts showed a reduction percentage greater than 70% against DENV-2 in the following concentrations: C. chamissoi carrageenan extracted with isopropanol at 1:5 and 1:10 dilutions, C. chamissoi carrageenan in sporophyte phase at 4 mg/ml, C. chamissoi carrageenan in gametophyte phase at 2 mg/ml, C. chamissoi carbohydrates in sporophyte phase at 0.135 μg/ml, C. chamissoi carbohydrates in gametophyte phase at 0.825 μg/ml, C. chamissoi carbohydrates in sporophyte phase at 0.825 μg/ml. On the other hand, the C. peruviana carbohydrates extract had antiviral activity against DENV-2 at 0, 4 μg/ml. Financial support: FINCyT-INNOVATE PERÚ

Palavras-chave: CHONDRCANTHUS CHAMISSOI, CHLORELLA PERUVIANA, DENGUE VIRUS
Resumo

Chikungunya (CHIKV) is an enveloped virus with a positive polarity positive RNA genome, member of the togaviridae family, of the genus alphavirus. Transmitted by Aedes aegypti mosquito. The main clinical manifestation is the severe pain in the joints. Lapachonas are compounds produced by semi-synthesis from lapachol with therapeutic efficacy against enteroviruses, Chagas disease and used as anti-malarial and anti-inflammatory agent. To evaluate the cytotoxicity (CC50) of the substance (H05) in VERO lineage cells; Evaluate the substance's ability (H05) to inactivate the viral particle (CHIKV); To evaluate the virucidal potential of the substance (H05) against CHIKV; To evaluate the synergistic effect of the substance (H05) together with Ribavirin; In the MTT assay, H05 showed a satisfactory CC50 result when incubated for 72h, exhibiting a CC50 of 1102.94 ± 201.22 μM. H05 presented values above 90% inhibition at the concentration of 20 μM and an EC50 of 0.96 μM. The virucidal effect was also observed, measuring how much the drug was able to inhibit the virus before its entry into the cell. Substance H05 was shown to be non-toxic to VERO cells; It was able to inhibit the viral particle of CHIKV; It showed a powerful Virucidal effect at 37ºC; It was able to act in synergism with Ribavirin, inhibiting the viral particles of CHIKV; H05 is a promising substance for the fight against CHIKV infections.

Palavras-chave: CHIKV, Antiviral, Naphthoquinone
The influenza virus causes acute respiratory infections, leading to high morbidity and mortality in groups of patients at higher risk. Antiviral drugs represent the first line of defense against influenza, both for seasonal infections and pandemic outbreaks. One class of anti-influenza drug is in clinical use: the neuraminidase inhibitors, such as oseltamivir (OST). However, because OST-resistant influenza strains have been described, the search for novel compounds with different mechanisms of action is necessary. Here, we investigated the anti-influenza activity of a fungi-derived natural product, aureonitol (AUR). MDCK cells were infected with different MOIs of influenza virus and treated with different concentrations of AUR for 24 and 48h to evaluate antiviral activity. Cytotoxicity assays through reduction of XTT salt were also performed. Inhibition of virus growth and cytotoxicity at 50% were calculated (EC50 and CC50). AUR inhibited influenza A and B virus replication in a MOI-, time- and dose-dependent fashion. Our compound was more effective against influenza A(H3N2), with an EC50 of 100nM and showed low cytotoxicity (CC50=1426µM). We performed cell-free assays to quantify hemagglutination inhibitory activity of AUR, and verified that it inhibited influenza hemagglutination with a minimum inhibitory concentration of 100nM. In functional adsorption inhibition assays, we noticed that AUR significantly impaired virus attachment/entry of different subtypes of influenza A and influenza B to different degrees. AUR had no effect on NA activity even when tested at 1000nM (10 times its EC50). To investigate AUR’s docking site, molecular modeling studies were performed using Arguslab software. The studies revealed that AUR binds to influenza hemagglutinin (HA) at the sialic acid binding site in the receptor binding site, with very low free-binding energies. AUR formed hydrogen bonds with highly conserved residues in influenza HA, which are involved in viral entry. AUR is a tetrahydrofuran derivative produced by different species of Chaetomium and organic synthesis of aureonitol has been proven to be successful. Because these characteristics indicate the feasibility of scaling up aureonitol production and our results show that this molecule inhibits influenza replication by targeting viral entry via conserved residues on HA, AUR’s chemical structure may be of interest for further development of anti-influenza drugs. Financial Support: CNPq and Faperj

**Palavras-chave:** antiviral, aureonitol, hemagglutinin, influenza virus
BIOPROSPECTING OF ANTIVIRAL ACTIVITY OF LEAF EXTRACTS OF MAYTENUS SP. AGAINST THE MAYARO VIRUS

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Resumo

The Mayaro fever whose symptoms are confused with those presented in Zika and Dengue Fever, including fever, myalgia and arthralgia, is caused by Mayaro virus (MAYV) (Togaviridae Family and Alphavirus genus). This virus was considered endemic in rural communities, however its expansion into urban communities has been reported, and MAYV was noticed as an emerging. No vaccine or antiviral therapy against MAYV and the search for antiviral was relevant. The aim of this study was to evaluate different extracts obtained from leafs of Maytenus sp against Mayaro virus. Before antiviral assay, cytotoxic assays were conducted to determine the cytotoxic concentration to 50% of the cells (CC50). For this purpose, mammalian cells (VERO) were added into 96 well microplates (5x10^4 cells/well), and after 24 hours, were treated with of four extracts at different concentrations. The revelation was obtained after 48h by MTT (methyl thiazol tetrazolium) colorimetric technique. We have detected the CC50 concentrations 491.46, 307.29, 246.1, 320.3 µg/ml for ethyl acetate (MIAE), chloroform (MIC), ethanol (MIE) and hexane (MIH) extracts, respectively. For antivirals assays, the same cells pre-treated with extracts were infected with MAYV at multiplicity of infection (MOI) of 0.1 virus/cell and 48 hours after infection (hpi) we found the protective/effective concentration to 50% of cells (EC50) and the results showed that MIAE, MIC, MIE and MIH were able to inhibit MAYV at 170.2, 6.6, 4.5 and 156.9 µg/ml concentrations, respectively. Finally, the selective index (SI) was calculated which refers to the ratio between CC50 and EC50 each extract, which should be above 4.0. The SI were larger than 4 for the MIC extracts (SI = 46.56) and MIE (SI = 54.69). The MIAE and MIH extract were considered toxic or non-selective. After that, mammalian cells were added into 24 well microplates and the virucidal effect was evaluated. The virus at MOI of 0.1 virus/cell were treated for 1 hour with the extracts and then was added to the plate containing cells and waited for 1 hour of viral adsorption. 48 hpi, cells were fixed and stained with violet crystal. Virucidal concentrations were 15.6, less than 3, 31.7 and less then 3 for MIAE, MIC, MIE and MIH, respectively. Therefore, our data indicate potentials antiviral and virucidal actions against Mayaro virus in compounds present in the plant studied that belongs to Maytenus genus. Financial support: FAPEMIG and CNPq.

Palavras-chave: Mayaro virus, Antiviral, Maytenus, Biotechnology
CHARACTERIZATION OF CLINICAL SIGNS AND SYMPTOMS OF THE CHIKUNGUNYA VIRUS IN BALB/C MICE

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Resumo

The Chikungunya virus (CHIKV) is a member of the Toaviridae family, genus alphavirus, with an enveloped capsid and positive, single stranded RNA. Transmitted by mosquitoes of the genus Aedes (aegypti and albopictus). Its main clinical manifestation consists in severe joint pains. The usual measures against CHIKV are the vector control (A. aegypti e A. albopictus), supportive measures and symptomatic treatment with the use of analgesic and anti-inflammatory medicines. Due to the increasing numbers of cases, the infection is considered a serious health problem in Brazil. In this sense, the need to research new antiviral drugs made it extremely important the conduction of an experiment that allows the characterization of the clinical signs and symptoms of a wild strain of CHIKV in murine models. Therefore, two years old BALB/c mice were infected subcutaneously with 104 PFU in 50µl of CHIKV. They were observed for a 14 days period and a score was developed to evaluate the presented symptoms, which are 0 – no illness; 1 – weakness and/or swelling of superior and/or posterior limbs; 2 – Moderate weakness with swelling of the limbs; 3 - Moderate weakness with swelling of the limbs, visible variation in the digits and red spots in the joints and paws; 4 – Severe weakness with swelling of the limbs, visible variation in the digits and red spots in the joints and paws; 5 – loss of movement of the hind limbs with swelling and/or digits variation; 6 – death or terminal stage. The animals developed symptoms from the third day post infection and its severity intensified throughout the 14 days of the experiment. Symptoms such as different levels of weakness and swelling were observed between the fifth and seventh day. Paralysis occurred in the ninth and tenth day. By the end of the experiment, after theirs deaths, the animal’s organs were removed for histopathological analysis. The experiment for the symptomatology characterization of the wild strain CHIKV in two days old BALB/c mice presented itself as a notable option in the creation of a standard score basis in future antiviral experiments.

Palavras-chave: BALB/c mice, Chikungunya virus, clinical symptoms, score
The acute respiratory infections (ARI) are an important problem in public health worldwide. Contributing for elevate the number of medical consultations and cost with hospitalization, has sudden onset, variable severity, accounting for about 2.2 million deaths annually, and it is estimated that 90-95% of cases have viral etiology. Among the etiological agents of ARI there are an expressive involvement of Influenza A and B viruses (FluA and FluB), Respiratory syncytial Virus (RSV) and Human Metapneumovirus (HMPV). The respiratory viruses reveal different circulation pattern around the world. In countries with temperate climate the peak of virus circulation occurs during the end of autumn and winter. However, at countries with tropical climate the circulation is related to rainy season and elevated air humidity. In this context, the main objective of this study was to verify the circulation pattern of FLU, RSV and HMPV in the North and Northeast regions of Brazil between January and June of 2017. 2178 respiratory samples obtained from patients with ARI in unities of health located in the states of the Acre, Amazonas, Amapá, Ceará, Maranhão, Pará, Paraíba, Pernambuco, Rio Grande do Norte and Roraima were analyzed. All the clinical specimens were sent to the laboratory for etiological viral investigation. The samples analysis involved the extraction of nucleic acid and detection of the viral genome by real-time Polimerase Chain Reaction preceded by Reverse Transcriptase (qRT-PCR), using specific primers and probes to FluA (H3N2 and H1N1pdm), FluB, RSV and HMPV. Of all patients analyzed, 985 (45.2%) were positive for any of the virus investigated, being 216 (21.9%) positive for FluA (H3N2), four (0.4%) for FluA (H1N1pdm), 156 (15.8%) positive for Flu B, 497 (50.4%) for RSV and 65 (6.5%) for HMPV. 47 co-detections were also identified, being five (0.5%) positive for FluA (H3N2) and FluB, 35 (3.5%) for FluA (H3N2) and RSV and also seven (0.7%) for FluB and RSV. The data revealed that the cases of viral infections were more frequent in children situated between 0 to 4 years and adults from 25 to 59 years old. Predominantly during the months of February to April with a peak circulation in March, RSV showed greater prominence with 50.4% of the positive samples. The constant monitoring of the studied viruses plays an important role in getting data to support interventions for prevention and control of infections by these pathogens in the population.
Clinical and epidemiological profile of the Sylvatic yellow fever outbreak in samples analyzed by Instituto Evandro Chagas in the first semester of 2017.

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Resumo

Sylvatic yellow fever (YF) is a disease transmitted by mosquitoes of the genus Haemagogus. This virus belongs to the family Flaviviridae and to the genus Flavivirus and is distributed in regions of South America and Africa, which correspond to the Amazon region, Paraná, Araguaia-Tocantins, Nile and Congo. Since January 2017, Brazil has confronted an outbreak of silvatic YF. Human serum samples from the spontaneous demand of the Instituto Evandro Chagas (IEC) in majority from states that is included in the coverage of Epidemiological Surveillance of the IEC were analyzed by the detection of anti-YF IgM antibodies by the IgM Antibody Capture Enzyme Linked Immunosorbent Assay (MAC ELISA). We used the EXCEL program (2007 version) for statistical analysis of the data referring to suspected YF samples received from 01/01/17 to 31/06/17, in the total of 333 samples from all Brazilian states, except SE, BA, CE, MS, DF, PR and SC. The states with the highest number of samples received by the IEC were PA with 66.9%, followed by TO and MT with 6.0%, AM and RO with 3.6%, GO with 3.3% and PI with 3.0%. The results of the antibodies detection showed that 18 (5.4%) were reagents for YF, 302 (90.6%) were non-reactive and 13 (3.9%) were indeterminate. The samples considered as reactive for YF were originated from PA (12), AM (4), RJ (1) and ES (1). The average age of the patients affected by the disease was 19.4 years, the majority being male (88.9%) and rural residents (76.5%) with occupations related to field activities. Patients who died (18.7%) presented classic signs and symptoms of the disease as jaundice, abdominal pain, fever and hemorrhages. The clinical-epidemiological profile of the Sylvatic YF outbreak in the patients analyzed by the IEC corresponds to the data observed in other outbreaks of this disease, where young male individuals, rural residents and non-vaccinated were the most affected. Financial Support: Instituto Evandro Chagas (IEC/SVS/MS).

Palavras-chave: Antibodies, MAC ELISA, Outbreaks, Silvatic, Yellow Fever
Dengue is a disease with a major impact on global public health, induced by 4 serotypes (DENV 1-4). However, research on this disease is scarce in Alagoas. Thus, this work sought to correlate the clinical profile of the patients with their immunological status in order to find biomarkers that aid in clinical management. For this we used 46 serum samples from patients infected by DENV-4, confirmed by RT-PCR. These patients were attended at the Hélvio Auto School Hospital, in Maceió city and we had access to their medical records. We also analyzed the presence of IgM and IgG against DENV and the avidity of IgG by ELISA. In addition, we quantified 6 proinflammatory cytokines (Interleukin 1&beta, 6, 8, 10, 12p70 and TNF) by CBA (BD Biosciences). As negative control of CBA we used 10 serum samples from healthy donors. The cytometric data were analyzed in the FCAP Array Program, v1.0.1 (Soft Flow Hungary Ltd.) and statistical analysis was performed using Prism 6.0 software (GraphPad), considering a significant P≤0.05. In the study group, 80% of the patients were responding to an infection secondary to the dengue virus. As a consequence, more than 60% of these patients had a diagnosis of severe dengue. Also, from all cytokines dosed only IL-10 presented levels above the minimum limit of detection of the kit and with significant difference in relation to the control group. It was also observed that IL-10 levels are significantly higher in the group of patients responding to a secondary infection than a primary infection (P <0.01). In addition, there was a significant negative correlation between IL-10 levels and platelet count (P <0.01), leukocytes (P <0.01) and monocytes (P <0.01). - Patients had high levels of IL-10 associated with severe cases of dengue, such as previously shown in other studies, for supposedly facilitating viral replication during the secondary response to DENV infection. Faced with this, an alert is being issued to health surveillance agencies to seek to reduce the number of dengue infections because the population is at high risk of developing severe dengue in Alagoas state. Financial Support: Ministério da Saúde/CNPq/SESAU-AL/ FAPEAL

Palavras-chave: IL-10, secondary response, severe dengue
Dengue virus (DENV) emerged from the sylvatic environment and colonized urban settings, being sustained in a human-Aedes-human transmission chain, mainly by the bites of females of the anthropophilic species Aedes aegypti. Herein, we sought evidence for fine-tuning in viral codon usage, possibly due to viral adaptation to human transmission. We performed analyses on the codon adaptation of DENV serotype 2 (DENV-2) genotypes from different habitats to humans and tried to correlate the findings with key evolutionary determinants. We found significant differences between humans and Ae. aegypti systems (p-value < 0.05), demonstrating that DENV-2 codons of endemic lineages become more adapted to humans than to Ae. aegypti. Moreover, we found no significant differences among epidemic American/Asian and sylvatic DENV-2 genotypes when comparing to human codon usage, which suggested a similar adaptive response on these two lineages. However, these measures were significantly different, when compared to Ae. aegypti codon preferences, with lower values for sylvatic than epidemic genotypes (p-value < 0.05), which is in line with serological in areas with circulation of sylvatic genotype and vectorial competence studies. Therefore, our findings provided a comprehensive assessment for the codon optimization of DENV-2 in different cycles and hosts. Such adaptive differences may constitute basic information with important implications for investigating the spillover and the transmission of new DENV-2 strains in endemic cycles.

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Palavras-chave: Dengue virus type 2, Endemic genotypes, Sylvatic genotype, CAI, Aedes aegypti
Infections with the Zika (ZIKV), Chikungunya (CHIKV) and Dengue (DENV) virus have been warning Brazil in recent times. However, the signs and symptoms are quite similar which make it difficult to clinical diagnosis. The risk factors that increase the morbidity and mortality of these infections are still poorly defined. This study aims to evaluate the occurrence of coinfection of ZIKV and CHIKV in patients assisted at the University Hospital in the Rio de Janeiro and the role of coinfection in disease severity. A total of 158 patients were selected for the study, and all had serum and urine samples collected. In all samples, we investigated the RNA of ZIKV and CHIKV through real time RT-PCR. All patients presenting amplification of the ZIKV and CHIKV cDNA in at least one of the serum or urine samples were tested for IgG and IgM against CHIKV and DENV. In the real-time RT-PCR we amplified the ZIKV cDNA in 25/158(15.8%) of the patients, and CHIKV cDNA were amplified in 29/158(18.4%), and ZIKV and CHIKV coinfection confirmed in 19/158(12%). In 85/158 (53.8%) of the patients, they did not obtain real-time RT-PCR amplification. In 19 patients who obtained amplification of the genome of ZIKV and CHIKV, 4(21%) were reactive for DENV IgM, whereas all 19 patients were reactive for DENV IgG. Anti-CHIKV IgM was detectable in 17/19(89.5%) and, anti-CHIKV IgG in 12/19(63.5%) patients. In this same co-infected group we also performed a semi-nested RT-PCR to detect DENV RNA and we obtained amplification of DENV serotype 1 in two samples. Among 158 patients of the study, 22(13.9%) were immunodeficient (ID), being 3/22(13.6%) were diagnosed with ZIKV, and 7/22(31.8%) with CHIKV infection. In 8/22(36.4%), ZIKV and CHIKV coinfection was confirmed. In the ID group, 6 out of 7 patients were hospitalized, being 4/6 with ZIKV and CHIKV coinfection, while 1/6 patients had ZIKV infections only and 1/6 with CHIKV infection only. The only fatal case occurred in the ID group with coinfection. In this study, we observed that the occurrence of ZIKV and CHIKV coinfection cases is similar to infections by ZIKV ou CHIKV only. In addition, ZIKV and CHIKV coinfected immunodeficient patients were more symptomatic than immunocompetent patients, which should be considered as risk factors for severe disease. Financial support: FAPERJ, CAPES, CNPq

**Palavras-chave:** Arbovirosis, coinfection, Immunodeficiency, laboratorial diagnosis
Computational Analysis and Identification of Amino Acid Sites in Zika Virus Structural Proteins Relevant to Development of Vaccines

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Resumo

Zika virus (ZIKV) infection is a specific public health problem, responsible for major epidemics and associated with neurological syndromes. ZIKV belongs to the Flaviviridae family, genus Flavivirus. Several evidences have been showing that the outcome of the infection may be influenced by an anti-flavivirus cross-reactivity immune status. The reverse vaccination is based in the use of computational tools able to predict peptides that have immunogenic potential, improving the development of new vaccines. In this way, the present work aimed to predict promising CD4+ and CD8+ T cell epitopes from the structural proteins of ZIKV (Capsid - C, Envelope - E and Membrane - M). The prediction started with the polypeptide sequence of the Brazilian isolate in Pernambuco (GenBank: KX197192.1), which was submitted to several analyzes, starting from the exploration of the Immune Epitope Database (IEDB) platform and proceeding through different approaches of immunoinformatics, such as sequence variability, conservation among different Flavivirus species, surface exposition and MHC binding. Several potential MHC class I and II epitopes were predicted using IEDB platform. Among the 278 candidates, we selected only the 89 potential epitopes that showed low similarity with other viruses of the FlaviviridaeFamily. From the previous step, only those that showed interaction with a greater number of MHC alleles were chosen, and finally, these peptides were subjected to an antigenic analysis of the VaxiJen server where 19 epitopes were annotated (6-C, 8-E and 5-M). The immunogenic profile of the selected epitopes in the last analysis was traced by evaluating the identity between different ZIKV strains and the population coverage of the MHC alleles. For the epitopes predicted for proteins E and M, the only ones that have their 3D structure resolved by cryo-electron microscopy (cryo-ME) on the RCSB / PDB server, were evaluated for the area of surface exposure. For E protein, the most promising epitopes for MHC class I and II interaction were respectively 374-MMILELPPF182 and 164-RAKVEITPNSPRAEA178 with exposure area of 1217.23 Å² and 1886.30 Å². The potential epitopes for M protein presented an exposure area of 1014.30 Å² – 1VTLPSSHSTR192 and 1430.80 Å² – 51WLLGSSTSQVIYL165. Therefore, the reported results may contribute to the development of vaccine candidates capable of destabilizing the structure of ZIKV and limiting the spread of infection. Financial support: FAPEMIG, CNPq.

Palavras-chave: IMMUNOINFORMATICS, VACCINES, ZIKA VIRUS
Introduction: Influenza A virus infects the upper respiratory tract of humans, causing acute respiratory illness; some cases may progress to severe lower respiratory-tract complications and death. Pathogenesis of severe cases is associated with a complex interaction between the host and the virus, including cytokine/chemokine responses and virulence factors. Objectives: To analyze cytokines/chemokines profile in patients infected with influenza A virus during the 2009 pandemics, according to severity. Methods: 45 patients sera from 2009 were assayed for 18 cytokines/chemokines (GM-CSF, IFN2-alfa, IFN-gamma, IL-10, IL12(p40), IL-13, IL-15, IL1-beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IP-10, MCP-1, MIP1-alfa and TNF-alfa) by multiplex immunology assay-xMAP microspheres in a Luminex System. Correlations between 3 groups (outpatients, severe hospitalized and deceased patients) were analyzed; 31 plasma negative for respiratory viruses were used as control group. Results: The age mean was 34.42y, 58% were females, and 50% of cases had underlying medical conditions. The majority (84.4%) of patients was hospitalized with severe influenza, and 7 patients died. Hospitalized group showed higher levels of TNF-alfa than non-hospitalized (p=0.024). The cytokines/chemokines response patterns in hospitalized and deceased patients showed increased expression of pro-inflammatory cytokines IL6, IL-4, IL-15 and TNF-alfa, and the chemokines IL8, IP-10, MIP1-alfa and MCP-1. Outpatients displayed a decrease in activation of IL-4, IL-8, TNF-alfa and IL-15 when compared with the control group, hospitalized and fatal patients. Comorbidity/risk factors were present in 40% of patients with available data. A lower expression of MCP-1 was independently associated with pregnancy; patients who were hospitalized showed higher levels of TNF-alfa concentrations than patients who were not hospitalized. Nevertheless, MCP-1 was found to correlate significantly with cough, MIP1-alfa with chills and sore throat, IL-15 with rhinorrhea and IL-8 with sore throat and dyspnea. This last symptom has been associated with severity and adverse clinical outcome in cases of influenza. Conclusion: There is a profile of innate and inflammatory responses associated to diseases severity, represented by IL-8, IL-4, IL15 and TNF-alfa. Cytokines IL-4, TNF-alfa, and chemokine IL-8 may be markers of disease severity because of their significant correlations with clinical outcome.

Palavras-chave: cytokines/chemokines, Influenza A virus, severity, profile
Human dendritic cells express the DC-SIGN receptor, a protein that recognizes carbohydrate present in the envelope of several viruses, including DENV. This receptor is encoded by the CD209 gene which undergoes alternative splicing to generate various isoforms of the protein. Some isoforms undergo changes in the carbohydrate recognition domain (DRC), which may lose affinity for the virus and thereby modulate its internalization. Therefore the objective of this work was to evaluate if sDC-SIGN1B type III, which undergoes a change in DRC, loses the ability to bind to mannose residues. The nucleotide sequence encoding the isoform-sDC-SIGN1B type III was obtained from Genbank and were used several bioinformatics software for physical and chemical analysis. The sequence of sDC-SIGN1B type III isoform was synthesized and cloned into the vector pET28a expression by the company Epoch Life Science. Heterologous protein expression was carried out at 37°C for 4 hours after induction with IPTG using E. coli strain Rosetta BL21 DE3. The results were analyzed by SDS-PAGE followed by Western blot. The protein was purified by affinity chromatography cobalt column and quantitated by Bradford assay. The functional assay to evaluate the binding ability of the sDC-SIGN1B type III isoform in mannose residues was performed on a mannose affinity column, previously equilibrated with buffer containing Ca2+ ions. All fractions recovered in the affinity column were measured by Bradford. The sDC-SIGN1B type III isoform (0.26ug/ul) was applied to this column and was fully recovered after washing. There was not observed the presence of the protein in EDTA elutions, indicating the loss of mannose-binding ability. The sDC-SIGN1A type III isoform, was used as positive control, since it does not has alterations in the CRD region. As expected, it was not verified the presence of sDC-SIGN1A type III isoform (0.17ug/ul) after washing the column and was completely recovered after elution. Thus, it was proven loss of capacity of the sDC-SIGN1B type III isoform to bind to mannose residues. Future in vivo infection assays using DENV will be conducted with the soluble isoforms with and without changes in the CRD for a better understanding of their role and participation in the process of DENV internalization in dendritic cells. Financial support: CNPq and FAPEMIG

Palavras-chave: DC-SIGN, sDC-SIGN1B type III, mannose
Zika virus (ZIKV), a mosquito-borne flavivirus, has become a public health emergency due to its rapid spread and occurrence of severe cases, including congenital complications and Guillain-Barré syndrome. ZIKV is related to other pathogenic arboviruses from the same family, including dengue and yellow fever virus. The clinical similarities, cross-reactivity, and cocirculation of those Flavivirus in Brazil have complicated the diagnosis of these infections, highlighting the need for a specific and sensitive test. A serologic test can be useful for acute detections as well as for monitoring and epidemiological studies. The envelope protein of ZIKV (ZIKV-E) has been shown to be immunodominant and associated with the generation of antibodies and, thus, is strategically important for the serological diagnosis of the infection. The aim of this work was to design a synthetic gene coding for the ZIKA virus envelope protein (sZIKV-E) to be posteriorly used in diagnostic assays. Initially, genes coding for E protein from 148 ZIKV sequences, deposited at GenBank, were aligned to generate a consensus sequence. Computational methods were used to predict the structure and physical properties of the sZIKV-E. The transmembrane domain was removed and the final sequence of the protein was analyzed regarding its antigenic potential. The nucleotide sequence coding for sZIKV-E was codon optimized and cloned into pET21. Subsequently, Escherichia coli strain XL10 competent cells were transformed with pET21a-sZIKV-E and positive clones were selected to subsequently transform the E. coli BL21(DE3) strain. The IPTG-dependent expression of sZIKV-E protein was optimized and posteriorly analyzed by SDS-PAGE. In silico analysis revealed that sZIKV-E secondary structure has predominantly coils and identified B lymphocyte epitopes in regions of structural disorder, which are advantageous for antigenic recognition. The overall antigenic prediction score in VAXIJEN_v2.0 was 0.58 (probable antigen) at a 0.4 threshold value. Best expression was obtained when cells were induced with 1 mM IPTG for 3 h. Coomassie Brilliant Blue staining showed the presence of sZIKV-E with the predicted molecular mass (~50 kDa). In conclusion, we were able to successfully design and express a ZIKV recombinant E protein with a potential to be used as an antigen in future diagnostic tests. Financial Support: CNPQ and CAPES

Palavras-chave: Flavivirus, Recombinant protein, Zika Fever, Zika virus, ZIKV
INTRODUCTION. Human papillomaviruses (HPV) are small double-stranded DNA viruses known as one of the most common sexually transmitted agents worldwide, infecting basal keratinocytes in mucosal and cutaneous epithelia. At present, over 180 types have been identified in distinct anatomical sites, most causing unapparent infections. However, there are strong correlations between particular types and persistent infection that can progress to different types of cancer such as cervical, vaginal, anal, vulvar, penile and oropharyngeal. Polymerase Chain Reaction (PCR) using consensus primers directed to L1 ORF is one of the most used procedures for HPV detection and genotyping. However, genotyping is usually done after PCR assay by using secondary laborious and expensive methods. OBJECTIVE. Developing a new PCR assay for detection and genotyping of HPV based on E1 ORF, generating amplicons with different sizes depending on the HPV type. MATERIALS AND METHODS. Complete genome sequences of all oncogenic HPV types available in the Papillomavirus Episteme database were aligned using MUSCLE algorithm and edited using the interface Jalview v2.9. Primers were designed and assessed using Geneious software and in silico specificity was checked with Primer-BLAST tool. HPV detection was performed using clinical samples of cervical intraepithelial neoplasia grade 3 (CIN 3) and compared to the gold-standard GP5+/6+ PCR assay. The two steps of the semi-nested PCR assay were performed containing 1.5 mM MgCl2, 1 U Taq DNA pol, 0.2 mM of each dNTP, 1 µl of sample DNA, 25 pmol of each primer in the first step and 10 pmol in the second. RESULTS AND DISCUSSION. A careful screening of the aligned sequences revealed the presence of a conserved region with variable length within E1 ORF, which encodes regulatory motifs that show a distinct arrangement at amino acid level among all HPV types. The in silico semi-nested PCR was able to predict a unique amplicon profile with distinguishable size bands for most types in both steps. Although using a low resolution method such as agarose gel electrophoresis, all the amplicons generated from the clinical samples showed an expected size indicating the presence of HPV 16, confirmed by direct sequencing. CONCLUSION. E1 was confirmed as a promising target for detection and genotyping of a large number of cancer-related HPVs by PCR, opening horizons to alternatives for improve diagnosis. FINNANCIAL SUPPORT. We thank CAPES for financial support.

Palavras-chave: HPV, cervical cancer, molecular diagnosis, typing, E1 gene
DETECTION OF BOVINE LEUKEMIA VIRUS (BLV) IN MILK FROM DAIRY COWS

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Resumo

Bovine leukemia virus (BLV) is the etiologic agent of enzootic bovine leukosis (EBL), a disease that affects cattle, which can lead to persistent lymphocytosis and to the development of fatal lymphosarcoma. BLV infects B-lymphocytes mainly, but also can infect CD8+ T-lymphocytes, monocytes and granulocytes, inducing a persistent infection for life. Cows that carry the virus can transmit it to their offspring through colostrum and milk, but the transmission of BLV to humans via milk remains uncertain. A study published in 2015 detected the BLV proviral DNA by in situ PCR in human mammary tissue samples with invasive carcinoma suggesting an association between BLV and human breast cancer. Although the consumption is mostly of pasteurized milk, the habit of consuming raw milk and milk products, potentially infected with BLV, is still common. The objective of this study was to investigate the presence of BLV provirus in milk of the BLV infected cows. Blood and milk were collected from 25 Gir x Holstein crossbred cows. DNA extraction was performed from milk cells after centrifugation using DNeasy Blood and Tissue kit (Qiagen), with adjustments in the manufacturer’s protocol. The same protocol was used for blood cells after collecting the buffy coat. Standard PCR amplification of the BLV tax gene was made along with amplification of the GAPDH normalizer gene to confirm the efficiency of DNA extraction. Milk somatic cell count (SCC) was performed by flow cytometry and the mean value of SCC from BLV negative cows was 563,000/mL compared to 959,000/mL for the positive ones. The BLV proviral DNA was detected in blood of 72% (18/25) and on the other hand, in none of the milk samples (0/25) analyzed. Determining the sensitivity of the test is required once the SCC were high in both positive and negative cows. The percentage of these cells that could be infected with BLV is not known. As the BLV has tropism for B-lymphocytes cells, a characterization and differentiation of the somatic cells should be done in further analyses. Previous studies have shown that the vertical transmission of BLV by milk is increased when the lactation phase is prolonged. Therefore, increasing the number of samples, as well as collecting milk from different days of lactation, can lead to a better evaluation of the release of BLV in milk and its viability. Financial Support: CAPES, FAPEMIG and CNPq

Palavras-chave: BOVINE, HUMAN INFECTION, LEUKOSIS, MILK, VIRUS
DETECTION OF IGA MARKER AS AN IMPORTANT TOOL IN DENGUE INFECTION DIAGNOSIS

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Resumo

In the last decades, dengue virus (DENV) infection has increased significantly in the world. Estimates indicate that approximately 390 million DENV infections per year occur globally. The World Health Organization (WHO) indicates that about 500,000 individuals develop into severe dengue and require hospitalization, being the majority children and about 2.5% of lethal cases. Thus, dengue is considered an arbovirus of global importance for public health, making it relevant to implement procedures that contribute to a more accurate and early diagnosis. In this context, the present study evaluated the IgA marker detection in serum samples from individuals with clinical suspicion of DENV infection of the 2012-2013 epidemic period, using commercial immunochromatographic test for IgA antibodies detection (MP Diagnostics (MPD) ASSURE®). Were analyzed 269 samples previously tested using commercial kit to the NS1, IgM and IgG serological markers, as well as the detection of viral RNA by RT-PCR for the C-prM region, to allow the evaluation of primary or secondary DENV infection. From the total samples evaluated, 117 (43.5%) were positive for the IgA marker. Was observed a positivity index of 32% (33/103) in the samples classified as primary infection and 75% (54/72) in cases of secondary infection. In relation to the other markers of infection, IgA was detected in 68.8% and 49.1% of IgM and NS1 positive samples, respectively. However, in 31.9% of the samples evaluated, IgA was the only marker of acute infection identified. These results demonstrate the importance of methodologies combination in the diagnosis of this infection. Furthermore, the use of the rapid IgA test is an important tool in early diagnosis as well as in different stages of the disease, helping to establish a complete database to be used for surveillance studies in health services.

Financial support: UFG.

Palavras-chave: dengue, detection, diagnosis, IgA, marker
084 - ÁREA: HUMANA E SAÚDE PÚBLICA

DETECTION OF NON-INFLUENZA RESPIRATORY VIRUSES IN A POPULATION SEGMENT OF THE METROPOLITAN REGION OF BELÉM, PARÁ, BRAZIL

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Resumo

Respiratory diseases are an important cause of illness and death in adults and children worldwide, with acute respiratory infections (ARI) being the most common manifestations. Viruses are among the major agents that cause ARI. In this context, in addition to the Influenza A and B viruses, other respiratory viruses also act as expressive agents that induce ARI, and may lead to severe conditions such as bronchiolitis and pneumonia. ARI present similar symptomatology, regardless the etiological agent, making clinical diagnosis and institution of correct treatment difficult, so laboratory diagnosis becomes a fundamental tool. Thus, the objective of this study was to detect non-influenza respiratory viruses in patients with ARI in the city of Belém, Pará, Brazil from August 2016 to July 2017. For this, clinical specimens (nasopharyngeal aspirate or combined swab) of 292 outpatients with ARI were collected. Sample analysis involved extracting the viral genome with commercial kit and real-time RT-PCR detection with probes and primers specific for Adenovirus (Adv), Human Bocavirus (HBoV), Human Coronavirus 229E, HKU1, NL63 and OC43; Human Metapneumovirus (HMPV), Human Orthopneumovirus (HOPV), Human Respirivirus 1 and 3, Human Rhinovirus (HRV) and Human Rubulavirus 2. Of the 292 samples analyzed, 138 (47%) were positive for at least one of the viruses tested. Among the viral agents investigated, HRV was detected in 101 (73%) positive samples, HOPV in 26 (19%), Human Respirivirus 1 in 7 (5%), Human Respirivirus 3 in 6 (4%), HCoV 229E Em 3 (2%), HCoV OC43 in 3 (2%), HCoV NL63 in two (1%), Rubulavirus human 2 in two (1%), Adv in one (1%), HCoV HKU1 in one (1%) and HBoV in one (1%). HMPV was not detected in the samples analyzed. There were also 14 (10%) cases of detection among two or more viruses investigated. The period of greatest viral circulation was between March and May. Our study demonstrated the expressive detection of non-influenza respiratory viruses in cases of ARI in the analyzed population, with HRV being the predominant agent, as well as in other studies conducted around the world. The peak of viral circulation coincided with the period associated with high precipitation rates in the city of Belém. Our data reinforce the need for the investigation of non-influenza respiratory viruses to assist in the better clinical management of ARI cases.

Palavras-chave: Respiratory viruses, Acute Respiratory Infection, Real-time PCR
085 - ÁREA: HUMANA E SAÚDE PÚBLICA

DEVELOPMENT OF SEROLOGIC METHODS FOR DIAGNOSIS OF INFECTIONS BY ZIKA AND CHIKUNGUNYA VIRUSES

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Resumo

Zika virus (ZIKV), isolated in 1947 in Uganda's Zika forest, is an Flavivirus genus (Flaviviridae). Chikungunya virus (CHIKV) is an alphavirus genus (Togaviridae) arbovirus isolated in Africa in 1952. ZIKV and CHIKV are single-stranded positive polarity RNA viroses, both transmitted by Aedes mosquitoes (A. aegypti and A. albopictus). ZIKV and CHIKV produce a dengue-like acute febrile illnesses with cutaneous rash, cephalgia, myalgia, headache, retro-orbital pain, conjunctival hyperemia. However, ZIKV infections have been recently associated with Guillain Barré neurologic Syndrome (flacid paralisis) and a congenital infection striking and destroying the central nervous system of the fetum leading to newborns with microcephaly. On the other side, CHIKV infections have been associated with acute (arthritis) and chronic severe joint disease. Our study aims to develop serologic tests for diagnosis of ZIKV and CHIKV including enzymme immunoassays using infected cultured cells as antigen and neutralization tests read by the enzyme immunoassays. For this study mosquito cells albopictus clone C6/36 will be used for the preparation of microplates for the enzyme immunoassay (EIA-ICC) specific for the virus to be standardized. Viruses are obtained from the serum and plasma of patients with suspected disease. Cell infection ZIKV and CHIKV will be confirmed by the detection of viral antigens using indirect immunofluorescence test. The title of the studied viruses will be determined by the formation of plaques (PFU/mL). The standardization of EIA-ICC will consist of two stages: the preparation of microplates and the test itself. In preparing the microplate infected bottles will be used and other bottles will be kept without infection, used as a negative control. After this step, cells are transferred to sterile polystyrene microplates containing 96 flat bottom holes in a quantity of 106 cells in 100 uL per well. Following this order: infected cells and uninfected cells in columns by alternating holes. After incubation, the plates will be fixed, washed and could be stored at -70 until used. The results for ZIKV presented 8,9% positivity for the presence of IgG antibody in 124 samples, but the presence of IgM and for CHIKV 15,3% of antibodies to IgG and 3% to IgM in for 65 samples tested. We conclude, therefore, that laboratory tests are essential tools for the diagnosis of patients and possible treatments. **Financial Support:**CAPES and FAPESP

Palavras-chave: ZIKA, CHIKUNGUNYA, VIRUSES
Arboviruses have become important and have been causing serious public health problems in tropical regions. The most prevalent arboviruses in Brazil are Dengue, Chikungunya and Zika, transmitted by the same vectors: A. albopictus and A. aegypti. Zika virus infection has symptoms such as arthralgia, rashes, conjunctival hyperemia, mild fever, headache and periarticular edema, also found in patients with other arbovirosis. For this reason, the specific diagnosis is critical. This study aims to evaluate the sensitivity of real-time RT-PCR diagnostic kits for Zika virus in samples sent to Funed. The sensitivity of four commercial kits was compared by analyzing 35 samples from previously analyzed patients using the Ministry of Health protocol - 24 Zika-positive, 3 Dengue-positive, 3 Chikungunya-positive, 3 Dengue/Zika-positive (coinfection) and 2 negative to these arboviruses. The RNA was extracted using the commercial QiAamp Viral RNA Mini Kit (Qiagen), and real time RT-PCR reactions were done following the instructions from kits developers, using 7500 Real-time PCR System (Applied Biosystems). The Ministry of Health protocol was done using a QuantiTect Probe RT-PCR Kit (Qiagen) to amplification reaction. All analyses were made in duplicate.Duplicates with divergent results were considered ‘inconclusive’ and were excluded from the study. It was obtained, to kits A, B, C and D, a total of 2, 0, 16 and 3 inconclusive results, respectively. The sensitivities observed were: Kit A 84%; Kit B 63,64%; Kit C 55,56%; Kit D 83,33%. All kits presented a specify of 100%, excepted by Kit C (50%). The obtained Kappa indexes were 0,7179 A, 0,5526 B, 0,03509 C and 0,7143 D. The number of analyzed samples, to kits A, B, C and D were, respectively, 33, 17, 22 and 32. As a result, it is possible to see that, when compared to reference method, kits A and D had a better performance. Comparing the kits A and B, the obtained Kappa index was 0,9296. Kits B and C did not show good results. However, a little amount of samples was tested to Kit B due to less availability of the kit in the lab. Looking to Kit C, it was observed a lot of inconclusive results, leading to a reduction in number of analyzed samples. These are preliminary results and further studies with more samples will be required for the use of these kits for diagnosis.

Palavras-chave: Zika, RT-qPCR, Sensitivity, Kit
Dengue fever (DEN) is an acute infectious disease caused by dengue virus (DENV), the most medically important arthropod-borne virus worldwide and a major public health challenge. In Brazil, several epidemiological studies have correlated DENV-3 infection to severer signs and symptoms, such as shock, abdominal pain and exanthema, compared to other DENV serotypes infection. The establishment of animal models to study DENV infections is of great relevance for research on pathogenesis, immunity, development and testing of drugs and vaccines. However, such studies have met numerous challenges, since circulating epidemic viruses do not naturally infect non-human species. Previous studies demonstrated that BALB/c mice, when infected experimentally with non-neuroadapted DENV -1 or -2 by the intraperitoneal or intravenous (iv) route, presented infection, clinical signs and tissue alterations similar to those observed in human cases of DENV infection. In this scenario, the main objective of the present study was to verify possible morphological and molecular alterations, as well as the presence of the DENV genome in different organs of BALB/c mice experimentally infected with an epidemic and non-neuroadapted DENV-3 strain. Groups of two months old male BALB/c mice were infected with DENV-3 by the iv route. Seventy-two hours post-infection, euthanasia was performed and brain, heart, kidney, lung, spleen and liver were harvested. Part of the samples was fixated and processed by standard techniques for morphological analysis by transmission electron and phase contrast light microscopy and part was macerated in Leibovitz medium for RNA extraction, detection and quantitation by real time RT-PCR assay. Morphological analysis of samples of all studied organs revealed alterations similar to those observed in DEN human cases including vascular congestion, signs of cellular degeneration and necrosis, focus of hemorrhage, edema, interstitial inflammatory infiltrate, presence of platelets, mononuclear and polymorphonuclear inflammatory cells inside blood vessels. Endothelial cells presented signals of activation and presence of cytoplasmic membrane phyllopodia could be observed. DENV-like particles were observed in kidney interstitial cells. The viral genome was detected in brain, lung, liver and spleen, and a suggestive title of viral replication was observed in the spleen. These results demonstrate the susceptibility of BALB/c mice to DENV-3 infection. Support: CNPq, IOC

**Palavras-chave:** dengue, mouse model, morphology, molecular biology
**EARLY PRENATAL ZIKA VIRUS INFECTION OF IMMUNOCOMPETENT MICE INDUCES NEURODEVELOPMENTAL AND OPHTHALMOLOGICAL ABNORMALITIES OF THE OFFSPRING**

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**Resumo**

*Zika virus* (ZIKV) infection is a global health emergency associated with serious neurological complications, like microcephaly in babies born from infected mothers. Recently, World Health Organization adopted additional criteria to diagnose ZIKV infection such as eyesight or hearing impairment and limb deficiency in addition to cephalic perimeter measurement, suggesting that severe microcephaly is only the “tip of the iceberg”. Indeed, it is known that several infections during pregnancy may be related to the development of different neuropsychiatric disorders secondary to changes in the neurodevelopmental process, suggesting a relationship between the immune system and the brain development. Those consequences on the offspring from ZIKV-infected dams are still not clear. Thus, our aim was to evaluate the effects of early ZIKV infection on the neurodevelopmental and ophthalmological abnormalities of the offspring born from infected dams. Additionally, we also evaluated the potential role of antibody-dependent enhancement in the exacerbation of those abnormalities. CS7BL/6 pregnant dams were inoculated with 1x10^6 PFU of ZIKV (HS-2015-BA-01 strain) by intraperitoneal (i.p.) route on gestational day 5.5 in the presence or absence of anti-envelope pan-flavivirus antibody (4G2). Negative and positive dam controls were injected with PBS or polynosinic-polycytidylic acid potassium salt (poly I:C) by i.p. route, respectively. Intraocular pressure (IOP), behavioral tests (social memory test, y-maze, open field test, sucrose preference test and others) and magnetic resonance imaging (MRI) started at four, eight and twelve-week age of offspring, respectively. A marked increase in IOP levels, indicative of ophthalmological abnormality, was detected in the offspring of 4G2-ZIKV and Poly I:C inoculated dams in comparison to ZIKV and PBS littersmates. Additionally, preliminary behavior analysis revealed slight alterations on basal locomotion, indicative of anxiety-like behavior, and a reduced glucose preference, suggesting anhedonia. Interestingly, MRI revealed reduction in whole brain volume of all infected groups in comparison to PBS control. Thus, our results reveal that early maternal ZIKV infection is associated to behavioral alterations of the offspring in adulthood. These results provide insights on clinical and neurodevelopmental consequences of early maternal ZIKV infection. **Financial support:** INCT Dengue, CAPES, CNPq, FAPEMIG, FINEP.

**Palavras-chave:** Zika virus, Arbovirus, Flaviviridae, Neurodevelopment, Immunocompetent mice
ENDOTHELIAL MODULATION DURING OROPOUCHE VIRUS INFECTION

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Resumo

Oropouche virus (OROV) is an emerging arbovirus in Brazil associated with rash fever and neurological disorders in humans. OROV have circulated since 1960, with more than 500,000 cases already reported in the Amazon region. It is believed that this virus can cross the blood-brain and placental barriers and cause neurological complications. OROV was showed to be neurotropic, detected in cerebrospinal fluid from patients with meningoencephalitis, and it was reported that the virus is able to induce fetal malformations in animal models. In addition, there were increased cases of abortions in Amazon during OROV outbreaks. However, the mechanisms associated with the disruption of endothelial barriers are poorly understood.

Thus, this project aimed to characterize the effect of OROV on endothelial cells, regarding the expression of molecules with important roles leukocyte endothelial transmigration or tight junctions disruptions between these cells. We observed that OROV was able to replicate in HUVEC cells with two different MOI (multiplicity of infection), such as 0.01 and 0.001. Also, supernatant analysis by ELISA showed that this virus induced the production of TNF-α in HUVEC cells after 4 hours post infection. OROV also induced the expression of IL-6 determined by qRT-PCR. These are vasoactive cytokines, associated with endothelial disruption in several models of infection. Finally, OROV infection in HUVEC cells promoted the transcription of genes essential to induce leukocyte endothelial transmigration or maintenance of the tights junctions. These data suggest that OROV can cross the endothelial barrier by 3 different mechanisms: 1. direct infection of endothelial cells and transport of generated viruses across basal lateral membranes; 2. spread of virus across endothelial cells tight junctions; 3. trojan horse pathway in which infected leukocytes in the blood migrate across endothelial membranes.

Financial Support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Palavras-chave: Arbovírus, Immune response, Neuroinvasion
Yellow fever (YF) usually occurs in the form of outbreaks with irregular intervals. In Brazil, after the elimination of the urban form, in 1942, there were only endemic foci of wild YF. Between July 2014 and December 2016, 38 suspected cases of AF were reported, without confirmation of the disease in Minas Gerais. In the same period, 119 epizootics were reported for this condition and only 5 were laboratory confirmed. In 2017, 1147 suspected cases of wild yellow fever in humans and 446 confirmed cases were reported as of June 30. YF confirmed epizootics were recorded in 121 municipalities of Minas Gerais, in all regions of the state, predominantly in the eastern region. 1352 epidemiological records that accompanied the biological samples received at the Ezequiel Dias Foundation from January to April 2017 for laboratory confirmation of Yellow Fever were analyzed. All the samples were tested for antiYF and anti-Dengue IgM (MAC-ELISA, CDC). In the analysis of the records, it was identified that the majority of the investigated cases occurred in the east of Minas Gerais, in the macroregions of Coronel Fabriciano, Governador Valadares, Manhumirim and Teófilo Otoni. About 86% of the confirmed cases belonged to the age group of 30 to 70 years and were male patients (85%). It was also noted that 262 patients suspected of having contracted the disease had been vaccinated against YF up to 10 days before the onset of symptoms. Of these, 139 presented Reagent anti-YF IgM results. It was also observed that 28 people had been vaccinated after the onset of symptoms. Of these, 14 samples were Reagent for anti-YF IgM. 57 samples were Reagent only for anti-Dengue IgM. In the region where the positive cases were identified there was no YF confirmed transmission in the last years. In the human population, the onset of cases is generally preceded by epizootics in non-human primates. The majority of the confirmed cases were in rural workers, which corroborates with the data of cases predominant in male patients and with no confirmed dispersion for urban area. Particular attention is needed in the state of Minas Gerais to cover greater vaccination coverage and to detect possible vaccine reactions, especially in the endemic area. Because the dengue virus and YF virus belong to the same family and present similar symptoms, differential diagnosis is important.

**Palavras-chave:** YELLOW FEVER, epizootics, vaccination, epidemiology, Minas Gerais
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EPSTEIN-BARR VIRUS DETECTION IN CASES OF CERVIX UTERI INJURY, ATTENDED IN THE OPHIR LOYOLA HOSPITAL, FROM 2008 TO 2012

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Resumo

*Epstein-Barr Virus* (EBV) belongs to the order *Herpesvirales*, family *Herpesviridae*, subfamily *Gammaherpesvirinae*, genus *Lymphocryptovirus* and species *Human gammaherpesvirus 4*. The virus latently infects more than 90% of the world’s population and is associated to various types of cancer both in lymphoid and epithelial tissues. Among the tumors of epithelial origin, the carcinoma of the uterine cervix may infected by EBV. The aim of this study was to investigate the prevalence of the virus in paraffin samples of lesions in the uterine cervix of patients attended in the Ophir Loyola Hospital, which is a reference for diagnosis and treatment of cancer in the State of Pará, in the period of 2008 and 2012. A total of 112 samples were obtained, and subjected to DNA extraction with the QIAamp DNA FFPE Tissue kit (Qiagen). Subsequently, qPCR was performed with the EBV PCRAlert kit (Nanogen). Among the analyzed samples, 22 were diagnosed as adenocarcinoma, 22 as invasive carcinoma, 11 as carcinoma *in situ*, 13 as squamous cell carcinomas, 1 as Leiomiiossarcoma, 19 as NIC III, 5 as NIC II, 2 as NIC I, 9 as other different types of benign lesions and 8 presented no histopathological evaluation. From the analyzed samples, 4.5% (5/112) presented a positive result for EBV by qPCR. Among the positive samples, only two presented a histopathological diagnosis, thus being diagnosed as adenocarcinoma. Adenocarcinoma was associated with positivity for EBV (X²=6.797; p=0.0091). The virus is already related to other types of adenocarcinoma, such as gastric adenocarcinoma. However, the evolution of infection-related neoplasia is not well understood. EBV is a class I carcinogen that is known to cause neoplasms in humans. The cervical cancer is one of the prominent tumor types among neoplasia of epithelial origin, which is related to the virus. Although there is no consensus on the pathogenic role of EBV in this neoplasm, the virus is present in several regions of the female genital tract. Hence demonstrating the need for further studies to test the virus relationship to cervical cancer.

**Palavras-chave:** EBV, cervical cancer, cervix uteri injury, qPCR
ESSENTIAL ROLE OF FOSFATIDILINOSITOL 3-QUINASE-GAMMA PATHWAY IN THE
PATHOGENESIS OF DENGUE VIRUS INFECTION

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Dengue is the most common arboviral disease that affects humans today and represents a serious public health concern. Currently, an estimated 400 million people are infected worldwide every year. Several molecules and signaling pathways are involved in the pathogenesis of inflammatory diseases. PI3Kγ, a member of the phosphatidylinositol 3-kinase (PI3K) pathway, has been a target of several studies because of its important activity during inflammatory conditions of medical importance. The aim of this study was to evaluate the role of PI3Kγ pathway activation in the pathogenesis of DENV-3 infection and to verify the therapeutic potential of the specific inhibitor of PI3Kγ pathway, AS605240, against infection. Two different mouse models of DENV-3 infection were used. A lethal model induced by an adapted strain of DENV-3 and a “dengue fever” like model induced by a clinical isolate, in the presence or absence of pan-flavivirus antibody (4G2), which mimics secondary infection. Infection of wild type (WT) mice with the adapted strain resulted in PI3Kγ pathway activation, through AKT phosphorylation. Severe disease manifestation, as represented by massive thrombocytopenia, hemoconcentration, leucocyte recruitment and elevated viral loads to target organs, followed by lethality were observed after virus inoculation. PI3Kγ-/- mice were massively protected from all DENV-3-induced parameters mentioned. In a model of mild disease using the clinical isolate, DENV-3 infected WT mice showed transient thrombocytopenia, hemoconcentration and increased vascular permeability at 24h after virus inoculation. Previous treatment of WT mice with 4G2 antibody exacerbated those manifestations. In this model, PI3Kγ-/-, PI3K kd/kd and AS60524-treated mice were protected from primary and secondary disease manifestations induced by DENV-3. Indeed, viral loads recovered from supernatant of primary culture of peritoneal macrophages of PI3Kγ-/- cells were reduced in comparison to WT littermates. Therefore, targeting PI3Kγ pathway seems to be beneficial to prevent the major outcomes of DENV infection. Financial support: CNPq, FAPEMIG , CAPES, INCT em dengue and PRONEX.

Palavras-chave: AS605240, DENGUE VIRUS, PI3Kgamma
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EVALUATION OF ANTIVIRAL ACTIVITY OF SYNTHETIC MOLECULES AGAINST CHIKUNGUNYA VIRUS

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Resumo

Chikungunya virus (CHIKV) is an enveloped virus with single stranded RNA genome and positive polarity. First isolated in Tanzania in 1952, this pathogen belongs to the genus Alphavirus, family Togaviridae, and its transmission occurs by vector (Aedes aegypti and Aedes albopictus). Since it was isolated, it has been associated with several outbreaks, until in 2014 the first autochthonous case was reported in Brazil. Chikungunya infection is characterized by severe joint pain, high fever, itching, and petechiae. This arthralgia caused by the virus can be a persistent and disabling symptom, but so far the treatment of the infection occurs only palliatively, since there are no approved antiviral drugs to treat it. The extreme severity of CHIKV and the high propagation of infection by the vectors demonstrates the need to search for and obtain anti-viral drugs against the pathogen. In this study, synthetic substances of the PFD series against CHIKV were tested. In order to observe the toxicity of the studied compounds (CC50), MTT assays were performed in VERO cells using concentrations of the substances (PFD01, PFD03, PFD04, PFD05, PFD07, PFD10, PFD14) varying from 50 to 1000μM, incubating the cells by 24 To 48 hours to analyze the cytopathic effect. The results showed that all the substances presented cell viability above 50% in the concentration of 200μM drug. Substances with the lowest cytotoxicity were PFD05, PFD07 and PFD14 with percentage of cell viability varying from 54% to 67% in the highest concentration tested (1000μM). To observe the possible effect of the substances against CHIKV (EC50), VERO cells were infected with this virus for two hours to adsorb virus particles, washed and then maintained with DMEM medium and the substances to be tested in different concentrations (0.65, 1.25, 2.5, 5, 10 and 20μM). PFDs 01, 04 and 14 were able to inhibit the virus in a dose-dependent manner, with inhibition above 90% at the concentration of 20 μM, except for the PFD07 derivative that inhibited 80% at the same concentration.

Palavras-chave: CHIKUNGUNYA VIRUS, ANTIVIRAL, SYNTHETIC MOLECULES
naphthoquinones of the series PD and H against Zika virus were tested. Thus for the Zika virus (ZIKV) is a positive-stranded RNA arbovirus belonging to the Flaviviridae family of the genus Flavivirus. Although generally asymptomatic, ZIKV can cause fever, petechiae, conjunctivitis and arthralgia, as well as microcephaly and Guillain-Barré syndrome. With the present severity of the infection, the development and development of antiviral drugs for the fight against the virus is extremely important.

In this work, Bis-naphthoquinones of the series PD and H against Zika virus were tested. Thus for the cytotoxicity assay procedure (CC50) MTT assays were performed on VERO cells using concentrations of the substances ranging from 25 to 400μM, incubating the cells for 24 to 48 hours to analyze the cytopathic effect. To observe the possible effect of the substances against ZIKV, VERO cells were infected with this virus, with MOI of 0.1, for two hours for adsorption of viral particles, washed and then maintained with DMEM medium and the substances to be tested in different concentrations (0.65, 1.25, 2.5, 5, 10 and 20μM). Subsequently the cells were fixed and stained with 1% violet crystal. In the obtained results, of the 21 substances tested, four (PD10, PD14, H06 and H08) present a high percentage of inhibition and high value of CC50. While PD20 and PD24 have a low CC50 and 100% inhibition. After the determination of the antiviral activity of the derivatives of the PD and H series, tests were made to determine the possible reduction of the infectivity of these substances directly on the ZIKV. Thus, the viral suspension was maintained for two hours at different concentrations of the derivatives (20, 10, 5 and 2.5 μM) and then added in VERO cell culture. The results showed that the derivatives PFD03, PFD04 and PFD07 were able to reduce the infectivity of ZIKV, the other derivatives tested did not present good inhibitory potential for this mechanism. Figure 3 shows that at the concentration of 20 μM the derivatives PFD03 and PFD04 reduced the infectivity of ZIKV above 80% while the drug PFD07 was able to reduce the infectivity in 62.2% in the same concentration.

Palavras-chave: ZIKA VIRUS, Antiviral , BIS-NAPHTHOQUINONES
Human adenovirus (HAdV) infections are distributed worldwide, accounting for 5 to 10% of all febrile diseases in infants and children, including lower respiratory tract infections, and may lead to morbidity, especially in immunocompromised patients. Given the background, secondary metabolites from plants are widely studied to assess their potential activity against different viruses. The aim of this study was to evaluate, in vitro, H. articulatus ethanolic (HaEE) and aqueous (HaAE) extracts antiviral activity against HAdV-5. HaEE and HaAE cytotoxicity was assessed by the MTT cell viability assay. A549 cell line (human lung adenocarcinoma) was exposed to serial dilutions of the extracts for 24 hours and 6 days. Assessment of antiviral activity was performed by plaque assay. A549 cells were grown in 6-well microplates, infected with the viral suspension and then treated with different non-cytotoxic concentrations of the extract. Viral suspension and culture medium were used as viral and cell control, respectively. The microplates were left incubating for 6 days at 37°C, and after the incubation period, all wells were stained with crystal violet dye. The 50% cytotoxic concentration (CC50) was >1228.58μg/mL for HaEE and >3685.68μg/mL for HaAE. Plaque assays demonstrated potential antiviral activity for HaEE, in a dose-dependent manner, with a 50% effective concentration (EC50) value of 573.43μg/mL. Therefore, the selectivity index (SI) of anti-HAdV-5 of HaEE in A549 cell was established to be 2.14 (SI=CC50/EC50). On the other hand, HaAE demonstrated possible pro-viral activity. It’s highest concentration had the highest HAdV-5 titer, with the viral titers decreasing as the extract’s concentrations were decreasing, compared the viral control. The difference observed between H. articulatus extracts regarding antiviral or pro-viral activity is possibly related to their composition. Thus, phytochemical profiles characterization will allow the identification of the substances with antagonistic, synergistic or additive activities. Furthermore, the ethanolic extract from H. articulatus showed antiviral activity against HAdV-5 and this finding may be useful in the development of anti-HAdV drugs, but further tests are needed. Financial support: CAPES, CNPq, Universidade Feevale.

**Palavras-chave:** Adenoviridae, Antiviral, Cytotoxicity, Plaque assay
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EVALUATION OF PROINFLAMMATORY CYTOKINES EXPRESSION IN CELLS INFECTED WITH COXSACKIEVIRUS A12

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Resumo

Coxsackieviruses (CV) are single stranded RNA viruses belonging to the Enterovirus genus. Human enteroviruses infections are wide spread and, generally, are asymptomatic or mild and self-limited. However, some can be life-threatening. The exact mechanism by which Enterovirus are able to escape the innate host immunity and cause tissue damage remains unknown, but it is anticipated to be a consequence of either the viral replication or from a dysregulated host immune response. The aim of this study was to determine the expression pattern of interleukin-1β (IL-1β), interferon β (IFN-β) and tumor necrosis factor α (TNF-α) in Adherent Epithelial cell line from Colorectal Adenocarcinoma (Caco-2 cells) infected with Coxsackieviruses A12 (CV-A12). For this purpose Caco-2 cells were seeded at a concentration of 2.5x10^5 cells per well in a 24-well plate and incubated for 24 h at 37ºC before infection with CV-A12. Cells were infected with 30 μl of CV-A12 and were harvested at 24 and 48h post infection. The total cellular RNA was extracted using the automatic extractor Magna Pure Compact System (Roche) and Magna Pure Compact RNA isolation kit (Roche) according to the manufacturer’s instructions. Reverse transcription (RT) was performed immediately after RNA extraction using the Transcriptor First Strand cDNA Synthesis Kit (Roche) with the random hexamer primer, according to the manufacturer’s instructions. Cytokines gene expression was evaluated by Real Time PCR using relative quantification of cytokines gene expression with normalization to the housekeeping gene GAPDH. Regarding the relative quantification of IL-1β, although gene expression was detected, the value was below the detection threshold, suggesting that IL-1β was expressed at different time point. In contrast, TNF-α expression both at 24 and 48 h post infection was suppressed and the IFN-β expression was induced at 24h post infection but was suppressed at 48h post infection. Given that TNF-α and IFN-β are important mediators of antiviral immune response our data support that CV-A12 can modulate the expression of these cytokines and consequently evade immune responses. Financial Support: University of Coimbra.

Palavras-chave: Viral infection, Coxsackievirus A12, cytokines, inflammation, Caco-2 cells
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EVALUATION OF THE HANDLING DAIRY PRACTICES ASSOCIATED TO VACCINIA VIRUS INFECTIONS IN A BOVINE VACCINIA ENDEMIC AREA IN BRAZIL

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Resumo

The Family Poxviridae comprise a fascinating group of complex DNA viruses, which replicate entirely in the cytoplasm of host cells. Orthopoxvirus is a genus within the Chordopoxvirinae subfamily with representatives of great importance in human and veterinary medicine, such as Variola virus, an obligate human pathogen responsible for the lethal and terrifying smallpox disease. Forty years after smallpox eradication, the increase of the susceptible population due to the discontinuation of routine vaccination against smallpox, among other important factors, is thought to have contributed to several OPV zoonotic outbreaks. In South America, Vaccinia virus (VACV) has been detected in recurrent outbreaks of vesiculopustular lesions (bovine vaccinia-BV), especially in Brazil, affecting mainly dairy cattle and milkers, being a burden to public health and dairy economy. Outbreaks usually occur in small rural properties, with basic infrastructure where milking is performed manually, without appropriated biosafety measures. In this study, we evaluated the dairy handling practices associated to VACV infections in a hyper-endemic area in Brazil and assessed the knowledge and practices related to VACV emergence and spread. In our survey, 123 individuals (51.3%) from a total of 240 have heard about BV before, from which 88 knew through outbreaks. Although most individuals involved in dairy activities (n=85/91) reported they had good hygiene practices, only 29.7% said they use adequate sterilizing products to clean their hands and 39.5% sterilize cows teats before and after milking. Furthermore, 46.7% of individuals reported they have contact with other animals besides dairy cattle, which could increase the exposure to VACV. Given the burden of BV on dairy economy and in public health as an occupational disease, we developed educational materials which target farmers and milkers and provide an overview and basic information about prevention measures against VACV infections. This would enhance surveillance and BV control and prevention efforts, especially for vulnerable populations located in endemic areas.

Palavras-chave: Poxviruses, Orthopoxvirus, Vaccinia virus, public health, educational outreach
EVALUATION OF THE HUMAN PAPILLOMAVIRUS TYPE 16 (HPV-16) PHYSICAL STATUS AND GENOME COPIES NUMBER BY USING A QUANTITATIVE REAL-TIME PCR (QRT-PCR) APPROACH.

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Resumo

Several epidemiological studies indicated that 290 million worldwide women are infected by genital human papillomavirus (HPVs), and that cervical carcinoma (CC) is the fourth most prevalent cancer worldwide, affecting nearly 290,000 women in 2016. Establishment of a persistent infection by oncogenic human papillomavirus (HPV) is thought to be central to the development of most cases of cervical cancer. Over 95% of cervical tumor specimens harbour HPV DNA. The classic high-risk types are HPV-16 and HPV-18. HPV-16 is classified in species 9 with related high-risk types 31, 33, 35, 52, 58, and 67, but HPV-18 is a member of species 7, which also includes high-risk types 38, 45, 59, and 68. HPV-16 and 18, alone account for approximately 50 and 14% of cervical cancers, respectively. Integration of HPV-DNA into the host cell genome appears to be an important event for cervical carcinogenesis given that the prevalence of integrated HPV-DNA increases as lesions progress. This growth advantage is thought to result from disruption of the HPV-16 E2 open reading frame, which may lead to increased cell immortalization capacity by increasing the levels of mRNAs encoding the viral oncogenes E6 and E7. The purpose of this study was to develop a system to properly examine the physical status and the genome number copies of HPV-16. Towards this end, a previously reported quantitative real-time PCR (qRT-PCR) was applied based on the assumption that the quantitative ratio of HPV-16 E2 to E6 gene targets would allow discrimination of the physical status of HPV-16. We examined 39 paraffin-embedded cervical tissue (PETs) samples with cervical cancer in order to identified cervical tissue samples positive for HPV-16. By using a conventional PCR protocol for detecting HPV-16, the HPV-DNA 16 was observed in 94% (33/35) of the cancer samples. We are currently dedicated to investigate the genome number copies of HPV-16 in our series of cervical cancer PET-samples harboring the most oncogenic HPV-16 genotype. Financial Support: CNPq and FAPEMIG

Palavras-chave: HPV-16 infection, cervical cancer, Paraffin- embedded tissues (PETs), Quantitative Real-time PCR, Physical status of HPV-16 genome
There doesn’t rule out ZIKV coinfection, verified in 18 sera from patients followed throughout the infection. Reactivity with DENV sera was observed, but reactivity with sera from CHIKV positive RT-PCR patients was lower than with ZIKV sera. Days after the first collection, which explains the number of sera from the same patients, but on different days showed that the detection of IgG became possible in 22.2% (4/18) in sera positive for DENV and 12.5% (2/16) for sera positive for CHIKV. It wasn’t possible with the substrate and reading at 405 nm. A positivity of 45.6% (21/46) was obtained in sera positive for Dengue virus. 43 negative sera controls were used from the laboratory from a mountain community and sequencing. In the standardization of the indirect ELISA test for antibody detection, recENV was used at 2.5 μg/mL and 80 sera from the laboratory were previously tested by RT-PCR for the Zika, Chikungunya and Dengue virus. 43 negative sera controls were used from the laboratory from a mountain community and previously collected before Zika and Chikungunya epidemic. Reactivity threshold was calculated with the mean of the results from control sera plus two standard deviations. The sera were diluted 1:100 and an alkaline phosphatase-linked anti-human IgG conjugate diluted 1:5000 was used, with a 20 minute reaction with the substrate and reading at 405 nm. A positivity of 45.6% (21/46) was obtained in sera positive for ZIKV, 22.2% (4/18) in sera positive for DENV and 12.5% (2/16) for sera positive for CHIKV. It wasn’t possible to define the time of infection of the sera tested, but in analyzes comparing the results of RT-PCR and ELISA of sera from the same patients, but on different days showed that the detection of IgG became possible in days after the first collection, which explains the number of negative samples in the ZIKV group. Cross-reactivity with DENV sera was observed, but reactivity with sera from CHIKV positive RT-PCR patients doesn’t rule out ZIKV coinfection, verified in 18 sera from patients followed throughout the infection. Therefore, even preliminary, the results point to the use of recombinant ZIKV protein as a useful tool in the standardization of immunological methods for the detection of antibodies against ZIKV. Finantion report: FAPERJ

Palavras-chave: Arbovirus, ELISA, Recombinant protein, Zika virus
Human herpesvirus 8 (HHV-8) is the most oncogenic human virus known in people living with HIV/AIDS (PLWHA), not treated by antiretroviral therapy (ART), which have 50% chance of developing Kaposi’s Sarcoma (KS), due to reactivation of HHV-8. In PLWHA, HHV-8 can remain latent for years with the expression of LANA specific antigen, which may activate HIV. However, in expressing the lytic gene, ORF50 may occur interaction with Tat protein, resulting in increased cellular susceptibility to HIV infection, and also reactivate the infection by HHV-8. The objective of the research was to estimate the frequency of latent and lytic HHV-8 in PLWHA. Subjects coinfected (HIV/ HHV-8) accompanied at the Clinical Hospital of the Federal University of Pernambuco (HC-UFPE) were analyzed. After signing the consent form, 5 mL EDTA tube were collected and the samples sent for Keizo Asami Virology Sector Immunopathology Laboratory (LIKA), being processed and stored at -80°C. To identify the lytic and/or latent infection, it was used the indirect immunofluorescence technique with anti-LANA antibody in BCBL-1 cells induced or not by TPA and, after labeling, the slides were examined with a fluorescence microscope. It was considered positive for lytic infection when 20% of the TPA treated cells had fluorescence and latent infection cells that showed dotted fluorescence in the nucleus. The results showed that 44.5% (61/137) were in the latent phase, 30.7% (42/137) in the lytic phase of the infection, 5.1% (07/137) were undetermined and 19.7% (27/137) no reagent. The frequency of patients in the latent phase was higher, suggesting that the presence of LANA specific antigen can activate HIV replication. Moreover, patients in lytic phase of the infection are at increased risk of HHV-8 reactivation which may be indicative of KS development.

**Palavras-chave:** FREQUENCY, HHV-8, HIV, LATENT, LYTIC
Viral infections are important causative agents in nephrotic syndrome and renal transplant patients and are responsible for significant morbidity and mortality. Some viral infections can produce active replication in the kidney, which often occur in immunocompromised hosts such as renal allograft recipients. Adenovirus, Epstein-Barr virus, cytomegalovirus, polymavirus (type BK) and herpesvirus (type 6 and 7) are members of this group causing specific diseases. The aim of this study was to recognize the etiological agent of the viral infections using laboratory molecular diagnosis to establish appropriate therapy and to guide the application of preventive approaches. Forty-seven pediatric patients, with a median age of 10 years old (range 2-18 years), 37 with chronic glomerulopathies and 10 renal transplant recipients, using at least two immunosuppressants, were enrolled in this study. Urine and plasma samples analyzed prospectively using Nested PCR for DNA detection of adenovirus (ADV), polymavirus (BK), Epstein-barr (EBV), cytomegalovirus (CMV), and herpesvirus (type 6 and 7). Eighty-eight collected samples were positive for the viruses studied (14%), from 39/47 (83%) of the patients. Fifteen out of 39 (38.5%) patients had positive CMV; 13/39 were HHV-6 positive (33.3%), 11/39 (28.2%) had positive HHV-7, 11/39 (28.2%) had BK virus, 9/39 (23%) had EBV, and 4/39 (10.25%) were ADV positive. Five out of 39 patients (12.8%) presented coinfection (infection by two or more viruses in the same sample) and 16/39 (41%) presented virus reactivation. Thirty-one of the 37 (83.8%) patients with glomerulopathies had infection, with CMV being the most frequent, with 11/31 (35.5%), followed by HHV-7 and BKV, which presented positivity in 10/31 (32.2%) and 9/31 (29%), respectively. HHV-6 was positive in 8/31 (25.8%) of patients, followed by EBV (7/31, 22.5%), and finally ADV, with 4/31 (12.9%). Eight out of 10 (80%) renal transplant recipients were positive for viruses. Four had CMV (40%), five had HHV-6 (50%), 1 had HHV-7 (10%), and 2 had BKV and EBV (20% each). With the implementation of virological surveillance in patients with glomerulopathies and renal transplant in this study, we can understand better the viral pathogenesis to optimize the treatment with the adequate administration of antiviral drugs and the reduction of immunosuppressive drugs, when necessary, avoiding episodes of decompensation and failure of graft and acute graft-versus-host-disease (aGVHD).

**Resumo**

Viral infections are important causative agents in nephrotic syndrome and renal transplant patients and are responsible for significant morbidity and mortality. Some viral infections can produce active replication in the kidney, which often occur in immunocompromised hosts such as renal allograft recipients. Adenovirus, Epstein-Barr virus, cytomegalovirus, polymavirus (type BK) and herpesvirus (type 6 and 7) are members of this group causing specific diseases. The aim of this study was to recognize the etiological agent of the viral infections using laboratory molecular diagnosis to establish appropriate therapy and to guide the application of preventive approaches. Forty-seven pediatric patients, with a median age of 10 years old (range 2-18 years), 37 with chronic glomerulopathies and 10 renal transplant recipients, using at least two immunosuppressants, were enrolled in this study. Urine and plasma samples analyzed prospectively using Nested PCR for DNA detection of adenovirus (ADV), polymavirus (BK), Epstein-barr (EBV), cytomegalovirus (CMV), and herpesvirus (type 6 and 7). Eighty-eight collected samples were positive for the viruses studied (14%), from 39/47 (83%) of the patients. Fifteen out of 39 (38.5%) patients had positive CMV; 13/39 were HHV-6 positive (33.3%), 11/39 (28.2%) had positive HHV-7, 11/39 (28.2%) had BK virus, 9/39 (23%) had EBV, and 4/39 (10.25%) were ADV positive. Five out of 39 patients (12.8%) presented coinfection (infection by two or more viruses in the same sample) and 16/39 (41%) presented virus reactivation. Thirty-one of the 37 (83.8%) patients with glomerulopathies had infection, with CMV being the most frequent, with 11/31 (35.5%), followed by HHV-7 and BKV, which presented positivity in 10/31 (32.2%) and 9/31 (29%), respectively. HHV-6 was positive in 8/31 (25.8%) of patients, followed by EBV (7/31, 22.5%), and finally ADV, with 4/31 (12.9%). Eight out of 10 (80%) renal transplant recipients were positive for viruses. Four had CMV (40%), five had HHV-6 (50%), 1 had HHV-7 (10%), and 2 had BKV and EBV (20% each). With the implementation of virological surveillance in patients with glomerulopathies and renal transplant in this study, we can understand better the viral pathogenesis to optimize the treatment with the adequate administration of antiviral drugs and the reduction of immunosuppressive drugs, when necessary, avoiding episodes of decompensation and failure of graft and acute graft-versus-host-disease (aGVHD).

**Palavras-chave:** HCMV, Nested-PCR, pediatric kidney transplants, chronic glomerulopathies, Adenovirus, Polymavirus (BK), betaherpe
Rotavirus is the major cause of Acute Diarrheal Diseases in the world. It is transmitted by the fecal-oral. Annually there were about 1.3 billion cases, with 4 million deaths worldwide. In Brazil, there are registered 3.5 million cases and 2,475 deaths per year. There are several factors that increase the potential spread of the disease. The antiviral research using natural products demonstrates greater acceptance by the population in using this type of therapy, besides it causes fewer side effects. In this study Arctium lappa L., Piper aduncum L. and Aristolochia cymbifera M. were tested as antiviral for Rotavirus A. For the cytotoxicity assay different concentrations of crude extracts were used in MA-104 cells in a 48 hours interval. Cytotoxicity was determined by cell viability using MTT assay. For the antiviral assay it was used the same cells and the same concentration with no toxicity for the cells during 48 hours and 4 μL virus were added per well (20 TCID₅₀). The MTT was used to evaluated concentrations where 50% of the cells remained viable even after infection with the virus. The material collected from the antiviral assay was amplified by qRT-PCR in order to detect the genetic nucleic acid from the virus. Using qPCR it has been observed that some concentrations reduced viral genetic material present in the samples. This method showed suitable for the screening of substances capable to act as an antiviral and inhibiting viral multiplication.

Palavras-chave: Extract of medicinal plants, Rotavirus, Cytotoxicity assay, Antiviral assay, Real time quantitative PCR
Acute gastroenteritis (AG) affects several individuals and rotavirus (RV) still remains a very common cause of severe diarrhea in children under five years old. The RV has dsRNA, divided into eleven segments that encode six structural proteins (VP1-VP4, VP6 and VP7) and six non-structural proteins (NSP1-NSP5/6). The RV has nine species (RVA-RVI) and in RVA was frequently used the binary combination VP7 and VP4 genes that designate the respective G and P genotypes. In 2008, there was a proposed classification system that assigns a specific genotype for each of 11 genes, with genogroup constellations 1 (Wa-like) and 2 (DS1-like) more detected. Due to their relevance, two RVA vaccines are licensed and introduced in universal immunization, including Brazil where was adopted in 2006. With this, the monitoring type RVA current is important. Therefore, the aim of this study is to characterize the NSP4 gene RVA circulating in the Southeastern region of the state of Pará, Brazil, from 2006 to 2015. We analyzed 45 fecal specimens from children and adults with AG who were attended in Parauapebas, Pará, Brasil. Samples were submitted to Reverse Transcription Polymerase Chain Reaction (RT-PCR) using specific primers, were sequenced and analyzed phylogenetically. Analysis of NSP4 gene demonstrated two distinct genotypes, 3 samples G1P[8] and 1 sample G9P[8] belonging to the E1 genotype (Wa-like constellation) and 41 samples G2P[4] belonging to the E2 genotype (DS1-like constellation). In genogroup 2, 8 samples presented similarities of 99.94%, 99.96% and 99.97% with samples from cat (G3P[9]), cow (G6P[5]) and roe deer (G8P[14]), respectively. Such results suggest possible human/animal reassortment for NSP4 gene. The other samples showed similarity with strains of human origin, and all presented high similarity over the time studied, occurring the association of the binary classification to the respective constellations already described. The similarity observation of these human samples with strains of animal origin emphasizes the importance of the characterization of the RV genes to help the detection of possible genetic variants, derived from zoonotic transmission, which may represent a challenge to the immunizers currently used.

**Palavras-chave:** NSP4 gene, reassortment, rotavirus A
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GENETIC TRANSFORMATION OF PICHIA PASTORIS FOR RECOMBINANT EXPRESSION OF NON-STRUCTURAL PROTEIN 1 (NS1) ZIKA VIRUS (ZIKV)

MARIANA FONSECA XISTO, INGRID MARQUES DIAS, JOHN WILLIANS OLIVEIRA PRATES, VINÍCIUS MEDINA PINHEIRO, SÉRGIO OLIVEIRA DE PAULA

Resumo

Yeast *Pichia pastoris* is a potent host for the production of large-scale recombinant proteins of great interest to the biotechnology industry because it has the ability to produce proteins in a stable manner and with post-translational modifications typical of eukaryotes. The Zika virus (ZIKV) belonging to the *Flaviviridae* family was first identified in monkeys in Uganda in 1947. Subsequently, in 1952 it was identified in humans. But it was in mid-2015 that Zika became known worldwide when Brazil notified the association of infections with Guillain-Barré syndrome and alarming number of cases of newborn babies with microcephaly. Transmission of the Zika virus occurs mainly through the bite of the vector mosquito (*genus Aedes*) but it is also possible that the virus is sexually transmitted. The ZIKV has a single-stranded RNA genome with positive polarity that encodes 10 final proteins, 3 of which are proteins that make up the viral structure and 7 are non-structural proteins (NS) that are related to replication, translation of the proteins themselves and virulence. Non-structural protein 1 (NS1) is used as a diagnostic marker for flavivirus infections. In this context, the focus of this work was to genetically transform the yeast *Pichia pastoris* to express the NS1 protein of ZIKV for diagnostic applications. To do so, the pPICZαA expression vector that had previously been cloned with the gene of interest (pPICZαA_NS1ZIKV) was used. The plasmid was amplified in *E. coli* TOP10F cells, extracted by the alkaline lysis method and linearized with SacI restriction enzyme for efficient integration into the yeast genome. Competent *Pichia pastoris* (KM71H) were prepared and the transformation was performed by electroporation. The yeast was plated in selective medium and the transformed colonies were selected through the zeocin antibiotic resistance gene. They were then grown and submitted to genomic DNA extraction for confirmation by PCR (polymerase chain reaction) with specific primers. The results showed efficient transformation by the presence of the exogenous gene in the yeast genome. The next steps are to cultivate and induce these recombinant yeasts to express the NS1 protein to be used in immunological tests. Financial support: FAPEMIG / CAPES / CNPq

Palavras-chave: Zika virus, NS1 protein, Pichia pastoris, recombinant expression
HANTAVIRUS CARDIOPULMONARY SYNDROME IN THE STATE OF MINAS GERAIS, 1993 TO 2014: A RETROSPECTIVE EPIDEMIOLOGICAL STUDY

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Resumo

Hantavirus Cardiopulmonary Syndrome (HCPS) is the most serious manifestation of hantavirus infection in the Americas and it is characterized by severe pulmonary involvement that leads to respiratory failure and cardiogenic shock. These viruses are maintained in natural cycles in rodents’ reservoirs and humans are infected through inhalation of aerosolized rodent excreta. Humans are accidentally infected and case fatality rate in Brazil ranges from 30% to 60% depending on the region and viral genotype. According to the Brazilian Ministry of Health, Southeast region accounted for 542 of 1995 cases described up to 2017, in which 287 (52.9%) were reported in Minas Gerais State. This work describes a retrospective study of the cases of SCPH in Minas Gerais state for the period of 1993 to 2014. We conducted a descriptive study based on the records of the Sistema de Informação e Agravos de Notificação (SINAN), analyzing the reporting data by the State. Two hundred and sixty six (266) notifications occurred in 66 municipalities of the state, in which, Araxá, presented 19,9% (53/266), Uberaba 11,2% (30/266), Patrocínio 10,9% (29/266), and Patos de Minas 10,5% (28/266) obtained the highest percentage of confirmed cases up to 2014. All municipalities with the highest reported disease are located in the Triângulo Mineiro and Alto Paraíba region. Case reports show that HCPS in Brazil primarily affects young adult males with an average age of 30 years (192/266, 72.1%) and it is related mostly to rural environment. The average coefficient of lethality for the period was 36.8% (98/266). Deaths were more frequently in males 26.6% (71/266) however, a higher lethality coefficient was observed in female individuals 45% (27/60). Regarding seasonality, cases were observed in all months of the year, although, in the driest months (April to August) a 62.4% (166/266) of disease notifications was observed. With the analysis of the data was possible to verify the need to prioritize actions aimed surveillance programs of hantaviruses epidemiology in the state. This study has demonstrated that this disease impacts the economically active population, mainly men who carry out rural activities. The results of this study may be used as an auxiliary tool for state epidemiological surveillance.

Palavras-chave: Emerging infectious deseases, Hantavirus Cardiopulmonary Syndrome, Epidemiology, Brazil, Minas Gerais
To the best of our knowledge, this is the first study correlating HCMV infection and standard chemotherapy viability in this type of cell lines, instead they suggest that HCMV can act together with chemotherapeutic agents to decrease cell viability. Our findings show that the virus does not increase chemoresistance in GBM cells subjected to chemotherapy treatment. We show that U251MG and TP365MG GBM cell lines, especially in glioblastoma multiforme (GBM). Furthermore, it was shown that the virus has oncomodulatory properties in GBM cells. GBM is the most common and malignant primary central nervous system tumor, despite the advances in tumor knowledge, the overall patient survival is no longer than 15 months. The standard GBM treatment is tumor resection followed by radio and chemotherapy. The chemotherapy treatment leads to DNA damage due to insertion of adducts in base-pairs. It is known that several HCMV proteins can alter the cellular DNA damage response, and therefore we aimed to analyze the HCMV effects in GBM cells subjected to chemotherapy treatment. We show that U251MG and TP365MG GBM cell lines have a decrease in cell viability when infected with clinical or laboratory adapted HCMV strains and treated with chemotherapeutic drugs Temozolamide or Carmustine. Intriguingly, antiviral ganciclovir treatment itself decrease cell viability in both cell lines, however, when infected cells are treated with ganciclovir the cell viability increased. Our findings show that the virus does not increase chemoresistance in GBM cell lines, instead they suggest that HCMV can act together with chemotherapeutic agents to decrease cell viability in this type of cells and the antiviral treatment can induce per se a possible increase in cell death. To the best of our knowledge, this is the first study correlating HCMV infection and standard chemotherapy treatment in GBM cell lines.

**Palavras-chave:** HCMV, Glioblastoma, GBM
HEPATITIS C VIRUS INFECTION IS INHIBITED BY BOTHROPS SNAKE TOXINS IN VITRO

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Resumo

Hepatitis C is a liver disease caused by the hepatitis C virus (HCV) and about 170 million people are estimated to be currently infected worldwide. The available therapy to treat chronically infected patients is based on interferon-α, ribavirin and direct acting antivirals (DAAs). However, this treatment is expensive, presents several side effects and antiviral resistance has been documented, demonstrating the need of new therapeutic approaches. In this context, natural compounds have demonstrated medical interest due to their biological activities. Compounds isolated from animal venoms have shown antiviral activity against a range of viruses such as Dengue virus, Yellow fever virus and Human immunodeficiency virus. In this study, were screened 15 toxins isolated from different snake species against HCV in vitro. The cell viability of the toxins was evaluated in Huh-7.5 cell culture by MTT assay and its activity on the HCV replicative cycle was analyzed in Huh-7.5 cells infected by HCVcc JFH-1 genotype 2a. The toxins effectivity was quantified by counting focus-forming units per milliliter of supernatant (FFU/mL) after 72 h of treatment. Four toxins from Bothrops genus significantly inhibited HCV replicative cycle up to 99.3%. In conclusion, these toxins can be useful for the development of future therapies against HCV after more studies about its mechanism of action. Financial Support: Capes, Fapesp, CNPQ, Fapemig, The Royal Society – Newton Funds.

Palavras-chave: Bothrops, HCV, Hepatitis C, Snake toxins, Animal venoms
HIGH CONTENT SCREENING FOR FLAVIVIRUS DRUG DISCOVERY

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Introduction: The Flavivirus is a large genus which comprises more than 70 different pathogenic arthropod-borne viruses with significant public health impact in different parts of the world and the potential of emerging in previously non-endemic regions. The Yellow fever virus (YFV) and Dengue virus (DENV) are some of the most important human pathogenic flaviviruses with similar symptoms that range from mild fever and malaise, haemorrhage, and rapid terminal events with shock and multi-organ failure. Despite the existence of a protective vaccine for yellow fever and dengue, the vaccine is contraindicated in individuals who are immunocompromised, for pregnant women and for infants and children. One alternative to vaccination would be the development of specific antiviral chemotherapies that could reduce the morbidity and mortality associated with these infections with less adverse effects. The objective of this study was to develop a high-content high-throughput screening assay to triage two commercial compound libraries (NIH Clinical Collection, National Institute of Health; and LOPAC1280, Sigma) with potential antiviral activity. Methods: Tests were performed in 384 wells plate. Huh7 cells and DENV or YFV-YFP were plated jointly with the candidate compounds and after 72h incubation, plates were fixed and stained. Images were taken in the automated high-throughput imaging system Operetta (Perkin Elmer). The acquired images were analysed in terms of cell viability and amount of infection. Results: From a total of 500 compounds, 48 were initially scored as positive hits, 20 for DENV2 and 28 for YFV-YFP. A secondary screening confirmed 14 compounds for DENV2 and 27 for YFV-YFP. Most of the confirmed hits are known for their antiviral activity for other viruses, however 2 compounds, which have never been reported as antivirals, presented thriving activity. Conclusion: Our assay allows us to screen a large amount of potential drug candidates in a short time span, and gives us multiparametric results, increasing the efficiency in the drug discovery process, saving time and cost. Funding: CNPq n. 141603/2017-8

Palavras-chave: Dengue, Yellow Fever, Drug Discovery, High-content screening
The human papillomavirus (HPV) is the main causative agent of precancerous lesions and cervical cancer (CC), responsible for many deaths in women worldwide each year. The precursor lesions of CC are categorized as cervical intraepithelial neoplasia grade I (CIN I), II (CIN II) and III (CIN III). According to their oncogenic potential, the different types of HPVs are classified into high (HR) and low (LR) oncogenic risk. Epidemiological studies on the prevalence of HR-HPVs are important for the development of strategies for prevention and treatment of precursor lesions and CC. In this study, the frequency of HPVs 16, 18, 33, 35, 45 was performed by PCR on 42 cervical biopsy specimens obtained from patients with CIN and cancer, and without cervical changes. In addition, the prevalence of HPVs 35 and 45 was investigated in 32 CC samples. The DNA of HPV16 was observed in 37.5% of CIN I samples, and 10% without alterations; HPV18 DNA was verified in 25% of CIN II samples, whereas DNA/HPV33 was positive in 12.5% of CIN I samples, 8.3% of CIN III samples, 12.5% of cancer and 40% of control samples. The DNA/HPV35 was observed in 62.5% of CIN I, 25% of CIN II, 91.6% of CIN III and 75% of CC samples. 75% and 37.5% of the CIN III and cancer samples showed the HPV45 DNA, respectively. Analysis of the prevalence of HPVs 35 and 45 in the invasive CC samples indicated the presence of these HPVs in 63.3% and 6.6% of these samples. In this study, a high incidence of HR-HPVs was observed in cervical biopsies analyzed, with the predominance of HPVs 33, 35 and 45 in patients with CIN III and cancer, indicating the importance of the genotyping of these HR-HPVs in the diagnosis and treatment of pre-neoplastic lesions and neoplastic lesions of the cervix. Financial Support: Pró-Noturno Program PROGRAD, UFMG; FAPEMIG; CNPq.

**Palavras-chave:** Cervical Cancer, Cervical Intraepithelial Neoplasia, Genotyping, HPV, PCR - Polymerase Chain Reaction
Infection with high-risk HPV types (HR-HPV) can lead to cervical cancer (CC) and HR-HPV16 and 18 are the most common types, responsible for about 70% of CC cases. In addition to the HPV type, variants, persistence and viral load also play a key role as adjuvants in cancer pathogenesis. Moreover, exposure to other sexually transmitted infections (STIs), especially HIV, can increase the risk of cancer development. The aim of this study was to determine the HR-HPV genotypes, HPV16 variants, HPV16 and 18 viral load of HIV-seropositive women attended at reference care centers for HIV/AIDS from eight Brazilian states to investigate the relationship with cervical lesion. Cervical samples of 647 HIV-seropositive women were obtained with cytobrush and stored in ThinPrep® PreservCyt transport media; Cobas® HPV test (Roche Diagnostics) detected 28.9% (187/647) of HR-HPV. Positive samples were referred to Laboratory of Virology-UFES to analyse the HR-HPV genotypes, HPV16 variants and HPV16 and 18 viral load. Viral DNA was extracted using the QIAamp DNA Mini Kit™ (QIAGEN); HPV was screened with the sets of PGMY09/11 primers and genotyped by Reverse Line Blot (RLB). HPV16 variants were described by sequencing using BigDye® (Thermo Fisher) reagent and the viral load of HPV16 and 18 was assessed by Real-time PCR, using TaqMan® protocol. Eighteen distinct probable and HR-HPV types were detected in 92% (172/187) of these samples and 40% (69/172) were mixed infection (up to six types). HPV16, the most frequent type, was detected in 29.6% (51/172) of samples, followed by 52>31>53>58; HPV18 occurred in 13.9% (24/172) occurred in 13.9% (24/172). European HPV16 variant predominate over non-European variants (Asian-American, African-1a and African-2a) (88% versus 12%). Moderate or high viral load (>10 copies/cell) occurred in 52.9% of HPV16 and HPV18 infected women and was significant in single infection versus in mixed infection with others probable or HR-HPVs (69.6%; p=0.05); even a higher significance occurred including LR-HPV (82.4%; p=0.007). HPV16 and HPV18 viral load were higher in cervical lesion cases (median 19.77 and 15.77 copies/cell, respectively) than in normal cases (9.16 and 5.82 copies/cell, respectively). In conclusion, there was a high frequency of HR-HPV, a predominance of European variant, and at least a moderate viral load of HPV16 and HPV18 in a population with greater risk of CC development. Financial support: Fundação de Amparo à Pesquisa e Inovação do Espírito Santo (FAPES)

**Palavras-chave:** HPV, HIV, Genotypes, Sequencing, Viral load
Human Bocavirus (HBoVs) are classified in the *Parvoviridae* family and are associated with infections of respiratory and gastrointestinal tracts. Viral infections are an important cause of morbimortality in immunocompromised patients such as allogeneic hematopoietic stem cell transplantation (HSCT) recipients. The aim of the present study was to evaluate the positivity rate and viral loads of HBoVs in clinical samples of patients who were subjected to allogeneic HSCT at a reference center for bone marrow transplantation in Goiânia, Goiás. A total of 105 fecal samples and 145 sera samples were collected from 21 patients, during October 2012 to October 2014. Samples were screened by qPCR TaqMan assay, with specific probe and primers targeting all HBoVs genotypes (HBoV-1 to -4), and viral loads were determined using serial dilutions of a recombinant plasmid. The results showed that 53.4% (11/21) of the patients were male, aged between 4 and 61 years-old (mean 35 years). The most observed hematologic malignancy was myeloid leukemia (acute or chronic), accounting for 57.1% (12/21) of the cases. The HBoVs were detected in 38.1% (8/21) of the patients. A positivity rate of 12.4% (13/105) in fecal samples was found, with a mean viral load of 4.83x10^7 copies/g (c/g). The virus were also detected in 6.9% (10/145) of sera samples with a mean viral load of 2.66x10^5 c/mL. Furthermore, 62.5% (5/8) of the patients were positive in both fecal and serum samples. Considering the symptoms presented by the patients, 28.5% (2/7) of the patients that were positive in fecal samples had diarrhea and vomit. Additionally, 62.5% (5/8) of the patients were co-infected with either Adenovirus, Norovirus or both. The samples will be sequenced to determine the HBoVs’ genotypes. The present data shows a high occurrence of HBoVs in allogeneic HSCT recipients. These results highlight the importance of monitoring patients undergoing HSCT for HBoVs. Financial Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Universidade Federal de Goiás (UFG).

**Palavras-chave:** Hematopoietic Stem Cell Transplantation, Human Bocavirus, Immunocompromised Patients
INTRODUCTION: Arboviruses are transmitted by arthropods, having a global distribution, affecting mainly population of tropical and subtropical countries. The geographical distribution of the vector and the virus is a fundamental factor for the emergence of arboviruses. In the last decades, several arboviruses have emerged and / or re-emerged in North, Central and South-America countries. Previously restricted viruses from regions of Africa and perhaps Asia, such as the ZIKV and CHIKV, have invaded new continents causing major public health problems. Several factors are related to infections caused by arboviruses today, among them the disorderly urban growth, the extensive process of international exchange and mostly theclimatic changes.OBJECTIVE: To identify and characterize circulating arboviruses in the Western Amazon Region. MATERIALS AND METHODS: 161 patients with a symptomatic characteristic of arboviruses were included in this study. The samples were collected during the first half of 2017 in the City of Porto Velho state of Rondônia and adjacent districts. In order to extract the genetic material, the QIAamp® Viral RNA Mini Kit was used, according to the manufacturer's standards. Afterwards, one-step RT-PCR was performed for the simultaneous detection of ZIKV, CHIKV and the four DENV serotypes, with the co-amplification of the internal control for all the samples tested, in order to increase the reliability of the reaction performed. RESULTS: From the 161 subjects included in the study 81 (50.31%) were male, 80 (49.68%) female, mean age of these participants was 36.68 years. The most frequent symptoms reported in the anamnesis were fever 81 (50.31%), headache 79 (47.20%), myalgia 43 (26.70%), exantheme 31 (19.25%). Was found 07 positive samples for the Zika viral RNA, showing a percentage of 4.26% of the total samples tested. CONCLUSION: Following this method, this systematic study demonstrates the importance of the clinical and epidemiological situation of infections caused by circulating arboviruses in the Western Amazon region, corroborating with new studies and the trends of prevention and control that have been developed in recent years. Other arboviruses are still being tested in this study to try to elucidate the prevalence of these viral agents in this region.

**Palavras-chave:** Arboviruses, CHARACTERIZATION, RT-PCR
Arbovirus develop part of their life cycle in arthropod hosts and can cause mammals disease, including in humans. The arboviruses constitute a serious public health problem due to their expressive morbidity and mortality. We highlight among arboviruses those caused by Flavivirus, such as dengue virus (DENV), Zika virus (ZIKV), Saint Louis encephalitis virus (SLEV) and Rocio virus (ROCV). Brazil has climatic and ecological conditions favorable to the proliferation of a great diversity of invertebrate vectors. The A. aegypti is the main vector of flavivirus in America, but A. albopictus and Culex spp. also presented vectorial competence for flaviviruses. The main objective of this study was to investigate the presence of Flavivirus in mosquito larvae of genera Aedes and Culex collected in 2016 and 2017 in Patos de Minas, MG. Pools of mosquito larvae (containing up to 15 larvae each) were collected by agents of the Dengue ServiceControl of Patos de Minas, in different neighborhood of the city. As larvae were sent to the Laboratory of Microbiology of UFU, where they were identified, processed and stored at -80°C. After extraction of total RNA, the samples were submitted to genus-specific RT-PCR. The viral specie of the positive samples was identified by a nested-PCR, usinginternal primersspecies-specific. A total of 115 larvae pools were collected between March 2016 and July 2017, with the majority identified as A. aegypti (247 larvae). 30 pools were tested and all were negative for Flavivirus. In addition to the continue of the molecular tests, the inoculation of the positive samples into C6/36 cell linage is predicted to allowing viral isolation. This work will contribute with information about the circulation of different Flavivirus species in Patos de Minas, since this information is not yet available. Financial support: FAPEMIG, CNPq, UFU.

Palavras-chave: Flavivirus, mosquito larvae, RT-PCR, epidemiology
Hepatitis B virus (HBV) infection represents a serious public health problem, considering cases of global morbidity and mortality. This pathogen presents a great genetic diversity, currently ten genotypes (A-J) have already been described. These genotypes are associated with variability in clinical evolution and therapeutic response, in addition to being broadly geographically distributed. In this context, the objective of this study is to identify the genotypic distribution of HBV in chronic hepatitis B carriers in a border region between two countries of South America, Brazil and Bolivia. To this end, during the first half of 2017, 19 individuals from the specialty consultations who had positive serology for hepatitis B, HBsAg and total anti-HBc reagents were invited to study. The genotypes were determined after extraction of the DNA according to the manufacturer's specifications of the QIAamp® DNA Mini Kit, after which the polymerase chain reaction (PCR) was performed to obtain a fragment of 416bp partially representing the region of the gene S. The DNA-HBV positive samples were sequenced and then the sequences were analyzed for the construction of a phylogenetic tree by the MEGA 6.0.6 Molecular Evolutionary Genetic Analysis software. The qualitative results for DNA-HBV were significant with a positive percentage of 57.89% of the samples (11/19). Based on the phylogenetic analysis, it was observed that the 11 individuals presented the genotype F. It is concluded that genotype F is characteristic of the native population of South America, with a high index of representation in the analyzed samples suggesting a homogeneity in the studied population, besides indicating that the means of transmission, allows the propagation of the genotype in the area investigated.

**Palavras-chave:** border area, genotypes, hepatitis B virus
The live attenuated 17DD Yellow Fever vaccines (YFV) are examples of a highly successful prophylactic intervention for controlling yellow fever (YF) disease expansion. However, YF vaccination in immune-compromised patients is contraindicated. One of the most common reason for contraindication of 17DD immunization is the frequent use of immunosuppressive medication in the treatment of rheumatic diseases. Our major goal is to investigate whether treatment with specific immunomodulators promotes a differential impact on the cellular immunity in patients with rheumatoid arthritis previously immunized with the YFV and who receive immunosuppressive medication. One hundred and eighty-three adults of both genders previously immunized with the YFV, age ranging from 23-86 years, were enrolled in this study and the subjects were categorized as healthy control group (CN; n=47), patients with rheumatoid arthritis who receive synthetic immunosuppressive medication (RA-Syn; n=83) and patients with rheumatoid arthritis who receive combined therapy, characterized by simultaneous use of synthetic and biological immunosuppressive medications (RA-Comb; n=53). To monitor the duration of immunity of the subjects, we analyze phenotypic-functional aspects of memory T and B-cells after 6 days of culture in the presence of YF-17DD virus (stimulated culture) or absence (unstimulated culture). The T-cells phenotypic analysis showed a higher percentage of naïve CD4 T-cells at RA-Syn as compared to CN. The early effector and central memory of CD8+ T-cells were increased and decreased, respectively, in both groups of patients (RA-Syn and RA-Comb) as compared to CN. Moreover, we observed a reduced frequency of effector memory at RA-Syn as compared to RA-Comb and CN. The B-cells analyses displayed decreased percentage of non-classical B-cells at RA-Comb as compared to RA-Syn and CN. The functional analysis showed reduced percentage of intracytoplasmic IL-5+CD4+ T-cells at both patients’ groups. In addition, there was increased percentage of IL-10+ CD8+ T-cells at RA-Comb as compared to RA-Syn. The results suggest that patients with rheumatoid arthritis have memory deficits, regardless of YFV vaccine stimulus, and the biological immunosuppressive medication employed at combination therapy may impair the duration of important memory biomarkers. **Financial Support:** CNPq, Instituto René Rachou/FIOCRUZ, Ministério da Saúde, Bio-Manguinhos/FIOCRUZ, FAPEMIG.

**Palavras-chave:** Adults, Duration of Immunity, Immunomodulatory Therapies, Yellow Fever vaccine
Several viral infectious agents have been affecting populations for centuries. Advances in molecular biology have enabled a better understanding of virology and identification of new viruses, such as HIV-1 and pandemic influenza. The wide dispersion of HIV-1 worldwide enables numerous clinical co-infections with other pathogens, such as influenza virus, due to the immunocompromised status of HIV-positive patients, especially during influenza pandemics. Surprisingly, in the 2009 influenza pandemic, several studies have described that the clinical outcome of influenza disease in most HIV-1 infected patients was very similar to that observed in immunocompetent individuals in the general population. The haemagglutinin (HA) of the influenza virus is essential for viral adsorption and is highly studied because of its importance in the pathogenicity of the virus and in the immune response of the individual. It is possible that during HIV-1 / influenza coinfection both positive and negative replication signals of both viruses may exist, therefore during coinfection a new equilibrium should be imposed on the host. In this context our results demonstrated that peripheral blood mononuclear cells (PBMC) from healthy donors was infected with HIV-1 and after treatment with influenza virus showed inhibition of HIV-1 replication. Likewise, when infected PBMC were treated with three influenza virus hemagglutinin subtypes (H1, H3 and H5), inhibition of HIV-1 replication was observed, and the H1 was the most potent subtype against HIV-1. It is worth noting that long term nonprogressor patients (LTNP), who do not progress to AIDS even in the absence of antiretroviral treatment, present an increase in TLR4 levels. Thus, PBMCs infected with HIV-1 were treated with specific antibodies in order to block TLR4 receptors and then treated with HA and LPS, classic ligand and TLR4 activator. Then we observed that treatment with HA, as well as LPS, inhibited HIV-1 replication, and we also noticed that blocking TLR4 prevented the inhibition of HIV-1 replication by HA. Taken together, our results demonstrate that the influenza virus, as well as its HA, inhibits HIV-1 replication, in vitro, which is dependent on TLR4.

Palavras-chave: haemagglutinin, HIV-1, Influenza virus
Resumo

Respiratory virus infections are the most common disease in humans, each adult presented in average one to three acute respiratory infection annually. An important risk population for acute respiratory infections (ARI) are immunocompromised patients, such as renal transplant recipients. This study aims to evaluate the incidence of acute respiratory infections of probable viral etiology and its impact on the ambulatory care of renal transplant recipients in the Hospital de Base of São Jose do Rio Preto, and assess the clinical/epidemiological profile of the patients. One hundred and seven medical records were analyzed from the period of January 2012 to July 2015. The data obtained were age, gender, suspicious episodes of acute respiratory infection and the probable viral etiology by clinical/epidemiologic parameters. The results showed that 58.9% of the transplant recipients were men (mean age of 44.14 years old, median of 45 years and standard deviation of 13.2). From these 107 medical records, 61.7% of the patients had some suspicious episode of acute respiratory infection, of these recipients, 60.6% were men and the infections were more frequent in the age group of 50-60 years old. There were a total of 133 respiratory manifestations with an average of 2.015 episodes by each patient in the post transplant period, among these episodes the most prevalent symptoms were cough (81.3%), coryza (36.6%) and fever (27.6%). It was also analyzed the distribution of the episodes over the months of each year (2012-2016), and no seasonal pattern was identified. Eighth nine episodes (65.4%) had medical prescription recorded, and of these, 70.1% prescribed antibiotics. The 133 manifestations of infection were analyzed for probable viral etiology, and among these, 29 (21.8%) presented clinical signs/symptoms of acute respiratory disease of probable viral etiology. Preliminary data analysis showed a significant impact of the viral respiratory infection among immunocompromised patients analyzed. Financial Support: CNPq, FAPEMIG

Palavras-chave: Respiratory viral infection, Transplanted patients, Acute Respiratory infection
Acute respiratory infections (ARIs) are a substantial cause of global morbidity and mortality. The Paramyxoviridae family has three important viral species that cause this kind of infection: respiratory syncytial virus (RSV), human metapneumovirus (hMPV) and human parainfluenza virus 3 (PIV 3). However, there is no preventive vaccine against these pathogens and the available treatments are expensive and liable to have adverse effects. The development of antiviral peptides (AVPs) derived from fusion proteins is an attractive option for the treatment of ARIs and bioinformatics tools are important for prediction of new AVPs, saving time and costs. This study aimed to predict AVPs derived from the fusion protein of RSV, hMPV and PIV 3. Nucleotide sequences of the fusion genes from different strains of these species identified worldwide in different years were obtained from GenBank. Multiple progressive alignments (Muscle and ClustalOmega) were performed to identify conserved regions in the nucleotide sequence of each viral species. The CAP3 Sequence Assembly Program and Weblogo tools (version 2.8.2) were used to obtain the consensus sequences for the fusion gene of each viral species and later translated with the Translate tool (Expasy). The conserved regions of the consensus sequence of the fusion protein were used in the AVPpred and AVP-IC50pred servers for the prediction of AVPs and determination of the IC50, respectively. Peptides that presented results >50% in the physical-chemical characteristics and amino acid composition models were considered a possible AVP and selected to determine the 3D structure with the PEP-FOLD software. In silico analyzes predicted 9 specific unpublished AVPs against RSV, 8 against hMPV and 5 against PIV3. In addition, the IC50 values for these AVPs ranged from 6.31μM to 0.3μM, being classified as effective or highly effective, respectively. The use of bioinformatic tools may be an alternative for the screening of AVPs against respiratory paramyxovirus, reducing costs and time of experimentation. Financial support: FAPEMIG, UFU.
Influenza is a globally distributed viral respiratory infection, which presents seasonal epidemic behavior. Genetic rearrangements involving different strains of influenza virus gave rise to highly transmissible swine-origin influenza A (H1N1), that produced the 2009 pandemic. Cumulative antigenic changes may enable the reintroduction of a given strain in a previously exposed population. In order to describe (H1N1)pdm09 circulation in Brazil since the pandemics, results of all respiratory samples tested for influenza detection in a private lab, from June 2009 to June 2017, using real-time PCR standardized according to CDC – USA protocol, were obtained from a database. During that period, 17,448 samples have been processed, of which 5,719 (32.8%) have been positive. Our data demonstrated a cyclic epidemic pattern of (H1N1)pdm09 circulation, which is indeed expected for a new influenza strain after its emergence. After 2009 pandemics, when 3,545 samples have been tested and 35% of them were positive, the demand was much lower from 2010 to 2012 (mean 649 samples/year). Although the proportion of positive samples remained stable (25% in 2011, 29% in 2012 and 22% in 2013), the frequency of (H1N1)pdm09 detection decreased along those years (89% of positive samples in 2009, 67% in 2010, 29% in 2011 and 27% in 2012). In 2013, however, another peak of circulation has been observed, when 2,122 samples were analyzed, with 22% of positive results and 84% of them corresponding to (H1N1)pdm09. A similar pattern has occurred in the subsequent years: in 2014 and 2015, we analyzed 794 and 619 samples, 21% and 26% of them were positive, and (H1N1)pdm09 corresponded to 13% and 3% of positive samples, respectively. A third epidemic wave became evident in 2016: 7,736 samples were processed, 40% of which were positive and (H1N1)pdm09 represented 94% of influenza strains detected. Interestingly, the epidemic waves have started earlier each year. Although, in 2009, the peak of confirmed cases was registered between July and August, in 2013 it occurred in May; in 2016, in turn, positive results increased progressively from February ahead, peaking in April. Together, our data show that epidemic waves A (H1N1)pdm09 are followed by periods of low circulation of this strain, probably because of natural immunization of population, besides increased demand for vaccination. However, the immunization has limited duration, which allows subsequent epidemics in a cyclic pattern.

Palavras-chave: viral respiratory infection, swine-origin influenza A, H1N1 pdm09, real-time PCR
Influenza: The Diagnosis Improvement in 2016 to 2017

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Resumo

Introduction: The Influenza Viruses, an orthomyxoviruses family, causes infectious respiratory disease outbreaks across the world since 1918. Normally they circulate throughout the whole year, but increases at specific cold periods. The aim of this work is characterize the Influenza suspected cases diagnosis by Real Time- Polimerase Chain Reaction (RT-qPCR) and Immunofluorescence Assay (IF) diagnostic results of the Brazil’s Public Health Laboratories in 2016 to June 2017. Materials and Method: Were analyzed the national data bank of the official laboratory software named “Gerenciamento de Ambiente Laboratorial” (GAL) with Excel®2010 and SPSS®v.20 during the period of 2016 to 2017 (First semester). Results: There are 173,960 influenza suspected exams in 2016 to June 2017. Comparing the first semester (Epidemiological Week 1 to 26) of 2016 (95,194) and 2017 (78,766) has been a decrease of 17.25% on the number of influenza exams requisitions. At this same period, the IF exam has been also decreased by 11.7% (14,530/16,461) but the RT-qPCR increased 49% (64,236/43,063). In 2017, the percentage of release of examination requests is 50%, so approximately 40,000 exams already have the final result. The median time of process and release those exams has been decreased 17% of the IF assays, 23 to 19 days, and 50% to RT-qPCR assays, 20 to 10 days. Both in 2016 and 2017 the positivity of the samples collected has agreed about in 20% but the data demonstrate that has not yet peaked. Conclusion: Was possible to observe that the sample collection of the suspected cases decrease can be a result of the seasonal wave displacement thus that the positivity tends to increase. Beyond that, it is certain that were an optimization of the laboratory diagnosis which is important once Influenza is a pandemic concern and requires a rapid surveillance response. Financial Support: Ministry of Health

Palavras-chave: Diagnosis, Influenza, Public Health, Virology
INVESTIGATION OF RABIES VIRUS IN NON-HUMAN PRIMATES DURING EPIZOOTICS OF YELLOW FEVER IN MINAS GERAIS, 2017

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Resumo

Rabies virus is the agent of a lethal encephalitis, and it causes approximately 55,000 human deaths every year. Rabies virus has a worldwide distribution, and primary reservoirs include foxes, bats, raccoons, skunks, dogs, cats, cattle, and others. The virus can be transmitted via saliva by bite, scratch, or abrasion. Rabies can cause infection in non-human primates (NHP), and in Brazil, rabies has been described in marmosets in Northeast Brazil in Rio Grande do Norte, Ceará, Piauí and Pernambuco States, in capuchin monkeys in São Paulo and monkeys in Mato Grosso state. At least 20 cases of human cases have been associated with contact with monkeys in Brazil. In 2017, Brazil faced many epizootics of Yellow fever (at least 642 epizootics have been confirmed, and 1448 are under investigation, and the estimative point to the death of more than 5,550 NHP). A total of 121 municipalities in Minas Gerais state confirmed YF epizootics and epizootics are suspected in other 351 municipalities. Laboratory of Zoonosis (LZOOON)- Belo Horizonte City Hall have already received 796 carcasses of NHP, including Callithrix sp, Alouatta sp, Cebus sp, Callicebus sp from rural and urban areas during YF epizootics in 2017. The increase in the number of NHP carcasses is more than 39 times when compared to previous years (based on data available of 2013-16). A total of 662 carcasses were in good condition to be tested for the presence of rabies virus, according to the standard protocols of LZOON. Brains samples from each animal were submitted to direct fluorescent antibody (dFA) test, and all animals were negative. Following, brain macerates are used for the biological tests, when they are used for intracranial inoculation of six Swiss albino mice and observation for rabies symptoms. A total of 60 animals has been double tested and were negative (in dFA and in the biological proof). The results, so far, do not indicate the circulation of rabies virus in NHP in MG, however, surveillance is essential to detect and control this disease. Financial support: FAPEMIG, CNPq, CAPES , Prefeitura de Belo Horizonte, MG.

Palavras-chave: Rabies virus, surveillance, Non-human primates
Zika virus (ZIKV) is an emerging mosquito-borne virus of the family *Flaviridae* and genus *Flavivirus*. This scenario of ZIKV infection linked to severe neurological complications as well as the establishment of ongoing ZIKV outbreaks in several countries in Latin America led to the WHO to declare ZIKV an international public health emergency. The transmission of ZIKV has been associated with *Aedes* spp. However, ZIKV also can be disseminated by sexual contact, perinatal transmission, and blood transfusion. Also, it was demonstrated ZIKV particles in urine, breast milk and saliva from infected patients. In this study, we showed an initial characterization of two viruses from the same outbreak: ZIKV Rio-U1 (Genbank: KU926309.2), isolated from a pregnant woman’s urine and ZIKV Rio-MB1 (Genbank: KY272991), isolated from a human breast milk. For this purpose, we compared ZIKV infection in four cell lines, Vero (African green monkey kidney), SK-N-AS (Human neuroblastoma), ST88-14 (Human malignant peripheral nerve sheath tumors) and ARPE-19 (Human retinal pigmented epithelium), using a viral multiplicity of infection (MOI) of 0.02. We hypothesized that these cells could be very susceptible to ZIKV due to its origin (SK-N-AS, ST88-14 and ARPE-19), and we worked with VERO cells because it's a well-established cell line to study flaviviruses. We observed that Rio-U1 has a higher viral titer compared to Rio-MB1 in all cells tested. Also, VERO and ARPE-19 cells demonstrated the higher viral titers peaks. Our results showed that there are differences in the biological aspects between these two ZIKV isolates. However, further studies in animal models would clarify if these differences will be associated with virulence. Financial Support: Oswaldo Cruz Institute/Fiocruz; MCTIC/FNDCT -CNPq / MEC-CAPES/ MS-Decit Nº 14/2016 – Prevenção e Combate ao vírus Zika.

**Palavras-chave:** Arpe-19, SK-N-AS, ST88-14, VERO, ZIKA VIRUS
Enteroviruses (EVs) are responsible for a wide spectrum of clinical diseases in humans, ranging from minor febrile illness to severe. Most infected persons are asymptomatic, mild presentations can include hand, foot, and mouth disease, respiratory or gastrointestinal symptoms, acute hemorrhagic conjunctivitis, and herpangina. Among the more severe syndromes associated with EV are myocarditis, neonatal sepsis, and infections of the central nervous system (acute flaccid paralysis, encephalitis and mainly aseptic meningitis). EVs comprise a large genus in the Picornaviridae family, order Picornavirales, with more than 100 serotypes delineated into four species known to infect humans (EV-A, EV-B, EV-C and EV-D) based mostly on their phylogenetic relationships. The aim of this study was to describe the occurrence of EVs infections in the São Paulo State, Brazil during 2015-2016. A total of 609 clinical specimens from patients (stool, eye-conjunctival-swat, rectal swab, biopsy, pericardial fluid, and cerebrospinal fluid) were analyzed by virus isolation and Real-time reverse transcription PCR for the presence of EVs. Conventional reverse transcription PCR, and VP1 partial sequencing were performed to determine serotype of EVs detected. Out of 609 samples, 29.7% were EV-positive. Fifty-four positive samples could be characterized with serotypes belonging to EV species A to C. The most commonly types during this period were Echoviruses 6 (E) (33.3%), followed by Coxsackievirus (CV) A16 (16.7%), and E-9 (16.7%), E-30 (13.0%), E-7 (5.6%), E-C99 (3.7%), CV-A6, CV-A24, E-11, E-13, E-20, and E-21 (1.8%). The emergence of CV-A16 was related to an outbreak of hand, foot, and mouth disease with neurological symptoms. Interestingly, several serotypes rarely identified in Brazil have been identified, e.g. CV-A16, CV-A6 and EV-C99. This study showed an extensive circulation of a variety of EV strains in São Paulo State. Our study of laboratory surveillance will serve as subsidies for the development of public health measures for the control and prevention of EVs infection in at-risk individuals in São Paulo State.

**Palavras-chave:** enteroviruses, serotype, coxsackievirus, echovirus, surveillance
8. In addition, from 2 samples positive in qPCR, 1 was sequenced, and phylogenetic analysis demonstrated that these samples were grouped together with the South American genotype I. The analysis demonstrated that these samples were grouped together with the South American genotype I. The analysis demonstrated that these samples were grouped together with the South American genotype I. The analysis demonstrated that these samples were grouped together with the South American genotype I. IL-10; IL-6 and IL-12 (CBA) was performed with patients serum (3 weeks post infection), and resulted in a significant increase of IL-10; IL-6 and IL-8. In addition, from 2 samples positive in qPCR, 1 was sequenced, and phylogenetic analysis demonstrated that these samples were grouped together with the South American genotype I. The recent outbreak of YF in Minas Gerais has great impact on regional hospitals, which are not prepared for the diagnosis of this agent and often do not have the capacity to support a large number of patients in ICUs. In fact, South American genotype I was associated a fatal case in ICU. The patients presented an intense inflammatory response, mainly mediated by IL-6 and IL-8, intense hepatic injury and acute renal failure. Financial Support: Capes, CNPq, Fapemig, UFVJM, Bioclin.

**Palavras-chave:** Yellow Fever virus, Epidemiology, Molecular characterization
LVBA-RECHTLV-1/2: A CHIMERIC MULTIEPIPOTE PROTEIN WITH POTENTIAL FOR DEVELOPMENT OF SCREENING AND CONFIRMATORY HTLV-1 AND HTLV-2 DIAGNOSTIC TESTS

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Resumo

The Human T-lymphotropic virus (HTLV) is the first retrovirus isolated in humans. HTLV-1 virus is responsible for causing Adult T-cell leukemia/lymphoma (ATL), HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and other important inflammatory conditions. Clinical manifestations with HTLV-2 are not very common, although there are some reports of a similar HAM/TSP syndrome and association with bacterial infections. According to the algorithm suggested by the Ministry of Health, the serological diagnosis is based on the detection of specific antibodies against HTLV-1/2. However, the occurrence of indeterminate results indicates the need to improve the currently tests already in use. Also, the development of alternatives and national inputs technology for screening of blood products is essential to ensure the implementation and maintenance as health procedure with full coverage by the state and also to be transferred to Brazilian blood banks after validation. The multiepitope proteins are a new approach in the development of tests for various infectious agents, with high sensitivity and specificity, resulting from increased epitope density. The aim of this work was to construct a chimeric protein (LVBA-recHTLV-1/2) with immunodominant antigens for HTLV-1 and HTLV-2 for the development of screening and confirmatory tests for HTLV-1/2 infections. This multiepitope protein was designed using p19, gp46 and Tax sequences of HTLV-1 and 2 and a histidine tail for protein purification in Äkta Start system by affinity chromatography using nickel preloaded sepharose columns after expression in Escherichia coli Rosetta-gami 2(DE3). In the Western blot assays the chimera was able to recognize in a specific manner pool of sera from HTLV-1+, HTLV-2+ (GIPH cohort) and commercial anti-histidine monoclonal antibody, but not the uninfected individuals pool of sera (GIPH cohort). Using an indirect in house ELISA, the LVBA-recHTLV-1/2 was able to specifically differentiate sera from HTLV-1 and 2 infected individuals and seronegative individuals (GIPH cohort and from a cohort containing subjects from São Paulo and Pará) with significant statistical difference (p <0.0001). The ROC curve analysis showed great accuracy with area under the curve 0.9662 and 0.9787 for clone 4.1 and clone 4.2 respectively. It was concluded that the chimera possesses great potential as antigen for the development of assays for HTLV-1/2 screening and confirmatory diagnostic.

Palavras-chave: HTLV-1, HTLV-2, Diagnostic, Multiepitope proteins
Meningoencephalitis caused by Flavivirus: situation of Minas Gerais State.


Resumo

Meningoencephalitis is the clinical condition in simultaneous inflammation of the encephalon and meninges occurs. Viral meningoencephalitis is the main cause of central nervous system (CNS) infections in the world, and viruses as Humanherpesvirus (HHV) and enteroviruses (ENTV), as well as flaviviruses are the main cause of these infections. The aim of this work is to detect and characterize the flaviviruses in cerebrospinal fluid (CSF) of patients with CNS infections. We analyze 94 CSF samples from children of the Hospital Infantil Joao Paulo II between the years 2014 and 2016. From these, 15 samples (16%) were positive for Dengue virus (DENV); 3 samples (3%) were positive for ENTV; 2 samples (2%) positive for HHV-1/2 and 1 sample (1%) positive for Zika virus (ZIKV). In two samples coinfection between ENTV and DENV was detected. The molecular diagnosis showed DENV-3 as the most prevalent among the dengue viruses with seven positive samples, followed by DENV-2 with four positive and DENV-1 with two positive samples. None sample was positive for DENV-4. We detected cases of double infection between DENV-1 and 3 and DENV-2 and 3, and cases of triple infection among DENV-1, 2 and 3. The samples were sequenced and the sequences of DENV-1 grouped with genotype V, that was related with other viruses which circulated in Brazil, and three samples grouped with genotype II that has never been described in Brazil before. The sequences of DENV-2 grouped with Asian II, and only genotype American Asian have been described in this country. The DENV-3 sequences grouped with genotype I and III that previously has been described here. The sample positive for ZIKV was related with post viral Guillain Barré syndrome. The detection of the agents involved in CNS infection allow the clinical therapy decisions antibiotics interruption and epidemiological information. Financial support: CAPES, CNPq and FAPEMIG.

Palavras-chave: Meningoencephalitis, flavivirus, Central nervous system
Acute gastroenteritis is the second cause of childhood mortality in worldwide. Several pathogens are related to this illness, especially group A rotavirus (RVA), which is responsible for 215,000 child deaths annually, mostly in developing countries. G12 RVA was first detected in 1987 in Philippines, and since this genotype has been described in several countries, being the sixth most prevalent RVA genotype in worldwide. Thirty fecal samples collected from children with diarrhea, previously genotyped as G12, were selected from Network Surveillance of Rotavirus Gastroenteritis, coming from Para, Amazonas and Acre States. Posteriorly, viral genome was extracted and subjected to polyacrylamide gel electrophoresis (PAGE) and polymerase chain reaction preceded by reverse transcription (RT-PCR) for the target genes. The obtained amplicons were purified and sequenced. PAGE showed that 50% (15/30) of G12 RVA had long electropherotype profile. With respect to RT-PCR, the amplicons were obtained in 18 (60%) of samples for VP4 gene, 22 (73%) for VP6 and 30 (100%) for VP7. Phylogenetic analysis showed that G12 RVA strains belonged to lineage III, both for VP7 and VP4 genes. There were aminoacidic substitutions between current samples in 2013 and 2014 in the three genes. Most of the samples (60%) presented the G12-P[8]-I1 genotype. In Northern Brazil, as reported in other regions of the world, G12P[8] RVA genotype is more associated with Wa-like genogroup, as described in this study. There was no identification of G12 RVA variants or atypical combinations and recombination events from the samples collected during the study period. Thus, there was probably no introduction of novel variants of this genotype in the population.

Financial support: Instituto Evandro Chagas (IEC/SVS/MS) e Conselho Nacional de pesquisa e tecnologia (CNPq)

Palavras-chave: Rotavirus, Gastroenteritis, Genes
The influenza viruses are important pathogens in public health, contributing for thousands of deaths every year worldwide. They present a high variability as main characteristic, due to its segmented genome and the antigenic diversity of the surface glycoproteins, Hemagglutinin (HA) and Neuraminidase (NA). The high diversity of the influenza viruses impact directly in the choice of vaccine strains and successful treatment. Thus, the aim of this study was to analyze the variability of the HA and NA of the influenza A viruses that circulated in the North and Northeast Regions of Brazil in the year of 2017. Between January and June 2017 were analyzed 1,980 samples collected from patients attended in Health Units in the States of the Acre, Amapá, Amazonas, Ceará, Maranhão, Pará, Paraíba, Pernambuco, Rio Grande do Norte and Roraima. The analysis of the samples involved the extraction of viral nucleic acid, detection by real time RT-PCR, amplification of the genes that encodes the surface glycoproteins HA and NA by RT-PCR and sequencing these genes. The influenza A viruses were detected in 239 (12,07%) samples, being 235 (98,3%) A(H3N2) and 4 (1,7%) A(H1N1)pdm09. Among the positive samples were selected 66 influenza A(H3N2) strains and 02 influenza A(H1N1)pdm09 strains for sequencing. The analysis of the HA gene of the influenza A(H3N2) strains revealed the circulation of the genetic subclade 3C2.a1, which is defined by the amino acid substitutions N171K in HA1 and I77V and G155E in HA2. Among the influenza A(H1N1)pdm09 strains all were genetically related with strains of the subgroup 6B.1, which is characterized by the substitutions S84N, S162N and I216T in HA1. The analysis showed the genetic similarity of the strains isolated in the North and Northeast Regions with the vaccine strains. Concerning analysis of the NA gene, no substitution described related to antiviral resistance was observed among strains analyzed at the moment. Our study show the predominance of the strains influenza A(H3N2) virus circulation, as observed in other places around the world. We observed that both influenza A(H3N2) and influenza A(H1N1)pdm09 strains isolated in the North and Northeast Regions of Brazil were genetically related to vaccine strains recommended by the World Health Organization. Thus, our study presents important data for epidemiological surveillance, which may help in the decision making of prevention and control of Influenza virus infections in Brazil.

Palavras-chave: Influenza A Viruses, Molecular Characterization, Diversity
MOLECULAR DETERMINATION OF VIRAL INFECTIONS IN CLINICAL SAMPLES OF PATIENTS AFTER HAPLOIDENTICAL STEM CELL TRANSPLANTATION

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Introduction. Hematopoietic cell transplantation (HSCT) is the treatment of choice for high-risk hematological diseases and in patients may not find compatible donors, there is the alternative of haploidentical HLA-HSCT (hHSCT), used when compatibility between the donor and the recipient is 50% or closer. Viral infection or reactivation remains a major cause of morbidity and mortality after allogeneic stem cell transplantation. The most common viruses are herpesvirus family, polyomavirus BK, parvovirus B19 and adenovirus and are the main viruses addressed in this study in relation to HSCT-haplo patients during the period of 100 days after transplantation or during the patient’s survival. Patients and Methods. Twenty HSCT recipients divided in two groups will be included in this study. The first is the study group, compound of haploidenical transplant recipients (hHSCT) and the other one is the allogeneic stem cell transplant recipients (HSCT), which will be the control group. Blood, urine and cerebrospinal fluid samples were collected so far when indicated by the physician. Diagnostic techniques such as PCR and Nested-PCR will be used to identify the viral infection and confirm the presence or absence of the viruses studied in the sample, according to the specific methodology for each virus. Results: Eight patients were included in this study. Seventy-eight samples of plasma and urine were collected from these patients. Forty-one out of 78 (52.5%) samples were positive for at least one virus (virus reactivation). HSV-1 was positive in 1/41 (2.4%) samples, VZV was positive in 2/41 (4.9%) samples, EBV in 2/41 (4.9%) samples, CMV in 2/41 (4.9%) samples, HHV-6 in 7/41 (17%) samples, Adv in 1/41 (2.4%) sample, BK in 24/41 (58.5%) samples and B19 in 2/41 (4.9%) samples. The most frequent virus was Polyomavirus type BK, which caused hemorrhagic cystitis. Conclusions. At the end of the study, it will be possible to analyze the clinical impact of these viruses infection in the period after transplantation. We will correlate both groups and in order to provide tools for better understanding and prevention of prognosis.

Palavras-chave: polyomavirus, hla-identical, haploidentical, stem cell transplant, virus infections
Resumo

Zika virus (ZIKV) infection has been linked to the occurrence of diverse neurological manifestations. Guillain-Barré syndrome, the most common neurological manifestation associated with Zika infection, is a neurological condition of probable autoimmune origin, considered the most common cause of flaccid paralysis in the world. This study aims to describe the reported cases of neurological manifestations in patients whose samples were sent to the Central Public Health Laboratory of the State of Minas Gerais for investigation of ZIKV infection. The data sheets of the National Information System for Notification Diseases (SINAN) received with serum samples for the diagnosis of ZIKV infection in patients with neurological manifestations from January 2016 to May 2017 were analyzed. From the analysis of the records, information was obtained regarding age, sex, symptoms, city of residence and type of neurological manifestations of the patients. Serological tests (anti-Zika IgM and IgG screening, as well as anti-Dengue IgM) were performed by ELISA using commercial kits and molecular analysis by real-time RT-PCR (RT-qPCR) using in house methodology. Of the 7004 samples received, 60 were of patients with neurological manifestations. Most of the patients (41) were notified for Guillain-Barré syndrome, and the others for various neurological manifestations. Serology was performed for ZIKV in 50 samples, with 2 IgM positive, 20 IgG positive, and 4 positive for both. The samples were also tested for anti-Dengue IgM, with 5 of them positive (3 positive for Zika and Dengue, suggesting cross-reaction). Patient ages ranged from 1 to 81 years, with a mean of 34. Regarding gender, 32 were male and 28 female. Of the 12 samples tested by RT-qPCR, none showed positive results. The most commonly reported symptoms of ZIKV infection were fever (20), headache (17), rash (9), and arthralgia (9). For 30 patients, there was no information about symptoms. Cases of neurological symptoms possibly associated with ZIKV infection were identified in 33 cities in Minas Gerais. Data from the serological tests, as well as the collection of information regarding the symptomatology, indicate a possible association of the infection with ZIKV and the development of neurological manifestations, but more studies are require to the better comprehension of this association.

Palavras-chave: ARBOVIRUS, DIAGNOSIS, EPIDEMIOLOGY, ZIKA
NEW DERMATOLOGICAL AND NEUROLOGICAL FINDINGS ASSOCIATED TO CHIKUNGUNYA OUTBREAK IN AREAS WITH ZIKA CO-CIRCULATION.

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Resumo

Chikungunya virus (CHIKV) is an Alphavirus transmitted to humans through Aedes mosquitoes. The infection persists for 1-10 days and results in a symptomatic condition characterized by intense arthralgias, myalgia, high fever, headaches and rash. During a Zika virus outbreak in the northeast of Brazil in 2015, it was observed a high rate of CHIKV co-circulation in pregnant women causing abnormal birth outcomes. The goal of this study is to identify the incidence of CHIKV in the inner of Paraíba state (Brazil) describing new clinical manifestations associated with the virus infection. We are following 1,000 pregnant women suspect to expose to arboviruses (BRAZIKA cohort) who resided in Campina Grande and surrounding municipalities (2015-2017). The clinical samples from mothers enrolled in this study were tested for CHIKV by reverse transcriptase polymerase chain reaction (RT-PCR) assay or serological test. Amongst the 650 samples tested so far, 56 (8.5%) were positive to CHIKV and the majority of samples collected were urine and serum followed by amniotic fluid and blood from umbilical cord from newborns. The presence of the CHIKV genome in the uterine environment such as amniotic fluid and blood from umbilical cord suggests that the virus could also be associated with teratogenic complications during the pregnancy. In fact, we observed imperforate anus, hydranencephaly, and romboencefalosinapse as developmental abnormalities and neurological damage possibly caused by CHIKV intrauterine expose. Besides this scenario, we identified dermatological manifestations in children such as vesiculobullous lesions, blister, and edema. To investigate if these lesions were associated with CHIKV, the viral genome was recovered from skin biopsies by Next Generation Sequencing. The whole virus genome sequencing is being produced to investigate virus polymorphisms associated with these severe cases of CHIKV infection observed. Finally, our results suggested a higher incidence of CHIKV among pregnant women and children at the same geographic region of ZIKV outbreak and its possible association with birth defects, showing the importance to consider CHIKV in congenital syndromes during pregnancy in epidemic areas. Financial Support: CNPQ.

Palavras-chave: Chikungunya, Dermatologic, Neurologic, Northeast Brazil, Symptoms
ONE STEP REAL-TIME RT-PCR DETECTION OF ALL DENGUE VIRUSES WITH A SINGLE PROBE AND INTERNAL CONTROL

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Resumo

Dengue is the most important arboviral disease worldwide, and human infection can be caused by any of the 4 types of Dengue virus (DENV1 to DENV4). The virus is transmitted by infected female mosquitoes from the genus Aedes. The disease can present asymptomatic, subclinical or symptomatic infections to more severe manifestations like hemorrhagic fever or shock syndrome. There are no specific antiviral drugs for dengue virus infection and effective vaccines are still a challenge. Diagnostic methods are based on virus isolation in cell culture, serological or molecular tests. However, molecular detection of DENV 1 to 4 aiming epidemiological surveillance currently demands genotyping, which can be laborious, time consuming and expensive. To the best of our knowledge, a single set of oligonucleotides able to detect all four viruses in a RT-PCR is also not available. In this scenario, we developed a one-step TaqMan real-time RT-PCR consisting of a single probe and single pair of primers, able to detect all four types of DENV. The protocol was developed with local reagents produced at Molecular Biology Institute of Paraná (IBMP/Fiocruz-PR), and contains an endogenous reaction control detecting the 18S ribosomal RNA gene. The protocol detected extracted RNAs from different lineages of DENV 1 to 4, previously diluted in DENV-negative human plasma, and showed no cross-reaction against the major human arboviruses (Yellow Fever, Zika, St. Louis Encephalitis, West Nile, Ilheus, Chykungunya, Mayaro, Oropouche, Equine Encephalitis of East and West, Mucambo, and Venezuelan Equine Encephalitis). We propose that the present protocol can be used for pre-screening during epidemiological studies or outbreaks. Further validation of the reaction shall be done in a larger number of patients in order to determine specificity and sensitivity. Financial Support: Molecular Biology Institute of Paraná (IBMP), BNDES.

Palavras-chave: Dengue virus, detection, real-time RT-PCR, internal control
PCR-DETECTION OF HPV TYPE 16 IN CERVICAL TISSUES OBTAINED FROM WOMEN WITH CIN AND INVASIVE CANCER.

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Resumo

Squamous-cell cervical cancer (SCC) is the second most common cause of cancer mortality in women worldwide. Among Brazilian women, cervical cancer is the third most common neoplasia with an overall incidence rate of 5.7%. In 2014, 15,590 new cases of invasive SCC cases were identified, with an estimative rate of 15.33 cases per 100,000 women. Cervical Intraepithelial Neoplasia (CIN) is a premalignant cervical lesion, which can progress to cervical cancer if untreated. Above 90 human papillomavirus (HPV) types have been officially classified, with over 40 types commonly infecting cervical epithelial cells. However, only a subset of these cervical HPVs, termed High-risk (HR) HPV types are involved with the development of cervical premalignant/malignant conditions, mainly the type 16 followed by the HPV types 18, 31, 33, 35, 45, 52 and 58. The establishment of a persistent infection by oncogenic HPVs is the most frequently detected cause of invasive cervical cancers and CIN. The purpose of this study was to investigate the prevalence of HPV16 in paraffin-embedded tissues (PETs) biopsies obtained from women from Belo Horizonte city, Minas Gerais state, Brazil, and who presented a histopathological diagnosis of cancer and CIN2 (grade 2). Therefore, we examined the HPV16-DNA prevalence in 49 PET-cervical biopsies diagnosed by means of a precise histopathological analysis as CIN2 (14) and SCC (35) cases. By using a conventional Polymerase Chain Reaction (PCR) protocol, the samples were initially subjected to a direct PCR with HPV16F/R primer-set to identify the presence of HPV16-DNA. Subsequently, negative samples were submitted to a more sensitive PCR protocol (Hemi-nested PCR) using the E6CF4/E7CR3 and E7CR3/16SF1 primers-sets to improve the virus detection effectiveness. The general prevalence of HPV16-DNA was of 92% (45/49) among the analyzed samples, with 86% (12/14) of positivity in CIN2 samples, and 94% (33/35) of positive cases in cervical cancer tissues. Our results pointed out a high incidence of HPV16 infection among the analyzed women, and reinforce the need of taking protective measures to prevent HPV infection, in special with the HPV16 genotype, particularly among sexually active women. If promptly diagnosed, cervical cancer presents one of the highest potentials of prevention and cure. Financial Support: CNPq and FAPEMIG.

Palavras-chave: High-risk HPV, HPV-16 infection, Paraffin-embedded tissues (PETs), Polymerase Chain Reaction (PCR)
Resumo

Rabies is an anthropozoonosis characterized by progressive and invariably fatal encephalitis, its main form of prevention is based on the immunoprophylaxis of animals and humans. Due to the high lethality and the lack of a specific and effective treatment for rabies, professionals who engage in risk activities and students who may experience some type of viral exposure should perform pre-exposure prophylaxis. This research refers to a qualitative study, carried out in the period of September 2016, where the calculations were plotted in a database and a descriptive statistics of simple and relative frequency was performed with the help of Excel software. A multiple choice questionnaire was applied to students of first (99), third (79) and fifth (34) year, totaling 212 students. The results show that although they are aware of the disease, few performed the immunoprophylaxis. Only 30% (65/212) performed the pre-exposure scheme, and the result varied according to the academic year. In the first year 23% (23/99) students performed pre-exposure prophylaxis, and 39% (9/23) took the serological test more than one year, in the third year the result was similar, 30% (24/79) performed pre-exposure prophylaxis and 52% (12/24) took the serological test more than one year, in the fifth year 53% (18/34) were submitted to pre-exposure prophylaxis and 17% (3/18) took the serological test more than one year. Of the 69,3% (147/212) students who did not perform the pre-exposure scheme, being able to choose more than one option as an answer: 78,7% (167/212) are not aware that they can perform prophylaxis being a student, 58% (123/212) stated that the site is inaccessible, 32,5% (69/212) have no interest in pre-exposure and 26,9% (57/212) say they were not attended at the immunization site. Although an increase in the percentage of vaccinated students has been observed according to the semester, the number of susceptible students to rabies virus infection is still significant.

Whether due to lack of knowledge or lack of interest of the students, these results show a significant deficiency in the pre-exposure of an important risk group. Because of the potential for viral exposure during the course and the lethal nature of the disease, it is crucial that students be made aware from the beginning of the course of the immunization possibility and of the risks associated with rabies. Financial Support: Faculdades Metropolitanas Unidas

Palavras-chave: Immunoprophylaxis, Rabies, Vaccine
Introduction. A neurological disorder caused by a viral infection is a fatal medical emergency. The approach to these complications requires a rapid neurological evaluation, since the sequelae may be irreversible, consequently, leading to death. Herpesvirus simplex (HSV-1 and HSV-2) and Varicella Zoster (VZV), members of the Herpesviridae family, are responsible for causing damage to the nervous system, especially acute encephalitis, meningitis, meningoencephalitis and myelitis in immunocompromised patients. The Polyomavirus (JC), member of the Polyomaviridae family, is highly associated with progressive multifocal leukoencephalopathy, a demyelinating infectious disease and it is considered a virus that has tropism for nervous system cells, as well as HSV-1 and HSV-2. Once these harmful agents infecting an organism, they establish latency for the rest of the host's life. Their reactivations may occur in immunocompetent and immunocompromised patients. This project aims to detect the presence of the DNA of HSV-1, HSV-2, VZV and polyomavirus JC in 100 samples of cerebrospinal fluid (CSF) of patients with suspected viral infection of nervous system using the Polymerase Chain Reaction (PCR) technique. Recognizing the true cause of the viral infection in the nervous system, it can provide a better treatment to patients, reducing the impact of these diseases. Patients and Methods. Two-hundred eighty-four molecular biology tests were performed in 71 patients (included so far) to verify the presence of viral genomes in the study samples. The technique used was Nested Polymerase chain Reaction (Nested PCR). Results and Discussion. Of these 284 tests, 8 presented positivity for one of the viruses studied (2.8%). Four patients (5.6%) presented positive results for HSV-1: 2 of them had encephalitis and had HSV-2; one had meningoencephalitis and had HSV-2 positivity; one of them had paresis of the cranial nerve. A patient with AIDS and neurotoxoplasmosis presented a positive result for VZV. There was no positivity for the JC virus. We observed that the occurrence of the HSV-1 virus is frequent mainly in cases of encephalitis. Coinfection with the HSV-2 virus can happen in most cases. The benefits of this project is a better diagnosis of viral infections and, concomitantly, a better treatment for the diseases caused by these viruses, allowing a better prognosis of the clinical case of these patients. Financial support: FAPESP

Palavras-chave: Herpesvirus, Polyomavirus, Cerebrospinal Fluid, Viral Infection, Polymerase Chain Reaction
Human cytomegalovirus is a ubiquitous infectious agent that affects mainly immunosuppressed, fetuses, and newborns. The virus has several polymorphic regions, in particular in the envelope glycoproteins. The UL55 gene encodes the glycoprotein B that has a variable region, containing a furin cleavage site and according to the variability different genotypes are characterized. Here we investigated variability and existence of selective pressure on the UL55 variable region containing the furin cleavage site in 213 clinical sequences from patients worldwide. We showed the occurrence of positive selective pressure on gB codons 461 and 462, near the furin cleavage site. Cleavage analysis of synthesized peptides demonstrated that most mutations confer better cleavage by furin, suggesting that evolution is acting in order to increase the efficiency cleavage and supporting the hypothesis that gB processing is important in the host. We also demonstrated that peptides containing sequences, that characterize genotypes gB2 and 3, are differentially cleaved by furin. Our data demonstrate for the first time that variability in the cleavage site is related to degree of gB processing by furin.

**Palavras-chave:** HCMV, Furin, Protease, Cleavage, Positive selection
POTENTIAL ANTIVIRAL USE OF SRC/ABL AND MEK/ERK INHIBITORS AFTER ENCEFALITE’S SAINT LOUIS VIRUS INFECTION

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Resumo

Several viruses of great importance in public health are included in the Flavivirus genus, such as Dengue virus (DENV), Saint Louis Encephalitis virus (SLEV), Yellow fever virus (YFV), and Zika virus (ZIKV). SLEV is widely distributed in the Americas and can cause diverse clinical signs varying from febrile syndromes to fatal meningoencephalitis. Similarly to other viruses, there is still no effective treatment against SLEV. A new approach for treatment lies in the search for cellular proteins as therapeutic targets, since the viruses as obligatory intracellular parasites, use various components of the cellular machinery for their replication. Previous studies, demonstrated that pharmacological inhibitors of cellular protein kinases exhibit a potential antiviral activity against different Flaviviruses, including pharmacological inhibitors of members of Src/Abl (W and Z) and MEK/ERK (X, Y and V) protein kinase families. Given the similarity in the multiplication cycles among members of this genus, the current work intends to investigate the potential antiviral effect resulting from the association of these pharmacological inhibitors, which can act at different steps of the SLEV multiplication cycle and its effect on virus multiplication in vitro. For this end, different combinations of inhibitors were tested aiming to find the minimum concentrations (MC) capable of promoting the reduction of at least one logarithmic unit in the viral titer. A Western blot assay was performed, where the MCs able to inhibit the phosphorylation of the respective kinases were determined and, subsequently, the MCs to be tested were chosen. Next, dose-response assays were performed to determine the combinations that showed a possible antiviral effect. No significant reduction in viral titer was observed in all combinations tested between the MEK/ERK (Y) and Src/Abl (Z) inhibitors. However, when the MEK / ERK (Y) was replaced with (V), the reduction was more expressive when compared to the individual antiviral action of both inhibitors. Drug association studies are widely used and aim to minimize side effects, by decreasing doses used and, consequently, reducing a potential drug resistance. Financial support: CNPq - CAPES - FAPEMIG.

Palavras-chave: Antiviral, Saint Louis Encephalitis virus, Src, MEK/ERK, Virus-host interaction
PREVALENCE OF EPSTEIN-BARR VIRUS (EBV) INFECTION IN PRESIDENTE FIGUEIREDO, AMAZONAS, BRAZIL.

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Resumo

The EBV infection affects over 90% of the world's human population and it is considered to be the major cause of Infectious mononucleosis. Currently, few studies regarding EBV prevalence had been conducted in Brazil, especially in the western Amazon region. The main goal of this study was to estimate the prevalence of EBV infection in the city of Presidente Figueiredo-AM. A total of 546 individuals residing at Presidente Figueiredo had blood sample and demographical data collected. The seroprevalence was determined using a commercial immunoassay and the social-demographical characteristics were assessed through an epidemiological questionnaire. All procedures were previously approved by The Research Ethics Committee (CEP) of the Federal University of Amazonas (CAAE 42203615.4.0000.5020). The results demonstrated a seroprevalence of 96.15% for EBV infection among the individuals studied. The most of infected people were elderly women (59.70%) and people with low education and income (53.29%). The high prevalence observed in this study showed that EBV infection strikes mainly elderly women and people with lower socioeconomic status. Thus, the findings bring attention to the need of more health promotion strategies to prevent of EBV infection and to better characterize its epidemiological profile in the Western Amazon.

Palavras-chave: PREVALENCE, EPSTEIN-BARR, INFECTION, PRESIDENTE FIGUEIREDO
DNA sequences. The employment of molecular techniques for analyzing paraffin DNA. Our results demonstrated a high prevalence of HPV infection among samples

β-globin amplification. Seventy-eight percent (38/49) of the analyzed samples were positive for HPV DNA. The DNA samples integrity was assessed by PCR using the β-globin gene primers, and all DNA samples exhibited the β-globin amplification. Seventy-eight percent (38/49) of the analyzed samples were positive for HPV-DNA detection, with 79% (11/14) of CIN2 samples, and 77% (27/35) of Cervical cancer tissues exhibiting positive amplification for HPV-DNA. Our results demonstrated a high prevalence of HPV infection among samples presenting cervical abnormalities as pre-invasive (CIN) and invasive cancer. Also, our results suggest that patients with CIN would require a strict clinical monitoring and/or treatment, in order to prevent a subsequent cervical lesion progression. Financial Support: CNPq and FAPEMIG.

Palavras-chave: Cancer, Cervical Intraepithelial Neoplasia, HPV infection, Polymerase Chain Reaction.
Dengue is a significant worldwide public health problem, especially in tropical and subtropical regions of the globe. Despite the recent licensure of a vaccine against all four dengue serotypes, limitations of this vaccine indicate that it is prudent to keep other vaccine options in the pipeline. In this study, we evaluated the vaccine potential of four viral-vectorized DENV vaccine candidates in a challenge model with DENV in mice. Four viral-vectorized DENV vaccine candidates (1NSALL, 2NSALL, 3NSALL, 4NSALL) have been created using Chimpanzee adenovirus (ChAd) and poxvirus (MVA). These were designed and created by The Jenner Institute, at University of Oxford. The vaccines were intramuscularly administered in C57BL/6 mice using a heterologous prime-boost regime. After boost, the animals were intracranially challenged with DENV1 (the Mochizuki neuroadapted strain) or DENV 3 (a natural neurovirulent clinical strain). Upon DENV1 challenge, the vaccines 2NSALL and 3NSALL delivered 100% protection whereas 4NSALL induced 85.71%. The 1NSALL vaccine, on the other hand, provided only 14.28% protection. Upon DENV1 challenge, protection rates of 42.85% were obtained in animals immunized with 2NSALL or 4NSALL, while 3NSALL vaccines delivered 28.57% protection. No animals were protected in the group immunized with 1NSALL. We analyzed clinical outcomes that suggest neurological damage like weight loss, ruffling fur, shivering, arched back and lower limbs paralysis. The analysis show a score decrease in some immunized and challenged groups comparing to control groups. The results indicate great potential for 2NSALL, 3NSALL e 4NSALL vaccines. Other experiments are being conducted to evaluate the protective efficacy in challenge with other DENV serotypes of and for a better characterization of immunogenicity in animal model. Acknowledgments: CAPES and University of Oxford.

Palavras-chave: dengue, vaccine, Dengue virus
INTRODUCTION. The more potent immunosuppressive therapy that has successfully reduced the incidence of acute rejection, improved graft outcomes in organ transplant, and prolong disease remission of glomerulopathies has also resulted in a higher incidence of viral complications. These complications are associated with different risks of morbidity and therefore need to be clearly defined. The principal viruses related to immunosuppression period are Epstein-Barr virus (EBV) and Cytomegalovirus (CMV). Thus, increasingly sensitive molecular methods allow for the detection of subclinical viral infection. OBJECTIVES. To apply strategies for the detection of viral infections, using Nested-PCR (NPCR) and quantitative Real-Time PCR (qPCR); standardization the viral load monitoring in pediatric patients with glomerulopathies and kidney transplant. METHODOLOGY. Samples of plasma and urine from forty-seven pediatric patients (37 glomerulopathies and 10 renal transplant) were included in this study. They were submitted to NPCR and qPCR techniques to assessed EBV and CMV DNAemia. Primers and Probe for qPCR technique were design according to the MIQE (Minimum Information for Publication of Quantitative Experiments). RESULTS. Using NPCR technique, 19/47 (40.4%) patients were positive. Fifteen out of 47 (31.9%) had CMV-DNA and 9/47 (19%) had EBV-DNA detected. These 19 patients, 5 (26.3%) had coinfection CMV+EBV and 2 of these patients had 2 episodes of coinfection. The standardization of the qPCR is under development. The sequence chosen for CMV is based on the UL83 gene and EBV is based on AG876. CONCLUSION: There are few studies evaluating viral infections in children with chronic kidney diseases in Brazil and with this one it will be possible to standardize a qPCR tests to know better about the occurrence of the infections caused by these viruses. Financial support: FAPESP

Palavras-chave: Chronic renal disease, Glomerulopathy, Renal transplantation, Human cytomegalovirus, Epstein-Barr virus
The concept of “One Health” encompasses an interdisciplinary network of knowledge, focused on the control of zoonoses and the spread of infectious diseases. In this context, environmental health provides crucial information and plays an important role in preventive health. Airports and bus stations are particularly favorable environments for microorganisms dispersion. Metagenomics and qPCR are tools frequently used in the study of environmental microbial diversity and potential pathogens. Thus, it seemed opportune to employ these approaches to investigate the microbial diversity of sanitary sewers of Carlos Drumond de Andrade Airport and Governador Israel Pinheiro Bus Terminal, Belo Horizonte-Brazil, which are public environments with a high concentration of people from different locations. In 2016, samples of sanitary sewage from the mentioned places were collected in two moments, with normal circulation of people and in period of increased movement. Samples were submitted to DNA and RNA extraction for enteric virus detection by qPCR (total enteroviruses (EV), adenovirus (HADV), rotavirus (RV) and hepatitis A (HAV) and E viruses (HEV)), as well as Bacteria and Archaea, by metagenomics methods, were studied. All enteric viruses studied were detected in at least one of the samples obtained at the airport (AP) and at the bus station (BS). HADV was presented the greater amount genomic copies (GC), $\geq 1,00E+06/L$ in all samples. Following by HEV ($\geq 1,00E+05$ GC/L, AP and BS), HAV, EV, RV ($\geq 1,00E+05$ GC/L, AP) and HAV, EV, RV ($\geq 1,00E+04$ GC/L, BS). The Bacteria domain was observed with far superior abundance compared to the Archaea domain. Firmicutes phylum was the most abundant, showing a higher relative frequency on days of intense movement of people. *Aeromonas* and *Arcobacter*, relevant potential pathogens, were found in all samples studied. The increasing movement and concentration of people between different geographical areas at the BS and AP should receive special attention because these areas can be a hot point spread zone of viral and bacterial pathogens. Environmental monitoring actions associated to the “One Health” context would help to prevent infectious diseases and constitute an important tool to subsidize public policy actions aimed at protection, promotion, and recovery of collective health.

**Palavras-chave:** qPCR, metagenomics, sanitary sewage, enteric virus, microbial diversity
Arboviruses are a worldwide, great concern regarding public health. They feature hematophagous arthropods as vectors. The maintenance of these viruses in nature occurs through enzootic cycles, in which the vertebrate animals that participate in this cycle are the non-human primates (PNH). These animals show an important epidemiological indicator for health surveillance, since they are indicators of circulation especially with regard to arboviruses. A retrospective study about Zika virus (ZIKV) and Alphavirus was performed on biological samples from PNH, which were received in the Section of Arbovirology and Hemorrhagic Fevers (SAARB) of the Evandro Chagas Institute as a spontaneous demand of surveillance of the yellow fever virus in Brazil in 2015. 135 samples (blood and / or tissues) of PNH from different regions of Brazil were analyzed. All samples were submitted to RNA extraction, where the method of the Trizol Plus kit (Ambion, Invitrogen) was used, following the instructions of the manufacturer. For genome research of the Alphavirus, the conventional method for PCR was adopted by using primers genus-specific, while the RT-qPCR was used to study ZIKV, Chikungunya virus and Mayaro virus genomes, these last two only under condition of positivity in the conventional PCR. Of the 135 samples, 62 were tested for ZIKV by the RT-qPCR method and 73 submitted to the RT-PCR method for Alphavirus investigation. All the samples tested showed negative results in said tests. The scenario of an epidemic caused by ZIKV has strengthened the studies and it has also suggested the possibility that PNH are acting as a reservoir for this virus. Furthermore, the Alphavirus are also treated as research targets in order to understand their relationship with the PNH and their performance as a reservoir. In this way, the retrospective research of these arboviruses in PNH was an opportunity to add more information and to assist in the elucidation of the dynamics of ZIKV and Alphavirus in Brazilian territory. The negative results obtained in this study do not eliminate the importance of continuous surveillance in PNH, since there is evidence that these animals may be acting on the maintenance cycle of ZIKV and some Alphavirus. Besides, the studies in surveillance help to better understand the maintenance cycle of these viruses and to take prophylactic measures. Financial Support: CNPq, Evandro Chagas Institute.

Palavras-chave: Arboviruses, Enzootic cycle, Non-human primates, Reservoir
Sapoviruses are important agents of acute gastroenteritis (GEA) in people of all ages, most often detected in samples from children and the elderly. They can be transmitted by the fecal-oral route, ingestion of contaminated food or water, through person-to-person contact and by fomites. The objectives of this study were to evaluate the occurrence and viral loads of sapovirus in fecal samples of patients subjected to allogeneic stem cell transplant recipients. The study included 19 patients, who were monitored from October 2012 to July 2014 in a transplant reference center in Goiânia, Goiás. Fecal samples were extracted using a commercial kit (Qiagen-Hilden, Germany), following the manufacturer’s instructions and tested by RT-qPCR Taqman, using primers (SaV124F, SaV1F, SaV1245R) and probe (SaV124TP) specific for genogroups I, II and IV, targeting the polymerase/capsid viral genome region. To determine the viral load of the samples a standard curve was constructed from serial dilutions of recombinant plasmid. Of the 19 patients monitored, two (10.53%) were positive for sapovirus. One patient, presented diarrhea and had four positive samples, he was first positive three days after the transplant, and excreted sapovirus for 13 days. The other had only one positive sample that was obtained one month after the transplant, and this patient did not present diarrhea, or any other acute gastroenteric symptom. The sapovirus copy numbers in fecal samples varied from 1.07x10^8 CG / mL to 5.56x10^8 CG / mL, with a mean of 2.40x10^8 CG / mL of sample. The data reveals the occurrence of sapovirus among allogeneic stem cell transplant patients and highlights the importance of including additional tests in the routine exams of immunossuppressed patients, such as the ASCT recipients. This is the first study to investigate and detect SaV in faecal samples of ASCT patients. Financial support: Fundação de Apoio a Pesquisa em Goiás (FAPEG) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

**Palavras-chave:** sapovirus, human calicivirus, allogeneic stem cell transplant, gastroenteric virus, immunossuppressed patients
Screening of compounds promising to antiviral against Chikungunya virus using the protein C in molecular docking studies

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Resumo

The Chikungunya virus (CHIKV), is a disabling disease associated to severe arthralgia. Although the articular symptoms usually resolve within weeks, several cases have been reported for months, and to date there are no specific therapies for CHIKV disease. In this context, the validation of antiviral targets becomes of great importance. The protein C, a constituent of the capsid, is a promising target for the development of antivirals. Thus, its 3D structure is not available in the literature. First, the 3D model of protein C of CHIKV was obtained by homology modeling using protein C of the Aura virus (4UON) complexed with glycerol as template through the program SWISS-MODEL (SM). The global quality of the model was evaluated by the Ramachandran Plot (RP), RMSD, Qualitative Model Energy Analysis (QMEAN), and ANOLEA method. The value for RMSD was 0.30 Å, QMEAN of -2.09 and 98% of the amino acid residues were in favorable regions according to the RP, confirming the quality of the model. Additionally, the ANOLEA showed that most amino acid residues were found in low energy zones. Thus, the constructed 3D model protein C showed viability for use in Molecular Docking studies. So, the glycerol was transferred to C protein of CHIKV through Discovery Studio 3.1 software. Then, in the AutoDock program was built a box centered in this ligand to define the region in which the molecule would be anchored. The box was defined as a cube with dimensions of 14x14x14 Å and coordinates X, Y and Z -3.639, 31.518 and 11.941, respectively. Glycerol is in a site that believes there is an interaction between tryptophan and protein c. Thus, the work aimed to investigate the interaction of glycerol and tryptophan with the active site of the protein C. The molecules obtained from the ZINC database and PUBCHEM had their charge calculated to physiological pH and 3D structure defined by the MarvinSketch 15.8.24 program. After preparation, the docking of protein C was done with AutoDockVina 1.0.2. We tested 403 compounds to check the interaction with protein C, of these only 8 compounds showed good interaction with binding energy values around -7.6 kcal/mol. These results showed promising compounds competing for binding to the tryptophan-occupied CHIKV protein binding site (-6.6 kcal/mol), since the affinity found for these compounds was higher, encouraging us to proceed with in silico studies to investigate the potential of these molecules. 

Financial Support: Capes e FAPEMING

Palavras-chave: Chikungunya virus, Antivirals, Homology modeling
SEROEPIDEMIOLOGICAL STUDY OF COXSACKIE B VIRUS INFECTIONS IN BRAZIL FROM 2010 TO 2016

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Resumo

Coxsackie B (COXB) is a RNA virus of Picornaviridae family that, like other enteroviruses belonging to this family, can infect several human tissues and organs. Although the vast majority of symptomatic infections presents as nonspecific febrile syndrome, exanthematous disease, meningitis, myocarditis, pericarditis, and other clinical syndromes of variable severity may occur. Six serotypes, B1 to B6, are known. The aim of this study was to describe seroprevalence of recent infections by COXB from 2010 to 2016. Thus, results of all samples tested for total antibodies against COXB at a private Brazilian lab during that interval, using cell culture neutralization assay, were analyzed. Recent infection was defined as positive results with titers equal to or greater than 1/32 for at least one of the serotypes; highest titer was used to identify the serotype involved. From 2010 to 2016, 13,633 serum samples, coming from six Brazilian states (SP, RJ, PR, RS, BA, PE) and the Federal District have been processed, of which 10,499 were reactive in titers equal to or greater than 1/32 for at least one serotype, which is equivalent to 77% of them. Proportion of samples indicative of recent infection was similar over the years, ranging from 75.8% in 2015 to 77.7% in 2013. The most frequent serotype was B4 in all years, which produced the highest titers in 30% of positive samples, ranging from 27% (2013) to 32% (2010). It was followed by serotypes B2 and B3, which represented 23% (23% to 25%) and 20% (17% to 22%). None of the serotypes presented an epidemic behavior during analyzed period. All serotypes circulated all along every studied year, though incidence increased during the Summer, starting in December and peaking between March and April. Although previous surveys indicate higher incidence of COXB infections in children up to 10 years old, frequency of recent infection in this age group was similar to those up to 65 years old (76.5%); the highest frequency was observed in individuals older than 65 years (90.7%). We conclude that, in our country, exposure to COXB is frequent, especially in the Summer, with predominance of serotype B4 and higher incidence in the elderly older than 65 years, when compared to other age groups. It is important to emphasize, however, that studied samples come from medium to high-income individuals who attended to private healthcare facilities; therefore, may not reflect enteroviruses prevalence of general Brazilian population.

Palavras-chave: Enterovirus, Coxsackie, Seroprevalence, Neutralization
Resumo

Renal transplantation is a widely indicated therapy for patients with chronic kidney disease. However, patients undergoing the procedure need to undergo immunosuppressive therapy to avoid rejection of the transplanted organ. This immunosuppression leaves patients much more susceptible to polyomavirus and herpesvirus infections, such as Human Cytomegalovirus (HCMV). To prevent this infection, prophylaxis with valganciclovir is usually done for three months. The objective of this study was to verify the results of IgM and IgG against HCMV of renal patients who underwent kidney transplantation at Ophir Loyola Hospital, Belém, Pará, Brazil, a regional reference in transplants. Two samples of 19 patients were collected: one immediately before transplantation and another 30 (± 5) days after surgery. For the determination of serological results, immunoenzymatic tests were performed with the VIDAS CMV IgG and CMV IgG kits (Biomérieux). Of the 19 patients, 18 had paired samples (one patient died soon after surgery). In pre-transplantation, 0% and 94.7% (18/19) of IgM and IgG positive results were observed, respectively. After transplantation, IgM positivity was 5.6% (1/18) and 94.4% (17/18) for IgG. The serological reactivity dynamics evaluation showed that there was no change in IgG results, however, one patient presented serologic conversion to IgM, a possible result of a reactivation of HCMV, favored by immunosuppression. This patient did not present symptomatology suggestive of active infection by the virus. Increasing the reactivation potential of HCMV in an immunosuppressive environment justifies the monitoring of infection by serological markers. It is suggested to monitor these results for a longer time after transplantation in order to gain a better understanding of the dynamics of the serological results after the end of prophylaxis with valganciclovir.

Palavras-chave: HCMV, immunosuppression, renal transplant
The genus *Flavivirus* consists of various disease-causing viruses called arboviruses, highlighting dengue fever, yellow fever, and chikungunya fever. Many of them may be transmitted to humans or other animals through the bite of mosquitoes of the genera *Aedes* and *Culex*. Dengue reveals itself as a high-impact disease in Brazil due to the favorable climate for breeding of the mosquito vectors and consequent spread of dengue virus (DENV). It is known the existence of four serotypes of this virus: DENV-1, 2, 3, and 4. It is important to note that the presence of more than one viral serotype is associated with severe cases of the disease (dengue hemorrhagic fever). Thus, the present work aims to detect and characterize *Flavivirus* present in larvae of mosquitoes of the genera *Aedes* and *Culex* collected in Uberlândia, Minas Gerais. For such, larvae of mosquitoes were collected by the Center for Control of Zoonoses (CCZ) of Uberlândia (in 2015) and, later, RNA was extracted from pools of approximately eight larvae. After this procedure, samples were submitted to One Step reverse transcription-polymerase chain reaction (RT-PCR) followed by multiplex nested-PCR by using primers targeting the four serotypes of DENV. Three RNA samples were tested and in one of them, from larvae collected in a single location in Uberlândia, amplification corresponding to RNAs of the serotypes DENV-1, 3, and 4 was observed. The identification of viruses present in larvae of mosquitoes in a region is of major importance to society so that effective measures of prevention can be taken, aimed at reducing the transmission of these arboviruses, not only to human population, but to vectors as well, to the impact on human health. Financial Support: CNPq and FAPEMIG.

**Palavras-chave:** Aedes, Culex, Dengue, Detection, Flavivirus
Smallpox, a human disease caused by variola virus (VARV), was declared eradicated in 1980 after WHO vaccination campaign using vaccinia virus (VACV). Because of the increasing concern about VARV use as a bioterrorism weapon, several developed countries have implemented guidelines for smallpox preparedness. In order to collaborate with the Brazilian Biodefense strategy we are evaluating the humoral immunity of a selected group of the Brazilian Army and analyzing the genome of the smallpox vaccine strain Wyeth. In a cross-sectional study, we are investigating the presence of total and neutralizing anti-VACV antibodies in sera from a cohort of 254 Brazilian Army militaries (16 women and 238 men) trained for Chemical, Biological, Radiological, Nuclear Defense. Of these, 235 serum samples have already been analyzed in a colorimetric ELISA test, using purified VACV strain WR as antigen. The cut-off was determined using sera from 33 non-vaccinated and 22 vaccinated individuals outside the cohort. Of the 235 sera, 14 samples were positive (5.96%), 15 samples were considered borderline (6.38%) and 206 were negative (87.66%) for the presence of anti-VACV IgG. We have also classified the volunteers in 3 age groups: ≤ 25 years-old, ≥ 26 and ≤ 39 years-old, ≥ 40 years-old. Except for one 21 years-old individual who tested positive, the other 28 positive and borderline individuals were ≥ 40 years old. These groups were then selected for testing the presence of neutralizing antibodies using a 50% Plaque Reduction Neutralization Test (PRNT50). Of the 28 sera, 15 have been already tested by PRNT50 and only one had a titer (1:70) considered to be protective according to the literature (≥ 1:32). Efforts for smallpox preparedness also include the research of smallpox vaccines used during WHO campaign. VACV IOC was the vaccine strain manufactured by Fiocruz-RJ at that time, but vials of VACV strain Wyeth were also part of the collection. However, it is unknown whether Wyeth strain was used in the manufacture of the Brazilian vaccine VACV-IOC. Therefore, we sequenced the whole genome of three clonal isolates of VACV Wyeth, using the NGS platform Illumina HiSeq 2500. Contig assembly generated genomes of 200,375 bp, 198,436 bp and 198,512 bp, with >2060x coverage. The phylogeny shows that all clones branch with clones of the American vaccine Dryvax, but not with VACV-IOC. This is an on-going study that will contribute with Brazilian smallpox preparedness.

**Palavras-chave:** smallpox, biodefense, brazil, immunity, vaccine
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SPREAD OF THE EMERGING EQUINE-LIKE G3P[8] DS-1-LIKE GENETIC BACKBONE ROTAVIRUS STRAIN IN BRAZIL

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Resumo

In 2013, a novel equine-like G3P[8] DS-1 like rotavirus (RVA) emerged as dominant strain worldwide. In 2016, this strain was reported in Brazil. The aims of the present study were to conduct a genetic retrospective investigation since 2013 in order to identify the possible entry of this novel G3P[8] strain in Brazil, describe its distribution across the country, help the understanding of evolutionary dynamics and potential implications in RVA vaccine programs. Between 2013 and 2016, a total of 3566 fecal specimens were collected from Southern, Southeastern and Midwest regions, and likely to be representative of Brazilian population. Specimens were screened for RVA using ELISA, and genotyped by RT-PCR. VP7 amplicons of positive G3P[8] samples were submitted to sequencing in order to identify the equine-like G3P[8] strains by nucleotide similarity search. Six equine-like G3P[8] representative samples were selected for investigation of the whole genome. G3P[8] represented 15.9% (114/720) of all RVA-positive samples, and further divided as equine-like G3 (5.3%; 38/720) and wild-type G3 (11.8%; 85/720). During 2013-2014, wild-type G3P[8] was the dominant strain across Brazil, and no equine-like G3P[8] was detected. Equine-like G3P[8] strain was first identified in March/2015 in Paraná State, suggesting that the strain stepped into Brazil through Southern region. The equine-like G3P[8] circulated only in Paraná till February/2016, but rapidly spread across the country after that, reaching Amazonas in March, Goiás in July and the Federal District in September. No wild-type G3 was detected since March/2015, therefore, it could be speculated that the atypical G3P[8] genotype was able to completely replace wild-type G3P[8] strains. Whole genome analysis revealed a DS-1 like genotype constellation (G3-P[8]-I2-R2-C2-M2-A2-N2-T2-E2-H2), and segments shared the highest nucleotide sequence identity with strains isolated in Brazil and Spain. This study highlights the emergence of the atypical equine-like G3P[8] genotype circulating in Brazil, and reinforces the potential for novel human/animal reassortant to arise within human population. Equine-like G3P[8] DS-1-like strains pose a challenge for the RVA surveillance, once G/P typing characterization by RT-PCR is unable to identify this intergenogroup reassortant without inclusion of sequencing analysis. Financial Support: Fundação de Amparo à Pesquisa do Estado de São Paulo (Grant nr. 15/12944-9)

Palavras-chave: rotavirus, surveillance, gastroenteritis
Zika virus (ZIKV) is a member of the flavivirus gene and its genome is a ~10.8 kilobase, positive strand RNA enclosed in a capsid and surrounded by a membrane. Studies on replication dynamics of ZIKV are scarce, which limits antivirals and vaccines development. In this study, Aedes albopictus mosquito lineage cells (C6/36 cells) and kidney epithelial cells of African green monkey (Vero cells) were inoculated with a ZIKV sample isolated from a Brazilian patient and the infection was characterized by immunofluorescence, phase contrast light and transmission electron microscopy and real time RT-PCR. The infection was observed in both lineage cells; ZIKV particles were observed in lysosomes, inside the rough endoplasmic reticulum as well as inside viroplasm-like structures. The susceptibility of C6/36 and Vero cells to ZIKV infection was demonstrated. Moreover, this study shows that part of the replicative cycle may occur within viroplasm-like structures, never seen in other flaviviruses’ structural replication aspects before. Financial Support: CNPq, IOC, Faperj

**Palavras-chave:** Zika virus, vero cells, C6/36 cells, viroplasm
The Zika virus (ZIKV), belonging to the genus Flavivirus and the family Flaviviridae, is associated with serious health conditions, such as neurological complications in fetuses and adults. Due to this, there is currently a great search for several ways of prevention and treatment of the ZIKV infection. Emodin has been shown to have a broad spectrum of pharmacological effects, showing that it has antiviral effects on several viruses. The aim of this study was to observe the possible virucidal effect of Emodin in ZIKV. For the preparation of the virus, the Brazilian strain of ZIKV (ZIKVBR) was inoculated in cells of the C6/36 cell line. The cytotoxicity of Emodin was tested on Vero E6 cells and the effects were evaluated after 24, 48 and 72 hours after addition of the drug. To confirm the possible interaction of Emodin with the virus particles, exact quantities of the virus were mixed with this compound and used to infect the Vero E6 cells and titrated by plate neutralization test, counting the number of lysis plates in each well after fixation with 10% formaldehyde and stained with violet crystal. The selectivity index was calculated to suggest the potential effect of this drug. It has also been tested whether this substance can influence the entry phase of ZIKV in the cell, with Vero E6 cells pre-treated with Emodin, without virus infection. Through the cytotoxicity assay, it was observed that concentrations above 40 μM were toxic and the calculated CC50 was 68.5 μM. For the EC50, the value was 6.70 μM, leading to a SI = 7.9. By incubating the virus with Emodin (40μM), after 96 hours, the viral titre reduction was approximately 85% over the control, which demonstrates that this compound is potentially virucidal. In addition, when the cell was pre-treated with the same, there was a 42.31% reduction of virus entry, which suggests the possibility of an action of the compound with cellular receptors, also leading to virucidal activity. It has therefore been found that this natural compound, Emodin, extracted from plants and widely used in eastern medicine, is a compound with potential virucidal activity against ZIKVBR in Vero E6 cells. Financial support: FAPESP.

**Palavras-chave:** Emodin, virucidal, Zika virus, ZIKV
SURVEILLANCE OF SEVEN RESPIRATORY VIRUS IN CHILDREN WITH ACUTE RESPIRATORY DISEASE IN SÃO PAULO CITY FROM 2014 TO 2017.

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Resumo

Acute respiratory infections are responsive for a high level of outpatient pediatric medical care and hospitalization, resulting in significant respiratory diseases in worldwide, particularly in developing countries. The viruses are related with 50 to 90% of lower respiratory tract infections in young children, being the most identified: Respiratory Syncytial virus (HRSV A and B), human Parainfluenza (HPV type 1, 2 and 3), Influenza virus A and B (FLU A and B), Adenovirus (HAdV). Our goals is to describe the circulation of respiratory viruses more frequent associated to acute respiratory infections in young children attended at Hospital Universitário HU-USP, in São Paulo, Brazil, during the last 4 years. As a methodology we did an exploratory descriptive research that required treatment quanti-qualitative, with data survey of period between 2014 and 2017. All virus detection were done at the University Hospital Laboratory using the Immunofluorescence methodology for detection of seven respiratory virus ( HRSV, HPV1, 2 and 3, FLUA and B and HAdV) using Kit Light DiagnosticsTM Respiratory Panel Viral Screening and Identification ( IFA Chemicon®, Merk Millipore Corp., USA). Of 5628 results analyzed, 2252 (40,1%) were positive for at least one of respiratory viruses studied. The HRSV was identified in 70,9% of the positive samples, followed by HAdV in 9%, HPV3 in 9%, FLUA in 6,6%, FLUB in 0,8%, HPV1 in 0,8% and HPV2 in 0,9%. Double viruses infections were detected in 0,8% of all the samples. This study reveals that there a high level of respiratory diseases mainly for HRSV.

Palavras-chave: Viruses, Surveillance, Children, Respiratory, Disease
Zika virus (ZIKV) is a member of the Flaviviridae family, along with other agents of clinical significance such as dengue (DENV) and hepatitis C (HCV) viruses. Since ZIKV causes neurological disorders during fetal development and in adulthood, antiviral drugs are necessary. Sofosbuvir is clinically approved for use against HCV and targets the protein that is most conserved among the members of the Flaviviridae family, the viral RNA polymerase. Indeed, we found that sofosbuvir inhibits ZIKV RNA polymerase, targeting conserved amino acid residues. Sofosbuvir inhibited ZIKV replication in different cellular systems, such as hepatoma (Huh-7) cells, neuroblastoma (SH-Sy5y) cells, neural stem cells (NSC) and brain organoids. In addition to the direct inhibition of the viral RNA polymerase, we observed that sofosbuvir also induced an increase in A-to-G mutations in the viral genome. Together, our data highlight a potential secondary use of sofosbuvir, an anti-HCV drug, against ZIKV. This work was supported by Conselho Nacional de Desenvolvimento e Pesquisa (CNPq), Fundação de Amparo a Pesquisa do Estado do Rio de Janeiro (FAPERJ).

**Palavras-chave:** Antiviral, Sofosbuvir, Zika virus
Human Papillomavirus (HPV) infection has been clearly shown to be a factor directly related to the development of cervical cancer. Despite the fact that most of the HPV infections are transient, an element highlighted by some studies specifically refers to persistent infections by HPV's of high oncogenic risk, also related as one of the causes in the development of cervical cancer and its precursor lesions. Even so, because of the great diversity of HPV types associated with genital infections, the real role of different molecular variants of HPV in this persistence still needs further clarification. The definition for variants refers to those HPV's that have a maximum sequence divergence of 2% in their gene structure correlating the population ethnicity. Today we have the following categories: Asian-American (Aa), African (Af1 and Af2), Asian (A) European (E). The present work determined the molecular variants of HPV-16 involved in persistent infections detected in a molecular study consisting of 1,642 women aged 15-50 years attended at Botucatu Health Units - SP. HPV-16 positive samples were amplified by a specific PCR reaction to the LCR region and submitted to sequencing and finally analyzed for the genetic variant check. A high prevalence of 33% HPV infection was observed, and 20% belonged to the HPV16 genotype. The mean age of women who participated in the cohort and had the HPV16 genotype was 22 years, the most common ethnic variable was white in both persistent infections 53.6% and transient 61.2% due to sexual behavior. General prevalence analysis showed that the European and Asian-American variants were the most frequent in the analyzed groups, 1st visit E 89%, Aa 4.5%; 2nd visit E 89%, Aa 8.3%; 3rd visit E 84.6%, Aa 5.2%; 4th visit E 87.1%, Aa 3.3%; 5th visit E 90%, Aa 10%. Regarding the persistence analysis, women with transient infection in the European branch were more prevalent, 79% (European/prototype + European/B-12), followed by the African branch 2 13.5%, and in the persistent infection the European branch 90.2% (European/prototype + European/B-12). Concluding, HPV16 was the most prevalent among the population studied, among HPV-16 positive women the majority were less than 35 years of age, the descriptive analysis suggests a higher prevalence of HPV16 in white women, single and with more than two partners. The most prevalent HPV16 variable in the study group was European, prototype lineage.

**Palavras-chave:** HPV-16, Persistence, Variant analysis
Acute respiratory infections (ARI) are an important cause of illness and death in humans worldwide. Among the pathogens that may cause those diseases, viruses account for about 50 to 90% when compared to other infectious agents. In addition to the influenza A and B viruses, other viral agents are able to induce ARI, such as: Human Respiratory Syncytial Virus (HRSV), Human Metapneumovirus (HMPV), Human Rhinovirus (HRV), Adenovirus (AdV), Human Coronavirus (HCoV), Human Bocavirus (HBoV) and Human Respirovirus (HV). In this context, clinical manifestations associated with ARI may range from mild cases such as colds, nasal congestion, rhinorrhea and pharyngitis, to severe cases such as bronchiolitis and pneumonia, the latter two being more common in children under five years old. Despite the diversity of agents, their symptoms are similar, making clinical diagnosis difficult, and leading to unnecessary prescriptions of antibiotics. The objective of the study was the detection of influenza and non-influenza respiratory viruses in patients diagnosed with ARI in the State of Acre, from January to March 2017. In this context, 81 samples of individuals of both sexes and different age groups were extracted using a commercial kit, following the manufacturer's instructions and the detection of the viral genome was through real-time PCR, using specific primers for HRSV, HMPV, AdV, HCoV (229E, HKU1, NL63, OC43), HRV, HBoV and Parainfluenza 1, 2 and 3. Among the samples analyzed, HRSV was the most prevalent, detected in 39 samples (48.1%). The influenza virus was the second pathogen most indentified, with 31 samples (38, 2%), followed by HMPV with 3 samples (4%). The other samples (9.7%) were negative. In this context, we conclude that in this study the non-influenza viruses are closely related to ARI cases. During the study period in the state of Acre, HRSV was the most prevalent among all cases, followed by influenza virus and HMPV, respectively. In this scenario, studies describing the circulation pattern of respiratory viruses in ARI cases are of great importance for the public health in order to better understand their epidemiological characteristics, as they generate data that provide subsidies to develop measures of prevention and control of viral respiratory diseases. Financial support: Instituto Evandro chagas, PIBIC, CNPQ.
VIRAL LOAD KINETICS IN BLOOD COMPONENTS OF PATIENTS CRONICALLY INFECTED BY HEPATITIS VIRUS C, BEFORE AND DURING TRIPLE THERAPY

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Resumo

Although hepatocytes are the major target of hepatitis C virus (HCV), it may still interact with blood components. The present study sought to evaluate viral kinetics in blood components of patients chronically infected with HCV before/during triple therapy (pegylated interferon-alpha/ribavirin and protease inhibitor-telaprevir or boceprevir). The goal of the study was to implement a real-time PCR using the Abbott RealTime HCV® kit to quantify HCV viral load in serum, whole blood, erythrocytes, platelets and leukocytes. In this approach, were analyzed peripheral blood samples from 20 patients with a confirmed diagnosis of chronic hepatitis C, infected by HCV genotype 1. This study population presented epidemiological/clinical-laboratorial profiles similar to those previous described. Abbott RealTime HCV® kit was optimized for HCV RNA quantification in research conditions and the standardizes method increased the number of reactions/kit in 4 times. Viral load was performed according to: treatment time, blood component, viral load levels and clinical outcome. It was observe that serum was the component with the highest levels of viral load and the red cells with the lowest level. During the therapy, there was a reduction in levels of HCV viral loads in all blood components analyze. This reduction was not observe at 24th week of treatment, when viral loads increased when compared to the 4th week. At 48th week, the viral load levels were decrease without reaching zero load in serum, whole blood, red blood cells and platelets. This persistent positivity was not able to change the clinical outcome of the patients, who maintained a sustained virological response (SVR). Moreover, HCV chronically infected patients present high HCV loads associated with blood components. The treatment of the infection was efficient and effective, causing a robust reduction of virus in the blood components. The increase in viral load at 24th week is indicative that this is a critical week in therapy. The finding of a persistent low viral load in serum, whole blood, red blood cells and platelets at 48th week of triple therapy was a surprising result of the study, since 85.7% of the patients obtained SVR. These data suggest that additional studies could improve the understanding of the mechanisms responsible for the blood components-HCV interaction during the kinetics of treatment for a chronic HCV infection. **Financial Support:** FAPEMIG, CNPq, Ciência Sem Fronteiras, HC/UFMG, CBER/FDA

Palavras-chave: Blood components, Hepatitis C, Triple therapy, Viral load
The etiological agents of precancerous lesions and carcinoma of the anogenital region are the high-risk human papillomavirus types (HR-HPV), among which HPV16 and HPV18 accounts for about 70% of the total cancers. The role of other HR-HPV types in carcinogenesis led to the incorporation of five HR-HPV (31, 33, 45, 52, and 58) in addition to the HPV6, 11, 16, and 18 VLPs of the quadrivalent vaccine, generating the nonavalent vaccine (9v-HPV). Persistence of the HR-HPV infection is the major risk factor to high grade lesions, and besides the viral oncogenes, other molecular determinants are still unknown. Therefore, we aimed to implement the real-time PCR methodology to evaluate the viral load of the oncogenic types contained in the 9v-HPV as a possible molecular biomarker for intraepithelial lesions in HIV-seropositive women. Cervical and anal scrapings of 140 women were obtained at a Reference Center on STD/AIDS. The results of viral load are normalized with the β-globin amplification of each sample. Standard curves are generated with dilution of plasmids containing specific HPV gene for each genotype. We tested two protocols for amplification of both HPV16/18 and β-globin genes: TaqMan® and in house method. The ideal parameters for viral load quantification were achieved with the in house method. CasKi and HeLa cells, which containing an integrated DNA of HPV16 and HPV18, respectively, were used as positive controls. The results of each sample were categorized as low (< 10 copies/cell), moderate (10-100 copies/cell), high (100-1000 copies/cell) and very high copies per cell (> 1000 copies/cell). Thirty-seven samples of HPV16 and 24 of HPV18 were quantified. Altogether, a moderate viral load was observed for HPV16 (median= 41.75 copies/cell) in contrast to low values for HPV18 (median= 9.6 copies/cell). While the median of HPV16 viral load did not differ between anal and cervical specimens, HPV18 viral load was moderate in anal specimens and low in cervical ones. The viral load of HPV16/HPV18 was not associated to normal (n=45) or altered (n=16) cytology in both sites. Quantification of the other five HR-HPV types and additional specimens will add more information to these preliminary results. Then, we may properly analyze the viral load as a possible biomarker of precancerous lesions in the population of HIV-seropositive women, which present a greater risk of anogenital lesions development. Financial support: FAPES, Fundação de Amparo à Pesquisa e Inovação do Espírito Santo; CAPES, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

Palavras-chave: anal, cervical, HIV, HPV, viral load
Zika Virus is an arbovirus of the family Flaviridae, of the genus Flavivirus and is transmitted by the mosquitoes Aedes aegypti and Aedes albopictus. This work was developed aiming to raise awareness of the population of Santa Inês-Ba, about Zika Virus, through the socialization of informative material made from data analysis of individuals diagnosed with viruses in the municipality. To that end, data from patients notified with the pathogen from January to June 2016 were provided by the Municipal Health Department, cataloged and analyzed, which guided educational actions to raise awareness of the population of the municipality. From a total of 609 notifications, 429 cases of Zika Virus were distributed in the months from January to June 2016. It was observed that almost 50% of the total cases were registered in the month of February, which is probably due to the summer period, warmer and rainy, and to the increase of the circulation of people in the municipality due to the event of the biggest festive event of the city. From the individuals reported, 64% were female biological sex, while only 36% were male biological sex, possibly due to cultural issues that make it difficult for men to seek health services. Among the patients with Zika, 36% were over 40 years of age, 34% were between 21 and 40 years and 26% were under 21 years of age. It is suggested that this imbalance in the age of the patients is due to the fact that the young population in Santa Inês is declining due to the low birth rate in recent years and the great migration of young people in search of work in other states, making the population in their majority. Of the total number of cases, 90% occurred in the urban area, of which 39% occurred in the central part of the city, where most of the population is concentrated. After searching for data, flyers were elaborated with explanations about the virus and the disease, as well as pamphlets with the research data. Both were socialized in the community, because it is believed that information plays a fundamental role in the control and prevention of the disease. Simple actions can have an impact on the public health of the municipality and increase knowledge about Zika Virus which can reduce the incidence of cases. Financial Support: IFBaiano

**Palavras-chave:** EDUCATION, PREVENT, ZIKA VIRUS
A peptide from Rabies Virus Envelope Glycoprotein, expressed as a fusion protein in insect cells, is able to stimulate the immune system of mice

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Resumo

Baculoviruses are circular double-stranded DNA viruses that infect insects and were initially used as biological control agents for being able to kill agriculture-important insect pests. In the early 1980s, its potential as a tool for the expression of heterologous proteins began to be explored. Today, baculoviruses have been used extensively to express antigens for vaccine production, diagnostics and even as potential vectors for gene therapy and DNA vaccines. Their biosafety is guaranteed by the fact that baculoviruses are not able to replicate in mammalian cells. However, they are able to enter these cells and show immunostimulatory abilities. The rabies vaccine currently consists of purified and inactivated virus suspensions with β-propiolactone, representing a health risk from its manipulation. Therefore, the use of recombinant proteins from viruses is an alternative to the development of safer diagnostic methods and vaccines. In addition, this strategy may reduce the cost of vaccine manufacturing and eventually, contribute towards disease control. In this work, we constructed two recombinant baculoviruses; one containing a recombinant immunogenic peptide derived from the rabies virus glycoprotein (Pept/G) fused to the polyhedrin protein (POLH) of the baculovirus Autographa californica multiple nucleopolyhedrovirus (AcMNPV) and a second one, containing the same peptide fused to the N-terminus of GP64, the major envelope protein of the AcMNPV. The recombinant protein fused with POLH was expressed in insect cells and in insects in the form of crystalline protein aggregates. This recombinant protein fused protein containing the Pept/G could successfully stimulate the immune system of mice. Therefore, recombinant proteins derived from Rabies Virus fused to the baculovirus POLH protein have the potential for the development of safer diagnostic tests and possible subunit vaccines.

Palavras-chave: Rabies Virus, Baculovirus, Expression, immune system, recombinant protein
Resumo

Dengue fever is a viral disease, considering endemic in more than 128 countries, with about 3.9 billion people worldwide living in infection risk area. Dengue virus is transmitted to humans through the bite of infected female mosquitoes of the genus Aedes. DENV is a RNA (+) virus belonging to the Flaviviridae family, Flavivirus genus. The development of a vaccine conferring immunity, safely and efficiently against all four serotypes of DENV is considered priority. The NS1 is a protein conserved among the serotypes of DENV (1-4) and it is related to the immunopathological process observed in the most severe forms of dengue, including the induction of inflammatory cytokine production and increased endothelial permeability via activation of Toll-like receptors 4 and 2. Researchers suggest the use of NS1 as a vaccine candidate against dengue, since vaccination with recombinant NS1 was able to protect mice against lethal doses of homologous and heterologous serotypes of DENV. Studies in our group have shown that bovine serum albumin nanoparticles (NPBSA) are promising vaccine systems for delivery antigen of DENV and other microbial antigens due to their adjuvant activity. This study aimed to verify the adjuvant potential of bovine albumin nanoparticles to induce an immune response in the presence of the NS1 of DENV-1. The results indicate that albumin NPs did not show significant cytotoxicity in cultures of macrophagic RAW 264.7 and BHK-21 fibroblast cells, however, the nanoparticles are captured more easily by RAW 264.7 cells than for BHK-21 cells. Subcutaneous administration of the NPs in mice in the presence or absence of the recombinant NS1 protein was able to induce the recruitment of an inflammatory infiltrate at the sites where the NPs were applied. Following immunization, it was observed that NPBSA were able to induce production of anti-NS1 IgM antibodies in 60% of the immunized animals and anti-NS1 IgG in 80%, these data may be related to the adjuvant profile of NP, which when administered associated with NS1, were able to induce a faster class exchange, leading to the production of IgG secreting plasmocytes. Future studies will be carried out to verify if the antibodies produced are able to inhibit the binding of NS1 to TLR-4 or TLR-2 receptor and thereby decrease the production of proinflammatory cytokines, responsible for increased vascular permeability and extravasation of plasma.

Palavras-chave: Dengue vírus, Nanopartículas, NS1, Vacina
ANTIVIRAL ACTION OF HYDROMETHANOLIC EXTRACT OF GEOPROPOLIS FROM S. POSTICA AGAINST RUBELLA VIRUS

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Resumo

Researches on chemical composition and biological activity of propolis had been carried out mainly on Apidae specie Apismellifera L, which exhibited activity against some important virus, like poliovirus, influenza, HIV, hepatitis and others. For the Meliponini species, known as stingless bees, there are few studies about their chemical composition and biological activities. This study evaluated the effect of geopropolis from Scaptotrigona postica on Rubella virus infected Rabbit Cornea cells (SIRC). Results on cell viability and cell proliferation assays indicated that geopropolis was non toxic to cultured SIRC cells. Viral binding, penetration assay, antiviral assay, real-time PCR, and transmission electron microscopy were used to demonstrate that geopropolis inhibit the production of infectious Rubella virus particles in cell culture. The chemical analysis of hydromethanolic extract of geopropolis from S. postica exhibited flavones-C-glycosides as main constituents, together with pyrrolizidine alkaloids and catechin derivatives, besides low content of hydroxycinnamic acid amide derivatives (triacylated spermidines). Hydroxycinnamate amides are used as a backbone for the synthesis of antiviral compounds. The antiviral activity of this geopropolis on herpes type I virus were reported. This work consists in a first report about the antiviral activity of geopropolis from Scaptotrigona postica against rubella virus

Palavras-chave: antiviral activity, rubella virus,, Scaptotrigona postica, geopropolis
The arboviruses Zika virus (ZIKV) and Dengue virus (DENV) have important epidemiological impact in Brazil and other tropical regions of the world. Recently, it was shown that previous humoral immunity to DENV enhances ZIKV replication in vitro, which may lead to severe forms of disease such as Guillain-Barré Syndrome in adults and microcephaly in neonates. Thus, traditional approaches of vaccine development aiming control of viral infection through neutralizing antibodies may induce cross-reactive enhancing antibodies. In contrast, cellular immune response was shown to be capable of controlling DENV infection independently of antibodies. The NS5 protein is a well conserved antigen among flaviviruses and is also the main target of CD8+ T lymphocytes. The aim of the present study was to design a flavivirus NS5 protein capable of inducing a cellular immune response against DENV and ZIKV. A consensus sequence of ZIKV NS5 protein was designed among isolates from Asia, Africa, and Brazil. Epitopes were predicted for the HLA class I and II alleles most prevalent in the Brazilian population. Epitopes predicted to be more reactive (percentile rank <1) and 100% conserved between ZIKV and DENV serotypes were selected. The distribution of the epitopes along the protein was shown on a three-dimensional model produced to predict the structural quality of the antigen and population coverage was calculated for different regions of the world. The designed protein was predicted to be stable by a modeling approach. A total of 23 peptides with high response-inducing potential and 100% conserved between DENV and ZIKV were selected. The distribution of such peptides along the protein model was shown to be homogeneous between domains. The population coverage of selected epitopes was shown to be 90.54% in Brazil, 77.34% in South America, 86.65% in USA, 94.95% in Europe, 80.39% in Africa, 80.54% in South Asia and 81.07% in Oceania. Such results indicate that the proposed antigen has the potential to induce protective cellular immune response to ZIKV and DENV in different human populations in the world.

**Palavras-chave:** Zika virus, Dengue virus, Vaccine, Epitopes, Bioinformatics
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DEVELOPMENT OF PEPTIDE BASED SEROLOGIC DIAGNOSTIC PLATFORMS TO DIFFERENTIALLY IDENTIFY DENGUE AND ZIKA INFECTIONS.

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Resumo

Dengue is one of the most important infectious diseases in Brazil, and early diagnosis is a determining factor for disease outcome, particularly for those afflicted with the most severe forms of infections. Co-circulation of viruses, such as Zika virus (ZV), that have serological cross-reactivity with Dengue virus (DV) further complicates diagnosis. One approach towards creating diagnostic tests able to differentiate between such viruses is to determine peptides that would lack immune response cross-reactivity. However, the immobilization of small proteins or peptides on surfaces has been a barrier to the development of tests based on these molecules. The goal of this work is to identify and select specific peptides in the non-structural protein 1 (NS1) of DV and ZV and evaluate their use in serological diagnostic platforms. To identify specific peptides we screened the DV and ZV peptide libraries with NS1 monoclonal DV1-4 and ZV antibodies (NS1 mAbs). Three peptides were identified with specific binding: one that is ZV-specific, a second that is DV-specific and a third that is non-specific (detected by antibodies to both viral NS1 proteins). These peptides were synthesized for use in three alternative diagnostic strategies. The first was an ELISA IgG/IgM assay in a flexible vinyl plate. We tested our peptide ELISA assay with 80 human samples from patients who had known a diagnosis of DV and/or ZV infection, using a whole protein NS1 IgG ELISA for comparison. The peptide ELISA showed a sensitivity of 40-62% and a specificity of 72-96%. The other two strategies we evaluated were a dipstick and lateral flow-based assays using gold nanoparticles. For the first, biotinylated peptides were conjugated with streptavidin and spotted onto a nitrocellulose membrane and nanoparticles conjugated with anti-human IgG were run with the human samples. For the second test, peptides synthesized covalently linked to lipoic acid were conjugated to the surface of gold nanoparticles. These peptide-nanoparticles were used to recognize anti-DV and anti-ZV antibodies captured from patient samples onto nitrocellulose. Both strategies were able to differentiate ZV and DV mAbs and patient samples. The techniques presented here are effective, fast and inexpensive tests and would allow in near future the rapid assessment of the exposure - very necessary, for example, in a vaccine campaign of both Zika and Dengue viruses. Financial support: CAPES, NIH AI100190

Palavras-chave: Diagnostic, Dengue, Zika, Peptides, Serologic
Dengue represents a world health problem with recurrent epidemics in tropical and subtropical regions. The development of a dengue vaccine able to protect against the four viral serotypes is considered a priority for the WHO. The envelope (E) and non-structural 1 (NS1) proteins are identified as promising antigens to integrate a vaccine against dengue virus (DENV). The E protein interacts with receptors present on cell surfaces, mediating virus endocytosis. Therefore, antibodies against this protein can be neutralizing, thus preventing virus entry inside host cells. Besides, other studies indicate that the NS1 protein is able to induce a protective immune response. Our group developed DNA vaccines encoding E and NS1 proteins from DENV2 isolated (pcTPANS1 and pE1D2, respectively) or concomitantly (pNS1/E/D2). Expression of the recombinant proteins mediated by these plasmids was evaluated, as well as the immune response and protection generated in immunized mice. BHK-21 cells were transfected with pcTPANS1, pE1D2 and clones of pNS1/E/D2 and protein expression was detected by immunofluorescence using specific antibodies. All plasmids were able to mediate the expression of E and NS1 proteins. Curiously, after transfection with pNS1/E/D2 we identified cells expressing both proteins simultaneously or only NS1 or E. BALB/c mice were inoculated with clones of pNS1/E/D2 as well as with pcTPANS1 and pE1D2 alone or in a mixture, in order to evaluate protection against DENV2. Two clones of pNS1/E/D2 (1 and 5) and the mixture of pcTPANS1+pE1D2 plasmids induced full protection, with 100% of survival and 0% of morbidity. Mice immunized with pNS1/E/D2 presented low levels of anti-NS1 antibodies when compared to pcTPANS1 immunized animals. On the other hand, antibody titers against the E protein raised in animals immunized with pNS1/E/D2 were similar to those detected in pE1D2-inoculated mice. Nevertheless, we observed significantly higher neutralizing antibody titers in the serum from animals inoculated with clones 1 and 5 of pNS1/E/D2 when compared to those detected after inoculation with pE1D2. In general, although the pNS1/E/D2 vaccine generated a protective immune response, the combination of the plasmids expressing the E and NS1 protein isolated (pE1D2 or pcTPANS1) seemed to be more efficient. Financial Support: FAPERJ, PPSUS/FAPERJ, CNPq, INCTV, PAEF/IOC.
The poultry vaccines are widely used and imply both economy and health animal. The commercial avian vaccines in Brazil are submitted to official quality control. The safety assay checks the property of the vaccine to be innocuous and does not cause any signal attributed to it. This study, aimed to evaluate the vaccines submitted to the official control during Jan to Jun/2017: Monovalent vaccines [Newcastle (ND), Infectious Bursal (IBD) and Infectious Bronchitis (IB) diseases], Combined vaccines (C) and Immune Complex Vaccine-antigen+antibodies of IBD (IC). For each safety test, two vaccines bottles diluted in PBS and 10-12 birds were weighed before being immunized with 10 doses of vaccines (according to manufacturer), and kept in BSL-3 isolator. Specific Pathogen Free birds were used as negative control. For 21 days, the birds are observed and any health signs recorded on form. At the end of the test, the birds are weighted, anesthetized and euthanized. Were tested 28 batches: 8 ND (B1, LaSota, C2 and CL/79 strains, 3 companies); 6 IBD (D78, GBV-8, S-760, CH80 and GM97 strains, 3 companies); 5 IB (H120 e Ma5 strains, 3 companies); 3 C batches (Clone 30+Ma5 and V4+H120 strains, 2 companies) and 6 IC (1 company). Weight gain were compared between vaccinated and unvaccinated birds, It was expected that would be statistically equal by Student’s t-test (mean of the independent samples at 5% of significance). During the observation, the birds did not show any signal attributed to vaccination. The comparing weights of vaccinated birds to control birds showed that the 8 vaccines (3 IB, 2 ND, 1 IBD, 1 C and 1 IC) presented significant difference. In the IB were observed that 60% of vaccine tested had weight loss. Factors such as microbiological contamination, fungi, salmonella and mycoplasma could be associated with weight loss, but no contamination was observed. However, only 4 vaccines were fully tested for the tests in question. No any extraneous agents were tested and it could explain the possible weight loss. It is suggested one better studied to verify the importance of these fact. It is emphasized that all 28 batches tested had the minimum values required by safety test under Brazilian law.

Palavras-chave: Avian live vaccine, safety tests, official quality control
IMMUNOGENIC AND PROTECTIVE POTENTIAL OF A DNA VACCINE MIX ENCODING DIFFERENT DENGUE VIRUS NON-STRUCTURAL PROTEINS

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Resumo

Dengue is the main arbovirose that affects humans and although it is known for more than 70 years there is no fully effective treatment for the disease. So far, the results obtained with the present available anti-DENV vaccine formulation demonstrated that the induction of high titer neutralizing antibodies does not represent a complete protection correlate suggesting that the study of cellular immune responses is particularly relevant in this field. In this context, this work aims to evaluate the immunogenic and protector potential of the major non-structural proteins of DENV (NS1, NS3 and NS5) as antigens encoded by DNA vaccines administered to mice as mix in a single vaccine formulation. C57BL/6 mice were vaccinated with two doses of a combination of 50 µg of each plasmid or 100 µg of empty vector, given intramuscularly at 2-week intervals. Two weeks after the last vaccine dose, immunized animals had a higher percentage of DENV-specific T cells compared to control animals. The mice were challenged with a lethal dose of DENV-2 JHA1 through the intracranial route two weeks after the last vaccine dose. Survival rates and the immune response profiles were followed both in spleens and infiltrated brain mononuclear cells. Analysis of the cytokine profiles generated by spleen cells or brain mononuclear cells, 4 days after challenge, revealed increased IFN-γ levels in vaccinated animals. In contrast, increased TNF-α and IL-10 levels were detected in non-vaccinated animals. Only the vaccinated animals developed full protective immunity to the lethal virus challenge (p=0.0057). The results obtained so far demonstrate the potential of non-structural proteins in the generation of protective immunity to DENV and contribute to the understanding of the immunological mechanisms associated with protection at experimental conditions.

Palavras-chave: Dengue, Vaccine, Non-structural Proteins, animal model
Dengue is a disease that in recent years has reached alarming incidence rates in the world. This disease is caused by the infection with the dengue virus (DENV) for which the main method of control based on the insect vector is not totally effective. This fact indicates that the search for safe and effective vaccines is a global priority. In this context, the use of recombinant proteins derived from the virus represents a viable alternative for the development of anti-dengue vaccines. The present study aimed the development and characterization of different vaccine formulations based on the non-structural protein 1 (NS1) administered by intradermal (i.d.) immunization route. As a strategy to improve the immune responses induced by the antigens, chimeric proteins generated after genetic fusion of DENV2 NS1 protein with monoclonal antibodies recognizing dendritic cell specific receptors (DEC205 and DCIR2). Vaccine formulations based on the NS1 protein or the chimeric proteins were administered by i.d. route in Balb/c mice and the specific antibody responses were measured as well as antibody subclass responses, antigen affinity and induction of potential deleterious side effects. Immunization with the chimeric proteins resulted in modulation of the serum IgG subclass responses and increased affinity of the NS1-specific antibodies against the target antigen but did not increase the magnitude of the response. In addition, no adverse effects associated with vaccine formulations were observed in vaccinated mice. Thus, the dataset obtained in this study demonstrates that, in a unprecedented way, that combination of a dendritic cell antigen targeting approach and use of the i.d. administration route may contribute for the improvement of anti-dengue virus subunit vaccine formulations. Financial Support: FAPESP and CAPES

**Palavras-chave:** Dendritic cell receptors, Dengue virus, Intradermal, Non-structural protein 1, Vaccine
The zika virus (ZIKV) is an arbovirus belonging to the genus Flavivirus, transmitted to humans by the bite of mosquitoes of the genus *Aedes* and can lead to clinical settings that can vary between asymptomatic and symptoms ranging from rash, conjunctivitis, headache and fever, being infection by this virus also associated with Guillain Barré Syndrome and cases of Microcephaly. The search for safe vaccines capable of conferring protection to ZIKV infection is a worldwide priority. In the present study, we evaluated an anti-ZIKV vaccine approach based on nanoparticles composed of multilamellar lipid vesicles (MLVs) combined with a recombinant ZIKV protein generated in a prokaryotic expression system. MLVs composed of anionic and neutral phospholipids were combined with a recombinant form of ZIKV domain III envelope glicoprotein (EDIII-ZIKV) produced in *Escherichia coli* BL21. BALB/c mice were immunized by the intramuscular route (i.m) with the vaccine formulation and antigen specific serum IgG titers were determined by ELISA. The formulation containing EDIII-ZIKV associated with MLVs proved to be effective in potentiating anti-EDIII-ZIKV antibody responses resulting in titers significantly higher than those observed in mice immunized with the EDIII-ZIKV protein combined with alum. These data demonstrate the efficiency of MLVs as an antigen delivery system and support further studies aiming determination of the protective immunity to the pathogen. Financial Support: FAPESP

**Palavras-chave:** Multilamellar Lipid Vesicle, Nanoparticles, Recombinant protein, Vaccine, ZIKV
PRELIMINARY RESULTS OF ANTIGENIC, PHYSICAL AND CHEMICAL CHARACTERIZATION OF HBSAG-VLPs

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Resumo

The recombinant Hepatitis B vaccine produced at Butantan Institute is composed of hepatitis B surface antigen (rHBsAg) expressed as VLP by Hansenula polymorpha yeast. The VLP based vaccines present advantages of security and effectiveness over live and attenuated vaccines, but it’s clear that maintaining the native conformation of the main epitopes within the VLP antigen in all stages of production and storage is critical for the maintenance of the antigenicity and immunogenicity of the vaccine. As stability study of the Butantan Hepatitis B vaccine, this work aimed to perform a physicochemical and antigenic characterization of the antigen in different steps of manufacturing where the protein is submitted to different conditions of pH and ionic strength (pH 5.5, 6.0, 6.4, 7.0, 8.5 with or without NaCl) and storage temperature (RT, 4°C and -20°C). The biological characterization was performed by antigenic content assessed by ELISA (Murex – ELISA) and by surface plasmon resonance (SPR) with specific mAbs. The purity and degradation patterns were analyzed by SDS-PAGE. The structure of recombinant rHBsAg particles was examined by circular dichroism (CD), synchrotron radiation circular dichroism (SRCD), Small-angle X-ray scattering (SAXS) and Dynamic Light Scattering (DLS). The preliminary results showed that rHBsAg is a very stable protein that does not show tendency to aggregate or change the secondary structure when the sample pH varied. In addition by SPR, pH or temperature of storage did not affect the rHBsAg recognition by three specific monoclonal antibodies. However the antigenic content of samples kept at 4°C and pH 5.5, 6.0 or 6.4 had decreased and presented degradation by SDS-PAGE. This was not observed when the sample was stored at 4°C and pH 7.0, the condition used for formulation of the vaccine. By SAXS, 90-95% of rHBsAg in samples form particles with a half spherical shell shape with a max diameter of 32 nm. When frozen at -20°C in different pH the DLS analyses showed the presence of 30% of volume in large aggregates (particles of 100 nm). These aggregates may perturb the stability and efficacy of the vaccine. The preliminary results indicate the better storage condition is 4°C pH 7.0 and the protein is sensitive to frozen temperatures. In vivo immunogenicity tests are planned to complement the results obtained so far. FINANCIAL SUPPORT - FUNDAÇÃO BUTANTAN

Palavras-chave: hepatite B, vacina, estabilidade
Influenza is a disease of global public health importance, due to its high transmissibility, morbidity and pandemic potential. Influenza pandemics take happens mostly when new influenza subtypes (heterosubtypic), to which the human population has no previous immunity, widespread around the world. Immunization is the primary way to reduce the impact of seasonal and pandemic influenza. Therefore, studies for alternatives to improve the heterosubtypic and to extend the memory immune responses generated by vaccination are promising fields of research. In this context, cytokines IL-7 and IL-15 are important targets to this aim, since they have been associated with the generation of a long-term memory response and the induction of a broad range cell mediate immune response, which would be able to protect against different strains and subtypes of influenza viruses. So, this study aims to evaluate the role of IL-7 and IL-15 cytokines in influenza virus infection in vitro and in the murine model. For this, we generated recombinant influenza viruses encoding the murine cytokine genes IL-7 (FLU-IL7) and IL-15 (FLU-IL15) by reverse genetics. Viral stocks were produced following successive cloning in cell culture. Further, the stocks were characterized by PCR, ELISA, sequencing and titration under agarose overlay. Recombinant influenza viruses have been found genetically stable and able to encode their foreign sequences in cell cultures, as well as in lungs of infected mice. Understanding the mechanisms of the immune response to viral infection under the stimulation of certain cytokines may support future studies aimed at new and more effective immunization strategies against influenza, as well as to be used as adjuvants enabling the development of new generation vaccines. Besides that, these recombinant viruses can be used as vectors against other diseases of medical interest. Financial Support: CAPES, FAPEMIG, CNPQ, IRR/FIOCRUZ

Palavras-chave: reverse genetics, recombinant viruses, long-term memory response, heterosubtypic response, new generation vaccines
TRANSCUTANEOUS ANTI-DENV VACCINES

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Resumo

Dengue is an arthropod-borne viral disease that threatens over half of the world population. At present, only one vaccine against the Dengue virus (DENV) infection is licensed for human use and several others are being tested under experimental and clinical conditions, but all of them require invasive administration procedures. The transcutaneous (TC) vaccine delivery route represents a non-invasive procedure that takes advantage of the specific cellular composition of the skin, which is enriched in antigen-presenting cells, such as Langerhans cells. In the present study, we evaluated the antibody responses generated in mice after TC immunization with regard to a parenteral (intradermal - ID) administration route using serotype 2 DENV particles (DENV2). Mice were submitted to a three doses immunization regimen in combination with a recombinant form of the heat-labile toxin (LT), originally produced by enterotoxigenic Escherichia coli (ETEC) strains, used as a vaccine adjuvant. After the immunization regimen, mice were intracranially (i.c.) challenged with the reference DENV2 New Guinea C (NGC) strain. The results indicate that vaccines administered via TC and ID routes were capable to induce strong humoral responses. Mice immunized via ID route elicited higher anti-DENV antibody responses than those immunized via the TC route. Incorporation of LT increased the production of specific antibodies and modulated the serum IgG subclass responses. Antibodies produced after the TC immunizations showed a prevalence of IgG1 subclass, with the IgG1/IgG2a ratio being modified after the introduction of LT. Mice immunized via ID route mounted a higher IgG2a response leading to a more balanced IgG subclass response. Furthermore, the antigen avidity of anti-DENV antibodies was increased, for both routes, after LT addition. Immunizations carried out via the two tested administration routes resulted in similar protection levels following lethal i.c. challenge with DENV2 NGC. The present results demonstrate that the TC vaccine administration route proved to have similar efficacy of vaccines administered via parenteral routes when measured by virus-specific serological responses following immunization with whole viral particles. Financial suport: FAPESP/GlaxoSmithKline

Palavras-chave: Dengue, Heat-labile toxin, Intradermal, Transcutaneous, Vaccines
The emergence and re-emergence of arboviruses such as Dengue virus, Zika virus and Chikungunya virus is considered a public health challenge due to the pathogenesis associated with these viral infections and their rapid spread. Strategies aimed to control these arboviruses include mosquito-vector control and the development of antiviral drugs and vaccines. Among the possible vaccine approaches that may be employed, the use of proteins, peptides and recombinant subviral particles expressed in different expression systems are highlighted. The co-expression of the Flavivirus capsid (C), membrane precursor (prM) and envelope (E) proteins can generate recombinant virus-like particles (VLPs) that resemble infectious virions in their structural and functional characteristics, but are not infectious. Adding these features to immunogenicity and safety in use as a vaccine antigen, VLPs are exploited as a platform for prophylactic vaccines against various viral infections. The use of yeast *Pichia pastoris* as an expression system has been explored for the production of VLPs and proteins of several Flaviviruses, such as TBE (Tick-borne encephalitis), Japanese Encephalitis and Dengue virus. In this context, the present work aimed the use of *P. pastoris* yeast for the production of VLPs based on the expression of the C, prM and E genes of ZIKV under the control of the constitutive promoter *PGK1*. Thus, the genes encoding the structural proteins were cloned in frame into a yeast expression vector. Once the cloning has been confirmed, yeast cells were transformed with the obtained construct and selected for the presence of the expression cassette. The selected clones were used in the induction experiment. Aliquots were collected at 24h, 48h, 72h, and 96h after the start of induction for further analysis. The verification of the recombinant polyprotein production was performed by western blot using anti-polyhistidine antibody. The analysis of different clones showed protein detection from 48h of induction. The obtained results confirm the efficiency of the system in producing the ZIKV structural proteins and the next step will be to verify the formation of VLPs and to evaluate the immunogenicity of the obtained structures. Financial Support: FACEPE.
The mosquito *Aedes aegypti* is responsible for the transmission of important public health problems, such as the four serotypes of dengue virus. Several products to control *Aedes* infestation have been developed, such as different types of adult traps and synthetic pesticides. The use of pesticides, however, can affect negatively the environment and select resistant lineages. Plant extracts have shown activity against immature forms of mosquitoes and produces less soil contaminants. The aim of this study is to assess the role of turmeric powder (*Curcuma longa*) extracts against *Aedes aegypti* immature forms. We have used commercial powder containing only rhizomes to prepare the extracts. The biologically active portions of *Curcuma longa* were extracted using water and lyophilized for further analysis. To assess larvicide activity, we have used 10 third-stage larvae and 10 fourth-stage larvae in 100 mL of different concentrations of the extracts. Different concentrations were tested in quintuplicates. Larvae from untreated controls were used to assess morphometric changes. Death rates of larvae were evaluated 24 hours after the exposition to different concentrations. The minimal inhibitory concentration (0,124 mg/ml) killed on average 10% of the larvae after 24 hours post exposition on third-stage larvae. The concentration that killed 62% of the larvae on this stage was 0,992 mg/ml, which was considered the cut-off concentration for the experiments with older immature forms. Concentrations ranging from 0,966 to 1,086 mg/ml killed, respectively, 72 and 80% of fourth-stage larvae. Morphometric changes, such as the augmentation of respiratory siphon, were noticed in treated larvae. No deaths were observed in untreated controls. We have used high performance liquid chromatography with diode-array detection in 250, 300 and 350 nm to assess chemical components of aqueous extracts of *Curcuma longa*. Phenolic acids, flavonoids, and curcuminoids were the major chemical components of the extracts. Steroids, alkaloids, tannins, and saponins were the minor chemical compounds of the extracts. Other studies have already demonstrated phenolic acid inhibitory effects against *Aedes aegypti*. The same has been observed for flavonoids and curcuminoids, however morphometric changes were not evaluated previously. Next step is to test the isolated action of each compound as well as to evaluate tissue changes in treated larvae.

**Palavras-chave:** Curcuma longa, *Aedes aegypti*, pesticides, larvic
Mato Grosso State presents a vast territory, tropical climate and diversity of biomes, vectors and vertebrate hosts favouring arbovirus circulation. Aiming to investigate the epidemiological circulation of Alphavirus and Flavivirus species, serum samples (n=454) of patients suspected of dengue (DENV), Zika (ZIKV) or Chikungunya (CHIKV) for up to five days were subjected to viral RNA extraction, duplex RT-PCR for Flavivirus (NS5) and Alphavirus (NSP1) followed by species-specific nested-PCR (DENV-1,2,3,4, yellow fever [YFV], Saint Louis encephalitis [SLEV], West Nile [WNV], Rocio [ROCV], Ilhéus [ILHV], Mayaro [MAYV] and Venezuelan, East and West equine encephalitis [VEEV, EEEV, WEEV] viruses). Additionally we performed RT-PCR protocols for ZIKV (NS5 and envelope) and CHIKV (envelope). Positive PCR products were confirmed by nucleotide sequencing. In total, 68 samples were positive for DENV-1 (4.4%), DENV-4 (29.3%), ZIKV (4.4%), YFV (4.4%), MAYV (47.1%), CHIKV (2.9%), EEEV (1.5%) and four were co-infected with MAYV/DENV-4, MAYV/ZIKV, DENV-4/DENV-1 and ZIKV/DENV-1. MAYV (n=8) and CHIKV (n=2) were isolated in Vero cells. Among these, 33.8% are man and 66.2% women, 20% of them were pregnant and 45.6% are 20-39 years-old. These patients are urban residents from Várzea Grande (26.6%), Cuiabá (23.5%), Campo Novo do Parecis (7.3%), Sinop (5.9%), Rondonópolis (5.9%), Nova Mutum (4.4%), Primavera do Leste (4.4%), Tangará da Serra (4.4%), Tapurah (4.4%), Peixoto de Azevedo (2.9%), Santa Carmem (1.5%), Nova Guarita (1.5%), Cláudia (1.5%), Mirassol D’oeste (1.5%) and Chapada dos Guimarães (1.5%); 10.3% of them have history of recent visit to rural and sylvatic habitats and 13.2% reported previous similar acute febrile disease. Most of these patients presented hyperthermia (50%), retro orbital pain (25%), exanthema (20.5%), myalgia (45.6%), arthralgia (35.3%), headache (35.3%), petechiae (17.6%) and pruritus (11.6%). A MAYV outbreak occurred in Várzea Grande during May-July, 2015. The first human cases of ZIKV Asian genotype and CHIKV ECSA genotype in the State were detected in Tapurah in August, 2015 and in Mirassol D’Oeste in July, 2015, respectively. One case of EEEV Madariaga was confirmed in a 10 year-old boy with history of constant visit to a rural area, without neurological signs, resident of Várzea Grande in October 2015. Here we demonstrate the importance of constant surveillance for arboviruses in the State.

**Palavras-chave:** flavivirus, alphavirus, RT-PCR, viral isolation, surveillance
CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF A NEW BACULOVÍRUS: MYTHIMNA SEQUAX NUCLEOPOLYHEDROVIRUS

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Resumo

The wheat caterpillar of the species Mythimna sequax is the main pest of some crops including wheat, oats, barley and rice in southern Brazil. The baculovirus insect viruses when applied in plantations may be an alternative method of control and that does not harm the ecosystem. However, there are no reports of baculoviruses isolated from M. sequax. The Embrapa-Soja virus database contains an extract from wheat lages with symptoms of baculovirus infection. Previous analyzes showed the presence of polyhedra in the extract. Thus, we aim to characterize at the molecular level the putative baculovirus found in the wheat caterpillar extract. We extracted the DNA by previously described methods and sequenced using the 454 platform (Macrogen, South Korea). After sequencing, the data obtained was submitted to de novo assembly and annotated the ORFs with the Geneious R9 program. Moreover, BlastX to identify homologous genes. We found the complete genome of a baculovirus, named Mythimna sequax nucleopolyhedrovirus (MyseNPV). The genome has a size of 148,403bp and a G+C percentage of 40.3%, with 169 CDS, and 13 ORFs are unique. The virus presents all core genes and after phylogenetic analysis, we found that the virus belonged to the genus Alphabaculovirus of Group II, which exclusively infect lepidoptera. The virus is close to the ancestor of the group containing baculoviruses that infect other pests of agricultural importance of MacoNPV-A, MacoNPV-B, MbMNpV and HaMNPV. We found a high degree of identity and syntenia between these genomes. A gain and loss reconstruction was performed within the group, and the main differences were: (1) absence of helicase-2 in MyseNPV and an independent loss in MacoNPV-A; (2) lef-7 is only present in MyseNPV and MacoNPV-A, whose absence in others suggests a loss in the ancestor of this group; And (3) Viral enhancing factor is absent in MyseNPV. In addition, we found that the BRO-A gene has similarity to an ascovirus, that suggests a horizontal acquisition during a co-infection between them in a host cell. Importantly, the position of chitinase and cathepsin are in contrast to other baculoviruses because they are more distant in the genome, but phylogenetic analyzes and tree reconstructions do not suggest an independent gain and more studies are needed. The analysis of this genome is of extreme importance to study the importance of these genes in viral infection and to develop a bioinsecticide. Financial support: Cnpq, FAP/DF

Palavras-chave: baculovirus, wheat pest, MyseNPV, alphabaculovirus, bioinsecticide
177 - ÁREA: VEGETAL E INVERTEBRADOS

CLARIFYING A DENSOVIRUS GENOME ANNOTATION, FIRST REPORTED IN BRAZIL

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Resumo

High throughput sequencing (HTS) has been related in many new papers describing new viruses or associating known ones with new hosts. The project objective was the prospection of new viruses using metagenomic and bioinformatics tools. For this purpose, total DNA was extracted from ten aphids (Myzus persicae) and sequenced by Illumina HighSeq 2500. The library were submitted to filtered and trimmed based in quality analysis, than were used in a 'de novo' assembly. A local Blastn was performed in all filtered contigs against a database containing all viral sequences, complete and non-redundant, present in Genbank site, downloaded in October 2016. A 5400 nt contig was found, high identity (95%) related to Myzus persicae nicotianea densovirus (MpnDV) and Myzus persicae densovirus (MpDV), both have already been reported in the aphid Myzus persicae and never related in Brazil. MpDV is reported in literature as containing 5 ORFs in genomic organization, whereas MpnDV is reported only 4 ORFs, despite their very high identity, around 97%. Due to the similarity among the contig found and the sequences of the viruses used as reference, a more detailed and in-depth analysis of their ORFs was done using the ORF finder tool, and manual inspection. Primers were designed for amplification of a 3400 nt fragment, involving the divergent ORFs sequences region. Cloned fragments were obtained and sequenced by sanger method. Based on the data obtained, we conclude that the sequence of Myzus persicae densovirus, reported in the literature with 5 ORFs, is probably annotated with errors and the correct ORF organization is that described within only 4 ORFs. Finnnancial support: CNPq, FAPDF

Palavras-chave: Densovirus, HTS, Bioinformatics, ORF, Annotation
The cultivated potato (Solanum tuberosum L.) is an important vegetable crop in Brazil with production concentrated especially in South, Southeast, and Central-West regions. In contrast, the wild potato (S. commersonii Dun.) is used as source of resistance in breeding programs. Potato crop has been affected by many viral diseases causing crop degeneration, and significant losses in production. Although begomovirus (family Geminiviridae) infections have been detected in low incidence in the field; however, these viruses represent a potential threat to potato production considering the high whitefly populations, as well as, begomovirus sources in the field. The objective of this work was to investigate diversity of begomovirus in cultivated and wild field-grown potato plants naturally infected. Leaf samples were collected from cultivated (47 samples) and wild (31 samples) potato plants showing yellow mosaic, leaf deformation and plant stunting. Total DNA was extracted from leaf samples using CTAB method and subjected to PCR-based tests with degenerated primers for begomovirus detection. DNA samples were also amplified by Rolling Circle Amplification (RCA) and digested with MSP I restriction enzyme for polymorphism investigation.

Natural begomovirus infections on both potatoes, cultivated and wild, in the field resulted in typical symptoms such as strong yellow mosaic and deformation of leaves. Out of 78 symptomatic potato plants PCR-tested for begomovirus, 95.7% and 64.5% were positive, for cultivated and wild potato, respectively. Total DNA obtained from thirty four (cultivated: 17; wild: 17) begomovirus positive plants were selected for polymorphism investigation through enzymatic digestion with MSPI. At least five enzymatic different profiles were identified for begomovirus isolates obtained from cultivated potato, while just one profile was verified for those isolates collected from wild potato. These data indicate the presence of variation in begomovirus samples from cultivated potato, suggesting the existence of different virus populations in the infected plants. In contrast, begomovirus isolates from wild potato which followed a homogeneous pattern indicate the presence of less variability. Cloning and sequencing of begomovirus isolates obtained from both potatoes, cultivated and wild, with species identification will clarify their genetic diversity. Financial support: Embrapa and FAPESP.

**Palavras-chave:** begomovirus, wild potato, diversity, cultivated potato
179 - ÁREA: VEGETAL E INVERTEBRADOS

ENTOMOVIROLOGICAL SURVEILLANCE OF THE YELLOW FEVER OUTBREAK IN BRAZIL IN 2017

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**Resumo**

Yellow fever is a disease caused by Yellow Fever Virus (YFV) belonging to the family Flaviviridae, genus Flavivirus. There are two cycles of viral maintenance: sylvatic and urban. In the sylvatic cycle, the main vector is the mosquito of the genus Haemagogus and in the urban cycle, the Aedes aegypti. In Brazil, the urban form was extinguished in 1942 while the sylvatic form is endemic in the Amazon Region. Also, sporadic cases of the disease occur in places where there is a susceptible population. In 2017, in Brazil, human cases of sylvatic yellow fever were recorded in the Southeast Region and, subsequently, in other states of the country. The aim of this study was to investigate the presence of virus in mosquitoes from areas where cases of the disease have been reported in 2017. The mosquitoes were identified and grouped in pools. Each pool was triturated with diluent solution composed by fetal bovine serum and antibiotics and submitted to viral RNA extraction with the QIAamp® MinElute®Virus Kit. Then, the Reverse Transcription followed by Polymerase Chain Reaction mediated by Quantitative Polymerase (RT-qPCR) procedure was performed based on the protocol developed by Domingo et al (2012) using the QuantiTect Probe RT-PCR® Kit. The samples were considered positive when the Ct (Threshold Cycle) value was lower or equal to 37.

Therefore, a total of 70 pools of mosquitoes from Minas Gerais, Mato Grosso do Sul, Bahia and Pará states were analyzed by RT-qPCR. Nine of these pools were positive to YFV, distributed as follows: AR843713 (15 females of Hg. janthinomys, Ct 32.23), AR843720 (25 females of Hg. janthinomys, Ct 29.08), AR843721 (25 females of Hg. janthinomys, Ct 28.44), AR843728 (19 females of Hg. janthinomys, Ct 32.09), AR843741 (2 females of Hg. leucocelaenus, Ct 24.09), AR843765 (6 females of Hg. janthinomys, Ct 32.51), AR843777 (1 female of Hg. janthinomys, Ct 27.60), AR843807 (7 females of Hg. janthinomys, Ct 34.81), all from Minas Gerais state and AR845803 (18 females of Hg. janthinomys, Ct 28.63) from Bahia state. The positive samples for YFV also were inoculated in C6/36 cells and just the sample AR843721 was positive for YFV. The detection of genome of YFV and viral isolation in potential vectors elucidated the circulation of YFV in Brazilian territory and showed the importance of entomovirological investigations in outbreaks.

**Palavras-chave:** Haemagogus, Yellow Fever, Flavivirus
FIRST REPORT OF PASSION FRUIT GREEN SPOT VIRUS IN THE STATE OF MATO GROSSO, BRAZIL

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Resumo

Passion fruit green spot disease, caused by Passion fruit green spot virus (PfGSV), affects plants of genus Passiflora and is characterized by chlorotic or necrotic lesions on fruits, leaves and branches. In severe cases, a general dieback is observed, leading to the death of the infected plants. The disease has been only described in few areas of SP, MA, BA and DF. PfGSV is a tentative cilevirus [ss(+)]RNA transmitted by false-spider mites of the genus Brevipalpus. In 2016, passion fruit producers from Tabaporã and Sinop, MT, reported severe necrotic symptoms and the death of passion fruit plants. To identify the causal agent of the disease, samples were submitted to transmission electron microscopy (TEM) analyses and RT-PCR for the detection of PfGSV. Brevipalpus mites occurring in the symptomatic samples were also collected for their morphological characterization. Total RNA was extracted from the lesions using Trizol® and three specific primer pairs were used to detect the putative presence of both RNA1 and RNA2 molecules from PfGSV. All samples tested positive for PfGSV, yielding the expected bands in 1% agarose gels. Amplicons were sequenced and exhibited more than 98% nucleotide identity with the partial sequence of PfGSV available in the GenBank (HM002746 and HM002747). Bacilliform particles (50-70 nm x 100-120 nm) and dense viroplasms, similar of those caused by cileviruses, were observed in TEM in the cytoplasm of infected cells. No other viral particles were detected by TEM. All mites collected in symptomatic plants (10 specimens) were identified as B. yothersi. This study is the first report of the disease in the state of Mato Grosso, Brazil. After the identification of the disease and notification of the results, passion fruit growers were able to proceed with the correct management of the disease through the control of the mite vector in the Tabaporã and Sinop areas.

Palavras-chave: Cilevirus, Brevipalpus mites, RT-PCR
182 - ÁREA: VEGETAL E INVERTEBRADOS

GENOMICS OF A NOVEL BACULOVIRUS ISOLATED FROM A LEGUMINOUS PEST, URBANUS PROTEUS (LINNAEUS, 1758) (LEPIDOPTERA: HESPERIIDAE)

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Resumo

Baculovirus are insect viruses that naturally controls population of insects including agricultural pests. They are divided into four genera, one them Alphabaculovirus are infectious to the larval stage of moths and butterflies. The Embrapa-Soja has a robust collection of viruses with almost 100 extracts of caterpillars that showed symptoms of baculovirus infection. We received part of an extract identified as Urbanus proteus NPV (UrprNPV) to analyze. Urbanus proteus belongs to the family Hesperiidae, one of the butterfly families that causes severe loss in beans, peas, and fava beans crops during the larval stage. In this work, we sequenced the genome of the putative virus using 454-pyrosequencing. The genome were de novo assembled and annotated using Geneious-R9 and BLAST-X. We also reconstructed the virus phylogeny using a set of 38 conserved genes. The genome of UrprNPV is 105 kbp long with a G+C content of 34.7%. We found 119 ORFs of at least 50 amino acid residues; 109 showed similarity to other baculovirus. We found the f protein, typical of group II alphabaculoviruses, iap-2 and iap-3, both related to the inhibition of cellular apoptosis. Nine ORFs were shown to be unique and only one presented no hit with any other organism in GenBank. The genome lacks chitinase, cathepsin, and typical homologous regions. Phylogenetic analysis based on the concatenated dataset of the 38 baculovirus core genes confirmed that UrprNPV is a group II Alphabaculovirus and belongs to a lineage that includes Adoxophyes honmai nucleopolyhedrovirus (AdhoNPV) and Adoxophyes orana nucleopolyhedrovirus (AdorNPV). UrprNPV presents the smallest genome among all alphabaculovirus sequenced to date. In order to understand such small genome, we investigated the loss and acquisition of genes and intergenic spaces in comparison to other closely related viruses. Regarding AdorNPV and AdhoNPV, UrprNPV did not present 17 genes, including 15 hypothetical proteins, one bro and the p43. The intergenic space was reduced as well. Two hypotheses could explain that genome shortening: UrprNPV belongs to a lineage that reduced independently its genome or this lineage resembled an ancestor that had not acquired genes and intergenic spaces. Importantly, most of the individual phylogenies of each core gene did not reconstruct the phylogeny based on the concatenated dataset, indicating high diversity in this virus lineage depicting resemblance to the ancestor or restricted dataset. Financial Support: CNPq

Palavras-chave: Alphabaculovirus, agricultural pests, biological control, Urbanus proteus NPV, genomics
Mycoviruses infect major of taxonomic Fungi spread around the world. In some associations, mycovirus are able to induce hypovirulence in the fungi host. This characteristic is important since it represents a potential tool to be explored as a control of fungal diseases. In this study, we identified a mycovirus infecting the phytopathogenic fungi *Sclerotinia sclerotiorum*, responsible for white mold disease. After sequencing, BLAST and molecular analyses revealed the presence of a Mitovirus (family Narnaviridae) with 2.7kb double-stranded RNA referred temporarily as *Sclerotinia sclerotiorum* mitovirus MV6/24 (SsMV6/24). The mitoviruses are no-virions that infect mitochondria’s fungi with unique ORF that encoded a RNA-dependent RNA polymerase in a genome with approximately 2.3 – 2.7 kb. In addition, tests were performed to test the pathogenicity of the fungus mitovirus-infected and compare with the mitovirus-cured. The presence of infection mitovirus on *S. sclerotiorum* generated changes such as a reduced growth rate, alteration morphology colony, reduction in the number of sclerotia, oxalic acid and reduced aggressiveness in plants. These results suggest that mitovirus is a potential candidate to be used as biocontrol agents against fungus. Besides, the relationship between SsMV6/24 with *S. sclerotiorum* is capable of generating hypovirulence, which is a source to future studies about the molecular mechanism evolved and ecological function. Financial Support: Fapemig, CNPq and CAPES

**Palavras-chave:** Biologic control, Hypovirulence, Mycovirus
The cell-to-cell movement protein (NSM) of tomato spotted wilt viruses (TSWV) has been identified as the avirulence determinant (Avr) of the Sw-5b-mediated resistance from tomato (Solanum lycopersicum L.). The expression of TSWV NSM in plants harboring the Sw-5b gene leads to a hypersensitive cell death response (HR). Apart from TSWV resistance-inducing (RI) isolates, resistance-breaking (RB) isolates have already been reported. This ability to overcome the Sw-5b resistance has been associated with two point mutations (C118Y and T120N) in the NSM proteins of RB TSWV isolates. Here we first show that the Sw-5b gene is sufficient for resistance not only against TSWV, but to members of five phylogenetically-related species classified within the so-called “American” evolutionary clade. Differently, Bean necrotic mosaic virus (BeNMV), a member of another clade circulating in the American continent, did not trigger Sw-5b-mediated HR. The NSM proteins of four (including TSWV and BeNMV) out of the seven tospovirus species tested for HR induction were then pointed mutated for Y118C and T120N. These mutations in all NSM proteins but BeNMV ended up in abrogating HR induction in both transgenic-N. benthamiana and tomato isolines harboring the Sw-5b gene. Finally, to investigate whether the viral movement functions and avirulence were attached, truncated versions of TSWV NSM lacking motifs associated with tubule formation, cell-to-cell or systemic viral movement were made. Upon co-expression of Sw-5b and NSM truncations in wild type N. benthamiana leaves, HR was still triggered when 50 amino acids (out of 301) were deleted from either amino- or carboxy-terminal ends like the full length NSM protein. Financial Support: CNPq, CAPES, FAP-DF, UnB, WUR and UPV.

**Palavras-chave:** Sw-5b, tospovirus, tomato, resistance, movement proteins
Mato Grosso State presents climatic and ecologic conditions favorable to arbovirus occurrence, associated with a great diversity of susceptible host and vector species. Culicinae subfamily comprises most of the vector species associated to arbovirus transmission. This study aimed to investigate the presence of viruses in the salivary glands of mosquitoes captured in a RAPELD area at Pirizal, North Pantanal with CDC light traps and Nasci aspirators during the rainy, transitional and dry periods of 2014-2015. The 1,657 specimens belonging to at least 16 species were allocated into pools according to climatic period and genus, and subjected to viral RNA extraction, double-strand of cDNA synthesis and viral randomic amplification through PCR, followed by high throughput sequencing (Illumina HiSeq 2500). Sequencing analysis revealed the presence of 15 putative novel viral species in these mosquito pools. Two viruses belonging to Rhabdoviridae and Iflaviridae families with 60% and 40% of identity with the closest viruses within these families were identified in three Psorophora albigena pools captured in the rainy period. In the transitional period, one pool of Aedes spp. presented one virus belonging to Flaviviridae (51% of identity) and one pool of Aedes scapularis presented a Bunyaviridae member (62% of identity). In the dry period, one pool of Sabethes gymnothorax presented one Flaviviridae species (45% of identity), one Chuviridae member (69% of identity), one Reoviridae (38% of identity), one Partitiviridae (56% of identity) and two contigs of Bunyaviridae members (41 and 56% of identity). Also in the dry period, one pool of Coquillettidía spp. presented one Rhabdoviridae (66% of identity) member, one pool of Psorophora albigena presented one virus belonging to Totiviridae (43% of identity) family, one Rhabdoviridae member (56% of identity), one Partitiviridae (54% of identity) and one Bunyaviridae species (54% of identity) and another pool of Psorophora albigena presented a Circoviridae species (70% of identity). The relatively low identity with known species indicates the presence of novel viral species associated to Culiciniae members in North Pantanal.

**Palavras-chave:** HIGH THROUGHPUT SEQUENCING, NOVEL VIRUSES, INSECT-SPECIFIC VIRUSES, ARBOVIRUSES, MOSQUITOES
6-METHYLMERCAPTOPURINE RIBOSIDE, A THIOPURINE NUCLEOSIDE WITH ANTIVIRAL ACTIVITY AGAINST CANINE DISTEMPER VIRUS IN VITRO.

OTÁVIO VALÉRIO DE CARVALHO, DANIELE MENDES FELIX, CLAUDIA DE CAMARGO TOZATO, CATARINA MARIA CATALDI SABINO DE ARAÚJO, JULIANA LOPES RANGEL FIETTO, MÁRCIA ROGÉRIA DE ALMEIDA, GUSTAVO COSTA BRESSAN, LINDOMAR JOSÉ PENA, ABELARDO SILVA JÚNIOR

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Resumo

Canine distemper (CD) is a widespread infectious disease that can severely impact a variety of species belonging to the order Carnivora, as well as non-carnivore species such as non-human primates. Despite large-scale vaccination campaigns, several fatal outbreaks have been reported in wild and domestic carnivore populations. This fact, in association with expansion of the disease host range and the development of vaccine-escape strains, has contributed to an increased demand for therapeutic strategies synergizing with vaccine programs for effectively controlling canine distemper. 6-methylmercaptopurine riboside (6MMPr) is a modified thiopurine nucleoside with known antiviral properties against certain RNA viruses. We tested the inhibitory effects of 6MMPr against a wild-type strain of canine distemper virus (CDV) infection in cells culture. We measured infectious particle production and viral RNA levels in treated and untreated CDV-infected cells. Ribavirin (RIB) was used as a positive control. Here, we report for the first time the antiviral effects of 6MMPr against CDV in vitro. 6MMPr was able to reduce viral RNA levels and to inhibit the production of infectious CDV particles. The selectivity index of 6MMPr was approximately six times higher than ribavirin. Our results indicate that 6MMPr has high anti-CDV potential and warrants further testing against other paramyxoviruses, as well as clinical testing of the compound against CDV. This project was funded by CNPq, CAPES, FAPEMIG.

Palavras-chave: Canine distemper, Antiviral, Azathioprine, Thiopurine, Nucleoside analogue
ANALYSIS OF CITOKYNE EXPRESSION PROFILE IN PBMC AND MACROPHAGES FROM DONKEYS AND EQUINES AFTER IN VITRO EIAV INFECTIONS

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Resumo

Equine infectious anemia virus (EIAV) is a lentivirus that infects all Equidae family members throughout the world however, the vast majority of studies have been conducted in horses (Equus caballus) with comparatively little information available for other equid species such as donkeys (Equus asinus). The aim of this study was to evaluate in vitro the cytokine profile related to EIA pathogenesis in equine and donkeys PBMC and, donkey’s macrophages after challenge with virulent and avirulent cell adapted EIAV strains. Cytokine expression profile was evaluated by real time qPCR, targeting TNF-α, IL-1α, IL-6, TGF-β and IFN-β genes using total RNA obtained from PBMC and macrophages cultures. After infection with high virulent EIAVWYO, the equine PBMC showed increased expression of TNF-α (24h), IL-6 e IFN-β (96 h) compared to donkeys PBMC. In the experiments of donkey macrophages infections with virulent and avirulent EIAV strains, it was not observed dysregulations in cytokine expression such as TNF-α, IL-1α, IL-6 e TGF-β (p<0,05). In this work, smaller or no evident dysregulation of cytokines by donkey PBMC and macrophages infected with EIAV may indicate that the pathogenesis of EIA in donkeys occur by different mechanisms from those established for the equine species. Thus, further studies are necessary to elucidate the cellular immune response mechanisms against EIAV in this species such as, retroviral restriction factors (RRF).

Palavras-chave: EIA, donkeys, pathogenesis
apothesis influencing the reactivation cycle of latent infection by bovine herpesvirus 1 in experimentally infected calves

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Resumo

Bovine herpesvirus 1 (BoHV-1) is the cause of respiratory and reproductive clinical signs in bovines leading to rhinotracheitis, vulvovaginitis, balanoposthitis and abortion. When in latent infection, the virus remains mainly in neuronal sites without significant induction of gene expression, genome replication or viral production. Generally, under stressful conditions, this expression is reactivated, leading to viral replication and reactivation of the infection. This study aimed to clarify the gene expression profiles on neuronal tissues in infection transition from latency to acute phase in bovines. Specifically, key genes associated with apoptosis signaling pathways were studied as their expression is likely affected by glucocorticoid levels that is increased during stress. To perform this study, we used dexamethasone, a potent synthetic glucocorticoid with immunosuppressive and anti-inflammatory activity. Dexamethasone was administered in animals experimentally infected with a BoHV-1 strain and under the latent phase of infection to reactivate the viral productive activity. For the first two months, animals were acclimated to the confined environment and monitored serologically every 15 days to ensure their viral negativity. The groups were identified as G1- mock infected treated with dexamethasone; G2- mock-infected not treated with dexamethasone; G3- BoHV-1-infected treated with dexamethasone (productively infected animals); G4- BoHV-1-infected not treated with dexamethasone (latently infected animals). Group 3 and 4 animals received 107.3 TCID50/ml BoHV-1 Brazilian strain EVI 123/98 in each nostril (total = 2 x 107.3 TCID50/animal). The animals were monitored for viral isolation and virus neutralization. The trigeminal ganglia were collected on experimental day 51 post-infection for total RNA extraction. Tissues were fixed in 10% neutral buffered formalin before processing to perform TUNEL assay. It was observed that pro-apoptotic genes, notably caspase 8 gene, are upregulated in trigeminal ganglia from cattle experimentally infected with BoHV-1, during the transition to a productive infection when induced by dexamethasone. Apoptosis in trigeminal ganglia was confirmed by TUNEL analysis. These results suggest the relation between of virus influencing apoptosis and the latency- reactivation cycle in experimentally infected cattle with BoHV-1. Financial Support: FAPEMIG, CNPq and CAPES.

Palavras-chave: Apoptosis, BoHV-1, Latency
Avian paramyxovirus (APMV) belongs to the genus Avulavirus in the family Paramyxoviridae. Members of family Paramyxoviridae are characterized by pleomorphic enveloped particles that contain a single-stranded, negative sense RNA genome. APMV are classified into thirteen distinct serotypes (APMV-1 to 13) by ICTV Virus Taxonomy 2017. Recently, six novel avulaviruses were described. The APMV-14, was discovered in a fecal sample of an unspecified duck sampled in Japan. Another avulavirus, APMV-15, was discovered in a cloacal swab of a white-rumped sandpiper (Calidris fuscicollis) sampled in Lagoa do Peixe National Park, Brazil. Another, tentatively also referred to as APMV-15 (and here renamed APMV-16), was discovered in feces of unspecified birds in South Korea. In addition, three distinct avulaviruses, Antarctic penguin virus A, B and C (here renamed APMV-17,-18 and -19) were detected in cloacal swabs of Gentoo penguins (Pygoscelis papua) sampled at Kopaitic Island in Antarctica. Here, we describe the biological and genetic finds of the new serotype APMV-15, first detected in the Rio Grande do Sul state, Brazil. Cross-hemagglutination inhibition tests indicated APMV-15 to be unique in the genus Avulavirus. Intracerebral pathogenicity index (ICPI) and mean death time (MDT) in eggs of APMV-15 showed that this isolate is a low pathogenic strain. The coding-complete genome of APMV-15 were compared to all other avulavirus coding complete genomes and performed phylogeny indicating its closest related to APMV-10, -8 and -2. Financial Support: FAPESP 2017/01125-2

Palavras-chave: Avian paramyxovirus, Avulavirus, Migratory birds, Paramyxoviridae
Viruses are an abundant and diverse group. Broad surveys of viral diversity are hindered by the inability to sample, from any location, a sufficient number of individual hosts, in terms of diversity and quantity, to provide access to this virome. This study utilized metagenomics to provide a snapshot of the diversity of RNA viruses present in ten samples taken from Non Human Primates studied during the surveillance for Yellow Fever in the States of São Paulo and Espirito Santo, Brazil, 2017. Viral metagenomics was used to analyze 10 cell culture specimens collected from non human primates sampled during the surveillance for yellow fever. Each specimens were clarified by centrifugation and then filtered. Viral nucleic acids were extracted using a Maxwell 16 automated extractor. Viral cDNA synthesis from extracted viral RNA/DNA was performed in a reverse transcription reaction and a 2nd strand cDNA synthesis was performed, followed by the use of a Nextera XT Sample Preparation Kit (Illumina) to construct a DNA library with each sample identifiable using dual barcodes. For size selection we used a Pippin Prep (Sage Science, Inc) to select a 400bp insert (range 200-600bp). The library was deep-sequenced using the MiSeq Illumina platform with 300bp paired ends. Datasets were then trimmed according the quality (99,9% coverage) and length (reads <30bp were removed) of each read using Geneious R9 software. Sequences obtained were examined to ensure that the mapping to a reference sequence did not generate a biased consensus sequence. Sequences identified as mosquito endogenous viruses were discarded as they likely comes from the cell culture used for virus isolation. The majority of the sequences obtained were from Yellow Fever virus, as the virus was isolated from all the studied samples, but other findings indicated a distinct viral community circulating in those primates. The virome observed in the samples included sequences related to the Bovine Viral Diarrhea virus, from the Pestivirus genus, a virus similar to the Simian Immunodeficiency Virus, a retrovirus, and a virus similar to the Feline Leukemia Virus, another retrovirus. The Non human primates may have obtained those viruses through spill over from other vertebrate hosts. Through sampling sylvatic animal, metagenomics may enable a broad survey of viral diversity and can significantly increase our knowledge of the RNA viruses present in sylvatic animals.

**Palavras-chave:** VIRAL DIVERSITY, VIRAL METAGENOMICS, NON HUMAN PRIMATES
Canine parvovirus type 2 (CPV) is recognized as the most common viral enteric pathogen in dogs. It is also known that CPV induces suppression of the immune system and thus may facilitate infection with other agents, but the role of bacterial enteropathogens in CPV-infected dogs is still poorly understood. The purpose of the present study was to detect and characterize *Clostridium perfringens* and *C. difficile* from CPV-infected dogs. A convenience sample of dogs was used and some selected epidemiological data were obtained: age, vaccination status, and clinical outcome, whole blood count. Stool samples were obtained at the moment the animals were admitted in the Veterinary Hospital and submitted to total DNA extraction. Stool samples positive for CPV by PCR (n = 82) were further submitted to *C. perfringens* and *C. difficile* detection. In addition, 20 random CPV-positive samples were selected for VP2 sequencing. For isolation of *C. perfringens*, stool samples were plated on SPS Agar and colonies were subjected to a previously described PCR protocol for the detection of genes encoding the major *C. perfringens* toxins (alpha, beta, epsilon and iota), enterotoxin (*cpe*) and NetB-, NetE-, NetF- and NetG- encoding genes (*netB*, *netE*, *netF*, and *netG*, respectively). One aliquot of each stool sample positive for *C. perfringens cpe*+ strains were subjected to CPE detection in a ELISA kit. For isolation of *C. difficile*, stool samples were inoculated on TCCF Agar and colonies were subjected to a previously described PCR for a housekeeping gene (*tpi*), toxins A (*tcdA*) and B (*tcdB*). Stool samples positive for toxigenic *C. difficile* were subjected to A/B toxins detection by an ELISA kit. The majority of animals included were puppies, unvaccinated or in intermittently vaccinated against CPV, and showed leukopenia. All samples submitted to CPV sequencing were typed as CPV-2b. Enterotoxigenic *C. perfringens* type A was isolated from three (3.6%) dogs. One (1.2%) strain, from an adult dog, was also positive for *netE*and *netF*. *C. difficile* infection was confirmed in one (1.2%) dog by isolation of a toxigenic strain and detection of A/B toxins. Therefore, the present study identified *C. difficile* and *C. perfringens* infection in CPV-positive dogs. Further studies are necessary to clarify if clostridial infections may predispose or potentiate CPV-infection in dogs or vice-versa.

**Palavras-chave:** *Clostridium difficile*, *Clostridium perfringens*, Coinfection, Dog, Parvovirus
The Caprine Arthritis Encephalitis - CAE is an important disease in goats that causes serious economic losses. It is caused by a retrovirus, subfamily Lentivirinae, and results primarily in chronic arthritis as early as in young adults and leukoencephalomyelitis in kids. In addition, it is associated with mastitis, interstitial pneumonia and weight loss. This disease is persistent, progressive and debilitating. The efficiency of CAE’s sanitary control programs depends on the sensitivity and specificity of the tests used in the initial diagnosis, and on the serological monitoring of the measures implemented, the frequency of their use and the management used in the herd. This study aimed to compare three diagnostic tests, two serological (Agar Gel Immunodiffusion - AGID and Western Blotting –WB), and one molecular (Nested Polymerase Chain Reaction - nPCR). The production of antigens for the AGID and WB was prepared on secondary cell cultures of goat synovial membrane infected with the CAEV-Cork standard strain. For the comparison of the tests, 222 samples from dairy goats were used. Relative sensitivity, relative specificity, positive and negative predictive values, and efficiency test were evaluated. The results were compared using the chi-square test with Yates correction (p2). The Kappa index was also calculated between tests. AGID, WB and nPCR detected 47 (21.2%), 71 (32.0%) and 108 (48.6%) positive animals, respectively. When comparing tests, it was found that WB detected more positive animals than the AGID (p<0.001). The results of nPCR paired with WB and AGID were compared and no significant difference was observed (p>0.05). Considering, as infected, the animal that had at least one positive result in any test, it was verified the detection of 138 (62.2%) total positive. Using all three tests together leads to 27.8% increase in detection as compared to the best (individual) test. For AGID, WB and nPCR diagnostic tests, the following sensitivities were observed: 34.1%, 51.4% and 78.3%, respectively. Taken as whole, it can be concluded that WB is more sensitive than AGID and that the combination of nPCR with a serological test (AGID or WB) significantly increases the immune response and detection. Financial Support: EMBRAPA; FUNCAP.

Palavras-chave: Goat, Lentivirus, Diagnostic tests
DETECTION OF PICOBIRNAVIRUS BY RT-PCR IN BIRDS FROM SANTA BÁRBARA, THE METROPOLITAN MESOREGIONS OF BELÉM, PARÁ STATE.

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Resumo

The anthropogenic actions have been presented as an important factor in the emergence of several emerging and reemerging diseases. In the state of Pará, the mesoregions of Belém and northeastern of the state are favorable to these events, since they have a high rate of deforestation in the Amazon, causing an imbalance of the ecosystem and providing a risk factor for the propagation of zoonotic diseases. Considered as emerging agents, opportunistic and suggestive of zoonotic potential, picobirnavirus (PBV) have been described in a wide range of animal species, with or without diarrheal signs. However, epidemiological and molecular studies of PBV still need to be better explored in order to prove the transmission between species and to show their zoonotic potential. In the period from February 2015 to April 2016, 30 fecal sample specimens of birds were collected, belonging to the municipality of Santa Bárbara, mesoregion of Belém, state of Pará. Those samples were suspended in 0.01M Tris Ca ++, submitted to the extraction of nucleic acid by the Boom method, and submitted to Polyacrylamide Gel Electrophoresis (PAGE). The Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) was performed for the smaller segment encoding RNA-dependent RNA polymerase (RdRp) using specific primers for genotype I and PBV genotype II. Among the fecal specimens tested, 13.33% (4/30) of positivity for genotype I were obtained, being these birds distributed between domestic birds (2/30) and wild birds (2/30). All samples showed negativity when tested by PAGE. Of the 4 positive samples for PBV, just one of them was characterized by genomic sequencing, showing homology with genogroup I and a proximity to strains of rabbit PBV. The results show the circulation of PBVs in birds from anthropic areas, in the city of Santa Bárbara, and indicate the requirement for genotypic characterization using primers that encode a greater number of base pairs, as well as a better phylogenetic characterization for transmission between species.

Palavras-chave: Picobirnavirus, RT-PCR, Birds
Differential expression of genes involved in bovine papillomavirus infection

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Resumo

Bovine papillomavirus (BPV) infects epithelial cells from bovine causing hyperproliferative lesions, which may progress to form malignant tumors. The virus interacts with host cells by altering the regulation of some genes, and thus triggering the lesion. However, it is not well known which genes are regulated by the virus. Therefore, this study is relevant in order to increase the knowledge on BPV pathogenesis, which could be important to identify possible novel drug targets against papillomatosis. The objective of this study was to make use of next-generation RNA sequencing methods to identify differentially expressed genes associated with the BPV infection, which can elucidate possible marker genes that could be used to control the disease, by identifying ligands that interact with the products of these genes. Adult bovines with papillomatosis and health animals was assessed. Several lesions were used for library construction. Histological and molecular assays were performed to confirm the infection by BPV. After sequencing, Galaxy-based command-line driven tools were used to analyze the sequences. Illumina output files were converted to FASTQ file format and sequence quality was assessed. Quality trimming was performed using Trimmomatic. Reads were aligned to the Bos taurus reference genome using Tophat as the alignment engine, and the mapped read were counted as fragments per kilobase of transcript per million mapped reads, which were generated using Cufflinks algorithm. Differential expression analysis was performed using Cuffdiff. RNA-sequencing generated a total of 121,722,238 raw paired reads with an average of 20M reads per library. It was possible to observe five very highly expressed genes and 110 highly expressed genes in our samples. Transcriptome adaptations revealed that 1355 genes were significantly differentially regulated. The comparison of the gene expression from infected and non-infected cows indicated that 652 genes were significantly up regulated while 694 genes were significantly down unregulated. Most differently expressed genes were associated in BPV infection pathways, which supports the hypothesis that the virus was the mechanism associated with this regulation. This is the first study that focused on a large-scale evaluation of gene expression associated with the BPV infection, which is important to identify possible metabolic pathways regulated by the host genes for the development of the lesion.

Palavras-chave: Bovine papillomavirus, RNA-seq, differential expression
EVALUATION OF SEROLOGICAL PROFILE FOR BLUETONGUE VIRUS IN GOATS AT SERGIPE STATE, BRAZIL.

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Resumo

Bluetongue (BT) is a viral disease caused by an orbivirus transmitted mainly by gnats of the genus Culicoides. The sexual transmission and intraplacental can also occur, while in the horizontal transmission it happens depending on the viral serotype. The virulence of BT virus varies quite markedly; even strains with matching serotypes have variable virulence. The notification is mandatory according to the list of the World Organization of Animal Health (OIE) and the Ministry of Agriculture, Livestock and food Supply of Brazilian Government. Goats can infect with the virus but rarely show any signs of clinical disease; it is a self-limiting disease in goats. The symptoms are characterized by salivation, congestion and inflammation of the mucous membranes, cyanosis and ulceration of the lips and tongue. It cause abortions, stillbirths and weak kids and fall in the productivity of the flock. The objective this work was to evaluate the serological frequency of goat for BV virus in the Sergipe State, Brazil. Three hundred and ten samples were collected from goats of seven municipalities (Canindé de S. Francisco, Gararu, Lagarto, Nossa Sa. da Glória, Poço Redondo, Poço Verde and Simão Dias) belonging to two mesoregions of the state. The serology diagnosis was Agar Gel Immunodiffusion - AGID by using the VMRD® Bluetongue Virus Antibody Kit. The results demonstrated that 16,45% (51/310) of the analyzed samples presented antibodies for the agent, and that 74,1% (7/27) of the properties presented positive animals. According to the animal category, the matrices presented more positivity (26.0% - 45/173) when compared to the young (3.5% - 4/115). Further studies are needed to better clarify the epidemiological situation of BT in Brazilian goat flocks, through the identification of viral vectors and serotypes circulating in different regions.

Palavras-chave: AGID, Bluetongue, Goats, Sergipe, Serological frequency
Recently, a novel viral genome, called porcine circovirus type 3 (PCV3) was reported in USA, China and Poland. This genome was identified in pigs with cardiac and multisystemic inflammation, respiratory disease, reproductive failure, porcine dermatitis and nephropathy syndrome and in aborted fetuses. Here, two full genome sequence of porcine circovirus type 3 (PCV3) recovered from sera of sows are reported. The sera were collected from sows who had just delivered litters with stillbirths, as well as from sows which delivered litters with no stillbirths, on the same farms. Serum samples were filtered (0.22 µm), ultracentrifuged and the pellet was treated with nucleases. Viral DNA was extracted using phenol protocol, enriched using φ29 DNA polymerase and subjected to high throughput sequencing (Illumina MiSeq). Paired-end reads were trimmed and de novo assembled using metaSPAdes genome assembler. The retrieved contigs were compared with sequences from Genbank database using Blastx and all assemblies were confirmed by mapping reads to contigs with Geneious software. Multiple sequence alignment was performed with additional 23 genome sequences of PCV3 by MUSCLE. Two full PCV3 genomes were recovered (PCV3-BR/RS/6 and PCV3-BR/RS/8), which displayed a >97% overall nucleotide similarity to others PCV3 genomes available at Genbank. The two genomes are 2,000 nt in length and contain two open reading frames oriented in opposite directions, that encode the putative capsid and replicase proteins. Rolling circle replication motifs and putative helicase domains were identified in the Rep coding region. The intergenic region contains a stem-loop motif. Phylogenetic inference was performed using a Bayesian method implemented in BEAST software with a strict molecular clock and the HKY+I nucleotide substitution model. PCV3-BR/RS/6 (MF079253) clustered more closely to genomes reported in China, whereas PCV3-BR/RS/8 (MF079254) clustered more closely to a PCV3 genome identified in South Korea. In view of the low number of full PCV3 genomes available, it would be premature to try to correlate genomic differences with possible origins of viruses. No PCV3 sequence was detected in pooled serum samples from sows with no stillbirth at the same farms. Further investigation is necessary to verify the association between PCV3 and stillbirths. This study reports the first identification of PCV3 full genome sequence in South America.

Palavras-chave: high-throughput sequencing, porcine circovirus, reproductive failure, swine
Bovine papillomavirus is the causative agent of papillomatosis in cattle. The disease causes cutaneous and mucosal lesions that can be minimized or lead to the appearance of malignant tumors. It occurs in Brazil and in several other countries, mainly affecting young animals. In addition to the unpleasant appearance of the animal affected by cutaneous papillomatosis, the problem can cause incalculable damage to the creative differences, especially in regard to the decrease of productivity. Knowing that Brazil is one of the great producers of meat and milk in the world, this study seeks to identify possible molecular mechanisms that are behind the pathological processes associated with bovine papillomatosis through the identification of genes related to the development of the lesions. For this, next-generation RNA sequencing was used to assess differentially expressed genes in infected and non-infected bovines. Functional annotation of the differentially expressed genes were performed using Gene Ontology, classifying genes based on biological process, cell component, and molecular function. UNIPROT databases were used to obtain functional information. The genes that presented functions associated with the progression of the papillomatosis lesion had their way mapped using the KEEG database. In total, 152 differentially expressed genes were identified between infected and non-infected libraries. Thirty one differentially expressed genes presented functions or they were related to metabolic pathways associated with the progression of papillomatosis lesions and cancer development in cattle. The functional annotation of these genes has shown that 80.6% were assigned to molecular function performed by the virus in the host, 90% were assigned to biological process, and 90% were assigned to the cell component in the host. 42% of the genes presented metabolic pathway, of these 5 are involved with the immune response triggered by the virus and 8 related to differentiation and cell cycle. Most genes were involved with immune response, transcription, cell cycle and proliferation, which possibly is involved in the development of papillomatosis lesions and malignant transformation in cows. Although more studies are needed, this is the first study that focused on a large-scale evaluation of gene expression associated with the BPV infection, which is important to identify possible mechanisms regulated by the host genes that are necessary the development of the lesion.

**Palavras-chave:** Bovine papillomavirus, functional annotation, RNA-Seq
The feline immunodeficiency virus (FIV) is a retrovirus with global impact, affecting both domestic and wild cats. This virus can cause a severe and progressive immunosuppression culminating in the death of the cat. Since the discovery of FIV, only one vaccine has been commercialized. This vaccine has its efficiency proven against FIV subtypes A and D, whereas subtype B - (FIV-B) which is the subtype circulating in Brazil - is currently not preventable by vaccination. Therefore, an effective vaccine against FIV subtype B is highly necessary in Brazil. The objective of this study was to develop and evaluate a vaccine against FIV-B using the Vaccinia Ankara Modified (MVA) recombinant virus as a vaccine vector expressing the variable region V1-V3 of the FIV-B envelope. In this work, we evaluated the immunogenicity of the vaccine in mice. For this purpose, mice C57BL/6 were immunized and after 21 days they received a booster dose. We demonstrated the generation of antibodies against the FIV subtype B envelope protein, which do not cross recognize the envelope protein of FIV subtype A, demonstrating that this vaccine generates a specific response against subtype B. In addition, it was observed that the sera from cats naturally infected with FIV were also able to recognize the envelope protein of FIV produced by the vaccine virus. We also evaluated the generated cellular immune response against epitopes present in V1-V3 region of the FIV envelope gene. Our results suggested that a good cellular response was induced, as measured by splenocytes proliferation and IFN-γ production in a murine immunization model. The data obtained in this study lead us to believe that this vaccine is a promising candidate to be evaluated in cats. Financial support: CAPES

**Palavras-chave:** FIV, MVA, RECOMBINANT VACCINE, REVERSE VACCINOLOGY, VETERINARY
199 - ÁREA: VETERINÁRIA

GENETIC CHARACTERIZATION OF SPECIES OF WILD CANIDS IN THE STATE OF CEARA AND ITS CORRELATION WITH THE RABIES VIRUS

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Resumo

Although it is one of the most ancient diseases related in human history, rabies still is a great challenge throughout the world. The rabies have in wildlife emergent importance in Brazil and the northeast region presents a unique epidemiology compared to the rest of the country. In this region were identified the two variants of rabies virus that are maintained and transmitted by wild terrestrial animals, with anual records of cases in the reservoirs species, in others wild and domestic species and humans. These viral variants were initially identified in the Ceara State, one in marmosets (Callithrix jacchus) and the other in wild canids, being restricted to the Northeast region until now. The aim of this study was to identify genetically, using mitochondrial DNA, the wild canids species of occurrence in the Ceara State, that act as reservoirs and transmitters of the rabies virus and the genetic characterization of isolated of the rabies virus from these animals. All samples were obtained from animals found dead or ill by passive surveillance of the agents of endemic diseases from the State secretary of health of Ceara. For this study were performed Reverse Transcriptase and PCR (RT-PCR) technique, genetic sequencing and phylogenetic studies. A total of 20 samples from wild canids was studied and of these, 04 presented positivity to the rabies virus. All samples of wild canids segregated with the samples of the Cerdocyon thous species, considered the main wild canid of occurrence in this region of the country. Among the 04 isolated of the rabies virus, all samples segregated in the genetic group formed by domestic and wild canids in a specific subgroup formed by wild canids from the northeast of the Brazil. This results suggest that the crab eating fox (Cerdocyon thous) is the main wild canid of occurrence in Ceara State, being responsible for the maintenance and transmission of the rabies virus variant previously described as related to these animals in northeast of Brazil. These results can contribute to a better knowledge of the animals species involved in this epidemiological cycle of the disease allowing the optimization of the actions for prevention and control of rabies in wildlife, considering the species characteristics and its interaction with humans and domestic animals. Financial support: São Paulo Research Fundation (FAPESP) and National research council (CNPq).

Palavras-chave: Rabies, Wild canids, Genetic characterization, Ceara, Brazil
200 - ÁREA: VETERINÁRIA

GENETIC IDENTIFICATION OF SPECIES AND RABIES DIAGNOSTIC IN BATS

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Resumo

Bats are important reservoirs of rabies virus (RABV). Different genetic lineages of RABV were identified in many species of bats. Morphology identification of these animals is rapid and efficient method however requires an able and specialist professional on the other hand this kind of professional sometimes is absent in institutes that are responsible for rabies diagnostic. Moreover, it’s common the sending of the Central Nervous System (CNS) already collected or degraded animal that not allow the identification of specie. The aim of this study was to identify the species of bats sending for the diagnostic of rabies in the Laboratory Virology of Institute Pasteur of São Paulo, through of the genetic sequencing partial of cytochrome C gene (COI) of mitochondrial DNA. Lung samples of these animals were subjected to extraction of total DNA from 128 bats and then was performed Polymerase Chain Reaction (PCR) using the forward primer LCO 1490 (5’-GGTCAACAATCATAAAGATATGG-3’) and the reverse primer HCO 2198 (5’-TAAACTTTGAGTTGACAAAAATCA-3’). Afterward, were submitted to genetic sequencing with the same primers previously described. The sequences were analyzed with the programs Chromas v. 2.23 (© 1998-2002 Technelysiumm Pty LTD) and Bioedit v. 5.0.9 and submitted at Basic Local Alignment Search Tool (BLAST) for the identification of bat species. Were identified 9 species belong 7 genre and 3 families. The RABV was detected in only one bat (Molossus molossus). This specie was the most common with 45 individuals followed by Eumops glaucinus (22), Glossopha ga soricina (20), Cynomops planirostris (19), Artibeus lituratus (11), Molossus rufus (7), Lasiurus ega (2), Eumops perotis (1) and Myotis riparius (1). Financial support: FAPESP- Fundação de Amparo à Pesquisa do Estado de São Paulo

Palavras-chave: Bats, mitochondrial DNA, genetic identification, rabies
Resumo

Equine pegivirus (Pegivirus E, EPgV) and Theiler’s disease-associated virus (Pegivirus D, TDAV) are newly classified viruses in the *Pegivirus* genus, *Flaviviridae* family. TDAV is the etiological candidate for Theiler’s disease or equine serum hepatitis. EPgV is associated with serum elevated liver enzymes. Information on origin, transmission route and pathogeny are scarce worldwide. The only prevalence study of equine pegiviruses conducted in Brazil described one EPgV infected horse (0.8%) and no evidence of TDAV. The aim of this study was to investigate the prevalence of equine pegiviruses in horses from the Midwest and Southeast geographical regions of Brazil. A total of 211 serum samples [Rio de Janeiro (RJ, n=175), Mato Grosso do Sul (MS, n=29) and Espírito Santo (ES, n=7)] were screened for the presence of EPgV and TDAV RNA by real time reverse transcriptase directed to the 5’ non coding genomic region (5’NC) of both viruses. As a reaction control, a synthesized double strand DNA comprising 196bp of the 5’NC region was employed: a dilution of 104 to 106 copies/reaction was added in each assay. In order to sequence the viral genome and differentiate between EPgV and TDAV, a nested RT-PCR directed to the helicase gene (NS3) viral region was amplified. Based on 5’NC detection, a prevalence of 23.7% (50/211) positive horses was found (RJ n=34, MS n=4 and ES n=2). Sequencing of 14 positive samples (151bp) revealed the presence of EPgV, but no evidence of TDAV. Nucleotide sequence variability was compared to 12 other EPgV sequences available on GenBank, including the previously identified Brazilian isolate. Overall nucleotide distance within Brazilian isolates was 6.4%. Nucleotide similarity between isolates from this study and the previous Brazilian isolate was 93.6-96.3%. Nucleotide similarity between Brazilian isolates and the United Kingdom (UK, n=10) and USA (n=1) isolates was 92.2%. Distance between all EPgV and TDAV was 37%. Maximum Likelihood tree (1000 replicates) showed that 7 isolates from the same farm in RJ clustered together, separated (bootstrap=99) from the other cluster which comprised all other EPgV isolates grouped with low statistical support (bootstrap less than 70). A high prevalence of equine pegiviruses was demonstrated in Brazil. Animals from both geographical regions were positive for EPgV and the three states investigated. Sequence analysis showed that all isolates from this study grouped to equine pegivirus with high similarity.

Resumão

Equine pegivirus (Pegivirus E, EPgV) and Theiler’s disease-associated virus (Pegivirus D, TDAV) are newly classified viruses in the *Pegivirus* genus, *Flaviviridae* family. TDAV is the etiological candidate for Theiler’s disease or equine serum hepatitis. EPgV is associated with serum elevated liver enzymes. Information on origin, transmission route and pathogeny are scarce worldwide. The only prevalence study of equine pegiviruses conducted in Brazil described one EPgV infected horse (0.8%) and no evidence of TDAV. The aim of this study was to investigate the prevalence of equine pegiviruses in horses from the Midwest and Southeast geographical regions of Brazil. A total of 211 serum samples [Rio de Janeiro (RJ, n=175), Mato Grosso do Sul (MS, n=29) and Espírito Santo (ES, n=7)] were screened for the presence of EPgV and TDAV RNA by real time reverse transcriptase directed to the 5’ non coding genomic region (5’NC) of both viruses. As a reaction control, a synthesized double strand DNA comprising 196bp of the 5’NC region was employed: a dilution of 104 to 106 copies/reaction was added in each assay. In order to sequence the viral genome and differentiate between EPgV and TDAV, a nested RT-PCR directed to the helicase gene (NS3) viral region was amplified. Based on 5’NC detection, a prevalence of 23.7% (50/211) positive horses was found (RJ n=34, MS n=4 and ES n=2). Sequencing of 14 positive samples (151bp) revealed the presence of EPgV, but no evidence of TDAV. Nucleotide sequence variability was compared to 12 other EPgV sequences available on GenBank, including the previously identified Brazilian isolate. Overall nucleotide distance within Brazilian isolates was 6.4%. Nucleotide similarity between isolates from this study and the previous Brazilian isolate was 93.6-96.3%. Nucleotide similarity between Brazilian isolates and the United Kingdom (UK, n=10) and USA (n=1) isolates was 92.2%. Distance between all EPgV and TDAV was 37%. Maximum Likelihood tree (1000 replicates) showed that 7 isolates from the same farm in RJ clustered together, separated (bootstrap=99) from the other cluster which comprised all other EPgV isolates grouped with low statistical support (bootstrap less than 70). A high prevalence of equine pegiviruses was demonstrated in Brazil. Animals from both geographical regions were positive for EPgV and the three states investigated. Sequence analysis showed that all isolates from this study grouped to equine pegivirus with high similarity.

Palavras-chave: Equine pegivirus, Prevalence, RT PCR, Sequencing
IN VITRO ACTIVITY OF ACYCLOVIR, GANCYCLOVIR AND FOSCARNET AGAINST EQUID HERPESVIRUS TYPE 1

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Resumo

Equid Herpesvirus type 1 (EHV-1) infects horses and asinine causing reproductive, respiratory and neurological disease. Prevention by using vaccines is the best way to avoid the EHV-1 related disease. Since the antiviral treatment of animal disease is still not widely used. Then, the objective of this study was to investigate the antiviral in vitro activity against EHV-1 of three antivirals used in treatment of human herpesvirus disease. The activity of Acyclovir [ACV], Gancyclovir [GCV] and Foscarnet [PFA]) was tested against EHV-1 by plaque reduction assay using Vero cells as virus replication indicator. The Human Herpesvirus type 1 (HSV-1) was used as control of antiviral effectivity. Different drug concentrations (10, 25, 75 and 100µg/ml) were tested against one hundred 50% tissue culture infectious dose (TCID50) of the virus. All tests were made in quadruplicate. GCV was the drug more effective against EHV-1 replication with 100% of virus inhibition with the doses used. PFA and ACV inhibited completely the virus replication at 75 and 100µg/ml concentration. At 25µg/ml the effectivity of PFA and ACV reduced 10 and 22% respectively; and using 10µg/ml, the effectivity of PFA and ACV reduced approximately 50%. All doses of GCV, PFA and ACV were efficient in reducing 100% of HSV-1 plaques in Vero cells. The results indicate that all drugs are promising candidates for experimental therapeutic testing in vivo against EHV-1. GCV is the most effective antiviral analyzed. Financial support: CAPES/CNPq

Palavras-chave: EHV-1, antiviral, HSV-1, herpesvirus, drug
Resumo

Bovine mastitis is one of the most common diseases founded on dairy cattle, consists in the infection of the animal’s mammary glands and mostly caused by bacteria of the genus *Staphylococcus* and specie *Escherichia coli*. The most used treatment consists in the intramammary antibiotics administration, aiming at suppressing pathogen proliferation. The formation of biofilm by these microorganisms is one of the great interferents for this treatment, since the extracellular material confers greater resistance to the antimicrobials agents. Continuous use of antibiotics strongly contributes for the emergence of super resistant bacteria, and it concerns over the transfer of antibiotic resistance genes to human pathogens. In this way, alternative treatments were search to contour the problem, like the therapeutic use of bacteriophage. On another hand, bacteriophages act on species or even specific strains, and this interferes on its affectivity against distinct groups of bacteria. So, it shows how necessary is using a cocktail, with different phages, that aim at increases the range of hosts. It was isolated 241 bacteria, from 27 animals during their dry period, and 89 demonstrated resistant for more than 7 antibiotics. For the present study, were select 6 bacteria that showed resistant for more than 50% of the 23 antibiotics. In order to evaluate the influence of the cocktail on bacterial biomass, 6 isolated were incubate on culture media at 37º C for 48 hours, in presence and absence of the phage cocktail, using MBEC® device following producer recommendations. Biofilm formed was analyzed in microplate spectrophotometer at 600 nm wavelength. It was observed that biofilm reduction on presence of cocktail phage for 15RA4 and 3017RA1 isolates; for isolates 3028RD1 and 8008RP1 there was no difference; and 2030RP1 and 2020RP1 happened a light increase on biomass, probably came from a stress caused to the bacteria, leading to an increase level of proliferation. Further assays will be carried out in distinct times, to establish a kinetic for phage action, besides to elucidate the action mechanisms about the evaluated bacteria.\textbf{Financial support:} FAPEMIG, CAPES, CNPq

\textbf{Palavras-chave:} bacteriophage, cocktail, .mastitis, dry cow therapy
The viral isolation in cell culture of clinical samples provide more in-depth studies about the characterization of a given virus. Senecavirus A (SVA) is a non-enveloped single-stranded RNA virus of the genus Senecavirus within the family Picornaviridae. SVA has been attributed with the vesicular disease. This way, the precise diagnosis is fundamental for be differentiated from other important illnesses in swine, such as Foot-and-mouth disease, swine vesicular disease and vesicular stomatitis. In May of 2016, a pig herd located in Santa Catarina State, Southern Brazil, presented diarrhea, followed by the appearance of vesicle in the skin and lesions in hoof and snout of suckling piglets. Out of 14 samples, including skin, snout, liver, hoof and vesicle, in twelve 75% (12/14) the presence of the SVA genome was detected by polymerase chain reaction (PCR) was performed with primers targeting the genomic 5’ untranslated region (UTR). In addition, the genomes were confirmed by the genetic sequencing, which would lead to the conclusion that the SVA is the responsible by this outbreak. The goal of this study is isolate SVA from clinical samples and compare two cell lines, a porcine kidney cells (PK-15) and lung carcinoma from human cells (A549). To viral isolation, the 11 samples were macerated and diluted in Eagle’s Minimum Essential Medium (EMEM) whit 5% Penicillin-Streptomycin (10,000 U.I./ml - 10 mg/ml). After 4 hours of incubation under refrigeration, the samples were filtered with a membrane (0.22µm) and 250µL of each sample was inoculated in the 6-well plates contain PK-15and A549 cells. In the first passage of the samples in the pk-15 and A549 cells was possible to detect the SVA genome all tested samples. However, in the following passages in PK-15 cells none evidence of cytopathic effect (CPE) and no genome was detected. In the fourth passage in A549 cells of a hoof sample was possible to views the CPE and to detected the SVA genome, being this recurring phenomenon in the passages subsequent this isolated. This study showed in vitro a greater susceptibility of the A549 lineage for the isolation and growth of SVA, when compared to PK-15 cells. Financial Support: Feevale; FAPERGS; CAPES; CNPq.

**Palavras-chave:** SVA, vesicular disease, A549 cells, viral isolation
MOLECULAR CHARACTERIZATION OF THE ENVELOPE PROTEIN OF EQUINE INFECTIOUS ANEMIA VIRUS FROM BRAZIL

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Resumo

Equine infectious anemia virus (EIAV) is a persistent lentivirus that causes equine infectious anemia (EIA). In Brazil, EIAV is endemic in Pantanal region and euthanasia is not mandatory in these areas. The envelope protein (Env) is the main viral component that induces host immune response after lentivirus infection. This gene codified the surface unit (SU-gp90) and the transmembrane unit (TM-gp45). The rapid mutation is also the major mechanism underlying lentivirus persistent infection and immune escape, and variations of EIAV Env are mainly concentrated in the gp90. This study aimed to analyze the evolutionary characteristics of the gp90 gene of Brazilian EIAV, by sequencing EIAV Env in naturally infected horses. Plasmas and leukocyte layer of infected horses from Mato Grosso and Mato Grosso do Sul States were collected and their proviral DNA was purified by Illustra blood genomicPrep Mini Spin Kit (GE Healthcare). The PCR primers were designed based on genomic sequence of two Brazilian EIAV, previously sequenced by our group. Three pairs of primers were used in different combinations to amplify 3kb, 2kb, and 210pb of Env gene, respectively. GoTaq® Green Master Mix or GoTaq® Long PCR Master Mix (Promega) was used in the reaction following the standard protocol and these PCR products were sequenced by Sanger method in a 3500 platform (Applied Biosystems). In two equine samples we sequenced all Env gene, using the 3kb product and internal primers of 2kb, and we got two different sequences from the same equine infected, with a deletion of four aminoacids in gp90, and only one from the other. In 8 equines samples we only amplified 210pb of the gp90 gene. These 8 equines are from the same farm and all sequences have less than 85% of identity with one another. Comparing this sequences differences, even horses from the same farm have an independent evolution of env genes of the current virus. We can observe some of the eight highly variable regions (V1–V8) previously described in the multiple alignment. Ten equines samples positive in AGID or ELISA and in our qPCR diagnostic of 5’LTR region could not be amplified by the Env PCR. These results show the high Brazilian variation of the current virus and it’s very important to detect because the ELISA test uses the gp90 purified protein. With this study it is possible to better characterize the virus circulating in Brazil and to cope with the challenges of the EIAV diagnosis in Brazil.

Palavras-chave: infectious anemia virus, envelope protein, gp90, Sanger sequencing
PCR using PathAmp FluA reagents. DNA libraries were prepared and

Palavras-chave: H1N1 influenza virus, Pandemic H1N1/2009 influenza virus, Swine, Reassortant

Influenza A viruses (IAVs) circulating in swine are of major economic concern for the swine industry and a pandemic threat for humans. The segmented RNA genome of IAV allows the occurrence of genetic exchange or reassortment among distinct influenza viruses during mixed infections. Recently, after spreading in humans, the 2009 pandemic H1N1 influenza virus (H1N1/2009) was re-introduced in pig populations globally, as well as in Brazil, and re-assorted with other virus lineages. Currently, H1N1/2009 and human-like H1N2 and H3N2 viruses circulate in swine in several Brazilian states. Herein, we describe the whole-genome sequencing of a novel human-like H1N1 IAV isolated from nursing pigs in 2014 in Santa Catarina State. Nasal swabs collected from pigs with respiratory clinical signs tested positive for IAV by reverse-transcription PCR targeting the matrix gene. Virus isolation was performed in SPF chicken eggs and the isolated virus was subtyped by RT-PCR. Virus RNA was extracted from nasal swabs and the eight gene segments were amplified by RT-PCR using PathAmp FluA reagents. DNA libraries were prepared and submitted for sequencing using Ion Torrent system at Embrapa Swine and Poultry. Influenza genome was assembled using Newbler V 2.9 with high coverage (180x). Nucleotide alignments of the NA and NA segments were generated for related human and swine IAVs, collected globally and downloaded from the Influenza Virus Resource available in GenBank. The phylogenetic relationships of the datasets were inferred by using the Neighbour Joining method. Analysis of the HA and NA segment of this virus revealed a novel introduction of human H1N1 influenza virus into swine in Brazil. Besides, the H1 and N1 probably represent the same human-to-swine transmission event in the early 2000s. The six internal gene segments (PB2, PB1, PA, NP, M and NS) of the novel virus showed a high similarity (98-99%) to H1N1/2009 virus. The detection of a novel reassortant human-swine influenza virus shows the very dynamic epidemiology of influenza virus in pigs in Brazil and highlights the importance of performing full genome sequencing of pig isolates in order to enhance genetic information about influenza virus circulating in pigs.

Palavras-chave: H1N1 influenza virus, Pandemic H1N1/2009 influenza virus, Swine, Reassortant
OCCURRENCE OF EQUINE INFECTION ANEMIA VIRUS (EIAV) IN THE NORTH OF CEARÁ: PRELIMINARY DATA

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Resumo

Equine Infection Anemia Virus (EIAV) is a lentivirus, Retroviridae family, which infects equids. EIAV is an important pathogen of equids that is spread worldwide. Prevalence rates of EIAV infection in Brazil have not been well evaluated and regional variations are largely unexplored. Greater surveys of Brazilian samples are required to determine EIAV isolates and subtypes. The occurrence of EIAV was investigated in 7 (seven) equines, 17 (seventeen) asinine and 1 (one) mule from properties in the north of Ceará, Brazil. Another aim of this study was to correlate the clinical parameters of equids and the EIAV. The blood serum was applied in the serological tests AGID (Brunch Laboratories, Brazil) and recombinant ELISAs, gp90 and p26. The animals were clinically assessed – CEUA-UNIINTA 2007.03.00. A total of 32% (8/25) of the sample was positive for EIAV, considering both ELISAs used (2 equines and 6 asinine); 20% (5/25) via gp90 and 20% (5/25) via p26. Only one equine was positive by AGID. None animal showed clinical signs compatible with EIA. This study, despite being insipid in the north of Ceará, confirms: a) the occurrence of the EIAV in the studied area, and b) shows the differences in consistency in the used tests. Our results underline the importance of more studies to improve the diagnosis tests in order to identify the EIAV in different species of equids. Financial support: FUNCAP/UNIINTA/CNPq.

Palavras-chave: asininos, equines, infectious, muares, retrovirus
PATHOGENESIS OF BOVINE HERPESVIRUS 2 IN CALVES FOLLOWING DIFFERENT ROUTES OF EXPOSURE

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Resumo

Bovine herpesvirus 2 (BoHV-2) as an alphaherpesvirus associated with mammilitis, a cutaneous and self-limiting disease of dairy cows. Biological and molecular properties candidate BoHV-2 as a potential vector for antigen delivery. In this study, we investigated the pathogenesis of BoHV-2 in calves following intramuscular (IM), intravenous (IV) and cutaneous (ID) inoculation. Animals inoculated by all routes – more pronounced in animals from group IV - presented an increase of body temperature between days 6 and 9pi. Virus inoculation after skin scarification (ID) resulted in mild inflammatory lesions characterized by hyperemia, small vesicles, mild exudation and scab formation between days 2 and 8pi. Virus or viral DNA was detected by PCR in the crusts/swabs collected from lesions of 3 out of 4 animals inoculated ID from day 2 to 8pi, and in the blood of 3/4 animals of the IM group, from day 4 to 8pi; and in 2/4 animals of the IV group, at days 6 and 8pi. No viremia was detected in the ID or in the control group. Calves from all inoculated groups seroconverted to BoHV-2 in titers from 4 to 64, as indicated by VN assays performed in serum samples collected at day 15pi. Administration of dexamethasone (DX) to the calves at day 48dpi, did not result in detectable virus reactivation as indicated by lack of virus detection by PCR in the blood and/or inoculation sites and absence of seroconversion. These results demonstrate that BoHV-2 is able to efficiently replicate in calves following different routes of exposure and is not easily reactivated by DX treatment, a desirable property of a candidate vaccine vector. Financial Support: CAPES/CNPq

Palavras-chave: BoHV-2 , Herpes mammillitis , Latency
PERFORMANCE VERIFICATION AND COMPARISON OF TWO COMMERCIAL ELISA KITS FOR SOROLOGICAL DIAGNOSIS OF AUJESZKY’S DISEASE

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Resumo

Susid alphaherpesvirus 1 (Aujeszky's disease virus [ADV] or pseudorabies virus), which belongs to the family Herpesviridae, subfamily Alphaherpesvirinae, genus Varicellovirus is the causative agent of Aujeszky's disease, a notifiable disease that causes substantial economic losses to the swine industry. Members of the family Suidae are the only natural hosts for ADV, although the virus can infect numerous other mammals. Only pigs survive after infection and serve as reservoir for the virus. The disease can be diagnosed by detection of the agent or by the serological response. Serological tests are indicated for the determination of subclinical infection and of animals in latency, especially in the determination of the health status of herds for international traffic. Normally, serological tests are only applied on pigs, since other animals die before seroconversion. For the credibility of the methods used in diagnostic laboratories, it is necessary to carry out the verification of the performance of the tests, to evaluate and provide evidence that the requirements have been fulfilled. The present study aimed to verify and compare two ELISA sets: the kit A detects the presence of anti-gB antibodies that bind to ADV antigens and kit B uses monoclonal antibody specific for the glycoprotein gE to detect anti-gE antibodies present in sera. To perform the test, we used 20 samples of ADV negative sera, 11 positive samples and three internal control (negative, weak and strong positive, respectively). The verification process underwent the following steps: selectivity test, repeatability and reproducibility, measurement uncertainty and analytical sensitivity. In the selectivity test, all the samples showed negative results, presenting 100% of specificity in both kits. When comparing the coefficient of variation of the repeatability and reproducibility, it was observed that for kit B the values were relatively higher for the positive and weak positive controls. Kit B presented lower uncertainty values at all stages: sample, control, combined and process expanded uncertainties. Kit B had slightly lower analytical sensitivity, since kit A detected antibodies at sample dilutions twice as large as kit B. Only kit A was used in a proficiency test and obtained satisfactory results. Both kits presented satisfactory results in all stages of this validation study and are adequate to the intended use, since they meet all the established criteria. Financial Support: MAPA.

Palavras-chave: AUJESZKY, DIAGNOSIS, ELISA, VERIFICATION
Picobirnavirus in production animals from state of Pará, Brazil

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Resumo

Introduction: In the State of Pará, Belém Metropolitan and Northeast mesoregions represent some of the areas where are concentrated the highest rates of deforestation in Amazon forest. In these areas, the strong anthropic pressure and high level of ecosystem degradation have led to a close relationship between the local wildlife and the rural population, resulting in an increase in the transmission of several pathogens, contributing to the emergence of various infectious diseases and the manifestation of new viral strains. In this context, diseases that affect production animals, such as the enteric disorders caused by picobirnavirus (PBV), are among the main causes that affect the rates of productivity, generating economic implications. Therefore, the objective of this study is to monitor the occurrence of this virus in production animals from areas of environmental change. Materials and Methods: The analysis was performed in 50 fecal samples from bovine and porcine, subjected to Polyacrylamide Gel Electrophoresis (PAGE) and Polymerase Chain Reaction preceded by Reverse Transcription (RT-PCR) using specific primers for PBV of genogroup I (GI).

Results: Fifty fecal specimens were tested, of these 9 showed positivity for PBV of GI genogroup by RT-PCR, which 8 samples were from porcine and one from bovine. However, all samples were negative in the PAGE technique. Conclusion: This research indicates the circulation of PBV in production animals that inhabit peridomestic areas with environmental change, where there is interaction with human beings. Thus, future studies are needed for genetic characterization of these positive samples, in order to verify the evolutionary relationship of genetic data along with the transmission factor between species. However, it will contribute to the assessment of its impact in relation to other enteropathogens, improving the understanding of enteric infections in production animals. Financial Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Palavras-chave: Picobirnavirus, Production Animals, Enteric Infections, Genogroup I
PORCINE CIRCOVIRUS TYPE 3 (PCV3) RECOVERED FROM BRAZILIAN WILD BOAR (SUS SCROFA)

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Resumo

In 2015, a novel member of genus Circovirus, designated porcine circovirus type 3 (PCV3), was reported in USA recovered from sows with chronic reproductive problems. After its first description, additional complete genome sequences have been recovered from pig in Korean, China, United States and Brazil. However, up to now, there is not investigation about PCV3 genome circulation in wild boars (Sus scrofa) population. This study reports a complete PCV3 genome sequence recovered from a wild boar. Retropharyngeal lymph nodes were collected from seven apparently healthy wild boars after slaughtering authorized by IBAMA (registration n° 723418) and SISBIO (registration No. 46998-1). Tissues were pooled, homogenized, filtered (0.22 µm) and centrifuged at 150,000 x g for 4 hours at 4 oC. The pellet was treated with nucleases and viral DNA extracted using a standard phenol chloroform protocol, and enriched by multiple displacement amplification (MDA). A DNA library was prepared with Nextera kit (Illumina®) and sequenced with Miseq v2 300 kit in a MiseqTM sequencing platform (Illumina®). A total of 304,406 paired-end reads were generated and de novo assembled with aid of the metaSPAdes genome assembler (v 3.9.1). All assemblies were confirmed by mapping reads to generated contigs using Geneious software (version 8.1.7). Multiple sequence alignment was performed with aid of the software MUSCLE, including the sequence reported here (PCV3-Ss/Br) plus another 27 PCV3 genome sequences available at Genbank.

Phylogenetic inferences were based on full-genome sequences by maximum-likelihood, with aid of Mega 6.06. The full PCV3 genome encompasses 2,000 nucleotides (coverage ~70-fold) and shares 98 to 99% nucleotide identity with previously reported PCV3 genomes. It contains two open reading frames (ORFs) disposed in ambisense orientation, which encode the putative capsid (215aa) and replicase (297 aa) proteins. Phylogenetic analysis showed PCV3-Ss/Br in a separated branch, despite its high identity similarity to other PCV3s. However, PCV3s. hares high identity with other PCV3s. These findings reveal that PCV3 can infect wild boars and emphasize the importance of investigations on the role of such animals as reservoirs for infectious agents which may infect domestic swine. Financial Support: CAPES, CNPq, Finep

Palavras-chave: Circovirus, Genome, Phylogenetic analysis
Samples were negative for OPXV. Although previous prevalence of VACV in bulk tank milk of the studied regions.

Virus on milk; 3 there was interfered with the detection of viral DNA; 2 (UG) of VACV per milliliter of milk, we have not identified any positive samples. Some hypotheses indicated that in experimental infection conditions, lactating cows secrete up to 10^6 genomic units of VACV per milliliter of milk. The samples were diluted 4-fold in PBS 1x. Viral DNA was purified using Wizard Genomic DNA Purification System (Promega, USA). Then, qPCR targeting the growth factor gene (vgf) of Orthopoxvirus (OPXV) genome was performed. The qPCR reactions were analyzed in duplicate. So far, we have not identified any positive samples. Some hypotheses to explain these results could be raised: 1 - the dilution factor of the bulk tanks and/or the milk volume collected may have interfered with the detection of viral DNA; 2 - subclinically affected cows eliminate lower amount of virus on milk; 3 - there was no VACV circulation in the farms of the studied regions during the analyzed period. We will continue to analyze the bulk tank milk samples until December, 2017, to estimate the prevalence of VACV in bulk tank milk of the studied regions.

Resumo

Bovine vaccinia (BV) is a neglected zoonosis caused by Vaccinia virus (VACV). Currently, BV outbreaks occur in all Brazilian territory, including Minas Gerais, the largest dairy producer in Brazil. Previous studies have revealed the presence of DNA and infectious viral particles of VACV in the milk of diseased cows as well as in experimentally infected cows that continued to eliminate the virus even after the lesions healed. In addition, VACV has been detected in milk and artisanal cheeses of properties without BV, showing that the disease may manifest subclinically in the herd, increasing the risk of transmission. Therefore, the consumption of raw milk or dairy products could be considered a risk factor for human VACV infection. Despite the impact of BV outbreaks, there are few epidemiological studies about the disease. In this way, we aimed to study the prevalence of VACV in milk samples collected from milk bulk tanks originated from mesoregions of Minas Gerais, Brazil. The Laboratório de Análise da Qualidade do Leite of UFMG (LabUFMG) receives monthly bulk tank milk samples from counties belonging to the Metropolitan, Triangulo Mineiro, Western, Northern, Northwestern and Vale do River Doce regions. We randomly selected 4.5% of all the bulk tank milk samples received per month, from January to April 2017. For each dairy farm, it was collected 100 mL of bulk tank milk. The samples were diluted 4-fold in PBS 1x. Viral DNA was purified using Wizard Genomic DNA Purification System (Promega, USA). Then, qPCR targeting the growth factor gene (vgf) of Orthopoxvirus (OPXV) genome was performed. The qPCR reactions were analyzed in duplicate. So far, we analyzed a total of 268 milk samples. All the tested samples were negative for OPXV. Although previous studies indicated that in experimental infection conditions, lactating cows secrete up to 10^6 genomic units (UG) of VACV per milliliter of milk, we have not identified any positive samples. Some hypotheses to explain these results could be raised: 1 - the dilution factor of the bulk tanks and/or the milk volume collected may have interfered with the detection of viral DNA; 2 - subclinically affected cows eliminate lower amount of virus on milk; 3 - there was no VACV circulation in the farms of the studied regions during the analyzed period. We will continue to analyze the bulk tank milk samples until December, 2017, to estimate the prevalence of VACV in bulk tank milk of the studied regions.

Palavras-chave: VACCINIA VIRUS, BOVINE VACCINIA, MILK, EPIDEMIOLOGY, PREVALENCE
The equine infectious anemia virus (EIAV) infection often results in initial febrile response, followed by recurrent cycles of the disease and finally, a prolonged asymptomatic period. These clinical signs variations are results of a number of factors including the strain of the virus, the specie of infected equid and the different susceptibility between the animals. In consequence of close relation between viral replication and the disease, studies about in vitro pathogenesis of EIAV in macrophages which are the target cells of the virus, depend on accurate measurement of viral load throughout the infection period. For the EIAV viral load determination, it was produced a control RNA, and afterward used in the qPCR system standardization. For this, primers were designed to a conserved EIAV genomic region, named gag, using the Primer3Plus program, based in the EIAVPV strain nucleotide sequence. The 520 base pairs region of the EIAVUK3 molecular clone, derivate of the EIAVPV strain was amplified by PCR, purified and cloned into the plasmid pGEM -T Easy Vector (Promega) with subsequent transformation in electrocompetent E. coli, DH5-α. The amplification of gag region from the pGEM -T Easy Vector with subsequent nucleotide sequencing confirmed the success of cloning. Bacteria with the construction pGEM -T Easy Vector_gag (520 bp) were spread and the positive molecular clones were confirmed by PCR. Large amounts of DNA template of the construction pGEM -T Easy Vector_gag were produced and linearized with the psfI restriction enzyme. After that, the RNA was synthesized in vitro from the DNA linearized product, using the T7 Ribomax Express Large Scale RNA Production System Kit (Promega). The RNA produced was used to construct an absolute standard curve and standardization of a quantitative PCR that will be used in studies of EIAV replication in equine macrophages in vitro. Financial Support: CNPq, CAPES, FAPEMIG, INCT-Pecuária.

Palavras-chave: CONTROL RNA, EIAV, VIRAL LOAD, STANDARD CURVE, qPCR
RESPIRATORY SIGNS, FEVER AND LEUKOPENIA IN CALVES INOCULATED WITH BRAZILIAN HOBI-LIKE PESTIVIRUSES

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Resumo

HoBi-like viruses comprise an unclassified group of bovine pestiviruses originally identified in commercial fetal bovine serum from Brazilian origin and, subsequently, isolated from diseased animals in several countries. Although frequently isolated from clinical cases, most HoBi-like isolates failed to reproduce overt disease upon experimental inoculation. In this study, we performed an experimental infection of seronegative calves with two Brazilian HoBi-like isolates. Four to six-months-old calves inoculated by intranasal inoculation (IN) with isolate SV757/15 presented viremia between days 4 and 12 post-infection (pi) and shed virus in nasal secretions up to day 12pi. Clinically, the animals presented transient pyrexia (days 4 to 8pi) and lymphopenia between days 4 and 8pi. In a second experiment, calves inoculated with isolate SV478/07 developed apathy, anorexia, mild to moderate respiratory signs (nasal secretion, hyperemia) and pasty diarrhea in the days following virus inoculation. These animals also developed hyperthermia (day 4 to 9 pi) and lymphopenia (day 4 to 7pi), presented viremia between days 2 and 9 and shed virus in nasal secretions up to day 11 pi. Both groups seroconverted to the inoculated viruses, developing virus neutralizing (VN) titers from 40 to 1280 at day 30 pi. These results confirm and extend previous findings showing that infections of susceptible cattle with HoBi-like viruses under experimental conditions are predominantly subclinical or mild, yet they also indicate virulence differences among field isolates. Financial Support:CNPq/CAPES

Palavras-chave: Bovine pestivirus, Atypical pestivirus, Experimental infection, Pathogenesis
Dehydrating diarrheal disease is a problem that affects pig herds worldwide and lead to increased morbidity and mortality of the herd generating economic losses, worldwide. Rotaviruses (RV) are identified as major infectious agents that cause diarrhea in pre- and post-weaning periods. Rotaviruses are members of the Rotavirus genus of the Reoviridae family, and are classified into ten species (A-J). RV belonging to species A (RVA) and, to a lesser extent, species C (RVC) and H (RVH) are often associated with diarrhea in pigs. Although the deleterious effect of these infections in swine herd productivity is well known, the same cannot be said about the frequency and distribution of these pathogens in Brazil, particularly in the State of Rio de Janeiro. Thus, this study aims to contribute to the monitoring of the dissemination of RVA, RVC and RVH in the Brazilian pig herds. To this end, we analyzed 105 fecal samples of pigs between 4 days and 2 months of age, from a commercial pig farm in the town of Barra do Piraí-RJ, whose herd has approximately 3,000 animals. The samples were classified into two groups according to the age of the animals: pre-weaning (4-20 days), 42 samples and post-weaning (21 to 60 days), 63 samples. Fecal specimens were collected directly from the rectum of animals with the aid of swabs or from the floor of the pigsty. At the time of sample collection 15 animals were presenting diarrhea: 12 pre- and 3 post-weaning. The fecal samples were analyzed for RVA, RVC and RVH through RT-PCR. Among the pre-weaning samples, 11 (26.2%; n = 42) tested positive for RV: 9 for RVA and 2 for RVH. For the post-weaning samples also, 11 (17.5%; n = 63) were positive: 4 for RVA, 6 for RVH and 1 co-infection of RVA+RVH. Six of the 15 animals that were presenting diarrhea tested positive, 4 for RVA and 2 for RVH. The results demonstrate the circulation of those pathogens among the herd, including among asymptomatic animals, which can be a source for environmental contamination that could result in the transmission of the virus to the flock causing diarrhea and consequently economic losses. In addition, because RVA is considered a zoonotic infection, the excretion of the virus in the environment could also result in the transmission of the virus to the farm workers causing interspecies infections and allowing the emergence of new virus mutants, a fact already demonstrated in the literature for RV.

Palavras-chave: A, especie, H, rotavirus, suino
RESUMO

Palavras-chave: AGID, Bovine, Deltaretrovirus, Lymphosarcoma, Persistent Lymphocytosis

Seroprevalence of Enzootic Bovine Leukosis (EBL) in dairy cattle from family agriculture in the State of Paraná, Brazil

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Resumo

In Brazil, the familiar agribusiness has been responsible for a large part of supply of the internal market. Around 70% of milk production in Paraná comes from family farming. The Enzootic Bovine Leukosis (EBL) is an infectious disease caused by a Deltaretrovirus with immunosuppressive potential, characterized by its chronic course. The virus primarily affects cattle, the disease in these animals results in economic losses due to the decline in production. The disease is characterized by excessive lymphocyte proliferation hemocytopoietic organs and tissue in reticulohistioytic tissue, and can develop in two forms: persistent lymphocytosis and lymphosarcoma. Vertical transmission, in utero, or through colostrum and milk, accounts for a relatively small proportion of infections. Iatrogenic horizontal transmission, through procedures permitting the contact of blood between cattle, has been shown to be a major route of transmission. The transmission through blood-sucking flies also shows to be an important route, since it the minimum dose infecting is 0,1µL of blood. Contact transmission stems from a mixture of natural sources of blood, exudates, and tissues that enter the body through mucosal surfaces or broken skin. The objective was evaluated the seroepidemiology of EBL in dairy cattle of familiar agriculture of 13 municipalities in the state of Paraná, Brazil. It were collected in 2016, 910 bovine blood serum samples, with age varying of 1 to 180 months, of different bovine dairy breed through the test Agar Gel Immunodiffusion (AGID) with commercial kit Tecpar® (gp51), according to manufacturer’s recommendations. The samples are derived of 71 properties of familiar agriculture, with semi-extensive creation located in 13 municipalities with Cfa subtropical climate. The statistical analysis used was the descriptive and the EPI Info™ 7. It was found that 100/910 (10,99%) of the animals were reactive to the AGID test, in which the Florestopolis municipality presented the highest rate with 22% of the reactive animals and in Candido Abreu no animal was seropositive. The risk factors found were: flies lack of control and drainage of flooded areas, as well as needles reuse. Unlike to different authors the age wasn’t detected as risk factors. Therefore, has been concluded the herd management is crucial for the control of Enzootic Bovine Leukosis, and despite the decreasing rates the care of management must to persist, aiming to keep it under control.

Palavras-chave: AGID, Bovine, Deltaretrovirus, Lymphosarcoma, Persistent Lymphocytosis
Enzootic bovine leukosis (EBL) is an infectious lymphoproliferative disease of cattle caused by bovine leukemia virus (BLV), a member of the Retroviridae family, genus Deltaretrovirus. The EBL is characterized by persistent lymphocytosis and lymphosarcoma and has an expressive economic impact due to losses in exportation, treatment of secondary infection, reduction in dairy production and also due to the special management required. The diagnosis of EBL can be done by molecular methods and by detecting specific antibodies in serum or milk samples. The infected animal develops a humoral immune response against viral proteins, mainly to the gp51 (envelope protein), and its identification is crucial to epidemiological surveillance, certification of disease-free areas and prevalence studies. The aim of this study is to develop an indirect ELISA using a gp51 synthetic peptide as antigen. After in silico prediction analysis, the chemical synthesis of the linear peptide KIPDPPQPDFPQL, named pgp51 was performed. To perform the indirect ELISA, the pgp51 was diluted in carbonate buffer (pH 9.6) and was evaluated at different concentrations (0.25μg/well; 0.5 μg/well and 1.0μg/well). Different serum dilutions, which the status of positivity or negativity to EBL were previously defined by agar gel immunodiffusion (AGID), were tested. The results revealed a better performance of the pgp51ELISA under the following conditions: antigen concentration at 0.5μg/well, serum diluted at 1:4 and conjugated (anti-bovine IgG whole molecule - Peroxidase, produced in rabbit) diluted at 1:5000. The pgp51 ELISA was efficient to segregate positive and negative serum samples indicating that it has a great potential to be used in the serodiagnosis of EBL, as it is faster than AGID test, less subjective and easily used to high-throughput screening. Financial Support: CAPES, CNPq, FAPEMIG, INCT Pecuária

Palavras-chave: EBL, GLYCOPROTEIN, SYNTHETIC PEPTIDE
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Small ruminant lentiviruses (SRLV) are members of the Retrovirus family whose prototypes are Visna/Maedi Virus (VMV) and the Caprine Arthritis-Encephalitis Virus (CAEV), are the causative agents of slow progressive degenerative diseases of goats and sheep, responsible for significant economic losses. These viruses cause lifelong infections with long periods of incubation and induce slow progressive inflammatory diseases primarily affecting the lungs, nervous system, joints and mammary glands due to viral replication in cells of the monocyte/macrophage lineage which is the main target cell. Infection by VMV and CAEV can lead to Visna/Maedi (VM) and Caprine Arthritis-Encephalitis (CAE) respectively. VM and CAE are distributed worldwide and development of a clinical disease takes a few months to a few years, and are acquired through ingestion of virus in milk or colostrum from infected does or ewes. Most infected animals remain subclinically infected, and detection of specific antibodies is the only sign of infection. For SRLV diagnosis agarose gel immunodiffusion test was used. As no vaccine is available, most often employed schemes to prevent spread of VM and CAE are based on the control of transmission, segregation and culling of infected animals associated with management practices, especially the offspring. The aim of research was the investigation of SRLV-Ab presence by agarose gel immunodiffusion test in goats and sheep in Paraná and Rio Grande do Sul. A total of 3339 (sheep-2353, goat-986) samples were collected, during 2015, from Parana (sheep-1618, goat-791) and Rio Grande do Sul (sheep-735, goat-195), were examined for the presence of antibodies against SRLV, using agarose gel immunodiffusion test with the kit AGID Biovetec (UFPE/DINE) according to the manufacturer’s instructions. The overall seropositivity was 5.48% (183/3339).

A total of 160 (sheep) and 791 (goat) samples from Paraná (sheep-160/791) and 21/1618 (goat) samples were collected from the states of Paraná and Rio Grande do Sul. SRLV antibodies were detected in 1.29% (21/1618) in goat and sheep, respectively. In Rio Grande do Sul antibodies against SRLV have been detected in 1.02% (2/195) in goat and no sheep were positive assessed by AGID. The infections did not appear to be related to the reduction in goats and sheep productivity. More research is necessary to understand the host-SRLV interaction and this supports the claim that the most CAEV/VM infected animals remains asymptomatic.

**Palavras-chave:** AGID, Caprine arthritis encephalitis, ovine progressive pneumonia, Retrovirus, Visna/Maedi Virus
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STANDARDIZATION OF DIGITAL PCR FOR SENECAVIRUS A, AN EMERGING VIRUS FROM PICORNAVIRIDAE

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Resumo

The new virus, Senecavirus A (SVA) belonging to the order Picornavirales, family Picornaviridae, genus Senecavirus was incidentally isolated in Gaithersburg, Maryland, USA in 2002 from the PER.C6 (transformed fetal retinoblast) cell line. However, currently, this virus is associated with vesicular disease in swine and the acute death of neonatal piglets and it has been reported in the United States of America, Canada and China. In Brazil, the SVA was reported in 2015 in an outbreak of vesicular disease in swine, clinically indistinguishable of Foot-and-mouth disease, a contagious viral disease, which is a worldwide concern because it generates substantial economic losses. We standardized a diagnostic tool for SVA based on RNA reverse transcription followed by Digital PCR (RT-ddPCR) (using one-step and two-step approaches). Assays such as analytical sensibility and specificity were done in parallel with real-time PCR, RT-qPCR (one-step and two-step) for comparison of sensibility and specificity of these methods. The one-step RT-ddPCR was considered more sensitive than two-step RT-ddPCR and both tests presented the same specificity. In addition, RT-ddPCR (one-step or two-step) indicated same results of analytical sensibility when compared with RT-qPCR (one-step and two-step), although RT-qPCR (one-step or two-step) was less specific than those assays described here. According to results and overall, the one-step digital PCR proved to be better than all methods analyzed and can be used as auxiliary diagnostic tool for SVA and for absolute quantification of this virus in biological samples. Financial support: MAPA, CNPq, FAPEMIG.

Palavras-chave: Absolute quantification, Diagnostic, Droplet Digital PCR, Senecavirus A
STOCHASTIC RISK ANALYSIS OF EQUINE INFECTIOUS ANEMIA INTRODUCTION TO BRAZIL. TIME TO REEVALUATE AGID AS SOLE TEST REQUIREMENT?

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Resumo

Equine infectious anemia virus (EIAV) is characterized as a RNA virus within the family Retroviridae and genus Lentivirus, is one of the 11 notifiable equine specific diseases to the World Organization of Animal Health (OIE). It is distributed over all five continents and has a great impact on the equids industry mainly because of movement restriction impositions. The importation of equids by Brazil constitutes a relevant source of genetic diversity and breeds improvement, however the risk of spreading diseases is inherently associated to the international transit of any animal species. Despite being the OIE’s recommended test, Agar Gel Immunodifusion (AGID) is known to have a low sensitivity producing many false negative results. Even so it is still the sole test requirement made by Brazil to assure that imported equids are free from the disease. The aim of this study is to assess the risk of importing equids that tested false negative in AGID to Brazil through a probabilistic stochastic analysis. The referred analysis was performed utilizing @Risk 7.5 to obtain the number of EIA equids introduced in Brazil, from January 2010 to July 2016. The data used in the inputs had different sources: number of equids exported to Brazil, by country of origin and date, was obtained consulting the System of Analysis of Foreign Trade Information (AliceWeb); World Animal Health Information System (WAHIS) was consulted in order to access EIA prevalence status of the origin countries on each exportations semester; and AGID test false negative estimate was calculated using previous studies. Three outputs were calculated for the whole period assuming binomial processes: number of false negative equids exported to Brazil “OP1” estimated in 23.0 (percentiles 15.8, 10% – 32.7, 90%); probability of not introducing any false negative equid “OP2” 1.1 E-11 (percentiles 1.2 E-16, 10% – 3.7 E-8, 90%); and risk of introducing EIA per imported equid (OP3) 3.0 E-3 (percentiles 2.1 E-3, 10% – 4.3 E-3, 90%). The analysis result is concerning, once all outputs indicate a strong probability of importing infected equids, also AGID predictive negative value had major impact (correlation coefficient > 0.7) on all outputs. This scenario does not only impairs the efforts to lower the prevalence of EIA in Brazil but also has the potential to cause more serious epidemiologic events due to arrival of virulent strains to which the local equids population has no previous contact.

Palavras-chave: Equine infectious anemia, Stochastic risk analisis, Importation risk, Agar gel immunodiffusion, Net analysis
Several different biological activities from naphtoquinones have been described, such as antitumoral, anti-inflammatory, bactericidal and fungicidal; however, antiviral activity is poorly reported. The aim of this study was to demonstrate in vitro antiviral activity of one synthetic naphtoquinone, C₆Cl₄O₂, (designated 3406) against bovine herpesvirus 1 (BoHV-1). Non-cytotoxic concentration was measured through MTT assay, after 72 hours on MDBK cells. In order to evaluate the antiviral activity, it was performed virus titration in the absence or presence of 3406 (at 0,244µg/mL, concentration previously determined as non-toxic), by Behrends & Kärber method. BoHV-1 titer was expressed as TCID₅₀/100µL after 72 hours of MDBK infection. The direct effect of 3406 on BoHV-1 infectivity was evaluated by incubation with the BoHV-1 at room temperature by 1, 2, 3 and 4 hours. The same volume of the viral inoculum without the compound was used as control. The presence of residual infectious virus was evaluated by titration on confluent MDBK cells immediately after the incubation. All assays described were performed in triplicate. Statistical analyses were performed using Fisher’s test, and values were considered significant when < 0.05. The virus titration in the absence or presence of 3406, show that addition of 3406 was able to reduce BoHV-1 titer from 105,5 to 101,25 (median), an inhibition of 99,9%. In the virucidal assay, significant alterations (p<0.05) on BoHV-1 titer were observed when virus was previously incubated with 3406 by 1 hour, as the titer were reduced from 104,5 TCID₅₀ to 102,25 (99,4%). After 4 hours of incubation with 3406 the BoHV-1 titer were reduced to 100,5. The protocol to search viral inactivation was able to demonstrate pronounced virucidal activity of 3406 compound against BoHV-1. The naphtoquinone derivative 3406 may be considered promising starting points for medicinal chemistry studies in antiherpessvirus chemotherapy. Financial support: Capes

**Palavras-chave:** antiherpesvirus, bovine herpesvirus, naphtoquinone, virucidal activity
Porphyridins are photosensitizers, compounds that absorb the energy of light to produce reactive oxygen species that can alter molecules and cellular mechanisms. The use of porphyridins for virus inactivation has been investigated and has demonstrated potential for in vitro and in vivo applications. Thus, the objective of this work was to evaluate the virucidal activity of two tetra-platinized porphyridins (3 and 4-TPyP) in selected bovine viruses. The cytotoxicity of the porphyridins was determined by the MTT test and the virucidal tests employed concentrations below the maximum non-toxic doses. DNA and RNA viruses, enveloped (bovine herpesvirus type 1 and bovine viral diarrhea virus) and non-enveloped (adenovirus and bovine enterovirus) were used in virucidal tests. Initially, viral suspensions were incubated with porphyridins and exposed to light for 0, 15, 30 and 60 min. After, virus titers in suspensions exposed to light for different times were determined by limiting dilution. The virucidal test with the 3-TPyP porphyridin at high concentration (13µM) resulted in total inactivation of DNA and RNA enveloped viruses even without exposure to light. In a lower concentration (1.82µM) this porphyridin reduced the infectivity of enveloped viruses after 15 min and led to complete virus inactivation after 30 min of light exposure. Incubation of enveloped DNA virus with 9.1 µM of porphyridin 4-TPyP resulted in complete virus inactivation even in the absence of light. No infectivity was detected when the RNA enveloped virus was incubated with 4-TPyP and incubated 15, 30 and 60 min in the presence of light. Furthermore, viral infectivity was detected decrease (p<0.05) without light activation, but the viral inactivation was not complete. The use of 4-TPyP at lower concentration resulted in gradual reduction of virus titers according time of light exposition. Neither porphyridins exerted effect over non-enveloped viruses. Thus, the results indicated that both porphyridins have virucidal activity on enveloped viruses, and they can affect in viral infectivity without photo-activation when used at high concentration. These results are promising in view of the use of even compounds as viral inactivators. Financial support: CAPES/CNPq

**Palavras-chave:** Enveloped viruses, Non-enveloped viruses, DNA, RNA, photosensitizers
Adenoviruses (AdV) and other enteric viruses are responsible for causing different diseases, mainly gastroenteritis. AdVs are double-stranded DNA viruses belonging to the Adenoviridae family. AdVs are shedded by the gastrointestinal tract and released into water bodies, these non-enveloped viruses remain for extended periods due to high resistance to environmental conditions and lack of sewage treatment. In this study, we evaluated the occurrence of HAdV-C and HAdV-F in water samples at distinct points within a water treatment plant and treated drinking water from the Peixe river, Joaçaba, Santa Catarina. The river is the water source for a population of approximately 55,000 inhabitants. Monthly collections of water in sterile bottles were performed for 6 months (July to December 2016), 50 mL for viral analysis and 100 mL for analysis of fecal bacterial contamination (Escherichia coli). Samples were collected in 4 steps within the water treatment plant (raw, decanted, filtered and treated) and in 4 points of treated water from taps of nursery schools. Viral concentration was performed by ultracentrifugation method, whereas the extraction of the viral nucleic acids was conducted using the commercial kit (Nuclear acid isolation kit, Biopur) and molecular detection by real-time polymerase chain reaction (Platinum Syber Green qPCR SuperMix-UDG, Invitrogen), using the equipment (Bio-Rad iQ5, Optical System Software - version 2.1) with primers to conserved regions of gene encoding for the viral hexon protein. The results indicate negativity for bacteriological contamination (E. coli) in all treated water samples. In general, HAdV was detected in 23% (11/48) of the samples, and for raw water, decanted and filtered 33% (6/18) of samples were positive with a mean concentration of 6.20x104 GC/l, in treated water HAdV was detected in 16% (5/30) and mean concentration 4.36x104 GC /l. For the different viral species, in general, HAdV-C was detected in 14.6% (7/48) with a mean concentration of 3.86x104 GC /l, HAdV-F in 8.3% (4/48) of the samples and a mean of 8.3% (4/48) of the samples and a mean of 8.3% (4/48) of the samples and a mean of 5.53x105 CG/l. Presence of HAdV in untreated waters denounces the deficiency of sewage treatment, as well as the detection of these viruses in treated water reveals resistance to the treatment processes, even in the absence of fecal coliforms. Thus, the data point to the need for insertion of virological treatment in surface waters from the Peixe River. Financial support: CAPES, CNPq, Fapergs, FEEVALE

Palavras-chave: Adenovirus, water sample, enteric virus, water treatment plant, water quality
Fecal contamination of hydrologic basins creates drastic consequences for public health and the environment. Fast and precise identification of the sources of fecal contamination is crucial for the development of remediation strategies. Enteric viruses are transmitted by the fecal-oral route through direct contact with an infected individual or with the consumption of contaminated food and water. Some of these viruses have been used as microbial source tracking since they are host specific and are very resistant in the environment. This study aims to determine the origin (human, bovine or porcine) of the fecal sources of contamination in waters from Santa Lucía and Uruguay rivers by using host specific viral markers as microbial source tracking. These rivers were studied since Santa Lucía River supplies drinking water to Montevideo (1,319,000 inhabitants) and Uruguay River supplies to Salto city which is the second most populated city in Uruguay (104,000 inhabitants). Monthly collections of surface water samples during one year was performed in six sites in Santa Lucía River and four sites in Uruguay River (120 samples) between June 2015 and May 2016. Viral concentration was performed by using an absorption-elution method with a negatively charged membrane and nucleic acids were extracted with the QiAmp Cador Pathogen kit. cDNA synthesis was carried out with random primers for Group A Rotavirus (RVA). Detection and quantification of human (HAdV) and porcine (PAdV) adenovirus, human (HPyV) and bovine (BoPyV) polyomavirus and RVA was carried out by quantitative PCR. RVA was the most frequent virus identified (37% - 44/120) followed by HAdV (18% -21/120), BoPyV (10% - 12/120) and HPyV (3% -3/120), with no detection of PAdV. The mean concentration of RVA was 1.5x10^5 genomic copies/L (gc/L), for HAdV was 1.5x10^4 gc/L, for BoPyV was 1.1x10^4 gc/L and for HPyV was 1.8x10^2 gc/L. This is the first study performed in Uruguay in order to get insight into the presence and the distribution of these host specific viral markers of fecal contamination. These results suggest that fecal contamination has a negative impact in the quality of the waters of these rivers, showing deficiencies in the procedure of sewage discharge from regional cities and in the breading of cattle. Financial Support: Comisión Sectorial de Investigación Científica, UdelaR, Uruguay
Several questions related to environmental pollution have increased with the inadequate management of domestic waste and industrial effluents. The lack of treatment of these effluents has contaminated soil and water in several ecosystems. And, consequently, providing a great dissemination of pathogenic microorganisms, generating an important impact on the environment and on the different species. Humans are subject to the action of pathologies caused by microscopic agents disseminated in all parts of the world. Among these agents, viruses are particularly noteworthy. Globally thousands of enteric viruses are released every day in the environment by the untreated effluents from the most diverse human activities, contaminating rivers, lakes and springs. Its transmission is through the fecal-oral route and are pointed out as the main enteric viruses causing acute gastroenteritis, being one of the most worrisome the human adenoviruses (HAdV). In addition, viruses are widespread in all environmental ecosystems around the world and act as indicators of fecal contamination in the environment. Rio Cai Watershed is one of the most polluted in the state of Rio Grande do Sul. The sediment is the result of soil erosion, such a particulate matrix is capable of harboring several microorganisms including viruses, which in turn have the ability to percolate through the soil reaching groundwater or dissociate from the sediment and return to the water body. The objective of the present study was to investigate the presence of enteric HAdV in sediment samples from Rio Cai. A total of 40 surface sediment samples were collected in June 2016 to March 2017 in 10 points. The viral genomes were extracted with the commercial extraction kit Biopur, following the recommended methodology. The molecular detection was realized by the qPCR technician, using specific primers for the HAdV types C and F detection. The results show that 70% (28/40) of the samples confirm the presence of HAdV type C and 25% (10/40) HAdV type F. The viral load ranged from $8.89 \times 10^3$ genome copies/gram (gc/g) to $1.55 \times 10^4$ gc/g for HAdV-C and $3.83 \times 10^5$ gc/g to $1.55 \times 10^5$ gc/g for HAdV-F. The results demonstrate contamination of fecal origin in the particulate matrix, possibly due to the discharge of sewage with human waste in the Rio Cai. This anthropic impact may reflect on the groundwater of the region due to the ability of the virus to percolate through the soil and to reach greater depths.

**Palavras-chave:** Adenovirus, environmental pollution, Rio Cai, Sediment
DETECTION OF ENTERIC ADENOVIRUSES IN SEDIMENT SAMPLES FROM THE PARANHANA RIVER IN THE RIO DOS SINOS WATERSHED, BRAZIL

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Resumo

Population growth, increased industrialization and land use for agriculture have contributed to aggravate environmental problems, directly impacting soil/sediment. Water pollution is a consequence of anthropic action and has become a major problem in densely urbanized areas. Viral bioindicators indicate environmental contamination of human / animal fecal origin and identify sources of pollution is critical to evaluate the implementation of best environmental management practices. The soil/sediment matrix is able to demonstrate the quality of the environment both momentarily and over time due to the ability of the viral particles to remain adhered to it, through the adhesion-desorption phenomenon by isoelectric potential. In turn, Paranhana River, belonging to Rio dos Sinos Watershed (RSW), one of the main tributaries of this river, in the State of Rio Grande do Sul, Brazil, is a recipient of agricultural residues and animal breeding sites, and may be contributing to the dissemination of residues from this agropastoral activity through soil/sediment. Analysis of enteric viruses is essential for the tracking of the pollution source, since these viruses are species-specific as the Adenovirus (AdV). The objective of this research was to identify viral agents through human fecal residues (Human Adenovirus type F 40-41) and animals (Adenovirus Bovine, Porcine and Canine - BAdV, PoAdV, CAV - respectively) present in soil / sediment dispersed in the environment. Six bimonthly sediment collections were carried out in May/2015 until March/2016, totaling 72 samples, distributed in 10 points of the rural and urban areas from the spring to the mouth. The sediment was eluted, followed by viral DNA extraction. Quantitative polymerase chain reaction (qPCR) was performed to detect DNA fragments from the HAdVF 40-41 as well as from the animal AdV. The results demonstrated 16.7% (12/72) presenting 2.02x10³ to 5.46x10⁵ gc/g of the HAdVF 40-41; 30.5% (22/72) for BAdV, from 1.67x10⁰ to 4.58x10⁶ gc/g; 8.3% (6/72) for PoAdV between 6.08x10³ and 5.68x10⁶ gc/g and 45.8% (33/72) for CAV with variation of 6.08x10³ to 2.85x10⁷ gc/g. Through this study, it was observed the dissemination of the viral genome in the soil / sediment matrix, which, through the adhesion-desorption phenomenon, can contaminate the water course of the Paranhana River, an important source and contributor to RSW, with higher viral load and presence in the month of November marked with lower rainfall in the region.

Palavras-chave: Adenovirus, Paranhana River, Pollution, Sediment
DEVELOPMENT OF MOLECULAR TOOL FOR PROSPECTING ORTHOBUNYAVIRUS GROUP C IN COLLECTIONS OF SMALL MAMMALS OF THE STATE OF MINAS GERAIS

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Resumo

The genus Orthobunyavirus, belonging to the Bunyaviridae family, comprises enveloped viruses with a tri-segmented RNA genome, the prototype being Bunyamwera orthobunyavirus. This genus is composed of more than 170 viruses, related to 48 species, grouped in 18 serogroups, which are divided according to their serological relations, being at least 30 related to diseases in humans. Serogroup C is composed of 15 viruses, four of which are recognized as species and the others as samples of these species. Viruses of this genus are associated with febrile diseases, febrile arthralgia, encephalitis and, in some cases, hemorrhagic fevers in humans, especially those who work or have close contact with closed forest areas. In Brazil, the Amazon region presents the genus' viruses as the second major cause of disease in humans, and viruses have been isolated also in regions of the most preserved forests in São Paulo and other regions of the country, coming from animals sentinels, animals Wild, arthropods and patients with febrile illness. Orthobunyaviruses are arboviruses mainly transmitted by mosquitoes and maruins, and their life cycle involves vertebrates, such as birds and mammals, which present with inapparent infections and can act as amplifying agents and disseminators of the virus through migration. It is known that these viral agents may be associated with wild rodents, which have great relevance for public health, associated with great diversity, remarkable reproductive potential and adaptability to different niches, the purpose of this work is the construction of primers for detection of Orthobunyavirus in samples of serum and viscera of small mammals, collected between the years of 2011 to 2013 in urban, rural and wild regions of Sabará, Serro, Rio Pomba, Ouro Preto and Contagem. For this, alignments of the Brazilian Orthobunyavirus Caraparu, Apeu and Itaya were made to define the best regions for initiator construction. Considering the great genetic variability and the capacity for rearrangement of these viruses, only three regions of the L-segment responsible for the polymerase were selected for the construction of degenerate primers capable of detecting Orthobunyavirus in conventional or semi-nested PCR amplifying regions of Approximately 1148bp and 422bp, which also allow phylogenetic inferences. Financial Support: CNPq, FAPEMIG, CAPES

Palavras-chave: Orthobunyavirus, rodents, molecular detection
228 - ÁREA: AMBIENTAL

EFFICIENCY OF CONSTRUCTED WETLAND ON ENTERIC VIRUS REMOVAL

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Resumo

Constructed wetlands (CW) are based on the natural wetland ecosystems where the interaction among the vegetation, soil, and their natural microbial population promote the removal of organic and inorganic pollutants, as well, pathogens present in water matrices, such as domestic wastewater. The efficiency of the enteric viruses removal from sewage by conventional wastewater treatment is a challenge regarding their high concentration and stability. The aim of this study was to evaluate the efficiency of two different CW configurations on human rotavirus (RV) and polyomavirus JC strain (JCPyV) removal. The CW evaluated consisted of: 1) one vertical saturated flow (VSF); and 2) one vertical flow (VF) followed by a horizontal flow (HF) (hybrid system) both representing a subsurface CW flows. The systems are located at UFSC and are regularly fed by domestic raw sewage from the university neighborhood using Thypha domingensis as a macrophyte plant. From April 2016 to February 2017, samples were monthly collected of each CW representing: i) raw sewage; ii) wetland entrance (septic tank); and iii) exit system. The samples were concentrated by polyethylene glycol 50% and quantified by real time PCR. The average of genome copies per mL (GC/mL) detected, respectively, in the raw sewage and septic tank was 3.30 x 10^6 and 2.60 x 10^8 GC/mL for RV and 2.90 x 10^5 for JCPyV. For RV, the VSF system showed the highest values of log10 reduction, 98.8% (1.95 log10), followed by the VF, 98% (1.70 log10) and the HF with 55% of reduction (0.35 log10). For the JCPyV, the log10 reduction values were 97.5% or 1.62 log10 in the VSF, followed by the VF, 98% (1.70 log10) and the HF with 55% of reduction (0.35 log10). The JCPyV system showed the highest values of log10 reduction could be under or super estimated by the fact that the samples in the entrance were not necessarily the same ones in the exit system. However, the considerable reduction of GC detected in the exit samples highlights the virus removal potential of these systems. In order to reuse the reclaimed water after the CW treatment, quantitative microbiological risk assessment (QMRA) studies should be employed to ensure the safety of this water for irrigation purposes.

Palavras-chave: constructed wetland, enteric virus removal, human rotavirus, poliominavirus, qPCR
**ENTERIC VIRUSES IN INDUSTRIALIZED SAMPLES COLLECTED AT THE POINT-OF-SALE IN SOUTHERN BRAZIL**

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**Resumo**

Enteric viruses can be transmitted by fecal-oral route and easily disseminated into the environment, water sources and food. Among these pathogens Hepatitis E virus (HEV), Rotavirus (RV) and Adenovirus (AdV) may be found in food due to low hygienic conditions therefore, cases have been reported frequently related to the consumption of contaminated food due to manipulation, transport, packaging and inadequate hygienic conditions. RV is a member of the Reoviridae family with non-enveloped ico-sahedral particles and its genome comprises 11 segments of double-stranded RNA. RV are a major cause of acute gastroenteritis in young children which may result in asymptomatic and symptomatic infections. HEV is an emerging enteric virus in industrialized countries and one of the major causative agents of acute hepatitis worldwide and has single stranded RNA genome of the genus Hepevirus belonging to the family Hepeviridae. AdV is a member of the Adenoviridae family with double-stranded DNA genome. AdV is widely found in the environment and, due its resistance is a good marker of fecal contamination. The goal of the work was to detect genomes of HEV, AdV and RV genomes in 96 meat samples (salami and sausage) collected at the point-of-sale Porto Alegre and Novo Hamburgo-RS and, 1g was macerated with 1mL of E-MEM and after extracted with TRIZOL and Biopur mini spin plus kit- Biometrix. The cDNA synthesis was performed with High-Capacity cDNA Reverse Transcription Kit following the manufacturer's protocol and submitted to conventional PCR using pan-specific primers for each viral family. RV and AdV genome was detected in 21 (21,8%) and 59 (61,4%) samples respectively and HEV genome was not detected. Enteric viruses are potential food contaminants and represent risks to human health. Despite the lack of specific legislation for viruses in foods Brazil, this survey points that viral analysis of food in Brazil may be required to determine the presence of contamination and its source complementary to bacterial analysis. Financial support: CNPq,CAPES, FAPERGS

**Palavras-chave:** HEV, food, PCR, environment
HEV tests. Therefore, the occurrence and characteristics of hepatitis E in Brazil are poorly understood and this study is the first initiative to obtain data from...

2.8 x 10^3 GC/L) from reclaimed water samples. All positive samples were... 

HEV in urban sewage, treated effluents and reclaimed water produced in four wastewater treatment plants (WWTPs) from metropolitan region of São Paulo city, totaling one hundred and forty one environmental samples. These WWTPs receive sewage from a population of 8 million people and the treatment processes consist of secondary (activated sludge) and tertiary treatments (coagulation, sand-anthracite filters, membrane bioreactor (MBR)/reverse osmosis and disinfection by chlorination). Raw sewage (500 mL) and treated effluents (1L) were concentrated by celite and reclaimed water samples (40 L) by hollow-fiber ultrafiltration system to a volume of 30 mL. Nucleic acids were extracted and detection was performed by Reverse Transcription Real-Time PCR assays (RT-qPCR). Results demonstrated a low circulation of HAV in urban wastewaters from São Paulo city (3.4% of the total samples), which 6.2% (3/48) from raw sewage (range: 3.6 x 10^3 - 6.4 x 10^4 genome copies-GC/L) and 4.4% (2/45) (6.1 x 10^2 - 2.8 x 10^3 GC/L) from reclaimed water samples. All positive samples were obtained in 2015. According to the São Paulo Health Surveillance Coordination (COVISA / SMS / 2016), 114 cases were reported in 2015 and no outbreaks were involved, with water being considered the least likely source of transmission, except when environmental disasters such as floods caused by excessive rainfall are the cause of outbreaks. HEV was not detected during all monitoring program. Data from Sinan Net (MS) reported only one case of disease caused by HEV infection in São Paulo State in 2015 and none report in 2016. However, should be emphasized that HEV is not routinely investigated in Brazil, and only a few laboratories perform anti-HEV tests. Therefore, the occurrence and characteristics of hepatitis E in Brazil are poorly understood and this study is the first initiative to obtain data from HEV in environmental samples in São Paulo city. Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) – Processo No 2013/26586-1

Palavras-chave: environmental surveillance, hepatitis A virus, hepatitis E virus, reclaimed water, urban sewage
Fecal contamination by *Escherichia coli* and *Enterococcus*, comparing with human adenovirus presence, as indicators of balneability in the Rio Grande do Sul coast

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Resumo

Monitoring of coastal water in RS occurs through the analysis of *Escherichia coli*, which is the indicative parameter of fecal coliforms (FC). However, traditional methods have been shown to be inefficient, since they do not reveal significant correlations of contamination by other pathogens. Some studies select *Enterococcus* as an alternative indicator of marine water contamination, due to their greater resistance. Among the fecal-oral transmission viruses, human adenovirus is responsible for infections gastrointestinal, respiratory and conjunctival. Goal of this study was to evaluate the hygienic-sanitary conditions of some beaches in the RS north coast, through out bacterial indicators as total coliform (TC), *E. coli*(FC), *Enterococcus* (ENT), in comparison with the detection of human adenovirus group C (HAdV-C). Eight points were selected for this study: Torres Praia Grande (P1), Torres Guarita (P2), Curumim (P3), Capão Novo (P4), Capão da Canoa (P5), Atlântida (P6), Imbé (P7) and Tramandai (P8). Samples were collection in July 2017 and represents the winter season condition, being the first in a planned two-year schedule. In each point 500 mL of water were collected and distributed in 100 mL aliquots for the detection of TC, FC and ENT, analysed by the Colilert® and Enterolert® kits (IDDEX). For viral analysis, 36 mL of each sample were concentrated using the ultracentrifugation method and then submitted to viral ADN extraction through the commercial Promega kit. Partial amplification of the HAdV-C hexon gene was performed by the real-time polymerase chain reaction (qPCR) using Promega GoTaq® qPCR Master Mix Kit. Was detect the presence of viral genomes in 7 sampled sites (except P8), with loads ranging from 1,08x10³ to 2,53x10⁸ genomic copies/liter. Analysis indicated TC at all points, ranging from 41 to 591 NMP/100 mL, absence of FC (<1 NMP/100 mL) at P2 and P3 and the presence at all others points ranging from 10 to 63 NMP/100 mL. ENT values obtained indicated a slightly higher quantification of this parameter in P1: 30, P4: 98 and P7: 74 NMP/100 mL and absent in P2, P3, P5 and P6. Despite greater quantification of ENT in some points, presence of this group remained constant at the same points in which there was detected of FC. This is a preliminary study which indicates HAdV-C distribution in most analyzed points evidencing the presence of human fecal contamination and absence of correlation with bacterial parameters evaluated. Financial Support: CNPq.

**Palavras-chave:** adenovirus, coastal water, Enterococcus, *Escherichia coli*, fecal coliforms
HUMAN BOCAVIRUS: DETECTION, QUANTIFICATION AND MOLECULAR CHARACTERIZATION IN DIFFERENT AQUATIC MATRIX OF URUGUAY

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Resumo

Human bocavirus belongs to family Parvoviridae, subfamily Parvovirinae, genus Bocaparvovirus. Four subtypes of the virus are described: HBoV1, 2, 3 and 4. HBoV1 is commonly associated with respiratory infections, while subtypes 2, 3 and 4 are associated with gastrointestinal infections. There are few studies describing the presence of HBoV in wastewater and superficial water around the world and these studies show high values of positivity. With the propose of known the state of situation of Uruguay about the presence of HBoV in different aquatic matrix, 68 wastewater samples of the cities of Salto, Paysandú, Bella Unión, Fray Bentos and Melo and 36 superficial water samples of the cities of Salto, Florida and Santa Lucia, were analyzed. The screening of samples was done by Multiplex qPCR, for the detection of the four subtypes, followed by qPCRs for the independent quantification of each subtype, and conventional PCR amplifications for the molecular characterization of the strains. HBoV was observed with high frequency (69%) in wastewater samples, founding only one positive sample (3%) in superficial water. In the wastewater samples, the HBoV1 was detected in 11 of 47 positives samples, with a media concentration of 8,21x104 copies/L, while the HBoV3 was detected in 35 samples with a media concentration of 4,13x106 copies/L and finally the HBoV2 and/or 4 were detected in 39 samples with a media concentration of 7,75x106 copies/L. Forty sequences of the virus were obtained. A phylogenetic analysis was perfomed by using the Neighbor Joining method and the kimura two-parameter model. The 4 Subtypes of HBoV were confirmed in the phylogenetic tree. With the present study we evidence the presence of HBoV in different aquatic matrix of the country, being the virus with the highest frequency in wastewater observed by our group until now.

Palavras-chave: Human Bocavirus, wastewater, superficial water, PCR and Phylogenetic analysis, Uruguay
INVESTIGATION OF ANIMAL ADENOVIRUS IN HYDROGRAPHIC BASIN OF THE RIVER CAI/RS

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Resumo

The precariousness of sewage systems, the large amount of industrial and domestic effluents compromises soil and water quality. With population areas if expanded these problems tend to increase. Enteric viruses are a heterogeneous group of viral agents associated with infections and subclinical diseases, such as Adenoviruses (AdV). These viruses are characterized by their stability both in the gastrointestinal tract and in the environment. They are excreted through faeces and can withstand soil and water contamination for long periods of time, and are therefore considered important indicators of contamination. The present research aims at detecting the main animal Adenoviruses, Bovine Adenovirus (BAV), Canine Adenovirus (CAV) and Porcine Adenovirus (PoAdV) in water and sediment samples from Hydrographic Basin of the Rio Cai/RS. Samples were collected quarterly in the period between July 2016 and March 2017 at different points. Forty samples of water (0.5L) and sediment (10g) were collected aseptically and conditioned at 4ºC. The viral recovery of the sediment occurred from the Technique of the Direct Method of Obtaining Viral Particles. The viral concentration of the waters was performed by the ultracentrifugation method. Viral genomes were extracted and molecular detection was performed by qPCR. The analysis of the samples revealed a chronic contamination along the Cai river during the whole investigated period. The most frequent virus in the water was PoAdV (45%) and in the sediment was the CAV (77.5%). In the water the quantification results ranged from 1.65x10^5 (CAV) in the fourth collection to 2.46x10^8 (BAV) in the second collection. In the sediment the results were of 7.73x10^5 in the second collection to 1,04x10^8 in the fourth collection. The fourth collection showed lower levels of contamination, as well as fewer contaminated sites. Thus, one can perceive both the impact caused by the lack of treatment of animal waste from small farms, and the effect of lack of urban sanitation, due to the constant presence of CAV observed along the Cai River Basin.

**Palavras-chave:** BAV, hydrographic basin of the river Cai, PCR real time, CAV, PoAdV
234 - ÁREA: AMBIENTAL

MOLECULAR CHARACTERIZATION OF AN ISOLATED MIMIVIRUS OF RUMINES FROM DAIRY COWS

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Resumo

The microbiota of bovine rumen is complex, possessing organisms of the three domains of life that are potential hosts of numerous viruses. These hosts are important, because they are involved in converting food into energetic products that are important for animal metabolism. Studies about viruses that infect bacteria present in the rumen have shown that they play an important role in maintaining the bacterial population and in the horizontal transfer of genes. However, there are few studies on viruses that infect fungi, protozoa and archaea present in the bovine rumen. Viruses that infect amoebas of the genus Acanthamoeba have received attention due to reports of new giant viruses that infect numerous species of amoebae. Viruses belonging to the Mimiviridae family are giant viruses associated with amoeba. Mimiviruses have a genome consisting of double-stranded DNA containing up to 1.2 Mb. Therefore, the aim of this work was the characterization of a virus that belongs to the family Mimiviridae and was isolated from ruminal liquid. We checked the isolation through the presence of cytopathic effect in cells of Acanthamoeba spp. and by amplification of the viral RNA helicase gene (a largely conserved gene amongst mimiviruses) by qPCR experiments. Transmission Electron Microscopy showed mimivirus-like particles with icosahedral capsid, with a diameter of about 400nm. Viral factories were observed occupying a large portion of the amoeba cytoplasm. Furthermore, distinct steps of virus morphogenesis were detected in association with the viral factories. In addition, we extracted the genetic material from the virus and sequenced using ion torrent in the Núcleo de Análise de Biomoléculas from Universidade Federal de Viçosa. In order to characterize the virus molecularly, we performed bioinformatics analyses. Giant viruses appeared to be present in different environmental samples and ecosystems, indicating that these viruses are common. Thus, further studies are necessary to elucidate the relationships between these giant viruses and their hosts. Financial suport: FAPEMIG, CAPES and CNPQ

Palavras-chave: Mimivirus, rumen, dairy , cattle
MOLECULAR DETECTION OF HEPATITIS E VIRUS IN ECOSYSTEMS IMPACTED BY SUINOCULTURE IN THE PARAENSE NORTHEASTERN AND METROPOLITAN MESOREGIONS OF BELÉM PARÁ.

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Resumo

Hepatitis E is an icteric disease caused by hepatitis E virus (HEV) and the World Health Organization estimates that there are around 20 million cases of HEV infection, with 3.3 million symptomatic cases each year and 56,600 deaths. The main form of transmission is through feces, besides reports of zoonotic cases. In the North, it is common to use surface and groundwater sources for consumption, since the supply of drinking water does not serve a significant portion of the population and most of the animal waste from nurseries is released directly into the environment, favoring a possible contamination of these. In this scenario, the objective of this study was to detect hepatitis E virus particles in ecosystems impacted by suinoculture in two municipalities of the mesoregions of Belém and Northeastern Paraense. During the period from September 2016 to June 2017, monthly collections of ecosystems were collected, washed in a pig farm located in the municipality of Capanema and during the months of June and July 2017 in an ecosystem located in the city of Castanhal, totaling 16 And 4 samples, respectively. The water samples were concentrated by the adsorption and filter membrane elution method. The viral RNA extraction was performed with QIAamp Viral RNA kit (QIAGEN) and then reverse transcription with SSIII-RT (Invitrogen). To detect HEV, amplification of the target sequences of the open reading regions (ORF1) and (ORF2) was performed by means of the nested-PCR technique. All the samples of the points studied were not positive for HEV, demonstrating that possibly such virus does not circulate in these regions of the State of Pará. Financial Support: CNPq

Palavras-chave: hepatitis E virus, suinoculture, environment
Giant viruses belonging to the Mimivirus genus (Mimiviridae family) were first described in 2003, during the characterization of a pathogenic amoeba-associated microorganism that was related to outbreaks of nosocomial pneumonia in a hospital in Bradford, England. Since then a huge diversity of related virus has been described from many different countries and environments. As soon as new giant viruses have been discovered, these organisms started to be phylogenetically classified based on sequences of the gene related to the DNA polymerase B protein. This gene is conserved among the different viral members but presents enough differences to separate them in three different lineages (A, B and C). These viruses, however, present a set of other conserved genes that are not so well explored in the literature. In this work we analyze the structure, syntheny and phylogenetic relationships of the major capsid gene (another hypothetically conserved gene shared by the Mimiviridae members) in different giant viruses isolates, including mimivirus KV, a recent virus isolated by our group. By using phylogenetic analyses and comparing the order of the intronic and exonic regions that compose the MCP gene of these giant viruses we observed a distinct pattern of genetic organization and gene evolution involving KV and other mimiviruses, even among members of the lineage A to which KV belongs. These differences also reflected in how the MCP mRNA is processed by splicing. By sequencing the mature transcripts of the MCP gene in both APMV (type-species among mimiviruses and member of the lineage A) and KV we have observed many differences in terms of content and organization, suggesting that in the Brazilian isolate this gene is processed in a different form. Finally, taken together, our results enabled us to highlight the MCP gene as a new interesting genetic marker that could also help in future studies involving the isolation and characterization of other giant viruses.

**Palavras-chave:** mimivirus, Acanthamoeba, capsid, phylogeny, evolution
Resumo

Triggering the amoebal phagocytosis process is a sine qua non condition for most giant viruses to initiate their replication cycle and consequently to promote their progeny formation. It is well known that the amoebal phagocytosis process requires the recognition of particles of >500 nm, and most amoebal giant viruses meet this requirement, such as mimivirus, pandoravirus, pithovirus, and mollivirus. However, in the context of the discovery of amoebal giant viruses in the last decade, Marseillevirus marseillevirus (MsV) has drawn our attention, because despite its ability to successfully replicate in Acanthamoeba, remarkably it does not fulfill the >500-nm condition, since it presents an ~250-nm icosahedrally shaped capsid. We deeply investigated the MsV cycle by using a set of methods, including virological, molecular, and microscopic (immunofluorescence, scanning electron microscopy, and transmission electron microscopy) assays. Our results revealed that MsV is able to form giant vesicles containing dozens to thousands of viral particles wrapped by membranes derived from amoebal endoplasmic reticulum. Remarkably, our results strongly suggested that these giant vesicles are able to stimulate amoebal phagocytosis and to trigger the MsV replication cycle by an acidification-independent process. Also, we observed that MsV entry may occur by the phagocytosis of grouped particles (without surrounding membranes) and by an endosome-stimulated pathway triggered by single particles. Taken together, not only do our data deeply describe the main features of MsV replication cycle, but this is the first time, to our knowledge, that the formation of giant infective vesicles related to a DNA virus has been described. Financial support: CAPES, FAPEMIG, and CNPq

Palavras-chave: Marseillevirus, Giant viruses, vesicles
Adenoviruses and rotaviruses are present in the environment and can be considered a great risk to the population health. The city of Macapá, for having currently one of the worst rates of sewage collection and treatment and because of its old water treatment system. The objective of this study was to evaluate the quality of the water collected and distributed in the city of Macapá-AP, evaluating the physico-chemical, bacteriological and virological parameters. The method for investigating the physico-chemical parameters was according to the standard methods, the bacteriological was done by the filtering membrane and the viruses (adenovirus and rotavirus) were identified by the concentration method, followed by the nucleic acid extraction using the Mini RTP Kit (Stratec Molecular) and the conventional PCR. The method was applied in water samples from the Amazon River, before the treatment, and in the samples of treated water at the distribution reservoirs exits, provided by Water Company from AP, in 14 points during the months of December 2015 to September 2016, totaling 91 samples. The examined samples revealed a situation of non-compliance with the Ministry of Health legislation for the physico-chemical parameters (turbidity, apparent color and pH), as well as for the presence of coliforms and adenovirus and rotavirus particles, both in the river and in some samples of the distribution network. Regarding viral positivity, the samples revealed 29.67% (27/91) of positivity. The most frequent virus was adenovirus-AdV, which represented 70.37% (19/27) of the positive samples, with rotavirus-Rv representing 29.63% (8/27). Surface water showed a higher positivity 94.51% (22/27) and distribution network 5.49% (5/27). The detection of the pathogens (viruses and bacteria) in these samples indicates that public water supplied by CAESA is not in accordance with the legal standards recommended by the Ministry of Health, and should receive appropriate treatments and intensified monitoring, so that it does not present contamination by these and other pathogens. The present study transcends importance because it contributed to the training of human resources in molecular biology techniques turned to the monitoring of water quality and basic sanitation at the state of Amapá and the effective contribution to the database of the Ministry of Environment and Secretariats of Health, where they can assist in the implementation of public and environmental health policies

**Palavras-chave:** Adenovirus, Rotavirus, concentration, PCR
ADAPTOR PROTEIN-1 COMPLEX IS INVOLVED IN SERINC5 DOWNREGULATION BY HIV-1 NEF

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Resumo

Nef is an accessory protein encoded by HIV-1 that enhances the infectivity of the HIV-1 virion particles. Nef subverts the protein trafficking machinery to robustly downregulate cell surface molecules (including CD4 and MHC-I) that are crucial in the host immune system and viral replication cycle. Among the components of the protein trafficking machinery required for Nef’s activities are the adaptor proteins (APs), which are heterotetrameric complexes that mediate cargo-protein sorting. Previous studies demonstrated that the host protein SERINC5 is an inhibitor of retrovirus infectivity and is counteracted by Nef. Virions containing SERINC5 have a diminished ability to infect target cells. Therefore, Nef removes SERINC5 from the plasma membrane preventing its incorporation into newly formed viral particles. The present study aims to identify molecules of the host intracellular trafficking machinery used by Nef to prevent SERINC5 antiviral action. Immunofluorescence microscopy analysis showed that in the presence of Nef, SERINC5 accumulates in the perinuclear region and colocalizes with Golgi markers in HeLa cells. We also demonstrate that SERINC5 accumulation in Golgi apparatus of Nef-expressing cells is sensitive to Arf-GEF inhibitor brefeldin A (BFA). Using bimolecular fluorescent complementation (BiFC) assays, we show that Nef interacts with the μ1A subunit of host adaptor protein 1 (AP-1) in intracellular sites containing SERINC5. Moreover, depletion of AP-1 μ1 subunit by siRNA compromises SERINC5 downregulation to the juxtanuclear region by Nef. We also show that perturbing the ESCRT pathway, by expression HRS or a mutant of VPS4 (VPS4-E/Q), redistributes Nef-downregulated SERINC5 to enlarged endosomes. Taken together, our results suggest that Nef removes SERINC5 from HIV-1 assembly sites by altering the intracellular trafficking of SERINC5 via the interaction with AP-1 and targeting SERINC5 to the MVB pathway using the ESCRT machinery.

Palavras-chave: SERINC5, HIV-1, Nef, AP-1
ANALYSIS OF ANTIVIRAL ACTIVITY OF EXTRACT AND AN ISOLATE OF PSYCHOTRIA SP. AGAINST THE ZIKA VIRUS

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The original isolation of Zika virus (ZIKV) was obtained in 1947 of a Rhesus monkey febrile exposed in the jungle Zika (Uganda). Faced with a pattern of sporadic occurrence in the following decades and without gravity, little importance was given to this arbovirose. However, in recent years, diseases caused by ZIKV infection have evolved to become a flaviviral threat, currently representing an emerging arbovirus in the world. To date, there are no antiviral drugs or vaccines against this virus, being attractive the research and prospecting of antivirals. In this context, natural products appear attractive as phytotherapics. The Rubiaceae family has more than 13,000 thousand species of plants, and within the genus Psychotria were identified bioactive extracts with antibacterial, antiviral, antifungal and anti-inflammatory activity in about 1000 species. Therefore, the objective of this work was to evaluate the cytotoxicity and antiviral activity in a methanolic extract of Psychotria sp and in a DX isolate from this extract. Initially, using Vero mammalian cells and technique of the methyl thiazole-tetrazolium (MTT), cytotoxicity tests were performed, giving the cytotoxic concentration to 50% of the cells (CC50). Then, the effective protective concentration was obtained for 50% of the infected cells (CE50). For the extract, no cytotoxic activity was detected until the highest concentration evaluated (500μg/mL). For the DX isolated, CC50 was 119.3μg/mL. From these values, antiviral tests were directed, adding the substances together with the viral infection, done at a multiplicity of infection (MOI) of 0.1 virus per cell. The CE50 was 47.85μg/mL for the extract and 31.87μg/mL for the isolated compound. Finally, from the ratio between CC50 and CE50, the selectivity index (IS) was calculated, which indicates how much a compound inhibits the parasite without affecting the host. Our data showed IS above 3, as recommended in the literature, indicating that the active dose is safe for the cells. The data obtained show that the extract of this plant has antiviral action against ZIKV, this action was also detected in at least one of its isolated compounds, which possibly acting in synergy with other compound. Studies are being conducted in our laboratory in order to identify possible mechanisms of action.

Palavras-chave: Psychotria sp, Zika virus, antivirals
ANALYSIS OF COINFECTION OF DENGUE VIRUS IN A MAMMALIAN CELL MODEL

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Resumo

Co-circulation of arboviruses such as Dengue virus (DENV), Zika virus (ZIKV), Yellow Fever virus (YFV) and Chikungunya virus (CHIKV) has been a common occurrence in many endemic regions of Brazil. This far, there is no treatment or therapy approved to treat any of these aforementioned infections. In this context, the prospection of antivirals must be evaluated in models allowing the analysis of antiviral activity during co-infections. DENV is the most important arbovirus posing threat to public health worldwide. World Health Organization estimates that almost four hundred million people are infected every year. The disease is caused by infection with one of the four different serotypes (DENV1-4). Thus, the first aim of this project is to investigate the in vitro co-infection with different DENV serotypes. Studies of Dengue co-infection are scarce and limited to mosquito cell lines thereby our proposal of a mammalian cell model more adequate for antiviral tests. Accordingly, VERO cells were co-infected with combinations of DENV serotypes and co-infected monolayers were processed for both conventional and quantitative PCR. The qPCR strategy employed here successfully amplified genomic segments of different DENV serotypes during co-infections in VERO cells. We were able to detect genome replication during co-infections with DENV serotypes 1 and 4, DENV serotypes 2 and 3, and DENV serotypes 3 and 4. We also did quantification and comparison of viral RNA of serotypes 3 and 4 between co-infected and individually infected samples and detected none to low variance in coinfected samples. These results seem to indicate that there is low interference in the genome replication between the two serotypes under the conditions analysed. Additional experiments are necessary for confirmation, however, especially regarding the viral morphogenesis. Once we validate this model, we will be able to pursue antiviral studies during co-infections. We foresee that this strategy will provide insights on arboviruses-host interplay, intrahost competition and ultimately therapy strategies that could be candidates to counteract arboviruses.**Keywords:** Dengue virus, Coinfection, Vero cells Financial support: CNPq, CAPES e FAPEMIG

**Palavras-chave:** Dengue virus, coinfection, Mammalian cells
ANTIVIRAL ACTIVITY OF SYNTHETIC ALKALOIDS ON CHIKUNGUNYA VIRUS

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Resumo

Chikungunya virus (CHIKV) is a positive single strain RNA virus transmitted by the mosquito Aedes sp. Infected patients can develop CHIKV fever which is characterized by clinical symptoms such as severe fever and incapacitating pain. In the last year Brazil suffered an outbreak of CHIKV with 61864 suspected cases and 13 confirmed deaths. There is no vaccine or antiviral treatment against CHIKV showing the necessity for studies to develop effective antiviral. Natural compounds, as Alkaloids, already showed antiviral activity against a range of viruses. However, the purification process is time consuming and difficulty to obtain the compounds from natural sources is limiting. To overcome these issues the synthesis of compounds based on natural molecules it is an alternative. This study aimed to evaluate the antiviral activity of 9 synthetic alkaloids, designed based on natural scaffolds, on CHIKV infection and replication in vitro. BHK-21 cells were plated in 24 well 24 hours prior to the infection with CHIKV ECSA which contained Gaussia luciferase reporter, at MOI of 0.01, in the presence or absence of compounds at nontoxic concentrations. Gaussia luciferase levels were measured 16 hours post infection to evaluate the antiviral activity of synthetic alkaloids. DMSO and Obatoclax were used as negative and positive control. Compound 1 inhibited 41.47% of CHIKV ECSA infection. Trying to find the specific step that the compound is acting the compound were tested in Huh-7.5 cells - expressing the subgenomic replicon CHIKV-NCT- for 72 hours to evaluate de CHIKV replication in vitro. Compound 1 decreased up to 75.55% of CHIKV replication, demonstrating that this compound might be a possible antiviral candidate for future treatment of CHIKV infections. Therefore, future analyses it is going to be conducted to understand the mechanism of action of this synthetic alkaloid on CHIKV lifecycle. Financial support: ROYAL SOCIETY – NEWTON ADVANCED FELLOWSHIP (NA150195), FAPEMIG (APQ-00587-14; SICONV 793988/2013), CNPq (445021/2014-4), FAPESP (2014/22198-0).

Palavras-chave: alkaloid, antiviral, chikungunya, synthetic
ANTIVIRAL EVALUATION OF VEGETABLE COMPOUNDS AGAINST HUMAN HERPESVIRUS 1 AND AICHI VIRUS

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Resumo

Introduction: The emergency and dissemination of viral diseases has been impacting significantly on the global health and economy throughout the years. This represents a challenge in matters of treatment, due to the onset of resistant variants to available drugs. In this context, there is an increasing interest on the development of new effective antiviral drugs. Among promising compounds, there are those derived from natural products, whose antiviral activity has been reported in several studies. Thus, this study aims to evaluate the antiviral activity of isolated medicinal plants’ compounds native from Middle West of Brazil against the human herpesvirus 1 (HHV-1) and the aichi virus, as well as determine its action mechanisms. Methodology: The substances 5,7-dihydroxy-6-methylflavanone (2), 5,7-dihydroxy-8-methylflavanone(3), 2’,4’-dihydroxy-6’-methoxychalcone (4) and 5,7-dihydroxy-6,8-dimethylflavanone (5), where obtained from the leaves of Campomanesia xanthocarpa and fruits of Campomanesia adamantium. Vero cells cytotoxicity was MTT evaluated. The antiviral activity against HHV-1 and the aichi virus was determined by viral titer reduction analyses according Reed & Muench, which is expressed in Viral Inhibition Index (VII) and percentage inhibition (PI). The index of selectivity (IS) was calculated as the ratio of CC50 and ED50. Mechanism of action assays were performed for substances with antiviral activity above 70% for HHV-1. Results: All evaluated compounds exhibited PI above 90% for both viruses. The substance 5 presented IS of 37.0 for both viruses, while substances 2 and 4 showed ISs of 139.8 and 36.0 for HHV-1. For aichi virus, those substances showed ISs of 15.1 and 11.5, respectively. Mechanism of action assays suggested that all compounds were capable of cause adsorption and penetration inhibition, besides being capable of intracellular action against HHV-1. The assays described were not performed for substance 3 due its high cytotoxicity. Conclusion: All the molecules tested presented viral inhibition with IS above 10 for HHV-1 and aichi virus, conferring potential antiviral action. It can be inferred that the interaction of these molecules with HHV-1 and the host cell possibly interferes in virus adsorption and penetration steps or at some biosynthesis stage leading to inhibition of viral replication.

Palavras-chave: Human herpesvirus 1, Aichi virus, Campomanesia sp., Antiviral drugs, Medicinal plants
In Brazil, the first suspected cases of microcephaly were reported in the beginning of 2015 and later ZIKV was confirmed as the etiological agent. The country was the first high cases reported of fetus and newborn with microcephaly and young and adults affected by the Guillain-Barré syndrome. From them on, ZIKV spread globally. ZIKV is an arbovirus and belongs to the Flavivirus genus, an enveloped ssRNA(+) and genome has circa eleven thousand nucleotides. It can be transmitted by 19 female species of Aedes. Urban and rural area A. aegypti is the main vector of ZIKV. Globally an estimated 2 billion people are in risk of infection. The project aims to characterize the basic cycle ZIKV infection in vitro using cells lines permissive by ZIKV African and Brazilian strains that their genome are on GenBank and demonstrate if the cells are susceptible and permissive to different viral strains through kinetics experiments. The ZIKV strains granted by Instituto Evandro Chagas (IEC) and MR-766 African strain are cultivated in Vero and C6/36 cells in a series of tree subcultures. The experimental kinects was tested in the cells lines A-253, KNS-42, ONS-76 and M059J. Viral titre, RT-qPCR, sequencing of the Envelope by the Sanger method and immunofluorescence with specific monoclonal primary antibodies by the High Content Screening System are used for virus characterization. The characterizations of established cell culture are determined by the growth curve and cell viability. Kinects experiments are series of time-point inoculation for definition of viral growth curve. The cells A-253, KNS-42, ONS-76 and M059J are infected by virus strains showing cytopathic effects and syncytia. In a series of tree passages (T1–T3) of both strains and the cells lines RT-qPCR shows that IEC is more infectious with lowers values of cycle thresholds (Ct) than MR766 in all cells. T1 IEC/A-253 Ct=18, MR766/A-253 Ct= 28; IEC/KNS Ct=15, MR766/KNS Ct=20; IEC/ONS Ct=17, MR766/ONS Ct=26, IEC/M059J Ct=12, MR766/M059J Ct=20. Comparison between passages T1 to T3 of the same cell and virus, only MR766/KNS T1 Ct=20, T2 Ct=18 and T3 Ct= 16; and MR766/M059J T1 Ct=18, T2 Ct=18 and T3 Ct=16 have elevation of number of viral RNA. These cells permissive of ZIKV and there are differences in infectivity between viral strains and in the same viral strain. Next steps will be continuous characterization of ZIKV strains and viral kinect experiments with one passage. Financial Support: FAPESP and CNPq.

**Palavras-chave:** Kinetcs, Permissive cells, Zika virus
CELLULAR PROTEIN ALIX IS A POSITIVE FACTOR FOR HIV-1 INFECTIVITY

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Resumo

Nef is an accessory protein early expressed in the life cycle of Lentiviruses HIV and SIV. This protein plays an essential role increasing viral infectivity and progression to Aids. It has been reported that Nef interacts with different cell partners to perform different functions, however the function related to increased viral infectivity is not yet elucidated. Among several roles, Nef mediates downregulation of CD4 receptors from the cell surface and target CD4 to lysosomal degradation through interaction with the cellular protein Alix. Nef site of interaction with Alix was previously mapped to YPLTF motif located at the positions 135-139 on the C-terminal of the Nef from HIV NL4-3 isolate. But the role of Alix during the life cycle of HIV-1 was not fully characterized. It was reported that Alix protein plays a central role in ESCRT machinery and this is essential for the budding of some enveloped viruses but not for HIV. Alix also plays different roles in cell such as regulating basal autophagy, incorporation of LBPA in late endosomes and the regulation of acidified endosomes. The objective of this study was to investigate the role of Alix on HIV-1 infectivity and the importance of interaction with Nef in this process. In our assays, we observed that silencing of Alix in Hek293T and HeLa cells with a specific shRNA against Alix followed by transfection of these cells with pNL 4-3 leads to a 3-4 decrease in infectivity of viral progenie produced from these cells but no impact on viral release and viral maturation was observed. Nevertheless, no impact on viral infectivity was observed on a NL 4-3ΔNef upon Alix silencing. This data confirming that Alix influencing HIV-1 infectivity of HIV-1 viral progeny dependent on Nef. These data were reproduced for SIVcpz, the precursor of HIV-1. Interestingly, lymphocytic MOLT4 cells silenced for Alix have been shown to be less permissiveness to HIV-1 infection. Alix-knockdown MOLT4 cells showed a lower expression of viral proteins after 72h of infection, confirming the phenotype of less permissiveness to infection. Thus, we confirmed that Alix plays a more important role during the replicative cycle of HIV-1 than previously reported in the literature when it was believed that Alix only played a secondary role on HIV-1 budding being important only on the absence of TSG-101, ESCRT-1 complex protein. We are investigating the mechanism by which Alix acts as a positive factor for HIV-1 and SIVcpz infectivity.

Palavras-chave: Alix, HIV-1, Nef, SIVcpz, Infectivity
CHARACTERIZATION OF THE ANTIVIRAL ACTIVITY OF PLANT SPECIES AGAINST ARBOVIRUSES

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Resumo

Infections caused by arboviruses affect thousands of people around the world, representing a global public health problem. Dengue virus (DENV), belonging to the Flaviridae family, as well as the Mayaro virus (MAYV) and Chikungunya virus (CHIKV), from the Togaviridae family, are responsible for outbreaks of febrile diseases which can result in severe hemorrhage or induce persistent joint pain, respectively. However, there are no licensed medications yet to treat these viruses. The aim of this work was to characterize the antiviral activity of selected plant species against different DENV, CHIKV and MAYV. The total leaves extract of Guazuma sp (GU), Piper sp (PP), Faramea sp (FM), as well as their different subtractions, was used to evaluate the activity in in vitro assays. After the adsorption period, the cells were treated with extracts at 50μg/mL, 75μg/mL, or 100μg/mL and incubated for 48 hours at 37°C in 5% CO2 atmosphere. The viral load was quantified in plaque assay and the cell viability was measured by the MTT assay. No significant cytotoxic effect was detected with tested doses of extracts of GU, PP, SI, FM. Under these conditions, these extracts promoted a preservation of cellular viability in relation to the infected and untreated conditions. Moreover, we also observed a decrease of up to 98% in viral load present at the conditioned medium in DENV and MAYV infection. Tests are currently under course to determine the concentration required to inhibit 50% of viral load (IC50). These data demonstrate the potential antiviral activity present in certain tested plant species. On this way, the next step is to fractionate the plant extracts of the species that have the most promising results and to evaluate the in vitro activity of these pharmacologically interesting fractions.

Palavras-chave: Dengue virus, Plant species, Mayaro virus, Antiviral activity
CHARACTERIZATION OF ZIKA VIRUS REPLICATION IN PRIMARY HUMAN MUSCLE CELLS AND MUSCLE TISSUE IN NEONATAL MICE

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Resumo

The ZIKA virus (ZIKV) is a single stranded positive RNA enveloped virus that belongs to the Flaviviridae family and flavivirus genus, transmitted to humans by Aedes mosquitoes. During ZIKV infection, severe conditions are associated with neurological complications such as Guillain-Barré syndrome and fetal malformation in after intra-utero exposition. However, in general, the infection promotes a mild fever with a high incidence of rash and muscle/joint pain. Viral replication in muscle tissue may be associated with tissue damage and contribute to viral spread, but the ability of ZIKV to replicate in muscle cells has not yet been demonstrated. Therefore, the objective of this study is to investigate the susceptibility of myogenic precursors and myotubes by ZIKV in vitro and in vivo using a neonatal mice model. In our in vitro study HSMN cells, primary human myoblasts isolated from skeletal muscle, were infected with ZIKV at multiplicity of infection of 1 and 5. The cells were incubated for 2 hours at 37 °C in a 5% CO2. At different hours post-infection the efficiency of viral replication was determined by plaque assay on Vero cells. Thus, we observed that ZIKV is able to replicate in human muscle cells, reaching the maximum peak of replication 24 hours after infection. The change in the viability of the myoblasts during infection was also determined by MTT assay. Cell viability analysis shows that ZIKV replication results in the death of myoblasts. Apparently, myogenic precursors are more susceptible to infection, since viral replication in HSMN cell differentiated into myotubes resulted in lower amplitude of ZIKV release and less percentage of positive cells when labeled with antibody against viral protein E (4G2). We also performed In vivo studies using wild type SV129 newborn mice (3 days-old) infected subcutaneously with 10^6 PFU of ZIKV. At different days post-infection, muscle tissues were collected and the viral titers were analyzed by PCR. The infection of SV129 mice with ZIKV results in a temporal crescent viral loads on muscle, consistent with viral replication. Furthermore, the animals developed clinical signs such as weight loss and difficult of locomotion. This study provides evidence that human myogenic precursor cells, as well the muscle tissue of neonatal mice are susceptible to infection by ZIKV, corroborating the skeletal muscle role in ZIKV pathogenesis. Financial Support: CNPq, Faperj and Finep

Palavras-chave: Zika Virus, Muscle, mice model
Introduction: Chikungunya virus (CHIKV) is a positive-sense single-stranded RNA Alphavirus which is an arbovirus considered an emerging/reemerging disease which has caused over a million cases in the world. Around 85% of infected patients develop fever, headache, myalgia articular, edema and rash in the acute phase. 40% of patients can develop chronic arthralgia from months to years. There are distinct genotypes, the East Central South African (ECSA) and Asian genotypes being responsible for the massive outbreaks. Dengue virus is the arbovirus hyperendemic in Brazil and normally causes mild clinical symptoms, which can evolve to severe conditions like hemorrhagic and neurological complications. In this study we detecting these arboviruses in samples of symptomatic patients in the city of Parnaíba, Piauí. Materials and Methods: The clinical samples were collected at health services in the city of Parnaíba, during 2016. Viral RNA was extracted and performed RT-PCR using previously described primers for CHIKV and DENV-1, 4. The PCR product was visualized in 1.5% agarose gel and excised for genome sequencing. Phylogenetics analysis was performed using maximum likelihood and Bayesian analysis. Results: CHIKV genotype ECSA and DENV-1 were identified circulating during the year of 2016 in the state of Piauí. CHIKV isolates clustered together with the CHIKV isolates from Bahia e Rio de Janeiro, from 2014 and 2016, respectively. The CHIKV isolates did not contain the E1 A226V mutation associated with more virulence. One patient infected with both CHIKV and DENV1 developed chronic arthralgia for 7 months. Conclusion: DENV1 and CHIKV genotype ECSA were identified circulating in serum from patients in Parnaíba, Piauí. The first report of ECSA genotype circulating in humans in the northeastern region of the country, after detection in 2014. Further studies are required to evaluate the economic and clinical impact of the circulation of these arboviruses, since high incidence of chronic arthralgia has been reported in other countries. Financial Support: We thank CNPq, CAPES, FAPEPI, and Prefeitura Municipal de Parnaíba for the scholarship and financing.

Palavras-chave: arbovirus, Northeast, CHIKV, DENV
DELAVERDINE ISOMERS LQFM 030 AND LQFM 182 STIMULATE HIV-1 BASAL TRANSCRIPTION

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Resumo

Since the first AIDS report, there is a constant search for therapies that prevent HIV transmission and/or replication. The combined use of different antiretroviral agents, known as highly active antiretroviral therapy (HAART), is the most effective therapy against AIDS. However, HAART is not able to completely stop viral replication and thus cure HIV infection. Therefore, it is urgent to develop new drugs with antiviral potential against HIV. In a study by our group to evaluate the efficacy and toxicity of ten compounds as new antiretroviral candidates, designed based on the NNRTI Delavirdine, 2 of these compounds, LQFM 030 and LQFM 182, showed capacity to increase viral replication. First, we determined the maximum non-toxic concentration (MNTC) of each compound in different cell lines. To test the antiviral potential of each compound, TZM-bl cells were infected with HIV-1 (M.O.I. = 0.5) and incubated in the presence of the compounds. Compared to untreated control, LQFM 030 inhibited replication of HIV-1 in 25%, whereas LQFM 182 stimulated viral replication by 2-fold. Despite this, the natural endogenous RT assay showed that both compounds inhibited RT activity by 100% compared to control. In viral replication assays performed on MT4 and MOLT4 lymphocytic cell lines, both compounds showed stimulatory effects on viral replication. Preliminary assays to establish stimulatory concentration of 50% of viral replication (EC50) on MOLT4 cells, indicated as EC50 for LQFM 030 and LQFM 182, 11.9 and 487μM, respectively. Finally, 2 preliminary assays were performed to evaluate the possible mechanism of viral replication stimulation. First, HEK293T cells were transfected with pLTR-Luc, pLTR-Luc+pTat or non-transfected and then incubated in the presence of the MNTC of the compounds or without drug. As result, LQFM 030 and LQFM 182 led to increased emission of relative luminescence units by 1.5-fold and 1.8-fold respectively. Second, the latently HIV-1 infected J-Lat 8.4 and J-Lat 10.6 cell lines were incubated with the MNTC of each substance with either 1μM PMA (control) or without drug. In this assay, there was no detection of GFP expression in the presence of both compounds, unlike control. Together, these results suggest that LQFM 030 and LQFM 182 lead to increased viral replication, probably acting in the cell transcription machinery. However, these compounds wouldn’t have the ability to remove the provirus from latency.

Palavras-chave: antiretroviral, isomers, compounds, HIV-1, therapy
Dengue fever is a systemic viral infection, self-limited, transmitted to humans through the bite of infected female mosquito from *Aedes* genus. It is currently the viral disease transmitted by mosquitoes with more rapid spread in the world and it is estimated that between 50 to 100 million new dengue infections occur annually in more than 100 countries. Dengue fever is caused by Dengue virus (DENV), a single strand positive sense RNA virus belonging to the Flaviviridae family. The DENV infection may be asymptomatic or present different clinical manifestations, such as dengue fever (DF), hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Published data indicate that some viral infections can cause epigenetic changes in their hosts, especially in the DNA methylation. Thus, the aim of this study was to investigate the influence of DENV infection in global DNA methylation of DENV infected patients (n=38) by MethELISA technique. The results obtained show a decrease of the relative DNA methylation percentage in these patients, suggesting an influence of viral infection in the epigenetic mechanisms of the host. Meta-analysis of DNA microarray indicates that patients infected with DENV shows an increase in the expression of Thymine DNA Glycosylase (TDG) and DNA methyltransferases 1 and 3 (DNMT1 and DNMT3) genes, all involved in epigenetic regulation. In vitro experiments using THP-1 cells infected with 1 and 0.1 moi of DENV-1 also showed a decrease of the relative methylation percentage after 24 and 48 hours post infection. So, these data indicated that DENV is able to interfere with the host epigenetic mechanisms. Further studies should be conducted to evaluate the mRNA expression of TDG and DNMT1 and DNMT3 genes in these samples.

**Palavras-chave:** Dengue virus, Methylation, DENV infection
Introduction: In previous work we performed molecular characterization of phospholipases A2 (PLA2) isolated from snake venom of Bothrops leucurus and demonstrated their in vitro activity against Dengue virus (DENV). Here we investigated the mechanism of action of the isoform Asp49-Phospholipase (PLA2D). This enzyme has a molecular mass of ~14KDa determined by mass spectrometry. Automated sequencing showed an active site of histidin and 14 cysteine residues important to stabilize the tertiary structure of the enzyme due to the formation of 7 disulfide bridges. Considering the great stability of this protein and its anti-dengue activity, our main goal here is to carry out some tests to better understand the mechanism of action of this molecule. Material and methods: In order to investigated if enzymatic activity would be involved in the antiviral role, LLC-MK2 cells were treated with PLA2D inactivated and native followed by incubation with DENV-2 at 37°C, 5% CO2. After 48h, inhibition of viral replication was measured by one step qRT-PCR. In addition, we performed cellular treatments for 48, 72 and 96h to assess the stability of PLA2D antiviral activity. Finally, to investigate if PLA2D was acting the cellular level or interacting directly with the virus, we performed immunoassay with different concentrations of cells and viruses fixed on substrate, followed by incubation with PLA2D, rabbit IgG Anti-PLA2D and peroxidase-labeled goat anti-rabbit IgG. Results: Interestingly, treatment by enzymatic inhibitors resulted in no inhibition of antiviral activity suggesting that catalytic activity is not involved in the antiviral mechanism. A great stability was evidenced for the antiviral action of PLA2D, since there was no significant increase of viral load when the incubation time was prolonged, unlike that observed in infected and untreated cells. Our immunoassay data suggest that PLA2D has indirect antiviral action interacting with cellular receptors and not directly with the virus, since we observed high absorbance proportional to the number of cells, what was not observed in reactions performed with viruses. Conclusion: PLA2D has antiviral activity against DENV probably acting at the cellular membrane level. Additional studies are being conducted to study this mechanism of action. These findings open perspectives for use of this molecule for development of diagnostic tools or prototypes for therapy against DENV. Financial support: FAPEMIG, CNPq, FUNED.

Palavras-chave: antiviral, Dengue, phospholipase, PCR
Oropouche virus (OROV) is an Orthobunyavirus that cause Oropouche fever, a febrile illness very common in Brazil. Despite its importance in public health, the replicative cycle of this arbovirus is poorly understood. In this work, we aimed to describe how OROV is assembled in mammalian cells. Toward this goal, we infected HeLa cells with OROV and the dynamics of viral one-step replication cycle was monitored at different time points post-infection (p.i.). Virus titter quantification was performed by TCID50 assay that showed the infectious profile of the virus. Then, the one-step replication cycle was monitored by Immunofluorescence and showed that after 24 h.p.i. large vesicle-like structures enriched in OROV proteins were detected, indicating viral factories. These factories were associated with an early endosome protein (HRS), a cis-Golgi protein (Giantin) and a trans-Golgi network protein (TGN46). Interestingly, Immuno-EM analysis of infected cells revealed large multivesicular body (MVB) structures that contained virus particles. Due to the resemblance with MVBs, we investigated the involvement of ESCRT (Endosomal Sorting Complexes Required for Transport) machinery in OROV replication. Knock-down of Tsg101 (ESCRT-I) and Alix led to a strong reduction in OROV budding (55% and 67%, respectively), reduced viral infectivity (~4 fold in both cases) and a decrease in the average diameter of viral factory compartments (~42% in both cases). Moreover, the overexpression of a dominant negative form of the AAATPase Vps4A (Vps4E/Q), which disrupts the MVB pathway, led to an enlargement in the area of the viral factories (~119%), where the Vps4A mutant accumulated. Importantly, we also detect a colocalization between Vps4E/Q-TGN46 and Alix-TGN46 with viral factories, suggesting that ESCRT machinery is recruited to the trans-Golgi network during virus assembly. Therefore, our data represents an unprecedented mechanism of how viruses hijack host cell components for coordinated morphogenesis.

Palavras-chave: Bunyavirus, Bunyavirales, Orthobunyavirus, Oropouche virus, ESCRT
EVALUATION OF AN ANIMAL MODEL TO STUDY THE ROLE OF OXIDATIVE STRESS IN MAYARO VIRUS PATHOGENESIS

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Resumo

Mayaro virus (MAYV) is member of the family Togaviridae, genus Alphavirus. The disease caused by MAYV involves myalgia, fever, rash and can induce arthralgia/arthritis. The mechanisms of MAYV-induced acute disease and associated long-term arthralgia remain undefined and little is known about the pathogenesis of MAYV infection. Recently, our research group demonstrated that MAYV induced significant oxidative stress in human hepatocyte and murine macrophage cells. Oxidative stress is established when there is a disruption/dysregulation of signaling and redox control caused by the increase of “Reactive Oxygen Species” (ROS) and/or a reduction in the antioxidant defence system. Many studies have shown that different viruses can induce oxidative stress, which can directly influence viral pathogenesis. Since the importance of oxidative stress on MAYV pathogenesis is still unknown, the aim of this study is to first establish a good mouse model of MAYV disease and then to evaluate the role of oxidative stress on viral pathogenesis. A model was developed in 21 day old C57BL/6 mice subcutaneously injected with 10⁶ plaque-forming units (PFU) of MAYV. Animal experimentation was carried out in accordance with the ethics regulations. Control and MAYV-infected C57BL/6 mice were examined for clinical abnormalities twice daily for 21 days. Five groups containing eighteen animals each (nine infected and nine uninfected animals) were anesthetized and euthanized on days 1, 3, 7, 10 and 21 days post infection (dpi). Blood samples were collected for determination of the anti-MAYV neutralizing antibodies by serum virus neutralization (SVN) assay, and the livers were removed to virus detection by titration using the methylcellulose plaque assay in Vero cell. MAYV-infected mice did not show any weight loss or signs of illness over the course of the experiment. Neutralization assay detected anti-MAYV neutralizing antibodies at 7, 10 and 21 dpi, with titers of 64,000, 128,000 and 1,024,000 neutralizing units per mL of serum, respectively. Yet, MAYV was detected in the liver of infected mice at 1 and 3 dpi, with title at 2,4 x 10⁴ and 1,5 x 10³ PFU/g, respectively. We suggest that in C57BL/6 mice the liver may be a possible site of MAYV replication, however, histopathology analyzes will be done to confirm this hypothesis. The application of an animal model to study MAYV infection is fundamental to understanding the mechanisms that contribute to disease, such as oxidative stress.

Palavras-chave: VIRUS, OXIDATIVE STRESS , VIRUS PATHOGENESIS
Biofilms are a microbial arrangement formed when suspended cells are attached to a surface and remain surrounded by a matrix composed of exopolysaccharides, proteins, nucleic acids, salts and water. The resulting agglomerate may contain multiple species, which are able to form a well-established and self-sufficient community. The prevention or combat of biofilms, if possible, involves use of chemical biocides. The exopolysaccharide matrix promotes a cellular protection, making the cells up to a thousand times more resistant to the action of biocides. Therefore, the use of bacteriophages mixes becomes an interesting alternative for biofilm control, once phages are natural bioremediators of bacteria, easy to handle, highly specific and production costs are economically favorable. The purpose of this study is to evaluate the synergy in the use of three different combinations of phages against biofilm formation. For evaluation of synergistic or antagonistic effects and the phages efficiency against the biofilm formation, we added in a reduced scale system 500 mL of LB medium, 45 mL of bacterial inoculum (Escherichia coli) with O.D at 0,7 and three different phages combinations A-C (A: phages 1 and 2; B: phages 3 and 4, and C: phages 1, 2, 3 and 4). The system remained under agitation for two days, and at the end of each day, three polypropylene coupons were removed and stained with Violet Crystal (VC) 0,1% during 30 minutes. After that, the VC was removed with distilled water and the coupons were dried at room temperature. Then, 0,7 mL of the moisture ethanol-acetone (4:1) was added, during 30 minutes, to dissolve the VC. The coupon biomass was quantified by optical density using microtiter plate, and the absorbance measured in 590 nm. In comparison with the control group, (without phages), the combination A was able to decrease biofilm formation in 50%, and the combinations B and C in 30%. According to the results the combination A was the most efficient in decrease the biofilm, the presence of all the phages in combination does not have a synergistic effect. Instead, their have a antagonistic effect, reducing the effectiveness of mix A. This results demonstrated that mixes with many different phages are not necessarily most efficient than combinations with reduced number of phages. Financial support: PETROBRAS, FAPEMIG, CNPQ and CAPES.

Palavras-chave: Bacteriophages, Biofilm, Bioremediation
EXPRESSION AND STRUCTURAL CHARACTERIZATION OF DENGUE VIRUS CAPSID MUTANT PROTEIN L81C/L92C

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Introduction: Dengue virus (DENV), a member of the Flaviviridae family, is the most important arbovirus in the world, but there are still no antivirals or vaccines to combat dengue virus infection. The mature viral particles are formed by three structural proteins: capsid (C), membrane (prM / M) and envelope (E). Protein C is a considerable stable dimer, composed of four α-helices and a flexible and structurally disordered N-terminal region. Rich in basic residues, the protein interacts with viral RNA, playing a crucial role in the packaging of genetic material. NMR dynamics studies have suggested that the sliding of the dimeric interface (α4-α4' helices) undergoing an open-close conformational equilibrium of protein C. Thus the interest in studying the behavior of protein dynamics has considerably increased. For this, it was designed a mutant named L81C/L92C which shows cysteine residues at positions 81 and 92, which allows the formation of disulfide bonds between the α4 and α4' helices of the protein. Thus, the closed conformation will be "trapped" by the extremities when the mutant is employed. In this way, the intention is to verify and understand how the presence of disulfide bonds can alter the motion of the protein. Methodology: To standardize the expression and purification of the mutant and to characterize its structure, circular dichroism spectroscopy (CD) was used to identify secondary structures elements and to monitor protein folding as well as the analysis of its thermal stability. Fluorescence spectroscopy was employed to analyze the chemical stability of the protein and thus it was possible to better observe the function of the disulfide bonds in limiting the movement of the protein. Measurement of the fluorescence signals provides a sensitive method of monitoring the biochemical environment of the single trytophan present in the structure, which acts as an intrinsic fluorophore. Results: The L81C/L92C mutant was successfully expressed and purified at the trapped closed conformation. Thermal and chemical denaturation experiments showed that the presence of the disulfide bond in the protein structure decreases its stability and hinders its refolding ability when compared to the wild-type. Conclusions: More experiments are needed to understand the dynamics of the trapped conformation and the role of the open-close conformational equilibrium in the stabilization of the protein. Financial Support: CNPq

Palavras-chave: Capsid, Dengue virus, Disulfide bond, Protein c, Thermal and chemical stability
HEPATITIS E VIRUS IN WILD RODENTS SUGGEST NOVEL SPECIE INTO ORTHOHEPEVIRUS GENUS (HEPEVIRIDAE)

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HEPATITIS E VIRUS (HEV) is a single stranded, positive RNA virus belonging to the genus Orthohepevirus into the family Hepeviridae. Hepatitis E disease is a zoonotic disease, which is the most common cause of acute sporadic hepatitis and fulminant hepatic failure in humans in the world. Despite the HEV has a wide range of host including rodents, to date the studies are limited to rodents from Muridae family. Thus, we hypothesize that Cricetidae family may also be reservoirs of HEV. Therefore, during 2008 to 2013 we collected blood samples from 647 wild rodents in rural area of Ribeirão Preto region, São Paulo State, Brazil. These rodents represent 5 different species (Akodon montensis, Calomys tener, Oligoryzomys nigripes, Necromys lasiurus and Mus musculus). Samples were distributed in 15 pools based on species, date and geographic location. Then, viral RNA was extracted and followed by synthesis of double-stranded cDNA prior to Illumina sequencing. Sequence reads were quality-filtered, removed the adapter sequences and remaining reads were assembled by the de novo strategy using the MetaViC pipeline. We identified a nearly complete genome of novel specie of HEV in Necromys lasiurus and partial genome in Calomys tener, which was tentatively been designated as Necromys HEV (NeHEV) and Calomys HEV (CaHEV). The NeHEV has a genome of ~7 kb that encodes non-structural polyprotein and capsid. Phylogenetic analysis with the complete coding sequences showed five distinct well supported clades that represent the orthohepeviruses A to D, and surprisingly the NeHEV and CaHEV forms a unique clade with kestrel HEV. In addition, the NeHEV and CaHEV shared 62% to 76% of amino acids identify with and kestrel HEV in non-structural and capsid protein, respectively. Our analysis suggests that NeHEV and CaHEV and kestrel HEV are a novel species into Orthohepevirus genus, which we have tentatively been designated as “Orthohepevirus E”. In addition, our results suggest that HEV reported in common kestrel and red-footed falcon are probably from dietary, especially because those birds are carnivores, which include in its dietary small mammals (i.e. rodents). Also, our results showed that the rodents presented viremia, which suggests a probably viral replication and evidence that rodents can play a role as a reservoir for this virus. Therefore, our study has expanded the ecology of HEV rodents in wildlife, which now includes as host rodents of Cricetidae family.

Palavras-chave: Hepatitis E Virus, Hepeviridae, Rodent-borne virus, Zoonotic virus
Human T cell lymphotropic virus type 1 (HTLV-1) is known to be a major agent of severe and fatal lymphoproliferative disease named adult T-cell leukemia/lymphoma (ATLL), and HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP), a neuroinflammatory disease. Moreover, HAM/TSP is very common in Brazil, and until the present time, there is no consensus on the specific treatment for HAM/TSP. For this reason, researchers have been attempting to isolate and characterize new extracts which can inhibit HTLV-1 replication and infection. Mussismilia braziliensis (phylum Cnidarian, class Anthozoa, family Mussidae) is endemic in Brazil and is found along the coast of Bahia state. The potential antimicrobial activity of several cnidarian species against different microorganisms has been shown previously by others. However, M. braziliensis has never been tested as an antiviral agent. The present study aimed to investigate the potential antiviral effect of M. braziliensis coral extract in MT-2 cell lines permanently infected with HTLV-1. The antiviral activity was also evaluated using electron microscopy. The TEM analyses of MT-2 cells were performed 24 h post-incubation in the presence or absence of 80 mg coral extract. The results demonstrated that M. braziliensis extract was able to inhibit the expression of tax/rex mRNA at concentration of 80 mg with significant p value (p < 0.05). Antiviral activity on gag/pol mRNA was not observed at any concentration tested (p > 0.05). The analysis of TEM was observed 419 virus particles quantified by TEM, 384 viral particles were measured in cells treated with coral extract and 35 in untreated cells. In the untreated cells, the virus particles ranged from 64 to 82 nm, and in the treated cells, they ranged from 38 to 65 nm. Comparison of treated and untreated cells by TEM shows that treatment with coral extract alters both the release of viral particles and the morphology, evidencing the production of defective particles. Hence, coral extract from M. braziliensis may inhibit viral replication in permanently HTLV-1-infected MT-2 cells, alters the morphology the particles viral it may be useful for development of new treatment for HTLV-1 infection.

Palavras-chave: anti-retroviral and coral extracts, HTLV and Mussismilia Brazilienzis, HTLV and anti-retroviral
HUMAN RESPIRATORY SYNCYTIAL VIRUS NUCLEOPROTEIN METHYLATION IS A POTENTIAL ANTIVIRAL TARGET

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Resumo

The Human respiratory syncytial virus (HRSV) is one of the most important pathogens of the respiratory tract. Despite the intense research, up to now there is no vaccine approved or an antiviral available for large-scale use. We reported previously that viral nucleoprotein (N) interacts with cellular metilosome and that a tagged N protein expressed by transfection is methylated at arginine and lysine residues. In this work we show that on N protein isolated from infected cells methylation also occurs in arginine residues, through reactivity with antibodies specific to these modifications and analysis by mass spectrometry. Previously found modified residues were confirmed and additional ones identified. These residues are located in N positions involved in interaction with the viral RNA and viral phosphoprotein, indicating functional meaning. With this evidence, we tested the effect of arginine methylation and demethylation, and lysine methylation inhibitors on viral protein synthesis. We obtained inhibition ranging from 73 (N protein) to 86% (G protein) with a lysine methylation inhibitor, UNC0646, on Hep2 cells 48 hours post infection. Our data indicate that N methylation has the potential to be exploited as a therapeutic target in developing antiviral drugs against HRSV.

Palavras-chave: Human respiratory syncytial virus, methyl transferase inhibitors, nucleoprotein, protein methylation, virus-cell interaction
IDENTIFICATION OF POLYMORPHISMS IN ADENOVIRUS SPECIES THROUGH NEXT GENERATION SEQUENCING

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Resumo

Adenoviruses (AdV) belong to the family Adenoviridae and are composed of five genera, including the genus Mastadenovirus, which includes the 57 human adenovirus (HAdV) serotypes which are subdivided into 7 species ranging from A to G. The HAdV-C species mainly affect the respiratory system and F species infect the gastrointestinal tract. The AdV genome is composed of a linear double stranded DNA molecule, with 36 to 44 kbp having between 48 and 61% guanine + cytosine. Currently, the use of next generation sequencing techniques has enabled the production of a large volume of sequences with many possible applications in research and diagnostic definitions, such as viral polymorphism identifications. The present study aimed to evaluate the different polymorphisms present in the sequences of HAdV-C 2 and 5 (Ad2 and Ad5 prototype strains) and HAdV-F 41 (Tak strain). All samples used are from passages in A549 cell line (human lung carcinoma). Sample preparation for further sequencing was performed using the Nextera XT® kit following the manufacturer’s instructions. Then all samples were submitted to next generation sequencing (MiSeq Illumina). In total, 105,521 (HAdV-2), 2,768,411 (HAdV-5) and 81,454 (HAdV-41) readings were generated and included in scaffolds after assembled onto complete viral genomes by de novo assembly and results confirmed using refseq. The frequencies and types of polymorphisms, as well as the regions of the DNA sequences that underwent changes, were evaluated by the SNPs/INDELS calling workflow in the Geneious 9.1.8 software. The total number of polymorphisms found were 54, 550 and 1681, respectively for HAdV-2, 5 and 41, being classified as: deletions, 7.40% (5/54), 9.27% (51/550) and 1.19% (20/1681); insertions, 22.2% (12/54), 4.36% (24/550) and 1.07% (18/1681); substitutions 70.37% (38/54), 86.36% (475/550) and 97.74% (1643/1681), all of them respectively for HAdV 2, 5 and 41. It is important to note that the total value of polymorphisms was higher for HAdVF-41 (probably due to lower passage levels), being that deletion was greater for HAdVC-5, insertion for HAdVC-2 and substitution for HAdVF-41. It should be noted that further studies, especially passages of HAdVF-41 in other cell lines, are required for a better understanding of viral replication, since a clear set of viral quasispecies in these highly conserved DNA viruses. Financial support: Feevale, CAPES, CNPq, FAPERGS

Palavras-chave: Adenovirus, HAdV-C, HAdV-F, Next Generation Sequencing, Polymorphisms
IN VITRO CELLULAR INNATE IMMUNE RESPONSE AGAINST ZIKA VIRUS

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Resumo

Zika virus (ZIKV) is an arbovirus transmitted by mosquitoes of the genus Aedes, but vertical and possible sexual transmission are also reported. Phylogenetic analyses of ZIKV reveal the existence of two major lineages: one includes the African isolates, and the other the Asian and American isolates. ZIKV infection was characterized by causing a mild disease presented with fever, headache, rash, arthralgia, and conjunctivitis, with exceptional reports of an association with Guillain-Barré syndrome (GBS) and microcephaly. Our objective was to compare the cellular immune response triggered by two Brazilian isolates of ZIKV (PE243 and SPH). For this, CHO cells transfected with TLR2 or TLR4 were infected with ZIKV and the activation of these TLRs were observed by flow cytometry. Beyond, intraperitoneal macrophages of C57BL/6 mice, KG-1 and THP-1 cells were infected with ZIKV, and the supernatants from these cells were collected on different days after infection. The supernatants were analyzed by Griess reaction, for the quantification of the nitric oxide produced by the cells and by CBA for the quantification of cytokines. It was observed that KG-1 cells were more susceptible to virus infection as THP-1 cells. Low viral multiplication occurred in mouse intraperitoneal macrophages. It has also been shown that the two ZIKV isolates trigger completely different immune responses. The ZIKV PE243 sample triggered a stronger immune response, with NO production, increased cytokine production and activation of TLR2, whereas the immune response triggered by ZIKV SPH was milder, with no production of NO, lower production of cytokines and without the activation of TLR2.

Palavras-chave: innate immune, zikv, in vitro immune, celular immune
Resumo

Conventional therapeutic approaches against cancer cause serious side effects that can significantly compromise the patient overall health. Oncolytic virotherapy work as an alternative treatment acting selectively on tumor cells. The in vitro oncolytic potential of *Bovine alphaherpesvirus 1* (BoHV-1) is already reported. Considering the biological similarities between BoHV-1 and *Bovine alphaherpesvirus 5* (BoHV-5) we hypothesized the possibility of share this oncolytic potential. This work aims to evaluate the in vitro permittivity of human tumor cells to BoHV-5, as well as the viral capacity to cause cytopathic effect in evaluated cells, comparing the results to that obtained using BoHV-1. Tumoral cell lines from skin (A431), cervix (HELA), brain (U251MG), liver (HEP-G2), kidney (HEK293T) and gut (HT29) cancers, and a non-tumoral control cell of skin (HACAT CLS) were infected by Mutum (BoHV-5) and Los Angeles (LA) (BoHV-1) samples. For permittivity assays, tumor cells were infected at the multiplicity of infection (moi) of 1. After 72 hours of infection (hpi) cells and supernatant were collected and titrated by End Point method using bovine kidney cells (CRIB-1). For cytotoxicity assays, tumor cells were cultured in 96-well plates and infected by Mutum and LA samples using moi of 10, 5, 2.5, 1 and 0.5. 48 hpi, cell monolayer was stained using violet crystal 4% and, after drying, the adhered dye was resuspended using a solution of SDS 1%. Cell viability was then evaluated using spectrophotometer at 570 nm wavelength. Each assay was performed at least in three independent experiments. As result, 100% of evaluated cells were permissive to BoHV-5, being this virus able to replicate even in HACAT CLS cells. 71% of evaluated cells were permissive to BoHV-1 infection, which was not able to replicate in A431 and in HACAT CLS, evidencing the greater promiscuity of BoHV-5. Although capable to replicate in all evaluated cells, only 28% of cells tested (HEK293 and HEP-G2) shows viability reduction (P <0.5) at all moi of BoHV-5, using the described evaluation methodology. BoHV-1, on the other hand, shows the same effect only in 14% of cancer cells tested (HEK293). These data indicate that cytotoxicity of these viruses in vitro is not directly related to the occurrence of viral replication. Although preliminary, these results indicates the oncolytic potential of BoHV-5 Mutum sample in vitro, overcoming cytotoxic potential of BoHV-1 LA sample in evaluated tumor cells.

Palavras-chave: Bovine alphaherpesvirus 5, Cancer, Oncolytic virus, Tumoral cells
INFECTION BY MAYARO VIRUS ALTERS GENE EXPRESSION OF SUPEROXIDE DISMUTASE AND CATALASE ENZYMES IN HEPG2 CELLS

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Resumo

Mayaro virus (MAYV) is a neglected tropical arbovirus belonging to the Togaviridae family and the Alphavirus genus. It causes the Mayaro Fever with acute symptoms including fever, myalgia, headache, rash, arthralgia, vomiting, and diarrhoea. The pathogenesis of MAYV has not been completely defined. Recent studies have demonstrated that oxidative stress via RNA virus infection can contribute to several aspects of viral disease pathogenesis. Oxidative stress is established when there is a disruption/dysregulation of signaling and redox control caused by the increase of “Reactive Oxygen Species” (ROS) and/or a reduction in the antioxidant defence system. ROS play important roles in fighting infections and are viewed as a protection mechanism of the host cell that contributes to its apoptosis. However, more ROS are formed with the advancement of viral multiplication, causing an imbalance in cellular homeostasis. The first ROS produced in the reduction pathway of oxygen is the superoxide anion, which is metabolized to hydrogen peroxide (H2O2) by superoxide dismutases enzymes (SOD). Glutathione and Catalase (CAT) then detoxify H2O2 by generating water and oxygen. Recently, our research group demonstrated that MAYV induced significant oxidative stress in infected HepG2 cells, as indicated by the increase of malondialdehyde (MDA) and protein carbonyl levels, and by a significant decrease of the reduced versus oxidized glutathione (GSH/GSSG) ratio. Additionally, MAYV-infected HepG2 cells also showed an increase in antioxidant defences, observed by increase in the total activity of SOD (6, 15 and 24 hpi) and CAT (15 hpi) enzymes. Therefore, the purpose of this study was to investigate if the MAYV infection also alters the gene expression of these enzymes. Thus, we evaluated by qRT-PCR the expression of the mRNA of the SOD1 (the most abundant isoform of SOD) and CAT enzymes in HepG2 cells infected or not with MAYV at 6, 15 and 24 hpi. We found an increase in SOD1 mRNA expression in infected cells at the 15 hpi. However, we found a decrease in CAT mRNA expression at all analyzed times, 6, 15 and 24 hpi. Then, we observe that there is an imbalance in the gene expression of the antioxidants enzymes SOD1 and CAT in MAYV-infected HepG2 cells. Our data shed light on some mechanisms that are operational in host cells following exposure to MAYV.

Palavras-chave: Mayaro virus, Oxidative stress, Antioxidant enzymes
INITIAL CHARACTERIZATION OF DESULFOVIBRIO ALASKENSIS PROPHAGES

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Resumo

Desulfovibrio alaskensis is found in environments associated with oil exploration and processing, where it is capable of generating negative impacts to the petroleum industry due to biofilm formation and H2S production. The anaerobic metabolism with sulphate reduction and H2S production fits this specie into the group of Sulphate Reducing Bacteria (SRB). Bacteriophages are called prophage when integrate their genetic material into the bacterial genome, lysogenic life cycle. The prophages can follow the lytic cycle when induced by some stress, which allows its isolation, characterization and application as a possible control tool for other bacteria. No study about bacteriophage is found for D. alaskensis, which makes this area of great interest for the control of SRB. The objective of this work was identify and characterize the prophages present in D. alaskensis. For this, two genomes of D. alaskensis deposited in the database, G20 and DSM16109, were used to identify prophages by PHASTER program. The sequence comparison was performed using the Mauve program and the morphology of the prophages identified by Transmission Electronic Microscopy (TEM). In order to prove what was founded by in silico analyzes, the DSM16109 strain was used and induced with 5 μg/mL of mitomycin C. Three complete prophages belonging to the Myoviridae family were identified by PHASTER program, with 2 prophages shared between the strains (mean size of 40kpb) and 1 prophage exclusive of the DSM16109 strain (24,4kpb). In addition to the 2 complete prophages, the G20 strain has a third very related prophage, with 48.6 kpb and 78.72% identity in relation to one of the others identified prophages in the strain. This third prophage may be masked in DSM16109 strain, whose genome is deposited in the form of contigs. The DSM16109 strain did not show complete lysis after the addition of mitomycin C, but presented a lower growth in relation to the control, characterizing the induction of the prophages present in the genome of the strain. The viral particles were identified by TEM in cut of bacterial cells impregnated with resin and in culture supernatant. These particles have an approximate size of 28 nm and no tail. Other works are needed to identify which of the 3 prophages are being expressed and the why the viral particles have no tail. This work is the first characterization of prophages in D. alaskensis. Financial support: PETROBRAS, CNPq, FAPEMIG and CAPES.

Palavras-chave: Bacteriophage, Desulfovibrio alaskensis, Mitomycin C, Prophage, Sulphate Reducing Bacteria
INVESTIGATION OF NATURAL VERTICAL TRANSMISSION OF CHIKUNGUNYA, DENGUE AND ZIKA VIRUSES IN Aedes aegypti AND Aedes albopictus IN AMAZON MUNICIPALITIES

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Resumo

Arboviruses are viruses transmitted to humans and other animals by the bite of hematophagous arthropods. Infections caused by Zika virus (ZIKV), Chikungunya (CHIKV) and Dengue (DENV) are current public health problems in the tropical and subtropical regions of the planet, especially in Brazil, which has climatic, environmental and economic characteristics favorable to the maintenance and emergence of these Arboviruses. One of the main prevention strategies continues to be vector control, with the elimination of breeding sites and surveillance of infested areas. The use of ovitraps for Aedes mosquitos monitoring has demonstrated promising results, and may help to increase the surveillance of DENV, ZIKV, and CHIKV in municipalities of the interior of Amazonas state. Therefore, this work aimed to detect the natural vertical transmission of these viruses in Aedes aegypti and Aedes albopictus. Mosquitos eggs collection were carried out using ovitraps in the municipalities of PresidenteFigueiredo, Guarajá, Tabatinga and Itacoatiara, in the state of Amazonas. Hence, eggs were taken to the Entomology Laboratory of the Health Surveillance Foundation of Amazonas - FVS-AM. Eggs were allowed to hatch, and larvae grouped into pools from 1 to 20 individuals were sent to ILMD/FIOCRUZ for viral RNA extraction and detection by RT-qPCR. A total of 2,239 larvae, in 94 pools of Aedes spp., were processed and tested by RT-qPCR targeting CHIKV, DENV and ZIKV. Results showed one positive pool for CHIKV and one positive pool for ZIKV, both samples from the municipality of Itacoatiara. The results were submitted to statistical analysis by Maximum Likelihood Estimation (MLE) and Minimum Infection Rate (MIR) with a Microsoft Excel plug-in provided by CDC. The MLE results showed 0.45 and 0.44 for CHIKV and ZIKV, respectively, whereas the MIR result showed 0.45 and 0.45 for CHIKV and ZIKV respectively. The positive ZIKV sample was successfully sequenced, confirming the viral species. The use of such methodology may contribute to health surveillance, corroborating with greater agility for the measures of transmission blockade.

Palavras-chave: Natural Vertical Transmission, Aedes aegypti, Aedes albopictus, Zika Virus, Dengue virus
INVESTIGATION OF POSSIBLE ALTERATIONS IN MEGAKARYOBLASTS DIFFERENTIATION CAUSED BY THE INFECTION OF YELLOW FEVER VIRUS

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Resumo

The Yellow Fever Virus (YFV) causes a hemorrhagic acute disease currently endemic in 47 African and Central and South American countries. Outbreaks have been recently reported in some of them, such as Brazil, where over 700 cases were confirmed from December 2016 to May 2017. The seemingly expansion of the endemic region in the country and the high circulation of the urban viral vector Aedes aegypti raise concern about the reinstallation of urban cases of yellow fever, which were last reported in 1942. Platelets are important in the coagulation process and produced by the cytoplasmic fragmentation of megakaryocytes, which arise from the differentiation of megakaryoblasts. Patients in severe cases of yellow fever can suffer hemorrhage associated to low platelet count in the blood, also called thrombocytopenia. A relationship between thrombocytopenia and the progression of the disease have already been stablished, which can lead to death of patients. However, the effects of YFV infection during the platelet production process is still unknown. Our goal is to investigate if the yellow fever infection alters megakaryoblasts under the differentiation process in such a way that could result in thrombocytopenia. To do this, we induced the differentiation of MEG-01 cells (human megakaryoblasts lineage) using Valproic Acid (VPA). We infected the megakaryoblasts with YFV17DD in a multiplicity of infection (MOI) of 1 in different days post treatment. We came up with this protocol having in mind that in an infected human being there are cells in different stages of the differentiation process and that thrombocytopenia itself induces the production of new platelets. Having previously verified that YFV infects and replicates in megakaryoblasts, we analyzed the cytotoxicity of VPA treatment by High Content Microscopy analysis and flow cytometry, and we did not observe cytotoxic effects using the 2 mM concentration until 72 hours post treatment. Using flow cytometry, we analyzed the viability of cells infected in different days post treatment with VPA. Using confocal fluorescence microscopy and anti-flavivirus antibody, we investigated the presence of viral particles in infected cells in various moments of the differentiation process. Our results suggest that cells induced to the differentiation process are less susceptible to YFV infection.

Palavras-chave: megakaryoblasts, differentiation, yellow fever
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MAYARO VIRUS INFECTION TRIGGERS OXIDATIVE STRESS, MITOCHONDRIAL DYSFUNCTION AND CELL DEATH IN MURINE MACROPHAGES.

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Resumo

Introduction: Viral arthritides are acute diseases characterized by inflammation of the joints. It has been demonstrated that arthritogenic alphaviruses are associated with rheumatic disease in humans, but the mechanisms of arthritis development induced by these virus are still unclear. Macrophages infiltration into the joints determines the severity and persistence of alphaviral infections. Recently, our group demonstrated that MAYV-induced ROS production triggered by TNF pathway in murine macrophages contributes to the inflammatory response observed during infection. However, the interrelationship between oxidative stress and mitochondrial function during MAYV infection as well as its correlation to the viral-induced inflammatory response are still missing. In this context, the aim of this work was to characterize the effect of oxidative stress triggered by MAYV infection on mitochondrial function, in murine macrophage Raw 264.7. Material and Methods: Virus replication was evaluated by plaque assay. Cell viability was accessed by MTT. The ROS production was measured by the fluorescent probe CM-H2DCFDA, the mitochondrial ROS (mtROS) using the probe MITOsox, and the nitric oxide (NO) using the probe DAF. Mitochondrial physiology was evaluated through high-resolution respirometry. Results and Discussion: We observed a significant 26% increase of NO and 31% of ROS in the beginning of infection (8h). At this time point, we did not observe significant alterations on cell viability, and mitochondrial function. On the other hand, at 15 h , we found that viral infection induced significantly alteration on cell viability, cell morphology and mitochondrial function. Infected cells present a decrease in the FCCP-induced maximum capacity of the electron transport system when compared to control, indicating that infection promotes impairment in the mitochondrial electron transport. Also, we observed a significant 30% increase in mtROS production 15h. Conclusions: The results altogether indicate that the oxidative stress observed 8h precedes the alterations in mitochondrial physiology, mtROS and cell death. It can be argued that these results suggest that oxidative stress could be an important mechanisms involved in alphaviral infection and this study may contribute to the elucidation of the correlation between oxidative stress and mitochondrial dysfunction during the inflammatory response involved in the development of viral arthritides.

Palavras-chave: cell death, alphavirus, Mayaro, oxidative stress, mitochondrial function
MOLECULAR CHARACTERIZATION OF A ZIKA VIRUS ISOLATE FROM MANAUS, AMAZONAS, BRAZIL

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Resumo

The Zika virus (ZIKV) is an arbovirus of the family Flaviviridae, genus Flavivirus, and two genotypes are recognized, one Asian and the other African. The virus is mainly transmitted to humans through the bite of Aedes aegypti mosquitoes. ZIKV was first detected in Brazil in 2015, in cases with classical symptoms of an arbovirus infection, but after that, central nervous system injuries were reported. This work aimed to characterize the BR/AM/16800005 sample isolated in Manaus, Amazonas, Brazil in January 2016. Initially, this sample was detected by real-time PCR during arboviral surveillance, and isolated in C6/36 Aedes albopictus cells. A viral metagenomic approach was used for sequencing in the Ion Torrent PGM platform. Briefly, the cell supernatant was filtered and treated with nucleases, following RNA extraction with Trizol, double-stranded cDNA production, random PCR amplification and nucleotide sequencing. A total of 418,103 reads were used for contig assembly with Geneious Pro 10.0.7, with at least 100 interactions using the GenBank ZIKV reference sequence KX369547. The complete genome has 10,731bp, and was analyzed to find variable sites using the Geneious Variations / SNPs tool. Twelve nucleotide differences were observed in relation to the reference sequence, three in the NS4B region, one in NS2A and other in NS2B, five in the envelope coding region and two in prM. Regarding the mutations types, 10 were transitions, two were transversions and only one was a non-synonymous mutation at position 2908 that led to an amino acid substitution, glutamic acid for lysine. The Phylogenetic analysis of the complete genome of isolate BR/AM/16800005 using all the complete genomes available on Genbank, demonstrated that it belongs to Asian genotype, as all samples characterized to date in Brazil. Further studies are on course to evaluate the impact of mutations in zika virus genome on viral competence.

Palavras-chave: Zika virus, Characterization, Next Generation Sequencing, Mutations, Phylogenetic
MORPHOMETRIC ANALYSIS OF HEPATIC TISSUE FROM SWISS MICE INFECTED BY DENGUE VIRUS

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Resumo

The Dengue virus (DENV) is currently a global public health issue, especially in tropical and subtropical regions. DENV infection can cause an intensity fever, clinically termed as Dengue fever. However, a group of patients who experienced a secondary infection, in the case of a different serotype, may progress to a more severe form of the disease clinically termed Dengue Hemorrhagic Fever. One of the consequences of DENV infection can be the impairment of liver functions, characterized by increased levels of liver enzymes and also several liver injuries. A quantitative morphometric analysis was performed using specific software in histological sections obtained from the hepatic tissue of Swiss mice infected with two DENV-1 strains (BR/Alfenas/2012 and Mochizuki). Several parameters were quantified, such as the number of binucleated hepatocytes, uninucleated hepatocytes and interstitial cells, percentage of the area occupied by hepatocytes, nuclei diameter, area of hepatocytes, area and volume of nuclei and also area of the cytoplasm. The number of binucleated hepatocytes per square micrometer, percentage area of hepatocytes and cytoplasmic area of hepatocytes showed significant differences between the infected groups when compared to the control group. This, in turn, evidences the presence of hepatocyte damage induced by the infection and also a regenerative ability of liver in response to such damage. Financial support: FAPEMIG.

Palavras-chave: Dengue, morphometry, liver injury, mouse
In 2003, the giant virus Acanthamoeba polyphaga mimivirus (APMV) was identified and some of the genes encoded by its large genome were shown to be related to protein translation processes. Some of such genes had never been observed in other viruses at the time of the discovery, being considered exclusive of cellular organisms. It is now known that other giant virus also have gene pooling that encodes elements involved with translation. In this study we prospected and characterized genes involved in translation in different giant viruses, including many Brazilian isolates, the recently described Klosneuviruses and we compared by network and phylogenetic tools those genes with cellular organisms. For this, the genomes of mimiviruses of A, B and C lineages; Cafeteria roenbergensis virus, Klosneuvirus, Acanthamoeba castellanii, Encephalitozoon cuniculi, Nanoarchaeum equitans and Candidatus Carsonella ruddii were analyzed for the presence of transfers RNA (tRNA), aminoacyl-tRNA synthetases (aaRS) and other factors involved in protein biosynthesis (TF). With this database, networks's analysis were made using the Gephi 0.9l.1 program; codon/amino acid usage was calculated using the Artemis program and phylogenetic analyzes were performed for the amino acid sequences of aaRS through the MEGA 7.0 program using the maximum likelihood method with 1000 bootstrap replicates. Comparison between the genes related to translation showed that the Klosneuviruses group had a larger tRNA set compared to the others, and aaRS for 20 known amino acids, as well as for A. castellanii and E. cuniculi. Phylogenetic analysis of aaRS revealed an independent origin of cellular organisms for the most of them. In relation to TF involved with translation, the giant viruses and small cellular organisms presented a much smaller set in relation to A. castellanii. The analysis of codon/amino acid usage for these viruses and A. castellanii revealed a similar profile of usage among the viruses, while it differs from the codon/ amino acid usage of the host cell. In conclusion, the data presented here contribute to a better understanding about the abundance, diversity and origin of genes associated with translation in giant viruses.

Palavras-chave: Giant viruses, Transfers RNA, Aminoacyl-tRNA synthetases, Translation
RECOMBINANT INFLUENZA VIRUSES CARRYING MURINE IFN\(\gamma\) GENE AS TOOLS TO EVALUATE THE ROLE OF THAT CYTOKINE DURING INFLUENZA VIRUS INFECTION.

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Resumo

**Objectives** It is known that cytokines, such as IFN\(\gamma\) play important roles during influenza virus infection, either improving or worsening its outcome. However, their role are still far from to be completely elucidated. Therefore, the aim of our study was to establish a new approach to evaluate the role of IFN\(\gamma\), when locally produced in lungs, to immunopathogenesis of influenza virus infection. To this goal, we constructed recombinant influenza viruses which are able to encode the murine IFN\(\gamma\) sequence. **Material and methods** Replication-defective influenza viruses encoding IFN\(\gamma\) sequence were generated by reverse genetics. Recovered viruses were purified twice by limit dilution on MDCK cells prior to the preparation of seed and work stocks. Further, they were characterized about their genetic stability by PCR and sequencing. Their phenotype were evaluated by lysis plaques on MDCK cells under agarose overlay and their ability to produce IFN\(\gamma\) in cell culture and in lungs of infected mice were assessed by ELISA. **Results** The recombinant viruses were found genetically stable and able to produce IFN\(\gamma\) in cell culture as well as in lung of infected mice at different time-points after inoculation. Moreover, we were able to demonstrate the biological activity of the viral-encoded IFN\(\gamma\) by using bone marrow differentiated macrophages (BMDM). **Discussion & Conclusions** Our preliminary findings strongly suggest that recombinant influenza viruses encoding cytokines would be remarkable tools to allow us better understand the role of those immunomodulatory proteins, when produced locally in lungs, to the immunopathogenesis of influenza virus infection. Financial Support: CAPES, CNPq, FAPEMIG e INCTV.

**Palavras-chave:** Recombinant Influenza, IFN\(\gamma\), cytokine, Reverse Genetics
SPIR-1 PROMOTES ACTIVATION OF IRF3 AND IS TARGETED BY VACCINIA VIRUS PROTEIN K7

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Resumo

The host innate immune response to infection provides a potent defence against virus replication and spread, and, consequently, viruses have evolved strategies to inhibit these anti-viral responses. Vaccinia virus (VACV) is a large double stranded DNA virus that encodes several immunomodulatory proteins, including many inhibitors of NF-κB or/and IRF3 activation. One such protein, K7, was described as a virulence factor that binds to interleukin-1 receptor-associated kinase-like 2 (IRAK2) and TNF receptor associated factor 6 (TRAF6) and suppresses nuclear factor-κB (NF-κB) activation. K7 also binds the DEAD-box RNA helicase 3 (DDX3) and inhibits the interferon regulatory transcription factor (IRF3) activation. Both NF-κB and IRF3 are essential pathways for an efficacious innate and adaptive immune response by the host. This study describes Spir-1 as an additional cellular protein targeted by K7. K7 and Spir-1 co-immunoprecipitated following ectopic expression and during VACV infection. Spir-1 belongs to a family of proteins involved in actin organisation. Nevertheless, K7 interaction with Spir-1 does not require its actin binding domains, suggesting a new function of Spir-1. To investigate if Spir-1 has any function in the host immune response, reporter gene assays were performed. Ectopic expression of Spir-1 in human embryonic kidney (HEK 293T) cells led to up-regulation of the IRF3 pathway, upstream of TRAF3 protein. In contrast, Spir-1 did not affect NF-κB activation. Furthermore, there was a reduction in the phosphorylation of IRF3 in Spir-1/- mouse embryonic fibroblasts (MEFs) cells upon stimulation by poly (I:C) when compared to the wild-type (WT) cells. Impairment of IRF3 activation correlated with decreased expression and production of cytokines in the Spir-1/- MEFs after stimulation with poly (I:C) or SeV infection, as measured by qPCR and ELISA. To further validate these results, Spir-1/- HEK cell lines were generated by CRISPR-Cas9. Preliminary data showed that these cells also had diminished IRF3 activation, which was restored after rescue of Spir-1 expression. Taken together, these data suggest that Spir-1 contributes to IRF3 activation and is targeted by VACV protein K7 to block this pathway. Financial support: Wellcome Trust

Palavras-chave: IRF3, K7, Spir-1, Vaccinia virus
THE ROLES OF OROPOUCHE VIRUS (OROV) NONSTRUCTURAL PROTEIN NSM IN VIRUS GROWTH AND PATHOGENICITY

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Resumo

Oropouche virus (OROV) is an emerging arbovirus in the order Bunyavirales, transmitted by the bite of midges and possibly other arthropods, which causes periodic outbreaks of febrile disease, predominantly in the Amazon. Recent report of human case outside that region raises concern that OROV may emerge in other parts of South America. OROV tripartite genome is composed of negative-strand RNAs designated L (large), M (medium) and S (small). The genome encodes three structural proteins (Gc, Gn and N), the RNA-polymerase, and two nonstructural proteins (NSm and NSs). NSm results from cleavage of a polyprotein precursor encoded by the M segment of RNA, and its function has remained obscure. Recombinant OROV knockout for NSm (rOROV-ΔNSm) and wild type (rOROV) were generated by reverse genetics and one-step replication cycle analysis revealed that the exponential replication phase of rOROV-ΔNSm was delayed in comparison to rOROV. The distribution of viral proteins and of key organelles participating in viral factories were studies by IF using confocal microscopy of fixed HeLa cells at 0, 12, 18 and 24 h post infection (pi). Double-labeling experiments were done with rabbit MAb for calnexin, TGN46 or giantin. Results showed that at 12h pi rOROV had a predominant localization suggestive of endoplasmic reticulum, while rOROV-ΔNSm was diffusely dispersed throughout the cytoplasm. From 18 to 24hpi rOROV glycoproteins were processed and transported through the trans-Golgi network (TGN) to viral factories, whereas rOROV-ΔNSm viral proteins were retained in the TGN indicating a delayed production of viral progeny. Suckling mice were subcutaneously inoculated with rOROV or rOROV-ΔNSm under similar conditions. Survival curves showed that rOROV-ΔNSm is more virulent for mouse, indicating that the delayed growth in cell cultures did not affect pathogenicity. To further investigate the role of NSm, human neutrophils were infected in vitro and production of reactive oxygen species (ROS), neutrophil extracellular traps (NET) and tumor necrosis factor (TNF) were evaluated. Cells infected with rOROV-ΔNSm produced more ROS, NET and TNF, which can contributetо viral immunopathology. We conclude that NSm affects mechanisms of OROVassembly in vitro, and may play roles as virulence factor in mouse and inducer of immunopathology.

Financial support: FAPESP, CNPq, CAPES.

Palavras-chave: Oropouche fever, Bunyavirus, arbovirus, NSm protein, organelles
Zika fever is an emerging infection disease, which has affected many countries, including Brazil. The causative agent is the Zika virus (ZIKV), a member of Flaviviridae family. To date, 76 countries have reported cases of ZIKV infection of which about 30 indicated the occurrence of serious complications such as congenital brain abnormalities and Guillain–Barré Syndrome. Understanding the behavior of the virus in the body is fundamentally important for appropriate prevention, management and containment of the infection. Cells respond to different types of stresses through mechanisms that control the expression of proteins that act efficiently against the accumulation of damaged macromolecules. The overload of protein processing in the endoplasmic reticulum (ER) during the viral multiplication cycle activates the unfolded protein response (UPR) signaling pathway, which encompasses three main branches: IRE1, PERK and ATF6. They act as sensors in the ER being responsible for monitoring stress level and activating the transcription of important proteins for the restoration of homeostasis. Previous studies have shown how other Flaviviruses control these pathways in ER. However, such aspects have not been demonstrated for ZIKV yet. The objective of this work was to evaluate the effect of ZIKV infection on the activation of the UPR pathway in mouse embryo fibroblasts (MEF). MEFs were infected with ZIKV and total RNA was reverse transcribed into cDNA. The cDNA coding for XBP1 (the main substrate of IRE1 sensor) was PCR amplified and the effect of infection on the splicing of the XBP1 mRNA in the presence and absence of ER stress inducers was evaluated by RFLP. Analysis of XBP-1 showed that during ZIKV infection the IRE1 sensor is activated and splicing of XBP-1 is time-dependent, with greater activation of the pathway taking place 5 days after infection. The findings indicate an important modulation of the IRE1/UPR pathway by the ZIKV. Nonetheless, other pathways of the UPR are currently being evaluated for a more comprehensive view of ER stress regulation during ZIKV infection. Financial support: CAPES, CNPQ e FAPEMIG

Palavras-chave: endoplasmic reticulum, IRE1, STRESS, UPR, ZIKA VIRUS
USE OF PROTEIN CAGES DERIVED FROM THE P22 BACTERIOPHAGE AS NANOCARRIER OF DRUGS FOR DISEASES OF THE CENTRAL NERVOUS SYSTEM.

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Resumo

Many diseases of the central nervous system such as Alzheimer's, Parkinson's and glioblastoma have no effective treatment as of today. The use of specific nanocarriers to diseased cells without affecting healthy cells is one approach that can solve this problem. Our nanocarrier model is based on the procapsid of bacteriophage P22 produced by heterologous expression of protein in E.coli as VLP (Virus-like-particle). It consists of 420 copies of coat protein (gp5) and 60-300 copies of the scaffolding protein (gp8). Previous studies from our group have showed the ability of this nanocarrier to be incorporated by astrocyte and glioblastoma cells. On the other hand, they are not incorporated by neuronal cells, which is of extreme importance for the continuity of the study, since to affect neuronal cells would be problem for a possible treatment given its importance and its low rate of proliferation and regeneration. To increase the incorporation of VLPs, in addition to inferring greater specificity for glioblastoma cells (model used in the study), we will use approaches such as introducing mutations to the VLP surface and/or fuse viral proteins derivates to the procapsid. First we tested R-G-D (arginine-glicine-aspartic acid) sequence added to the outer surface of protein gp5. This peptide has affinity for integrin, which is a membrane protein super expressed in some glioblastoma lineages and in other types of cancer. We also tested a 10 amino acid cell penetrating peptide (CPP) synthesized after an analysis of possible CPP candidates from the scaffolding protein sequence. Experiments were done with this peptide at 1x, 2x, 10x, 50x and 100x dilutions from a stock containing 130 µg / ml to evaluate possible cytopathic effects on SNC cells. Using the MTT methodology, it was observed that there was no cell death at any of the concentrations used when compared to the control. Also, it was observed by optic microscopy that none of the CPP concentrations used resulted in morphological changes. In conclusion, the present study showed that using viral-derived proteins and VLPs could be potentially used as nanocarries, and have great potential to be explored and tested as possible aids in the treatment of SNC diseases. Financial support: CNPq and FAPERJ.

Palavras-chave: Virus Like Particle, P22, Bacteriophage, Nanocarrier, Glioblastoma
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VACCINIA VIRUS GROUP CO-INFECTION IN A DAIRY WORKER WITH OCULAR VACCINIA INFECTION

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Resumo

The Vaccinia virus (VACV), the prototype virus of the genus Orthopoxvirus (OPV) and was used during the smallpox eradication campaign. Several exanthematic VACV outbreaks have occurred in Asia and South America, affecting mainly the dairy cattle and rural workers. Brazilian VACV (VACV-BR) have been isolated and characterized biologically and phylogenetically. These studies demonstrated that circulating viruses belonged to at least two distinct genetic clusters. In September 2015, an unvaccinated, 45-year-old man that worked on a farm located in Carangola County, Minas Gerais State, Brazil, developed the typical manifestations of bovine vaccinia infection, including fever and painful vesiculo-pustular lesions. He had lesions on the left hand, right arm, and nose, as well as an atypical manifestation in the left eye with aches in the ocular globe and periorbital region. The clinical condition progressed to significant visual acuity losses in the affected eye. Dried swab specimens from his lesions were used for molecular diagnosis using OPV-specific PCR that targeted the C11R gene and the A56R gene. All samples were positive for both OPV targets. Vero cells were infected with the specimen supernatants to isolate the virus. The VACV was isolated from the hand, nose and eye samples from the patient. These isolates were tested for their plaque phenotypes in BSC-40 cells, which demonstrated the presence of at least two types of viral populations composed by small and large plaques in an estimated ratio 2:1. The viral clones were propagated and their DNA was isolated. The molecular markers genes A56R, B5R, C23L and A26L commonly used in the molecular separation of VACV clusters confirmed the molecular dichotomy of the isolated viruses. In summary, our study demonstrated the genetic and phenotypic variability between two viruses isolated from the same sample in a natural human co-infection with VACV. The viruses belong to two distinct VACV-BR groups, reinforcing and expanding previous works with other hosts. This study is the first to prove the association and isolate VACV samples from a natural ocular vaccinia infection case. The effort to understand singular aspects of VACV-BR co-infections should be increased and further molecular and biological characterizations of these samples should be carried out to identify and better understand the natural dynamics and symptomatology caused by VACV-BR. Financial support: CNPq, CAPES, PRPq-UFMG, FAPEMIG

Palavras-chave: Vaccinia virus , ocular infection, OCULAR VACCINIA
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VIROME ANALYSIS OF RNA VIRUSES IN TICKS IN THE SOUTH REGION OF BRAZIL

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Resumo

Tick-borne viruses are transmitted to human and animals by tick bites and are related to emerging and re-emerging diseases. Currently, 28 species of ticks from the Ixodidae family have been described in South America, belonging to Amblyomma, Boophilus, Dermacentor, Haemaphysalis, Ixodes and Rhipicephalus genus. Despite a wide spread of ticks in South America, and the great importance of tick-borne viruses in public health worldwide, currently there are only two viruses associated with ticks described in South America, the Cacipacoré and Mogiana tick viruses. Therefore, we applied a high-throughput sequencing (HTS) approach to determine the viral RNA diversity in ticks of the South region of Brazil. To this end, we sampled ~600 ticks (Rhipicephalus microplus) and 36 serum samples from cattle collected in six farms between 10/2015 06/2016. Samples were distributed in 12 pools based on the sample (ticks or cattle serum) and site of collection. Viral RNA was extracted, followed by synthesis of double-stranded cDNA and high-throughput sequencing using Illumina platform. The reads were cleaned and assembled with de novo methods using the MetaVIC pipeline. We identified and characterized the complete genome sequences of three species of RNA viruses, which were classified into the families Flaviviridae, Phenuiviridae and Chuviridae. We identified three nearly complete genomes of new strains of Jingmen tick virus (JMTV) from Flaviviridae, and we detected a frequency of ~57% in ticks and ~19% bovine serum samples by RT-PCR. Interestingly, the phylogenetic analysis reveals that JMTV strains circulating in Brazil are probably a different genotype from the previously described in Africa and Asia. We identified the complete genomes of Lihan virus (Phenuiviridae) and Wuhan Tick virus 2 (Chuviridae), and we detected a frequency of these viruses in ~83% and ~66% of tick samples, respectively. On the other hand, none of the cattle serum samples were positive for these viruses by RT-PCR. Additionally, we propagated Lihan and Wuhan tick virus 2 by successive passages in insect cell lines (C6/36) and we observed characteristic cytopathic effects three days after inoculation. Also, the partial genomes were detected by RT-PCR in two passages of both viruses. Collectively, these results showed the circulation of three species of RNA viruses in Rhipicephalus microplus, as well as JMTV, a potential tick-borne virus infecting cattle in the south of Brazil. Support: FAPESP.

Palavras-chave: Virome, Tick-borne viruses, Metagenomics, Emerging viruses, Arbovirus
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Zika virus (ZIKV) is an arbovirus member of the Flavivirus genus and the Flaviviridae family. This virus was first isolated in the Zika forest in 1947, Uganda, from a Rhesus monkey. In 2015, the first cases of ZIKV infection in the American continent, especially in Brazil, were reported. Zika disease is characterized by fever, headache, joint pain or rash and conjunctivitis. The possible relation between ZIKV infection and neurological disorder such as Guillain-Barré syndrome and microcephaly led the World Health Organization to declare "Zika Fever" as an emergency public health problem of global concern in 2016. Previous studies have suggested that oxidative stress, as part of the host cell response, might play an important role in the pathogenesis of a variety of RNA viral infections. Oxidative stress is established when there is a disruption/dysregulation of signaling and redox control caused by the increase of "Reactive Oxygen Species" (ROS) and/or a reduction in the antioxidant defence system. Here, to better understand the some mechanisms that are operational in host cells following exposure to ZIKV, we investigated whether ZIKV induced oxidative stress in HepG2 cells. We monitored ROS production and the oxidative stress marker Malondialdehyde (MDA) at different time points after infection. ZIKV infection of HepG2 cells induced an increased in ROS generation at 15, 24 and 48 hours post infection (hpi). Additionally, ZIKV infection of HepG2 cells resulted a significant increase in the MDA level at 15, 24 and 48 hpi. Since MDA is a by-product of lipid peroxidation, using this marker, we demonstrated that oxidative stress occurred during ZIKV infection. Different viruses may alter cell homeostasis in various ways, which can generate oxidative stress and its deleterious effects on the host cell. From the results presented in this work, we can infer that ZIKV infection induces oxidative stress and that this event may be important for its pathogenesis. Therefore, we still intend to perform more experiments to better understand the relationship between ZIKV pathogenesis and oxidative stress as well as to explore the usage of other cell lines.

Palavras-chave: HepG2 cells, Oxidative stress, Reactive Oxygen Species, Zika virus
Since 2015, incidence of GBS in Brazil has increased simultaneously to Zika virus (ZIKV) outbreak. However, it is not clear the real impact of ZIKV in the occurrence of the disease, once that syndrome can be the result to other infectious, immune events or even other endemic arboviruses, as Dengue virus. Besides being the gold standard to ZIKV diagnosis, viral isolation gives subsidies to further understanding the relationship of ZIKV with neurological complications. In this context, the goal of this study is the isolation of ZIKV from GBS patients samples to further studies of neurovirulence. Cerebrospinal fluid (CSF) was used to isolate ZIKV in C6/36 cells and neonatal mice. Cell monolayers with 80% confluence were inoculated with CSF dilutions (1:10), and 20 µL of CSF were inoculated, by the intracranial route, in neonatal mice. Cell cultures as well as neonatal mice were observed daily to cytopathic effect analysis (7 days) and symptoms development (10 days), respectively. After this period, real-time PCR with Syber green dye, to NS5 gene was performed for virus detection. Isolation of ZIKV from CSF was possible to one sample in mice, with PCR positive, however, no symptoms were observed. The brain was macerated, diluted (1:50) and inoculated in C6 / 36 culture, testing PCR positive with melt curve compatible to ZIKV. The virus isolation from GBS patients, especially from LCR is important to strengthen the relationship between ZIKV and GBS, giving subsidies for neurovirulence studies. This is one between a few reports about ZIKV isolation from LCR.

Financial Support: CNPQ

Palavras-chave: Guillain Barré Syndrome, Zika Virus, Isolation
Zika virus (ZIKV) was first identified in 1947 in Uganda and has been associated with sporadic cases of human disease in the endemic areas of Africa and Asia. In 2007, a large outbreak of ZIKV was reported in Yap Island, Micronesia, where 73% of the population was exposed and 2015 arrive in Brazil. Different studies have demonstrated the viral excretion between 3 at 6 months from the semen and 30 days from de urine, detectable by qRT-PCR, however anything study showed infectious viral particle in this long shedding ZIKV patients. Here we describe the isolation of ZIKV present in saliva, urine and semen specimens were collected weekly from two patients with ZIKV prolonged infections. For virus isolation, Aedes albopictus mosquito cells (C6/36) were inoculated with 500 µl of saliva, urine or semen. After 8-12 days of incubation, cells were collected and tested for ZIKV presence by qRT-PCR. All samples were blind-passages at least 3 times before being considered negative. Molecular assays were utilized to measure virus presence in the different body fluid and in the infected cell line. As it has been widely reported presence or viral RNA in a biological specimen is not equivalent to demonstration of infectivity. Thus, we attempted virus isolation of ZIKV in insect cells from positive semen, saliva and urine samples collected from both male patients to confirm presence of infectious virus. For patient 1 we obtained viral isolates from urine and semen samples collected 18 days after symptoms onset; from urine, semen and saliva samples at day 25; and from the semen samples at days 32, 53 and 117. For patient 2, we isolated virus from semen samples collected on days 19, 26, 40 and 82 days after symptoms onset. In conclusion we demonstrated that in our patients studied, infectious virus and complete viral particles were detected up to 120 days post syndrome onset. Crucially, the lasting presence of infectious ZIKV in semen and in sperm cells may considerably increase the risk of sexual spread. This type of transmission could potentially have a synergistic role in virus spread along with the more typical route of vector borne transmission. Financial support: Fapesp – 2016/ 08727-5 (Oliveira D. B. L.)

Palavras-chave: ZIKV, Cultivo celular, C636, Semen, Urina
ZIKA VIRUS NEUROPATHOGENESIS: A STUDY OF CELLULAR MIRNAS AND TARGETS MODULATED BY VIRUS INFECTION.

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Resumo

Zika virus (ZIKV) is an arthropod-borne flavivirus that recently brought a lot of concern due the development of the congenital ZIKV syndrome. RNA viruses, such as flaviviruses, have been reported to exert a profound impact on the host MicroRNAs (miRNAs) expression profile. Cellular miRNAs mediate the post-transcriptional control of gene expression and play important regulatory roles in many physiological and disease processes, including viral infections. The unveiling of the cellular miRNAs modulated by ZIKV may aid in the identification of possible cellular pathways modulated by viral infection. With this aim, we assayed an infected neuroblastoma cells (SH-SY5Y) with the Brazilian ZIKV strain PE on a TaqMan® OpenArray® microRNA panel. In order to enrich our findings, we also measure the mRNA levels of two critical predicted targets: the neuronal transcription factor SOX2 and the antiviral kinase EIF2AK2. Seven host miRNAs (miR-99a*, miR-126*, miR-190b, miR-361-3p, miR-522-3p, miR-299-5p and miR-1267) were seen to be significantly downregulated during ZIKV infection (p-value ≤ 0,05), and one (miR-145) was seen to be upregulated. It is relevant to report that eleven miRNAs were found to be exclusively expressed in either ZIKV-infected or mock groups. Further bioinformatics analysis indicated that the predicted targets were overrepresented in some central nervous system function-related Gene Ontology (GO) terms and biological processes. The possible downregulation of genes involved in Astrocyte development (GO:0014002) could be observed, which favors an immature and static cellular state. Also the downregulation of genes classified in Chromosome separation set (GO:0051304) could be responsible for the disruption in the cell-cycle and reduced proliferation of forebrain-specific progenitor cells observed during ZIKV infection, resulting in decreased neuronal cell-layer. Overall, 1207 gene transcripts were predicted as possible targets for the modulated miRNAs, including genes involved in neuronal development and homeostasis, cellular migration, cytoskeletal organization, immune and antiviral response and cell cycle regulation. Among these, we could verify that SOX2 (predicted as target for both miR-145 and miR-126*) and EIF2AK2 (predicted as target for miR-190b) were upregulated during ZIKV infection. Here, we present for the first time ZIKV-induced changes in host miRNA populations during infection in a neuronal model.

Financial Support: CNPq/CAPES

Palavras-chave: ZIKV, ZIKA, miRNAs, NEUROPATHOGENESIS, FLAVIVIRUSES
ZIKA VIRUS REPLICATES IN ADULT HUMAN BRAIN AND INDUCE COGNITIVE DEFICIT IN ADULT MICE

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**Resumo**

Zika (ZIKV) is a flavivirus associated with neurological complications mainly in brain development after intrauterine exposure. ZIKV infection is known to affect human neural precursor cells impacting neurogenesis. While some cases of ZIKV infection have been associated to important neurological manifestations in adult patients, including memory impairment, it is largely unknown whether and how ZIKV can induce abnormalities in the adult brain. In this study, we aimed to determine whether ZIKV infects adult brain tissue from human and/or mouse. Human temporal lobe cortical slices were infected with 10e7 pfu of ZIKV for 1 hour and both the kinetics of replication and cytokine production were assessed after that. Over 24 hours post-infection (hpi), the viral titers in the slices became approximately 10 times higher than the starting inoculum and kept increasing up to 72 hpi. The infection led the slices to secrete significant amounts of two pro-inflammatory cytokines, IL-6 and IL-1β. In the in vivo experiments, adult Swiss mice were intracerebroventricular inoculated with 10e5pfu of ZIKV. Infected animals exhibited a lower weight gain than control group, but with no lethality. In the brain of these mice were detected increasing contents of ZIKV RNA with a peak of replication in the 6th day, and viral persistence of more than 60 days. Analysis by qPCR of mice-infected brain regions showed that ZIKV diffuses preferentially into the hippocampus and frontal cortex, brain regions key for learning and memory. In these regions, no co-localization of ZIKV and glial cells was detected, suggesting that neurons are the primary target of infection at this tissue. However, no signs of neural cell degeneration were present. The infection led glial cells to become activated and elevated the expression of IL-6, IL-1β and TNF-α concomitant with virus replication. To evaluate the consequences of these findings in ZIKV pathogenesis, memory tests were performed in infected mice, and two markers of memory consolidation, synaptophysin and CREB phosphorylation were analyzed. Zika infection impairs learning and memory, and reversibly decreases hippocampal synapses related to memory. We describe for the first time that ZIKV replicates in ex-vivo human adult cortical tissue, a region non-related to adult neurogenesis, which highlights the importance to further investigate prolonged consequences of ZIKV infection in adults. Financial Support: CNPq, Faperj and FINEP.

**Palavras-chave:** Zika, Memory, Human, Mice
The dengue virus (DENV) is maintained in an urban transmission cycle: man - mosquito – man. The aim of this study was to evaluate the DENV IgG seroprevalence in patients from a prospective cohort held in a certain neighborhood of São José do Rio Preto, a medium-sized city and which is endemic to DENV. For this, 1519 patients, recruited from December 2015 to March 2016, were interviewed about their demographics information and they had blood samples collected. The patients’ sera were analyzed by ELISA. The results showed that 1092 patients (73%) were positive to DENV IgG, 396 patients (26%) were negative and 31 (1%) of the patients were inconclusive. From the patients who reported not having dengue (506/34%) during the interview, 414 patients (82%) were positive to DENV IgG. Among women, (596/74%) were positive, while among men, (892/73%) were positive. In our cohort, Dengue affected more the 60 yr age group (219/84%), followed by the 18-59 yr age group (773/73%) and for last the 10-17 yr age group (101/58%). In relation to ethnicity, black people were positive in 77% (464 of the 600) of the cases in comparison with the white people, which were positive in 70% (539 of the 767) of the cases. Regarding the type of domicile, 75% (943 of the 1251) of the cases occurred in people who lived in the house while 64% (150 of the 234) of the cases occurred in apartment dwellers. The average minimum wage between the participants was from two to four wages. It was previously described that regions with lower financial conditions are more affected by DENV. Also, our data showed that at least 73% of the studied patients in this cohort had contact with the DENV at least once in life. São José do Rio Preto is hyper-endemic region to dengue which corroborate with our data. Some studies show that people over 60 are less affected by dengue, while, young people are most affected. Our data show the inverse of this scenario. The type of domicile also collaborates in the transmission of the disease, since the mosquito does not reach high altitudes. Thus, data obtained in this prospective cohort study may provide crucial information on the real incidence of DENV in the population, collaborating in the planning of a better strategy for combating, treating and preventing infections with a high impact on public health. Financial support: FAPESP, CAPES
Since the emergence of Zika virus (ZIKV) in Brazil in 2015, many countries and territories in the world have confirmed cases of the disease caused by the virus. The ZIKV-associated neurological manifestations and congenital defects make the development of safe and effective antivirals against ZIKV of utmost importance. Here, we evaluated the antiviral activity of a thiopurine nucleoside analog derived from the prodrug azathioprine, against the epidemic ZIKV strain circulating in Brazil. In all the assays, an epithelial (Vero) and a human neuronal (SH-SYSY) cell line were used to evaluate the cytotoxicity and the effective concentrations of drug against ZIKV. The levels of ZIKV RNA, viral infectious titer and the percentage of infected cells at the presence or absence of drug was used to determine the antiviral efficacy. After the treatment, zikv production decreased by over 99% in both cell lines in a dose- and time-dependent way. Interestingly, the drug was 1.6 times less toxic to SH-SYSY cells compared to Vero cells, presenting 50% cytotoxic concentrations of 460.3 µM and 291 µM, respectively. The selectivity index of the drug for Vero and SH-SYSY cells was 11.9 and 22.7 µM, respectively, highlighting the safety profile of the drug to neuronal cells. Taken together, our results identify, for the first time, this specific thiopurine nucleoside analog as promising antiviral candidate against ZIKV that warrants further in vivo evaluation. Financial Support: CNPq, FACEPE, CAPES, IAM-FIOCRUZ/PE.

**Palavras-chave:** Antiviral, Zika virus, cytotoxicity, Vero, neuronal cells
Dengue is the most important arboviral disease worldwide. It is estimated an occurrence of 400 million infections by one of the four Dengue virus (DENV) serotypes every year. Severe disease is associated with a systemic inflammatory response, thrombocytopenia, hemoconcentration and increase in vascular permeability. Resolution of inflammation is an active process leading to restoration of tissue homeostasis. Here, we studied the role of Annexin A1 (AnxA1), a glucocorticoid-regulated protein that has anti-inflammatory and proresolving actions, in resolution of acute Dengue virus (DENV) infection. ELISA analysis of AnxA1 levels in serum of patients infected by DENV revealed reduced levels this protein in comparison to heath individuals. Similarly, AnxA1 levels were significantly reduced in the serum of wild type (WT) mice infected by a clinical isolate of DENV-2. Accordingly, DENV-2 inoculation to WT mice resulted in some signs of disease manifestation, as represented by thrombocytopenia, hemoconcentration and increased vascular permeability in liver after 24hs and 48hs of DENV-2 inoculation. Interestingly, all those parameters of disease were massively exacerbated in Anx-A1/- mice and most interestingly, disease lasted longer, remaining until 72hs after infection. To confirm the phenotype, FPR2/- mice, which are deficient to the Anx-A1 receptor, were infected by DENV-2 and also developed a markedly disease manifestation in comparison to WT-infected littermates. Finally, pretreatment of mice with AnxA1-active N-terminal peptide (Ac2–26) significantly decreased all DENV-induced parameters of disease manifestation. AnxA1 plays a crucial role in the context of acute DENV infection by promoting timely resolution of inflammation.

**Palavras-chave:** Annexin A1 , Arboviruses, Dengue virus, Inflammation
Resumo

The Mayaro virus (MAYV) is an arbovirus found in the Amazon Forest region and in some South American countries, such as Peru, Bolivia and Venezuela. The arboviral cycle of the MAYV is similar to the cycle of other arboviruses, which leads to an underestimation of the actual number of cases. To date, there are no therapies or vaccines available for Mayaro fever, and it is necessary to develop drugs that interrupt its progress. Phytotherapics, especially of the flavonoid group, are good attractives to research as antivirals due to their diversity, low cytotoxicity and numerous reports showing antiviral activity in this class of molecules. In silico studies previously performed by our group demonstrated favorable binding energy of the flavonoid ANT2, isolated from Salacia sp., to protein C of the viral capsid. In vitro studies have confirmed great antiviral activity and low cytotoxic effect (CC50> 500µg/mL). Thus, the present research investigates possible mechanisms of the antiviral action promoted by the flavonoid AN2, determining the stage of the viral cycle affected. Mammalian cells, Vero, were implanted in 96 wells microplates (5x10^4 cells/well) and, after 24h, were treated with different concentrations of the ANT2 compound (250 to 7.8µg/mL) and at different times of the cycle, using a multiplicity of infection (MOI) equal to 0.1 virus/cell. The results obtained so far suggest that the ANT2 compound does not interfere in the adsorption step and has moderate activity in the viral penetration stage. When ANT2 was used to pre-treat only the viral inoculum, pronounced antiviral activity was observed, with reduction above 6 log units in the virus titers produced. On the other hand, when the compound was added with the virus already adsorbed to the cells or after its penetration, little or no action was detected, suggesting a virucidal effect. The virucidal concentration of ANT2 (VC50) was 75µg/mL, consistent with the value obtained previously for the effective protease concentration for 50% of infected cells (EC50), which was 71.66 µg/mL. The results, together, give evidence that ANT2 acts primarily to bind to viral components, although its binding to cellular receptors is not ruled out. Financial Support: FAPEMIG and CNPq.

Palavras-chave: Mayaro virus, Antiviral, Salacia sp
Antiviral Activity of Diterpenes from Canistrocarpus Cervicornis Against Human Herpesvirus Type 1

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Abstract

Human alphaherpesvirus 1 is an etiologic agent of infection endemic in the world transmitted by oral contact, oral-genital contact. Bovine alphaherpesvirus 5 is an important agent of meningoencephalitis in cattle and has been identified in outbreaks of neurological disease in bovine in several Brazilian States. The aims of this work were to evaluate cytotoxic effect, antiviral properties of the (4R,7R,14S)-4α,7α-diacetoxy-14-hydroxydolast-1(15),8-diene (diterpene 1) and (4R,7R,14S)-4α,7α-diacetoxy-14-hydroxydolast-1(15),8-diene (diterpene 2) from Canistrocarpus cervicornis. The crude extract was dissolved in pyridine and treated with acetic anhydride. The extraction of the reaction mixture in the usual way afforded (addition using H2O Mili-Q® and extraction by CHCl3) and was subjected to silica gel chromatography elution by increasing the polarity of pure n-hexane/ethyl acetate mixtures in the fraction 9 was identified diterpene 1. Dolastane diterpene 2 was isolated by silica gel chromatography after acetylation of crude extract. To evaluate diterpenes 1 and 2 cytotoxic effect VERO and MDBK cell were treated with 12.5, 25, 50, 100, 200 and 500 μM. The CC50 results obtained by MTT assay to diterpene 1 (650 μM ± 7.3 and 1124 μM ± 12) and diterpene 2 (910 μM ± 8.9 and 1171 μM ± 8.5) respectively. Both compounds were able to inhibit Human alphaherpesvirus 1 (KOS) with effective concentration required to achieve 50% protection of the Vero cell (EC50) were to diterpene 1 (6.25 μM ± 7.5) and diterpene 2 (120 μM ± 5.7) and selectivity index were 104 and 7.5 respectively. On the other hand, both compounds were not able to inhibit Bovine alphaherpesvirus 5 (BoHV-5RJ42/01) replication into the cell but they were able to interact directly with Human alphaherpesvirus 1 (KOS) and BoHV-5RJ42/01 reducing their infectivity on VERO and MDBK cells. The inhibition of the virus infectivity was determined by plaque assay using untreated and treated KOS and BoHV-5RJ42/01. Virus suspension contained 1x10⁴ PFU were mixed with 25 and 50 μM of each diterpenes at 24 ºC for 1 h, diluted and percentage of the inactivation results for diterpene 1 at 25 μM (50%; 43%) and for diterpene 2 were 45%; 40% for KOS and BoHV-5RJ42/01 respectively and result of infectivity of virus after treatment for diterpene 1 at 50 μM were 100% and 67% and diterpene 2 100% and 59% for KOS and BoHV-5RJ42/01 respectively.

Keywords: Antiviral, Diterpenes, Human Herpesvirus Type 1
Dengue is the most important arboviroses in the World, in terms of morbidity, mortality and economic losses. The severe dengue is associated with changes on the endothelial barrier function due to the production of inflammatory mediators by several infected cells. Immune cells, such as monocytes and dendritic cells, have been considered primary targets for the replication of Dengue virus (DENV), however, many studies have demonstrated that hepatocytes also contribute to increased viremia. Furthermore, these infected cells produce factors, such as cytokines and chemokines, which contribute to the increased endothelial permeability. Natural products, which have immunomodulatory properties described in ethnopharmacology, can promote the control of viral replication and a reduction of cytokines that induce endothelial permeability, which could result in milder clinical manifestations. Lauraceae, a family of the Amazon flora, has been evaluated for its biodynamic activities, due to its almost complete chemical and pharmacological uniqueness. The anti-inflammatory activity of this family has been described by some authors. Therefore, the aim of this work was to characterize the antiviral and immunomodulator potential of a Lauraceae species. To do so, human hepatocyte line (Huh-7) was infected with DENV-2 and then treated with different concentrations of hydroalcoholic extract of the Lauraceae species - for 72 hours. The antiviral activity was measured by ELISA detecting viral non-structural protein (NS1) levels in the supernatants of infected and treated cultures. These supernatants were also assayed to evaluate the immunomodulatory activity of the extract through the dosage of IL-8 by ELISA. At 48 h and 72 h post infection, some concentrations of the extract were able to significantly reduce the levels of NS1 and IL-8 cytokine, indicating antiviral and immunomodular activity of the Lauraceae species. Although these results are satisfactory, further studies are required in order for the Lauraceae extract to be considered a potential candidate for the treatment of dengue. Financial support: FAPEAM, UEA and INPA.

**Palavras-chave:** ANTIVIRAL, IMMUNOMODULATORY, LAURACEAE, DENV-2, HEPATOCYTE
The arthropod-borne have been caused a thousand of cases in the entire world. In the last years, Sao Jose do Rio Preto city (SJRP) notified many cases of the Dengue virus (DENV), Zika virus (ZIKV) and Chikungunya virus (CHIKV). Aedes aegypti is the main mosquitoes responsible for the urban transmission at these viruses. In this study, we evaluated the relationship between the Breteau index (BI) and the adult mosquito infestation and the arbovirus occurrence. This study was performed in Vila Toninho, neighborhood in the city of Sao Jose do Rio Preto, Sao Paulo State, Brazil, between October/2015 and July/2017. The field team realized the BI during the first three week in each month and the traps for the capture of adult mosquitoes were installed once a month, always in the last week of the month; the mosquitoes (immature or adult) were identified using the specifics Taxonomic keys. More than one thousand traps installation were realized, 1,530 mosquitoes were collect, 579 Aedes aegypti (181 males and 398 females), 9 Aedes albopictus (only females) and 942 Culex sp. (361 males and 581 females). 169 Aedes pools were tested by molecular assays for DENV, ZIKV and CHIKV. 12 were positive by DENV (only Aedes aegypti - 4 males and 8 females), 6 was positive by CHIKV (1 Aedes albopictus female and 5 Aedes aegypti female ) and 23 were positive by ZIKV (only Aedes aegypti – 11 males and 12 females). The month infestation maps were build using the TerraView and ArcGIS softwares al index. The association between the mosquitoes infestation and arbovirus occurrence can be seen in the maps. These maps are important, because it can be used to monitoring the control vector and the epidemiological surveillance at the arbovirus occurrence in the city during the outbreak. Financial Support: FAPESP, BUTANTAN, SUCEN.

**Palavras-chave:** ARBOVIRUS, SPATIAL ANALYSIS, DENGUE VIRUS, ZIKA VIRUS, CHIKUNGUNYA VIRUS
AUTOCHTHONOUS TRANSMISSION OF EAST/CENTRAL/SOUTH AFRICAN GENOTYPE CHIKUNGUNYA VIRUS IN RIO DE JANEIRO, BRAZIL

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Resumo

Chikungunya virus (CHIKV) is an alphavirus that causes a fever characterized by headache, intense polyarthritis, rash and joint swelling and is transmitted by Aedes aegypti and Aedes albopictus vectors. There are three main CHIKV genotypes circulating nowadays, which are: Asian, West African, and East/Central/South African (ECSA). CHIKV first entered in Brazil in 2014, on the Amazon region and autochthonous transmissions have been reported in Northeastern Brazil since then. In Rio de Janeiro, since the end of 2015, autochthonous transmissions have been settled and 1868 cases of CHIKV infection were confirmed during the 2016 outbreak. In an attempt to characterize genotypes circulating during the 2016 outbreak in Rio de Janeiro, patient samples were analyzed through whole genome sequencing. Blood samples obtained from patients attending an emergency laboratory at Rio de Janeiro were obtained, and CHIKV infection was confirmed by Taqman-based RT-qPCR. Samples were tested negative for Dengue and Zika virus infection by specific TaqMan-based RT-qPCR. For this purpose, CHIKV was successfully isolated from two blood cell-fraction samples (named RJ-IB1 and RJ-IB5) in Vero cells. After RNA isolation, cDNA synthesis and confirmation by qPCR, 4 overlapping fragments were PCR amplified and sequenced on an Illumina MiSeq platform after tagmentation-based library construction and de novo genome assembly was done with Geneious. We observed that CHIKV isolates from both patients had similar growth kinetics in cell culture, reaching highest yields at 48 h after infection. Phylogenetic analyses showed that CHIKV RJ-IB1 and RJ-IB5 mapped within the ECSA genotype together with other isolates from northeastern Brazil and no evidence of intergenotypic recombination was observed. We detected unique mutations in RJ-IB1 and RJ-IB5 that are absent in other CHIKV isolates: NSP4-A481D in the viral RNA polymerase and E1-K211T in an E1 polymorphic site. Furthermore, the Brazilian ECSA subgroup shares 2 exclusive mutations (E1-M407L and E2-A103T) that are absent in other ECSA strains. These mutations might impact adaptability to Ae. Aegypti or alter CHIKV fitness in vertebrates and their real impact must be better investigated. Financial Support: CNPq, FAPERJ, CAPES

Palavras-chave: Chikungunya virus, vector-borne infections, ECSA genotype, mutations, Brazil
Chikungunya virus (CHIKV) is an arbovirus, member of the Alphavirus genus in the Togaviridae family. It causes a disease in which common symptoms are fever, rash, myalgia, and arthralgia. CHIKV has already caused several outbreaks in different continents, with thousands of cases. Therefore, CHIKV is recognized as a notable public health problem, with no vaccine or antiviral treatment available. The CHIKV outbreaks in 2015 and 2016 in the northeast Brazil, corroborate to the risk of an epidemic outbreak in North of Brazil. In this work, we report the complete genome of a CHIKV isolate obtained in July 2015. Initially, CHIKV was confirmed by RT-qPCR and further submitted to a random PCR amplification protocol for viral metagenomics studies. The NGS sequencing was performed using a 318v2 chip with 200bp chemistry, in an Ion Torrent Personal Genome Machine, according to the manufacturer’s instructions. A total of 522,269 reads were generated and used for contig assembly with the CHIKV reference sequence NC_004162, using the map to reference tool embedded in Geneious 10.0.7. The cleaned final sequence has 11,826bp, 90X mean coverage and a Q30 score of 74.9%. Subsequently, all complete Brazilian CHIKV genomes deposited in GenBank were aligned using the MAFFT algorithm for nucleotide and protein sequences comparisons. Furthermore, a previously published dataset of CHIKV genomes was used for phylogenetic analysis with MrBayes 3.2, using the General Time Reversible model with a proportion of invariable sites and a gamma-shaped distribution of rates across sites. The alignment of the Brazilian sequences reveals a few number of amino acid substitutions throughout the polyprotein. Phylogenetic analysis shows that the Amazonas isolate sequenced in this study belongs to the ECSA genotype, and it is closed-related to sequences from samples of Feira de Santana, Bahia, Brazil. Further studies are being conducted to characterize the phylodynamics of CHIKV in Amazonas State completely.

Palavras-chave: ECSA, Chikungunya, Metagenomic, Amazonas
Hantavirus is a genus of viruses belonging to the *Bunyaviridae* family. Some Hantavirus strains are capable of causing human diseases, such as a Hantavirus pulmonary syndrome and hemorrhagic fever with renal syndrome, while others are not associated with human diseases. Hantavirus can be transmitted to humans by inhalation of particulate matter eliminated in the secretions of infected rodents. The aim of this work is to describe the main clinical and epidemiological variables of patients positive for hantavirosis in the State of Minas Gerais in the years of 2015 and 2016. A total of 1335 samples were received at the Central Public Health Laboratory of Minas Gerais for the diagnosis of hantavirus in the period from January 2015 to December 2016. Samples were tested for IgM and IgG anti-Hantavirus by ELISA. Of the samples tested, 24 tested positive for Hantavirus. The mean age of the affected patients was 36.5 years, with a predominance of male patients (18). The mortality observed among the positive cases was 45% (11 patients). The most commonly reported signs / symptoms were fever (87.5%), dyspnea (79.2%), myalgia (70.8%), headache (62.5%), acute respiratory insufficiency (54.2%), cough (50%) and hypotension (41.7%). Positive cases were distributed in 16 municipalities, with a higher prevalence in the Uberaba, Uberlândia and Patos de Minas regions (6 cases in each), located in the western part of the state. The other cases are from the regions of Itabira, Divinópolis and Ubá. Of the 24 positive samples, 11 also went through the Hemorrhagic Fever protocol, in which the sample is also tested for other diseases (Dengue, Yellow Fever, Spotted Fever, Leptospirosis and Hepatitis A). 2 samples showed positive results for leptospirosis (although they were negative in the confirmatory test, suggesting false-positive results for leptospirosis), 1 for Spotted Fever and 1 for Dengue (borderline result, suggestive of false-positive for Dengue). A sample was also tested for Influenza in RT-qPCR, with a positive result, suggesting co-infection (the sample was IgM and IgG positive for Hantavirus). Based on the results obtained, it was observed that the cases of Hantavirus in Minas Gerais present high mortality and are characterized mainly by respiratory manifestations, rather than hemorrhagic symptoms.

**Palavras-chave:** EPIDEMIOLOGY, HANTAVIRUS, SEROLOGY
Methods

Introduction and Aims: To characterize the immunological profile related to HCV treatment. Patients and Methods: 20 patients with chronic HCV infection GT1 treated with PR and telaprevir (TVR) or boceprevir (BOC) were included. The MCP-1/CCL2, RANTES/CCL5, IL-8/CXCL8, MIG/CXCL9 and IP10 / CXCL10 chemokines and microparticles (MPs) were analyzed by flow cytometry before (BT) and during treatment (AT) at weeks 2 (n=20), 4 (n=20), 8 (n=19), 12 (n=18), 24 (n=16) and 48 (n=12). The protocol was approved by Ethical Board of UFMG/Brazil. Results: 14/20 completed treatment and 12/14 (86%) achieved SVR. CCL2 and CXCL8 were increased at week 12 AT compared to BT. CXCL9 and CXCL10 decreased at week 24 compared to week 12 AT. The MPs from lymphocytes TCD3+ decreased at weeks 12, 24 and 48 AT compared to BT and decreased at weeks 24 and 48 compared to week 4 AT. The MPs from lymphocytes TCD4+ decreased at the weeks 12 and 24 compared to BT and decreased at week 12 compared to week 4 AT. MPs from monocytes decreased at weeks 2, 12 and 24 AT compared to BT and increased at week 48 compared to week 24. The MPs from neutrophil decreased at the weeks 2 and 24 AT compared to BT decreased at week 24 compared to week 8 AT and increased at week 8 compared to week 2 AT. Of note, there was a progressive decrease in liver enzymes and microparticles after treatment and peak of chemokines at week 12 of treatment. Most biomarkers reduced at week 24 post-treatment, except for CCL2 and CCL5. Conclusions: CCL2, CXCL8 are associated with recruitment of immune cells involved in mechanisms against viral infection. In contrast, the chemokines CXCL9 e CXCL10 exhibit reduction throughout treatment. The reduction of microparticles during the treatment suggests that it promotes a favorable environment for more effective immune response against the HCV virus. This immune scenario suggests an immunomodulatory modulation during HCV treatment. Financial Support: FAPEMIG, CNPq, Ciência Sem Fronteiras, HC/UFMG, CBER/FDA

Palavras-chave: Hepatitis C, Chemokines, Microparticles, Triple therapy
PCR to ZIKV/CHIKV and was observed the increase in the incidence of the arboviruses infections over the years. The outbreak of ZIKV infections has been associated with the increase of microcephaly cases and others neurological disorders and it was considered an international health public emergence. The CHIKV infection has similar symptoms to those of other arboviruses besides presents polyarthritis/ arthralgia, which in some cases may be debilitating, lasting for months or even years after the fever, and interferes in quality of life having economic impacts due to reduced productivity. Then, the aim of this study was to detected the circulation of different arboviruses in acute febrile patients from a DENV prospective cohort in São José do Rio Preto. For this, 52 serum samples from patients presenting dengue-like symptoms, collected from November/2015 to March/2017, were analyzed by RT-PCR to DENV and qRT-PCR to ZIKV/CHIKV and was performed using primers targeting the ZIKV envelope (E) gene; CHIKV NsP1 gene and DENV NS5 gene. From the 52 samples analyzed, 07 were positive to DENV, being 01 sample to DENV1, 04 to DENV2, 01 to DENV4 and 01 showed coinfection with DENV1 and DENV4. Four samples were positive to ZIKV and none showed positivity to CHIKV. In 2016, the São Paulo state faced a ZIKV outbreak, with 9845 suspected cases notified (4032 confirmed) and our results showed this circulation in São José do Rio Preto. Although, no samples were positive to CHIKV, but the circulation was previously reported in human and mosquitoes in the city, suggesting the possible future epidemic. The molecular surveillance of arboviruses has been necessary to improve the knowledge about the spread and the evolution of these viruses in Brazil and around the world. Financial support: CAPES, FAPESP, BUTANTAN.

**Palavras-chave:** DENGUE, ZIKA, CHIKUNGUNYA, PROSPECTIVE COHORT
The JC Polyomavirus (JCV), a ubiquitous virus, infects most of the worldwide population. In health individuals, the infection shows no symptoms, and remains latent mostly in the kidney epithelium. Although, in immunocompromised patients, the virus can reactivate, migrate to the brain, and cause progressive multifocal leukoencephalopathy (PML), a fatal demyelinating disease of the central nervous system (CNS), which is caused by the lytic infection of oligodendrocytes. JCV can transform cells in culture and is oncogenic in laboratory animals. The human cytomegalovirus (HCMV) is a ubiquitous virus that infects most of the human population and, is able to establish lifetime latency. However, under immunosuppressive conditions, it can reactivate and cause various diseases, such as microcephaly, in newborns, meningitis, and hepatitis, among others. The most severe cases occur in patients under treatment for organ transplant, AIDS and cancer, where the virus could induce organ rejection, or increase cancer malignity. Glioblastoma multiforme (GBM) is the most prevalent and malignant tumor of the CNS. For some time now, accumulating evidence has suggested an association between the HCMV infection and GBM. Also, studies have demonstrated that, when a cell infected by HCMV, which was non-permissive to JCV, is introduced with JCV DNA, it can cause the latter to reactivate and successfully replicate. Been possible the association of these two viruses, it is hypothesized that this association could be linked to tumor malignancy or even oncogenesis. In this work, tumor tissue and peripheral blood of patients with GBM were examined for the presence of HCMV and JCV DNA. Six fresh surgical tissue brain and 6 peripheral blood samples were analyzed by real-time PCR (qPCR) for the presence of the VP1 JCV gene and (glycoprotein B) gb HCMV gene. HCMV DNA was detected in half of the tumor samples (50%) and half of the peripheral blood samples (50%). JCV DNA was detected in four of the tumor samples (66%) and in one blood sample (16%). Only one tumor sample detected DNA from both viruses (16%). This work next step is to test a larger amount of samples, so it could be measured the prevalence from both viruses associated in GBM from Brazilian patients.

Palavras-chave: Glioblastoma, HCMV, JCV, qPCR
CONSTRUCTION OF A RECOMBINANT MVA CODIFYING A MULTIEPITOPE CHIMERIC PROTEIN BASED ON HTLV-1-HBZ PROTEIN

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Resumo

Human T-cell lymphotropic virus 1 (HTLV-1) is a retrovirus that belongs to the genus Deltaretrovirus and to the family Retroviridae. It is estimated that 10 to 20 million people living worldwide are infected with the virus. The HTLV-1 causes a chronic infection, and about 5% of the patients will develop severe diseases. Two distinct diseases are associated with the HTLV-1 infection, adult T-cell Leukemia (ATL) and HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP). Although both diseases have poor prognosis, there is no specific treatment for them. Recently, some studies have shown that the T-cell mediated cytotoxicity against the viral protein, HBZ, is able to control the infection through specific cell lysis. Due to the impact of the HTLV-1 infection, this project aims to construct a recombinant Modified Vaccinia Ankara (MVA) virus carrying a gene that codes a chimeric protein containing HBZ epitopes, as a potential candidate for HTLV-1 therapeutic vaccine. The epitopes contained in the chimera were selected through in silico analysis of T-cell epitope prediction. The chimera gene was commercially synthesized in the plasmid pCloneEZ and was amplified in E. coli XL10-Gold. The chimera gene was obtained from the pCloneEZ by restriction digestion with SmaI and PstI, and it was then subcloned in the transfer plasmid pLW44, which was also amplified in E. coli XL10-Gold. To generate the recombinant MVA, primary chicken embryo fibroblasts (CEFs) were infected with wild type MVA (m.o.i=1) and after adsorption the cells were transfected with 1000ng of pLW44. Clones of recombinant MVA were selected through detection of GFP, PCR and RT-PCR. Two chimeric proteins (HBZ-CHI and HBZp-CHI) were constructed; the presence of a signal peptide (p) is the difference between them. Fragments of size of 294bp and 366bp, for the HBZ-CHI and HBZp-CHI, respectively, were observed after the digestion of the pCloneEZ and the subcloned pLW44. The PCR of the viral DNA has shown bands of 624bp and 692bp for HBZ-CHI and HBZp-Chi, respectively. Also, as expected, the RT-PCR of the viral RNA has shown a band of 267bp for both constructions. These results confirmed that recombinant MVA viruses carrying chimeric HBZ gene were built. Future studies should test the efficacy of these viruses as potential therapeutic vaccines for HTLV-1. Financial Support: CNPq, FAPEMIG and PRPq-UFMG

Palavras-chave: HTLV-1, MVA, HBZ, Chimeric protein, Therapeutic vaccine
DENGUE VIRUS MODULATE ANTIVIRAL RESTRICTION FACTORS THAT TURNS MONOCYTE ACTIVATED CELLS MORE PERMISSIVE TO VIRUS INFECTION

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Resumo

The flavivirus genus is composed of enveloped virus with positive-sense single-stranded RNA. This genus includes viruses with public health concerns, such as Dengue virus (DENV), Yellow fever virus (YFV) and Zika virus (ZIKV), which affects especially subtropical regions. After virus transmission by vector bite, the first sites of viral replication are cells present under the skin, such as monocytes and macrophages. These cells are involved in the production of new virus particles and inflammatory mediators that are related to disease outcome. Due to the importance of these cells in pathogenicity, we evaluated the susceptibility of monocyte and macrophage cells in different stages of differentiation to DENV. First, we differentiated THP1 cells with phorbolmyristate acetate (PMA) during 24 hours (THP1 stimulated) and 6 days (macrophages) and we observed an increase of virus susceptibility in early stages of monocytes to macrophage differentiation. All the monocytes and macrophage receptors markers (CD14, CD11b and CD206) were evaluated. To determine whether the monocyte to macrophage lineages susceptibility to flavivirus infection could be associated with cellular antiviral restriction factors we tested a panel of 46 factors already described for other RNA viruses. The restriction factors investigated include innate immune response, interferon response factors and others related to virus RNA detection, RNA genome edition, translation control and virus particles release. Our results showed down regulation of some restriction factors such as APOBECs; the translation initiation factor 2-alpha kinase 2 (EIF2AK2); ISG15–protein ligase HERC5; SAMHD1; Interferon-induced transmembrane protein 1-3; tripartite motif-containing protein 56 (TRIM56) and radical S-adenosyl methionine domain-containing protein 2 (RSAD2). These findings could explain the higher virus susceptibility to THP-1 stimulated cells. We also observed an increase of CCR5 receptor expression and its ligand CCL5 that have been described important for Dengue replication. We also found that some miRNAs are differently expressed during monocytes activation. In conclusion, the results suggest that the infection of DENV triggers cellular miRNAs in THP-1 stimulated cells and its decrease the levels of cellular restriction factors establishing the virus infection. Financial Support: CNPQ.

Palavras-chave: Dengue virus, THP1, miRNA, monocyte, Zika virus


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Abstract

Acute gastroenteritis (AG) is a second major cause of childhood mortality, resulting annually in 578,000 deaths. The most important viral agent associated with AG is Rotavirus A (RVA) that is responsible for approximately one third of these deaths. Usually RVA infection in adults is harmless, however in solid organ transplant recipients it can progress with severity, even though, to date, available data on RVA infections in transplant recipients are scarce. Another viral agent that has been presenting an emerging character is Human Bocavirus (HBoV), even though their role in the context of viral gastroenteritis has not yet been fully elucidated. However, there is increase evidence on the detection of HBoV in fecal specimens of individuals with AG. This study aimed to detect RVA and HBoV in fecal samples of immunosuppressed patients submitted to renal transplantation during the first year after transplantation. Two hundred and four fecal samples were collected from 33 patients submitted to renal transplantation. For RVA detection a commercial ELISA kit was used, while for HBoV detection Polymerase Chain Reaction (PCR) and Nested-PCR were used. Nucleotide sequencing was performed in positives samples for viruses characterization. One hundred ninety samples were tested for RVA and the positivity rate was null. With regard HBoV, 105 samples were tested and were observed a positivity of 30.4% (32/105). HBoV3 was the most frequent genotype detected in 93.8% (30/32) of samples, followed by HBoV1 (3.1%; 1/32) and HBoV2 (3.1%; 1/32).

The findings obtained suggest that RVA was not an important cause of AG among patients submitted to renal transplantation. On the other hand, this represents the first detection of HBoV on faecal samples of immunosuppressed patients in Brazil, with high prevalence of HBoV3. Further studies are needed in order to understand the role that such viral agents have in the AG etiology in this population. Financial Support: Instituto Evandro Chagas/Fundação Amazônia de Amparo a Estudos e Pesquisas (FAPESPA)

Keywords: Bocavirus, Renal transplantation, Rotavirus
DETECTION AND GENETIC CHARACTERIZATION OF HUMAN BOCAVIRUS IN CHILDREN HOSPITALIZED WITH ACUTE GASTROENTERITIS IN NORTHERN REGION OF BRAZIL.

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Resumo

Gastrointestinal infection is considered one of the major syndromes associated with morbidity and mortality among children aged under five years. The etiological agents of this disease may be parasites, bacteria or viruses. Human Bocavirus (HBoV) has been reported in association with acute gastroenteritis (AG), although its role in this infection is not completely elucidated. Studies have shown the association of HBoV with other pathogens, assuming that such viruses are not a true pathogen but a passenger innocuous. This pathogen can infect individuals of all ages, although the frequency is lower. This study aimed to detect and describe HBoV genotypes among children hospitalized with AG collected during 2013 from National Network of Surveillance of Rotavirus Gastroenteritis. It was selected 130 fecal samples for HBoV detection and submitted to polymerase chain reaction (PCR), followed by Nested-PCR for VP1/VP2 gene. HBoV frequency was 4.6% (6/130). Among positive samples, four samples were subjected to phylogenetic analysis, which belong to HBoV-1 (25%, 1/4), HBoV-2 (50%, 2/4) and HBoV-3 (25%, 1/4) genotypes. Co-infection with other gastroenteric viruses occurred in 33.3% (2/6) of positive samples. The present study suggests the relevance HBoV as a pathogen of gastrointestinal tract, being more frequent in children under 1 year of age and showing a low frequency of co-infection with other viruses. Financial support: Instituto Evandro Chagas (IEC/SVS/MS), Conselho Nacional de Ciência e Tecnologia (CNPq).

Palavras-chave: Human, Gastroenteritis, Bocavirus
DETECTION AND IDENTIFICATION OF VIRUSES IN MOSQUITOES AT NOVO HAMBURGO, RIO GRANDE DO SUL

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Resumo

The abundance of arbovirus vector Aedes aegypti has been increased in Brazil. In Novo Hamburgo, RS, programs as LIRAa (Rapid Index Survey of A. Aegypti) confirmed high mosquito infestation. Between 2014 and 2015, an increased number of focuses are reported, representing 255% (225 mosquitoes focuses in 2014 and 638 in 2015). Until June, 2017, 2427 mosquitoes focuses were identified at city. Of these, 270 outbreaks of A. aegypti were identified in LIRAas conducted in January and March of 2017 and the municipality was categorized as a state of imminent danger to public health, according the Predial Infestation Indexes (IIP). The present work aims to identify virus circulating in culicidae in Novo Hamburgo, by molecular methods. From April to November, 2016, passive collection methods were tested (Adultrap®, Victrap), arranged in intervals ranging from three days to one week, at sites identified as “highly infested” in the Novo Hamburgo’s urban areas. Between April and December 2016, 14 mosquitoes Culex quinquefasciatus, 7 Aedes albopictus and 13 Aedes aegypti were collected (November and December were the most representative months for the genus Aedes). Samples of C. quinquefasciatus were submitted to high performance sequencing (MiSeq Illumina). 10,400 readings were generated, resulting in 1833 contigs larger than 75 bp, (MIRA) Genominc screening was performed in a general database (BLAST) and specific for viral genomes (BLAST2Go). 237 sequences from the original 1833 contigs showed similarity to adenovirus genomes, specifically the genes encoding hexon and the DNA binding protein (DBP). In sense to confirm the presence of Adenovirus (AdV) in these samples, quantitative PCR for human adenovirus (HadV) F and C was conducted. HadV-C was detected in A. aegypti samples (2,34 E05/5µL genomic copies) and A. albopictus (6,83 E04/5µL genomic copies). These preliminary results can suggest adenoviruses circulation in mosquitoes but it is not possible to identify the source of contamination (human blood viremia or contaminated water reservoirs that are potential breeding sites to Aedes). Additional analyzes will be conducted to confirm these findings and to test another adenoviruses groups. Financial Support: Universidade Feevale.

Palavras-chave: Adenovirus, Aedes aegypti, Arbovirus, qPCR
DETECTION OF DIFFERENT SPECIES OF POLYOMAVIRUS IN TWO STATES IN THE NORTHEAST REGION OF BRAZIL

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Resumo

The poliovirus that infect humans (HPyV) are non-enveloped viral particles with double stranded DNA genome that belongs to the Polyomaviridae family. Numerous studies have been published on the pathogenic role of BKPyV and JCPyV, however, information about the transmission, pathogenesis, and epidemiology of other species of HPyV are scarce or even nonexistent. In this way, studies that will contribute to a better understanding of the epidemiology of HPyV in the population are fundamental for the understanding of ecology and diversity of these viruses. This study aimed to evaluate the frequency of BKPyV, JCPyV, KIPyV and WUPyV infections in the states of Bahia and Pernambuco in Northeast Brazil. Saliva samples were obtained from immunocompetent volunteers with ages between 8 and 87 years and were analyzed by real-time PCR. Of the 185 saliva samples analyzed, 84 (45.4%) were positive for at least one of HPyV species tested: 31.4% (58/185) were only positive for BKPyV, 10.3% (19/185); were only positive for KIPyV and 0.5% (1/185) was only positive for JCPyV. WUPyV was not detected in any samples. Co-infections between these viral species were detected in 3.2% (6/185) (BKPyV + KIPyV = 5; BKPyV + JCPyV + KIPyV = 1). There was no significant difference regarding viral detection between the female and male genders. Also, there was no significant difference in the excretion of HPyV between the different age groups. Twenty-one BKPyV-positive samples and 9 KIPyV-positive samples were analyzed for genotypes identification. Phylogenetic analysis showed that the strains of BKPyV belonged to genotype II and KIPyV strains belonged to genotype B, with the exception of PE25 strain which formed a separate clade of strains from genotypes A and B. The percentage of infections in the study population was 67.4% in Bahia and 22.2% in Pernambuco. BKPyV, JCPyV and KIPyV, were detected in both populations, however, the frequency of detection and distribution of these species was quite distinct. In Bahia, it was observed a significantly higher positivity than Pernambuco; there was a predominance of BKPyV species in Bahia and KIPyV in Pernambuco. This is a pioneer study and the results revealed HPyV circulation in the Northeast region of Brazil, provided unprecedented information about the KIPyV genotypes and supports the hypothesis about the role of polyomavirus as markers for human migration.

Palavras-chave: Polyomavirus, Brazil, Saliva
Orthopoxviruses (OPV) are emerging viruses with great importance in human and veterinary medicine, such as Vaccinia virus (VACV), which causes outbreaks of bovine vaccinia (BV) in South America. The classical route of VACV transmission is featured by direct contact between milkers with infected dairy cattle. However, VACV has been detected in milk samples even when submitted to different thermal treatments, as well as during artisanal cheeses preparation. The role of milk and its subproducts in VACV epidemiological cycle are poorly explored. Therefore, we investigated whether commercial artisanal cheese could be a source of VACV, and consequently an alternative route of virus exposure. Thirty-eight commercial artisanal cheese samples were obtained during June 2015–May 2017. These samples are commercial, obtained in Belo Horizonte, Minas Gerais state. However, the places of origin of these cheeses are state dairy basins, being 21 samples from Serro, 4 samples from Alto do Paranaíba, 12 samples from Araxá and 1 sample from Canastra. To detect VACV DNA, an OPV-specific nested-PCR targeting C11R gene and real-time PCR targeting A56R gene were performed. Eight samples (21%) were positive for C11R and three (7.9%) were positive for A56R. Only one sample (2.6%) was positive for both genes. The fragments amplified in three positives samples for C11R and two positives samples for A56R were sequenced, confirming the presence of VACV in artisanal dairy products. Previous studies have revealed the contamination of milk with VACV, and also analyzed viral viability in experimentally contaminated milks and their subproducts. The consumption of dairy food has already been pointed out as a possible new route of VACV transmission, during an outbreak of Buffalopox virus, a VACV related strain known to circulate in Asia. However, the dynamics of infection through this alternative route is still unknown. The presence of VACV in artisanal cheese could favor human exposure to VACV and be a burden on dairy economy, once the artisanal cheeses produced in the Minas Gerais dairy basins are commercialized throughout Brazil and even in other countries and are recognized as Intangible Heritage of the State. Further studies are necessary to clarify the role of commercial artisanal cheese as an alternative route of VACV transmission in its epidemiological cycle. Also, cheese producers belonging to the affected dairy basins should be educated regarding VACV circulation and its risks.

**Palavras-chave:** Vaccinia virus, artisanal cheese, alternative transmission routes, epidemiology
Development of a Cell-Based High Content Screening Strategy for Mayaro Virus Drug Discovery

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Resumo

The Alphavirus are a large genus which occur all over the world and can be divided into those of the New World and the Old World. Most of these Alphavirus may cause encephalitis or diseases with joint involvement. In South America, there is an Alphavirus that can cause arthralgia and even arthritis, the Mayaro virus, which is transmitted mainly by Haemagogus mosquitoes. The Mayaro virus has been producing in Brazil and neighboring countries outbreaks of febrile illness, such as the 33 cases of Mayaro fever described in the city of Manaus in 2007 and 2008. There is no specific vaccine or antiviral treatment for the Mayaro virus, although one alternative to vaccination would be the development of specific antiviral that could reduce the morbidity associated with this infection with less adverse effects. The objective of this study was to develop a high-content screening assay to identify compounds with potential antiviral activity. In this study, we used MAYV strain Be Ar 20290 and tests were performed in 384 wells plate. The human-derived cell line (Huh7) and MAYV were plated and after 60h incubation, plates were fixed and stained. Images were taken in the automated high-throughput imaging system Operetta (Perkin Elmer). The acquired images were analysed in terms of cell viability and infection ratio. With this trial we could find potential drug candidates in a short period of time. At the moment, drugs previously approved for treatment of Hepatitis C, such as Sofosbuvir and Ribavirin, are being tested for the Mayaro virus.

Financial Support: FAPESP

Palavras-chave: Mayaro, High-Content Screening, Drug Discovery
Introduction. Parvovirus B19 has been linked with various clinical syndromes including neurological manifestations. However, its role in the latter remains not completely understood. Human Parvovirus B19 (PVB19), the etiological agent of the fifth disease, is associated with a large spectrum of pathologies, among which is encephalitis. Since it has been detected from the central nervous system in children or in immunocompromised patients, its causative role in serious neurological manifestations is still unclear. The detection of PVB19 DNA in cerebrospinal fluid supports the hypothesis that this virus could potentially play a role in the pathogenesis of neurological complications. Objectives. The aim of this study is to detect the presence of Parvovirus B19-DNA in cerebrospinal fluid (CSF) from patients suspected of viral infection in the nervous system through a Polymerase Chain Reaction technique (PCR) together with clinical findings. Methodology. Sixty-four samples of CSF were analyzed by virological investigation for all nine herpesvirus (HSV-1, HSV-2, VZV, EBV, HHV-6A, HHV-6B, HHV-7 and HHV-8), adenovirus and polyomavirus JC, using Nested-PCR in CSF from patients attended in Emergency Room Units from Clinics Hospital, Unicamp, São Paulo, Brazil, since January, 2017. Cerebrospinal fluid was collected and an aliquot was sent to Virus Laboratory, Faculty of Medical Sciences, Unicamp, Campinas, São Paulo, Brazil, to analyses the molecular tests. Results and Conclusion. Four out of 64 (6.25%) patients with neurological symptoms presented positive DNA-B19 in CSF. One of them presented HIV+syphilis; one had positive for oligoclonal bands; other had sepsis and the last one had seizure. We hope with the results obtained to encourage and open doors to new research on the subject, emphasizing the importance of the scientific engineer in the search of solutions for the detection and treatment of infections. In the presence of neurological disorders, especially when there are no specific signs, but seizures are present, it is important to search for PVB19 both in immunocompromised and immunocompetent patients. Moreover, the introduction of the PVB19 DNA test into diagnostic protocols of neuropahties, especially those undiagnosed, could clarify the etiological agent that otherwise could remain unrecognized. Financial Support: FAPESP

**Palavras-chave:** Parovirus, Cerebrospinal Fluid, Neuropathies
Yellow fever (YF) is an infectious viral hemorrhagic disease endemic in parts of Africa and South America. The clinical picture ranges from a mild febrile illness to a severe infection leading to renal and hepatic failure, cardiac damage, bleeding and shock. It is estimated that about 10% of YF cases develop into severe forms, associated with high lethality, ranging from 20-50% of cases. The most effective form of prevention is the yellow fever vaccine. Recently, in early January of 2017, the Minas Gerais State in Brazil, faced the largest outbreak of the YF with more than 1,000 suspected cases and more than 80 deaths confirmed by the disease. Owing to the scarce studies evaluating the immune response in patients with yellow fever, the goal of this study was to evaluate the cellular immunity of adult patients with confirmed YF infection during the acute phase of infection and the convalescence period. Thus, 55 patients hospitalized at the Eduardo de Menezes Hospital, Belo Horizonte, Minas Gerais state, Brazil were enrolled in this study. The data obtained were compared with those from a control group (CT) of ten healthy individuals who were immunized with the 17DD yellow fever vaccine. Circulating cytokines and chemokines were quantified in the serum by the Cytometric Bead Array method. The data showed that Yellow Fever patients (YFP) presented increased levels of circulating IL-6, IL-10 and CXCL8 besides decreased levels of IL-1-beta and CCL5 as compared with the CT group. Moreover, during the convalescence period, YFP with late hepatitis presented higher circulating levels of IL-10 and CXCL8 than patients who did not present hepatitis. Interestingly, death in the YFP group is associated with increased circulating levels of the inflammatory mediators such as TNF, CXCL8 and CCL5 as well as decreased levels of the IL-10, a regulatory mediator. Overall, the data suggest a differential profile of immune response during the acute phase or the convalescence period of YF virus infection and indicate that may altered profiles of circulating inflammatory/regulatory mediators are associated with distinct clinical outcomes. Financial Support: FIOCRUZ, FAPEMIG e CNPq.

**Palavras-chave:** Clinical outcome, Cytokines and Chemokines, Hepatitis, Yellow fever outbreak
EFFECTS OF THE NATURAL COMPOUND AC.O ON THE HEPATITIS C VIRUS REPLICATION IN VITRO

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Resumo

The Hepatitis C Virus (HCV) infection is one of the major causes of liver diseases. It is estimated that approximately 3% of the population is infected with this virus. There is no vaccine and the current treatment is based on direct-acting antivirals (DAAs) in monotherapy or administered in combination with interferon and ribavirin. During the replication process and/or under therapy new resistant variants are generated due to the lack of proof-reading activity by the RNA polymerase of this virus. Additionally, therapy is expensive and presents many side effects, demonstrating the need of developing alternative approaches to treat infected patients. In this context, compounds extracted from plants have demonstrated to possess several biological activities including antiviral properties. Brazil has a large plant biodiversity and bioactive compounds isolated from its flora may act as antivirals. Here we evaluated the effects of the long-term treatment with the natural compound AC.O on HCV replication in vitro. Huh-7.5 cells stably expressing subgenomic replicon genotype 2a (SGR-FEO-JFH1) were treated with AC.O at the effective concentrations of 50% (EC50 = 0.9 µM) and 80% (EC80 = 2.8 µM) for 30 days. DMSO was used as non-treated control. Culture medium containing AC.O or control was replaced every 3 or 4 days and cells were harvested for analysis. Replication levels were obtained by normalizing the luminescence levels from luciferase assay with the amount of proteins in the lysates quantified by BCA analysis (Pierce BCA Protein Assay Kit). Therefore, cytotoxicity did not interfere with the results. The data showed that AC.O significantly reduced viral replication up to 84% (EC80) after five days of treatment, progressively increasing viral replication obtaining highest levels on day 26, judged by the expression of the viral proteins. This result demonstrates the lack of antiviral activity of AC.O over time which may be due to the appearance of resistant variants to this treatment. Further assays are being performed for a better understand of the mutation acquired by the variants which resisted to the AC.O therapy. FINANCIAL SUPPORT: ROYAL SOCIETY (NA150195); FAPEMIG (APQ-00587-14; SICONV 793988/2013), CNPq (445021/2014-4), FAPESP (2014/22198-0).

Palavras-chave: Antivirals, Hepatitis C Virus, Natural compounds, Replication in vitro
Chikungunya is a mosquito-borne infection caused by an Alphavirus, mostly characterized by fever, intense arthralgia and arthritis. The aim of this study was to describe epidemiologic features of this infection in Brazil, where it has recently been introduced, at the end of 2014. Thus, we analyzed the results of 1158 samples tested for CHIKV detection using real-time PCR and 5082 samples tested for anti-CHIKV specific antibodies using immunoenzymatic assay, from January 2015 to June 2017, in a Brazilian private lab. Regarding the molecular test, 5.8% of the samples were positive, while 32.9% were positive for specific IgG and IgM. In 2016, the greatest proportion of positive samples has been observed, 8.5% of those analyzed by PCR and 43.3% of those tested for IgG and IgM. There was no significant difference in gender as far as the molecular test is concerned (proportion of positive samples: 6.1% in women and 4.7% in men); however, when serology was considered, frequency of reactive samples for IgG and IgM tend to be greater among women than in men (35.7% versus 26.4%, respectively). Frequency of positive serology results varied considerably from one Federal State to another: positive IgG and IgM was verified in 52% of samples coming from Rio de Janeiro, 28% of those coming from Pernambuco, and 17% of those coming from Bahia, and 4.8%, 3.8%, and 1.2% of those coming from São Paulo, Paraná, and Rio Grande do Sul, respectively. Although any seasonal pattern had been observed in results of molecular test, positive results in serology tended to occur from May to August of 2016, reflecting mostly clustering cases from Rio de Janeiro. Both the molecular and the serologic tests had the greatest proportion of positive tests among adults (19 to 59 years old) and the elderly (60 years or older): regarding PCR, 5.3% of the adults and 10.2% of the elderly had positive results, while 38.33% and 47.42% of them had positive IgG and IgM, respectively. We concluded that serology is more often requested for Chikungunya investigation, probably due to timing when patients seek for medical assistency. Our data reflected mostly the outbreaks registered in Rio de Janeiro and Pernambuco in 2016, when the disease occurred mainly in adults and women.

**Palavras-chave:** Chikungunya, epidemiologic, real-time PCR, immunoenzymatic
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EPIDEMIOLOGY OF HEPATITIS B VIRUS INFECTION IN USERS OF ILLICIT DRUGS IN MACAPÁ, AP.

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Resumo

Hepatitis B is an infectious and contagious viral disease caused by the hepatitis B virus (HBV). The etiologic agent is a DNA virus, hepatovirus from Hepadnaviridae family. It is an immediate notification disease per represent public health problem in the country. The transmission occurs through unprotected sexual act; intravenously (shared needles and syringes during tattoos, piercings, dental or surgical procedures, etc.); objects for using of injectable or inhalable drugs; vertical transmission (mother/son); breastfeeding and accidents with contaminated sharp objects. In adults infected by HBV, 90 to 95% get healed; 5 to 10% remain with the virus for more than six months evolving to the chronic form of the disease that may result in cirrhosis and/or hepatocellular carcinoma. The suspicion of diagnostic may be forwarded by clinical and/or epidemiological data and the confirmation of the diagnosis is laboratory, performed by serological markers of HBV. The treatment differs depending on the hepatitis B stage. The work aimed to investigate the presence of hepatitis B in illicit drug users resident in Macapá-AP city. A standardized quiz was applied on socio-demographic data and 10 mL of blood collected from 49 drug users in 2015, were obtained from Mount Tabor community and Psychosocial Attention Center for alcohol and other drugs-AD CAPS; tested for the detection of HBsAg, anti-HBc and anti-HBs serological markers, by enzyme-linked immunosorbent assay (ELISA) using commercial reagents. Of the investigated, 40.82% had positive results for HBV infection. Most male (38.78%); single (65%); born in AP (65%); distribution according sex and age range, 34 to 48 years (50%) both sexes; monthly income less than 1 minimum wage (45%); incomplete elementary school (45%), and those with college degree incomplete/complete, feature the lowest percentage (5%). Of positive results, 95% were from Mount Tabor and 5% of the CAPS. The socio-demographic characteristics converged with other studies found, being predominantly male population, consisting of young adults, singles and with low salary and education level. The results pointed to the need for more effective public health actions, in health promotion, prevention and control of hepatitis B over this population, such as vaccination campaign against hepatitis B, available in SUS and, raise awareness and sexual education. Financial Support: CNPq.

Palavras-chave: Hepatitis B, Epidemiology, illicit drug users
Meningitis is a disease with a global distribution that constitutes a worldwide burden, with viruses as the primary etiologic agents. The range of viral meningitis severity depends mainly on age, immune status and etiological agent. The aim of this work was to investigate the suspected cases of viral meningitis using molecular techniques to confirm the viral infection. The diagnosed virus was correlated with clinical findings and cytochemical parameters in cerebrospinal liquid (CSF) of patients. CSF of 70 children with the presumptive diagnosis of viral meningitis was analyzed by real time PCR (qPCR). Viruses were identified by qPCR in 44 CSF samples (62.9%). Among them, 31 were identified as Enterovirus (ENTV) (70.4%), six as Human herpes virus 3 (HHV-3) (13.6%), five as Dengue virus (DENV) (11.7%), one as Human herpes virus 1-2 (2.3%) and one as Human herpes virus 5 (2.3%). Patients in the HHV-positive groups had increased percentage of polymorphonuclear neutrophils (PMN) (mean of 81%) while the groups of patients with DENV and ENTV had a mean of 30.9%. Results from the present study contribute to knowledge about the epidemiology of viral agents in pediatric CNS infections. The detection of DENV in 11% of CSF samples raises the relevance of DENV as the etiological agent in CNS infections occurring endemic areas. The correlation between the etiology of infection and the associated clinical features demonstrated the weakness of classical biomarkers (cytochemical CSF parameters) for diagnosis of pediatric CNS viral infections. Thus, molecular diagnosis is essential for the management of patients with CNS infections, especially now as Zika virus emerges in this population. Financial Support: Capes, CNPq, Fapemig, UFVJM

Palavras-chave: Meningitis, Dengue virus, enterovirus
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ETIOLOGY AND CLINICAL COURSE OF INFECTION IN PEDIATRIC CANCER PATIENTS WITH FEBRILE NEUTROPENIA

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Resumo

Patients with malignancies have long periods of severe neutropenia and are particularly vulnerable to infectious complications. The treatment with chemotherapy and the underlying disease promote depletion of immune system activity. Most episodes with febrile neutropenia are treated with empiric broad-spectrum antibacterial therapy without identifying the site or agent of infection, such as unexplained fever. Conventional diagnostic methods such as blood cultures are usually negative. Given the urgency of care to cancer patients and febrile patients needing rapid infection diagnosis, the search for new laboratory tests in order to promote high accuracy in identification of pathogens in these patients is imperative. Objective. To identify in blood samples the genome of EBV, CMV, HHV-6, HHV-7, PVB19, BKV and AdV in episodes of febrile neutropenia from pediatric oncology patients. Methodology. Fifty-eight samples of blood and serum were collected from patients with episodes of fever ≥38°C and absolute count of neutrophil ≤500/mm³ or ≤1,000/mm³, with an expected decrease below 500/mm³ within next 48 hours, using qPCR, nested-PCR or PCR methods. Blood and urine culture were collected with an internal protocol. Results. Positive viral DNA was found in 6/58 (8.6%) CMV; 10/58 (17.2%) PVB19; 2/58 (3.4%) BKV; 6/58 (10.3%) EBV, 12/58 (20.7%) HHV-6, 14/58 (24.1%) HHV-7 and none to AdV. Blood culture was positive in 4/58 (7%) patients and urine culture in 1/58 (1.7%). Signals and symptoms of infections diseases were found in 53/58 (91.4%) episodes. Roseola infantum was found in 6/58 (10.3%), symptoms in respiratory tract 18/58 (31%), nervous system 1/58 (1.7%), liver changes 28/58 (48.3%) and no signals of gastrointestinal tract. The presence of two or more microorganisms were found in 7/58 (12.1%) and 5/7 (71.4%) with two viral DNA detected and presence of symptoms of infection disease for respiratory system, 1/7 (14.3%) detect viral DNA CMV and HHV-6 with signal of roseola. Conclusion. This results shows that virus DNA detections, mainly EBV, CMV, HHV-6, HHV-7, PVB19, BKV and AdV, are frequent in children receiving cancer treatment with fever and neutropenia and that the virological monitoring is important to know the real etiologic agent of infection to avoid desnecessary antibiociocotherapy. Financial Support: FAPESP

Palavras-chave: Hematology-oncology, Herpesvirus, Neutropenia, Infection, Fever
Zika virus (ZIKV) is a positive-stranded single-stranded RNA arbovirus belonging to the Flaviviridae family of the genus Flavivirus. The virus arrived in Brazil in 2015 and has spread freely throughout the Americas. Although generally asymptomatic, ZIKV can cause fever, rash, myalgia, conjunctivitis and arthralgia, as well as microcephaly and Guillain-Barré syndrome. Due to the increase in cases of infection, the search and development of efficient antiviral drugs against Zika is extremely important. In this work, synthetic substances of the PFD series were tested. Thus, for the cytotoxicity assay procedure (CC50), VERO cells maintained in 96-well plates were treated with the substances tested at different concentrations (25, 50, 100, 200 and 400 μM) and incubated for 72 h at 37 °C in the atmosphere with 5% CO2 and after this time the supernatant was discarded and 100μL of MTT diluted in DMEM medium was added and re-incubated under the pre-conditions for three hours. After this period, the medium was discarded and 100μL of DMSO was added to the wells, followed by a new incubation of 30 minutes. Following these procedures, the plates were analyzed on an ELISA reader at 520 nm. In order to evaluate the lowest concentration of substances capable of inhibiting viral particle production (EC50) by 50%, VERO cells in 24-well plates were infected with ZIKV using 0.1 MOI for two hours at 37ºC in the atmosphere with 5% CO2. The plates were then treated with 40μM of the tested substances and incubated for 120h at 37 °C in 5% CO2 atmosphere. After this period, the cells were fixed and stained with 1% violet crystal in 10% formalin. To evaluate the concentration of substances with virucidal potential, the substances of interest were used. Using 1 μM eppendorf, a mixture of each drug at 10 μM concentration was made with Zika virus, one eppendorf of each mixture went to the oven at 37 °C and another to the freezer at 4 °C, being incubated for 2 h each, VERO cells were then used which were maintained in 24-well plates, and the plate was subsequently incubated at 37 °C in an atmosphere with 5% CO2 for 72h. After this period, the cells were stained with violet crystal solution for PFU count. In the obtained results, of the 21 substances tested, four present a high percentage of inhibition and high value of CC50. While PD20 and PD24 show a low CC50 and 100% inhibition the results obtained from the virucida were also satisfactory.

Palavras-chave: ZIKA VIRUS, ANTIVIRAL, SYNTHETICS MOLECULES
EVALUATION OF ANTIVIRAL EFFECT OF EXTRACTS OF TONTELEA SP. AGAINST ZIKA VIRUS

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Resumo

**Zika virus** (ZIKV) is a flavivirus that belongs to arbovirus (Arthropod-borne virus) group, and was able to infect humans. ZIKV infection can be transmitted in parallel ways to vector transmission, such as vertical transmission, which occurs in pregnant women who transmit to the fetus, and sexual transmission. No vaccine or antiviral therapy against ZIKV, and the search for antiviral was relevant. The aim of this study was to evaluate different extracts obtained of *Tontelea* sp against *Zika virus*. Before antiviral assay, cytotoxic assays were conducted to determine the cytotoxic concentration to 50% of the cells (CC50). For this purpose, mammalian cells (VERO) were added into 96 well microplates (5x10^⁴ cells/well), and after 24 hours, were treated with of four extracts at different concentrations. The revelation was obtained after 48h by MTT (methyl thiazol tetrazolium) colorimetric technique. We have detected the CC50 concentrations 181.912, 370.777, 224.959 and 351.472 µg/mL for hexane twigs (TGH), chloroform twigs (TGC), ethyl acetate leafs (TFAE) and methanolic leafs (TFM) extracts, respectively. For antivirals assays, the same cells pre-treated with extracts were infected with ZIKV at multiplicity of infection (MOI) of 0.1 virus/cell. 48 hours after infection (hpi), we found the protective/effective concentration to 50% of cells (EC50) and the results showed that TGH, TGC, TFAE and TFM were able to inhibit ZIKV at 38.66, 38.52, 74.19 and 83.05 µg/mL concentrations, respectively. Finally, the selective index (SI) was calculated which refers to the ratio between CC50 and EC50 each extract, which should be above 4.0. The SI were larger than 4 for the extracts TGH (SI=4.70), TGC (SI=9.60) and TFM (SI=4.23). The TFAE was considered toxic or non-selective. The TGC showed one excellent activity against ZIKV since its SI was much larger than 4. After this we can determined the virucidal concentration for TGH, TGC, TFAE and TFM extracts and its showed less than 15 µg/mL for TGH and TGC and less than 7 µg/mL for TFM and TFAE. The concentration able to block the viral adsorption were 7 µg/mL for TFAE and TFM and 62.5 µg/mL for TGC. Therefore, our data indicate potentials antiviral and virucidal actions against Zika virus in compounds present in the plant studied that belongs to Tontelea genus. We believe in deepening research with plant isolates. This step could be started in our laboratory. **Financial support:** FAPEMIG and CNPq.

**Palavras-chave:** Celastraceae, ZIKV, Virucidal, Antiviral
EVALUATION OF EPSTEIN-BARR VIRUS PRESENCE IN SAMPLES OF SALIVA FROM PATIENTS PRE- AND POST-RENAL TRANSPLANT

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Resumo

Epstein-Barr Virus (EBV), also known as Human gammaherpesvirus 4, infects about 95% of the world’s adult population and is transmitted primarily through saliva, which gives the possibility of further virus detection. The number of viral genome copies in saliva is high in HIV-infected and transplanted individuals. The main signs and symptoms related to EBV infection are headache, lymphadenopathy and fever. Renal transplant patients are more susceptible to the development of clinical complications related to EBV infection, once they use immunosuppressive medication. The objective of the present study was to investigate the presence of EBV in samples of saliva before and after renal transplantation and to correlate the positivity with the main signs and symptoms of the infection. One hundred saliva samples were collected from 50 patients transplanted and followed at Ophir Loyola Hospital, one sample being pre-transplant and another 30 days after transplantation. DNA extraction (Qiagen DNA mini-Qiagen Kit) followed by qPCR (Kit qPCRAlert EBV®-NANOGEM) was performed. Data were collected from the patients’ medical records in order to relate clinical symptoms to the positivity of the samples. Of the pre-transplant samples, 64% (32/50) presented positivity. Among the post-transplant samples, 74% (37/50) were positive. Positivity was not related between pre- and post-transplantation (McNemar - p=0.0574). To date, 28 hospitalization records have been analyzed, of which 11 patients presented clinical symptoms related to EBV. The relationship between positivity and symptomatology was analyzed for both periods and it was observed that all patients who presented clinical symptoms were positive in the post-transplant sample and those who did not present positivity in this period were defined as asymptomatic (Fisher bi - p=0.0001). A greater number of positive results were observed in post-transplant samples and there was an association of symptoms with positivity in this period. Saliva is a non-invasive collection sample and EBV detection results have been shown to be related to the patient’s clinical condition. Therefore, we suggest the use of this biological specimen for the monitoring of infection in renal transplant patients.

Palavras-chave: EBV, immunosuppression, saliva, renal transplant, qPCR
The main prophylactic form for animals susceptible to rabies is the immunization, whereas when clinical signs manifest, there isn’t effective treatment. Some European countries require an International Animal Health Certificate for the entry of the animals into the country of destination, therefore the animals must have an titer of rabies virus neutralizing antibodies (VNA) ≥ 0.50 IU/mL for adequate protection against rabies. A serological evaluation was carried out at the Pasteur Institute between 2006 and 2011, where was demonstrate a titer 10 times higher than the minimum required in the serum samples of felines. The inactivation of feline’s serum is important because the complement system can continue to active after inactivation at 56 ° C for 30 minutes. The objective of this study was to evaluate the usual method of inactivation and the complement system interference in samples of feline’s serum for the determination of neutralizing antibodies to rabies virus. Twenty-three feline’s serum samples immunized against rabies were analyzed, aliquoted and inactivated at 30 minutes in a water bath at 56°C and 65°C and submitted to the Rapid Fluorescence Inhibition Microtest, to quantify rabies neutralizing antibodies. The results showed significant differences of the titles obtained with inactivation for 30 minutes at 56°C (GM: 0.75 IU/mL, Max: 477.96 IU/mL, Min: 0.01 IU/mL), and the same samples when tested at 65°C (GM: 0.25 IU/mL, Max: 47.8 IU/mL, Min: 0.03 IU/mL). At both temperatures nine serum had results <0.5 IU/mL. In conclusion the serum heat-inactivation at 65°C demonstrate efficient to inactivate complement system showing a decrease of VNA titers. Financial Support: Instituto Pasteur - SES

Palavras-chave: CATS, COMPLEMENT SYSTEM, NEUTRALIZATION, RABIES VIRUS
EVALUATION OF THE ANTIVIRAL ACTIVITY OF COMPOUNDS EXTRACTED FROM PLANTS AGAINST AICHI VIRUS AND HUMAN HERPESVIRUS 1

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Resumo

Researchers have been looking for nature substances capable of inhibit microorganisms, which can be extracted from various sources such as animals, plants, invertebrates and bacteria. Drugs for the viral diseases treatment are less than the drugs available for the treatment of other infectious diseases. This generated interest in synthesizing new antiviral drugs, bringing benefits to society as a whole. In this context, it has been widely reported the use of natural products as a material for the antiviral drugs development. This study aims to evaluate the antiviral activity of compounds isolated from medicinal plants native to the Brazilian region “Centro Oeste”. Three substances extracted from the leaf of the medicinal plant *Schinus terebinthifolius* Raddi, popularly known in Brazil as "aroeira da praia", were studied. The extraction was performed with 100% methanol, producing two phenolic compounds (STF-1 and STF-2) and one flavonoid (STF-4). The antiviral assay was performed against *Aichi virus* and *Human herpesvirus 1* (HHV-1). Cell cultures of the Vero lineage were performed and the cytopathic effect was observed by inverted microscope. The cytotoxic assay was performed by the MTT method and the antiviral activity was determined by the viral titer reduction using the Reed and Muench statistical method, expressed as Viral Inhibition Index (VII) and percentage inhibition (PI). The assays showed that the phenolic compound STF-2 had inhibition above 90% against both viruses (HHV-1 and *Aichi virus*) at concentrations between 62 μg/mL and 15 μg/mL, and the flavonoid compound STF-4 showed a PI of 90% against *Aichi virus*, at concentrations of 62 μg/mL to 31 μg/mL. However, no inhibition was obtained against HHV-1. The phenolic compound STF-1 presented no inhibition against any of the viruses. The results indicate that two substances tested showed antiviral activity, allowing potential as antiviral drugs.

Palavras-chave: Aichi virus, antiviral drugs, Human herpesvirus 1, Schinus terebinthifolius Raddi
In Brazil, after the introduction of Chikungunya virus (CHIKV) and Zika virus (ZIKV) from 2014, the country lives a simultaneous circulation of different arboviruses. The Central-West region is considered an endemic area for arbovirus of global impact such as dengue and yellow fever, with the metropolitan region of Goiânia presenting high infestation of the genus *Aedes* mosquitoes and the highest dengue incidence in the country. In this context, a molecular investigation of the CHIKV and ZIKV viruses was proposed in cases of individuals with exanthematic disease, treated in the public health system of Goiânia from April to June 2016. Serum and urine samples were collected from 48 patients, eight to sixty years old, that was submitted to viral RNA detection by real-time RT-PCR, using the Path-ID™ Multiplex One-Step RT-PCR Kit.

Of the 48 evaluated individuals, none showed CHIKV positivity, however, an overall positivity index for ZIKV of 64.6% was observed (31/48). No statistical difference was observed in relation to age and gender, although higher positivity among males 83.3% (10/12). Considering the female population, 28.6% (6/21) of the women with ZIKV infection were pregnant. The symptoms most frequently reported in the investigated population were myalgia, conjunctivitis and fever, and a variation was observed in the infected individuals in relation to the age groups. In the individuals up to 39 years of age the most frequent symptom was myalgia (45.4%), followed by conjunctivitis (27.2%) and fever (22.7%), whereas in individuals older than 40 years, conjunctivitis (55.5%) was followed by myalgia (22.2%) and fever (11.1%). Considering the similarity of the clinical picture between the different endemic arboviruses in the region and the high risk of unfavorable outcomes in pregnant women and concepts exposed to ZIKV, molecular investigation for the different viral agents becomes essential for confirmation of cases, contributing with data for the virological and epidemiological surveillance. Financial Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)

**Palavras-chave:** Arbovirose, Exanthematic, Real-time, Zika virus
Dengue is the most prevalent viral vector-borne disease worldwide, with around half the world’s population estimated to be at risk of infection. Currently, is the most important infectious disease in Brazil in terms of epidemiological impact. Interactions among patient’s immune status, age, comorbidities, and many other factors contribute to the disease’s complexity. Despite the efforts, there is no effective vaccine against the four dengue serotypes yet as well as efficient medicines in the treatment of patients. Therefore, several studies have been conducted to search for substances with antiviral activity against Dengue virus (DENV) and other flaviviruses and medicinal plants have been demonstrated as an important source of bioactive compounds. Bauhinia holophylla (Bong.) Steud. is a woody species from Cerrado, of which leaves are used in traditional medicine on treatment of infections and Diabetes Mellitus. Phytochemical studies indicate the presence of steroids glycosides, triterpenes, lactones and flavonoids in its leaves. So, the aim of this study was to evaluate the anti-dengue activity of extraction / fraction / subfraction of B. holophylla leaves. Bioassays were performed with DENV-2 strains and Baby Hamster Kidney cells (BHK-21). Initially the cells were exposed to increasing concentrations of extraction / fraction / subfraction, ranging 3.12 - 200 µg/mL, to determined the concentration that decreases 50% of the cell viability (CC50). Subsequently, in the global activity antiviral assay, infected cells were DENV-2 (MOI 0.01) and treated with the extraction / fraction / subfraction to determine the effective concentration of 50% infected cells (EC50), by MTT method. Selectivity Index (SI) was calculated by the ratio of CC50 to EC50. The CC50 results obtained were 89.59 to EHE; 98.90 µg/mL to FrAcOEt and 112.98 µg/mL to sFrAcOEt. The antiviral activity was observed in all extract / fraction / subfraction evaluated. The hydroethanolic extract had promising activity with EC50 of <3.25 µg/mL an SI 27.56. These preliminary data showed that B. holophylla bioactive compounds can are potential candidates for anti-DENV drugs development.

**Palavras-chave:** Bauhinia holophylla, Dengue virus, Extract, Antiviral
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Resumo

The most severe Yellow Fever epidemic recorded in Brazil in recent decades was reported in 2017 with 446 confirmed cases and 159 confirmed deaths in Minas Gerais (MG) state. The aim of this study was to do a prospective study of yellow fever virus in 96 patients admitted to the Eduardo de Menezes Hospital (HEM)-MG, with suspicion of YFV infection, in Jan/17. The patients were from eight mesoregions of MG, mainly from Vale do Rio Doce. The majority of patients were men, mostly from 22-59 years old. Sixty-six patients lived in a rural area and 21 lived in an urban area. Sera were collect and used in routine YFV and DENV IgM tests, RT-qPCR, and in viral isolation. Twenty-three patients were YFV negative, four of them had arbovirus infection. A total of 73 patients had the diagnosis of YF based only on PCR (38), on viral isolation (03), serology (42) or serology and PCR (22) Among the YFV positive patients, 12 received YFV vaccine before the recent infection. Two of these patients died, and both took YFV vaccine 15 days before de onset of the symptoms. Nineteen patients evolved to death, mainly alcoholics, men, 30-60 years old, who lived in rural area. The 54 patients who recovered were followed up for at least four months after the medical release. Interestingly, after approximately 60 days after medical release, 11 patients reported asthenia and tiredness, and the values of hepatic enzymes AST and ALT that were previously decreasing, presented an increase. During this time, we tested urine and serum from these patients, using YFV RT-qPCR,and all were negative. All these patient’s samples were tested by PCR for DENV, ZIKV, CHIKV, and others viruses that can cause hepatitis, and all were negative. One patient (male, 51 years old and farmer) has a liver biopsy collected 60 after the medical release, and it was tested positive in PCR. Liver enzyme values were also altered in this patient (AST 987U/L; ALT 1285U/L). As far as we know this condition has never been described after acute yellow fever and demonstrate the importance of the follow up of the patients after de medical release. Further studies are needed for better understanding of the disease course including the convalescence period. YELLOW FEVER GROUP: other members: HEM-MG: G.L.Milanez, T.Drummond, FUNED-MG: T.E.R.Adelino; G.Carvalho, M.Oliveira; IRR: M.Pascoal, C.E.Calvazarra, J.G.de Oliveira, UFMG: M.S., Arruda, G.S.Trindade, E.G.Kroon. FINANCIAL SUPPORT: FAPEMIG, CNPq, CAPES, SES-MG

Palavras-chave: Arbovirus, Hepatitis, Yellow fever, Yellow fever virus
Introduction: Respiratory infection by influenza A virus is a major health problem worldwide. Studies about the molecular evolution of influenza A support vaccine recommendation and help the understanding of viral determinants of infection. Objectives: To analyze the mutations related to virulence and the evolution of influenza A/H1N1pdm09 from clinical samples collected during the 2009–2015 seasons in Rio Grande do Sul, RS. Methods: Phylogenetic analysis was conducted on 56 strains with full-length nucleotide sequences for all 8 segments and compared with other sequences of South America obtained in an influenza database (Fludb.org). A modeling test was performed to show de amino acid changes in the HA and NA molecular structures. Results: HA presented 45 substitutions sites in post-pandemic strains versus 17 sites in pandemic strains, and NA presented 30 versus 13 substitutions sites, respectively. No resistance mutations in NA were found and there was no association between any specific amino acid substitution and death. Nevertheless, mutations observed only in some fatality cases might be associated with disease outcome, such as HA-D239G, which was found in two of nine fatality cases. All sequences from the 2009 pandemic AH1N1pdm09 viruses exhibited a ladder-like topology, characteristic of viruses subject to continuous antigenic drift, typical of human seasonal influenza viruses. Specific mutations that characterized the different seasons were observed, as well as an accumulation of mutations mainly in the 2013 strains. During this period distinct clusters of isolates from Rio Grande do Sul were observed, according to each season, with more than 80% of isolates within the cluster sampled in this geographical region. This temporal segregation of Brazilian samples may suggest a tendency of positive selection of AH1N1pdm09 with better adaptation to propagate than the predecessor strains. Conclusion: Genomic comparison of IAV strains reconstituting its genetic evolution may contribute to understanding the seasonality of outbreaks, the range of disease severity and for the development of vaccines.

**Palavras-chave:** Influenza AH1N1pdm09 virus, viral mutations, phylogenetic analysis
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GENETIC DIVERSITY OF CURRENT INFLUENZA B VIRUS STRAINS DETECTED IN THE NORTH AND NORTHEAST REGIONS OF BRAZIL.

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Resumo

Influenza B virus is one of the etiological agents of respiratory disease. Nowadays, there are two antigenic and genetically distinct lineages of this virus (Yamagata and Victoria), co-circulating among human population, and causing seasonal epidemics with significant proportion associated with morbidity and mortality. The viral particle has two surface glycoproteins: hemagglutinin (HA) and neuraminidase (NA). These proteins undergo a big selective pressure that may induce the occurrence of mutations supporting the virus escape from the immune system and the resistance to antiviral drugs. Thus, the genetic diversity of influenza B virus may impact in the vaccine formulation and also a possible treatment failure. In this context, influenza B virus strains circulating in the North and Northeast regions in Brazil during 2017 were genetically characterized. The methodology involved extraction of viral nucleic acid followed by RT-PCR (polymerase chain reaction preceded by reverse transcription) for HA and NA genes amplification and their sequencing. A total of 43 strains were analyzed, with 19 belonging to the Victoria lineage and 24 to Yamagata lineage. The circulation pattern analysis showed that the spread of two lineages of influenza B virus were detected in both regions of Brazil. An amino acidic substitution (E105K) was identified in the NA gene, representing a mutation that confers resistance to the antiviral used in the treatment of influenza. Therefore, in view of the circulation of the two lineages and the occurrence of a resistant strain of the lineage that is not contained in the vaccine, the adoption of a quadrivalent immunization against influenza would be more efficient, as it would contain strains for influenza A (H1N1 and H3N2) and both strains of influenza B virus, thus ensuring better protection. This disagreement between circulating strains and vaccine strains demonstrates the real need for virus surveillance for the acquisition of a vaccine suitable for the southern hemisphere.

Palavras-chave: Influenza B virus, genetically characterize, respiratory infections
The cytomegalovirus (CMV) is the most common agent of congenital infection in the world. It is not clear why some newborns with congenital CMV infection are asymptomatic and others have symptoms. One of the major glycoproteins of the virus is the glycoprotein B (gB). This protein is polymorphic and highly immunogenic. Most of the wild viral strains are grouped into four major gB genotypes. Recognizing the vital role of this glycoprotein in virus-host interaction, it is likely that different genotypes are associated with virulence and different clinical outcomes. In fact, it has been suggested that these genotypes may be associated with the risk of developing the disease due to a specific tissue tropism or some mechanism to facilitate viral replication. In this study, we have performed a neonatal screening for detection of asymptomatic congenital CMV infection. We included all newborns under three weeks who were admitted to the Intensive Care Unit of the Hospital Manoel Novaes, in Itabuna, Bahia. Urine samples are being collected in collectors bags, and subsequently, saliva samples also have been collected with sterile swabs. Samples of saliva and urine collected are subjected to PCR without prior DNA extraction. Children with congenital infection will be assessed and monitored by a medical team until the second year of life. If necessary, they will be treated. The genotypes of CMV glycoprotein B will be determined by RFLP. By the time, we collected samples of 206 children. There was only two positivity in the samples, incidence of 1%.

Palavras-chave: CMV, Glycoprotein B, genotype, ICU
HANTAVIRUS SEROSURVEY IN CENTRAL AND SOUTHERN BRAZIL, 2012-2016 SENT TO INSTITUTO ADOLFO LUTZ – NÚCLEO DE DOENÇAS DE TRANSMISSÃO VETERINÁRIA.

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Resumo

Hantavirosis are anthropozoonosis of compulsory notification, caused by a virus from the Bunyaviridae family, belonging to the genus Hantavirus. The human infection can vary from asymptomatic or acute nonspecific febrile disease to its classic forms, known as Hemorrhagic Fever with Renal Syndrome (FHSR) and Hantavirus Cardiopulmonary Syndrome (SCPH), the later, occurring in Brazil. Hantaviruses circulate naturally among different groups of mammals, and wild rodents of the family Muridae and Cricetidae are particularly effective in transmitting the virus to humans. Viral infection in rodents is not lethal and the virus is eliminated for all life. Humans can be infected by contact with aerosols of saliva, urine, and rodent feces. Cases of Hantavirus are associated with rural workers, with a higher incidence in male. In order to evaluate the occurrence of Hantavirus infection in the Central and Southern regions of Brazil, and the seroprevalence of infection by gender, we survey samples from patients sent to the reference laboratory for the serological diagnosis of hantavirus. In the period January/2012 to December/2016, a total of 5,353 samples were sent for enzyme-linked immunosorbent assay (ELISA) tests for the detection of M-class antibodies (IgM) against Hantavirus. The laboratory data obtained were as follows: 93.6% (5,012/5,353) of the samples obtained non-reactive results, while 5.4% (288/5,353) were reagents and 1% (53/5,353) were inconclusive. The reactive samples, 73% (210/288) were from male patients and 27% (78/288) from female patients. Regarding the states of occurrence, 32.65% (94/288) were from Santa Catarina, 24.75% (71/288) from São Paulo, 16.67% (48/288) from Rio Grande do Sul, 9% (26/288) from Mato Grosso, 5.55% (16/288) from the Federal District Area, 4.17% (12/288) from Paraná, 3.47% (10/288) from Minas Gerais, 2.43% (7/288) from Goiás, 1% (3/288) from Mato Grosso do Sul and 0.34% (1/288) from Espírito Santo. The data obtained in this work are in agreement with the data of the literature, since the occurrence of reagent samples was higher in the South region, traditional zone of grain production in small family farms, and in male individuals, which are majorities in this economic activity. Financial Support: SES

Palavras-chave: Hantavirus, Rodents, Serosurvey
Hemagglutinin (HA) is a protein that makes up the influenza virus viral capsid, responsible for viral adsorption of the host cell. It is the primary component of the annual vaccine against a complaint. Studies in which the products are innovative are capable of promoting a significant stimulus in the immune response, and HA is a ces products. In influenza infection, there is an uncontrolled immune response with a production of several cytokines that culminate, in addition to cellular apoptosis, in a cell death pathway, a new one, a necroptosis. The literature demonstrates that it is a necroptosis and is dependent on the activation of RIPK1. In addition, our data revealed that TLR4 is important for necroptosis and its activity related to the production of TNF-α, a cytokine produced mainly by macrophages. We evaluated an ability of HA in the activation of TLR4. Murine macrophages were stimulated with LPS; THERE IS; Inhibitor pan-caspases (zVAD); Inhibitor of TLR4 (CLI095), inhibitor of RIPK1 (Nec-1) and anti-TNF-α antibody. Macrophages were labeled with Anexin V and PI for analyzes of cell death by Flow cytometry. The extracellular LDH was quantified by the LDH DOLES kit. A production of TNF-α was quantified by the ELISA technique. Through Western Blot they evaluate a phosphorylated RIPK1 activation. Treatment with macrophages induced by TNF + zVAD (positive control of necroptosis) was similar in the HA group. Pre-treatment with Nec-1 inhibited the necroptosis phenomenon in HA-stimulated macrophages, and in addition, we found that HA-induced necroptosis in macrophages occurred in a manner dependent on the production of TNF-α by TLR4 activation, once That as cells pretreated with CLI095 also fear inhibition of TNF-a production and necroptosis. Our data demonstrated that it is capable of inducing a necroptosis, and that this phenomenon occurs in a way dependent on the production of TNF-α through the activation of TLR4. Since several studies demonstrate that necroptosis can trigger a series of events that contribute to a deleterious non-host outcome, and if our data to date show that the induction of the necroptosis phenotype can be stimulated by HA, one can To conclude, to date, that this cell death pathway may act as a mechanism for potentiating the infection. However, new methods are needed to establish a better understanding of the mechanisms involved between HA and necroptosis.

**Palavras-chave:** HEMAGGLUTININ, NECROPTOSIS, MACROPHAGES, TNF, PRODUCTION
Current protocols that use peripheral blood samples may provide important data regarding the vaccine-induced immune response. However, a range of distinct components of whole blood samples are required and the different anticoagulant systems employed may impair some properties of the biological sample and interfere with functional assays. Although the interference of heparin in functional assays for viral neutralizing antibodies such as the functional plaque-reduction neutralization test (PRNT), has been well characterized, the development of pre-analytical treatments to remove heparin is still required for the establishment of optimized protocols. The present study intended to optimize and evaluate the performance of pre-treatment of heparin-collected blood samples with ecteola-cellulose (ECT) to provide accurate measurement of anti-Yellow Fever Virus (YFV) neutralizing antibodies by PRNT. Our data confirmed the interference of heparin on PRNT reactivity in a dose-responsive mode. Distinct sets of conditions for ECT pre-treatment were tested to optimize the heparin removal and all sets were able to reduce heparin interference on PRNT titers. The optimized protocol was pre-validated to determine the effectiveness of heparin plasma:ECT treatment to restore the PRNT titers as compared to serum samples, confirming the interference of heparin on the PRNT test as well as the effectiveness of heparin plasma:ECT 1:2 treatment to restore the original PRNT titer observed in serum samples. The validation and comparative performance were carried out by using a large range of serum (1,190 samples), vs heparin plasma:ECT 1:2 paired samples obtained from unvaccinated and 17DD-YFV primary vaccinated subjects and showed the ability of ECT treatment for accurate measurements of PRNT titers using heparin plasma samples. Furthermore, these data demonstrated that the use of heparin plasma:ECT 1:2 and serum samples yielded a similar pattern of PRNT reactivity distribution for the study population. Altogether, these findings support the use of heparin plasma:ECT samples for accurate measurement of anti-YFV neutralizing antibodies. Financial Support: FAPEMIG, CNPq, Programa Nacional de Imunizações - PNI, Secretaria de Vigilância em Saúde - SVS, Bio-Manguinhos/FIOCRUZ, Instituto René Rachou/FIOCRUZ e CAPES.

Palavras-chave: Ecteola-cellulose, Heparin, PRNT, Vaccinology, Yellow Fever Virus
Dengue is a major global public health problem, which is caused by infection with Dengue virus (DENV). DENV adheres to host cells through interaction of viral glycoprotein E and the DC-SIGN receptor (Dendritic Cell-Specific ICAM-3 Grabbing Non-integrin) by its Carbohydrates Recognition Domain. This receptor is encoded by the CD209 gene which undergoes alternative splicing generating several different membrane and soluble protein isoforms. Membrane isoforms are present mainly on the surface of human macrophages (Mac) and immature Dendritic Dells (iDC). Despite the knowledge of the mDC-SIGN envelopment in DENV infection, the role of soluble isoforms in this process is not well known. Therefore, the aim of this study was to evaluate the role of three soluble DC-SIGN isoforms in DENV infection process. The nucleotide sequence of sDC-SIGN1A type III (iso8), sDC-SIGN1B type I (iso10) and sDC-SIGN1B type III (iso12) isoforms were obtained from GenBank, synthesized and cloned into expression vectors pJ414-SR, pD454 and pET28a, respectively. The heterologous expression of iso8 was carried out at 37 °C for 18 hours and of iso10 and iso12 at 37 °C for 4 hours, with IPTG 0.5 mM. Escherichia coli BL21 DE3 Rosetta strain was used for heterologous expression. The three proteins were purified by affinity chromatography on a cobalt column and results analyzed by SDS-PAGE and Western blot. The isoforms were produced and the concentrations were 0.51 mg/mL (iso8), 0.45 mg/mL (iso10) and 0.56 mg/mL (iso12). DENV-2 was grown in Aedes albopictus C6/36 cell line and quantified by the TCID50 assay in BHK-21 cells. In order to obtain human (Mac) and iDC, THP-1 cells were stimulated by Myristoyl Acetate (PMA) at 100ng/mL for 48 hours to differentiate into iDC. Differentiation was analyzed by flow cytometry through respective antibodies in the titers: CD11c- 1:75; CD80- 1:75; CD86- 1:25; CD14- 1:75 and CD209- 1:50. THP-1 cells were standardized as a model to iDC differentiation by expression of CD86, CD209, CD11c and non-expression of CD80 (DC maturation marker). Once these results were obtained, in vitro functional assays will be performed in monocytes (THP-1 cell line), Mac and iDC with different concentrations of the three recombinant proteins to evaluate the influence of the isoforms in the DENV infection process.

Palavras-chave: DC-SIGN, CD-209, Dengue virus, heterologous expression, Immunophenotyping
HIGH INCIDENCE OF ERYTHROPARVOVIRUS (B19) IN PATIENTS WITH CLINICAL EXANTHEMATIC SYMPTOMS IN SÃO PAULO CITY.

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Resumo

Parvovirus B19, also known as B19 virus or erythrovirus B19, was recently renamed as Primate erythroparvovirus. The virus that was discovered in 1975 belongs to the Parvoviridae family, Parovirinae subfamily, Erythroparvovirus genus. It is a small virus measuring only 23-26nm, the name reflects his size, since parvum means small in Latin. B19 is a non-enveloped icosahedral virus that contains a linear positive or negative ssDNA genome of approximately 5.6Kb that encodes three proteins: a nonstructural protein, NS1, and two structural proteins, VP1 (83 kDa) and VP2 (58 kDa). B19 transmission occurs by infected respiratory droplets, by blood-borne and by mother-to-child transmission. Infection with B19 is common in schoolchildren (5 to 9 years of age) but can also affect adults. Although asymptomatic cases are common, several diseases are related to B19 infection, as the fifth disease (or erythema infectiosum) in children, which B19 is the etiological agent. In adults arthralgias and arthritis are frequently reported associated with B19, as a result of deposition of immune complexes. Outbreaks can arise in schools and nurseries from time to time. Our objective with this study was to monitor the clinical incidence of B19 in the city of São Paulo between 2013 and 2017 in different samples from patients with exanthematic disease attended in different hospitals in São Paulo City. These samples were extracted by automatic platform NUCLISENS® easyMag® (BioMerieux Lyon, France), then a small NS1 region was amplified by Semi-Nested PCR reaction in an One Step reaction with In House primers (PCR: nt1395-1422 to nt1657-1684; Semi-Nested: nt1579-1599 to nt1657-1684) and analyzed in agarose gel electrophoresis to detect the viral genome. Result: Of the 56 patients with clinical exanthem 11 (19.6%) were positive by PCR B19. These results show the high incidence of the virus in São Paulo and the importance of B19 surveillance. Financial Support: Research funded by: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

Palavras-chave: Erythroparvovirus B19, Diagnosis, Exantema, Epidemiology
The HTLV-1 (Human T-lymphotropic virus 1) induces myelopathy (HAM/TSP), adult T-cell leukemia (ATL) and other inflammatory and rheumatologic diseases. The HTLV-1 Tax protein is a regulator of gene expression, highly immunogenic being suggested in several studies that anti-Tax antibodies are involved in the pathogenesis of HAM/TSP. The aim of this study was to evaluate the anti-Tax-IgG reactivity in HTLV-1 infected individuals from a Brazilian and Argentinean cohorts and to correlate the levels of anti-Tax antibodies with clinical condition and sex. A total of 154 individual were enrolled from Brazilian cohort: 34 seronegative (NP-Br), 48 HTLV-1 asymptomatic carriers (AC-Br) and 47 with HAM/TSP (HT-Br); and 25 from Argentinean cohort: 10 symptomatic (Sym-Ar; 9 female and 1 male; age mean 52.1) and 15 asymptomatic (AC-Ar; 4 female and 11 male; age mean 38.9). The anti-Tax-IgG reactivity was assessed using an in house ELISA based on a prokaryotic recombinant C-terminal Tax. The Brazilian group with HAM/TSP installed showed the greatest reactivity for the Tax recombinant protein, with the highest O.D mean, followed by the symptomatic group from Argentina (Mean O.D: HT-Br 1.335 > Sym-Ar 1.091 > AC-Ar 0.824 > AC-Br 0.6742). It is interesting to highlight that the difference between mean O.D for individuals asymptomatic and symptomatic from Brazilian cohort was significant (p<0.0001), while these difference among individuals from Argentinean cohort was not (p=0.5579) and that 73.3% of the individuals asymptomatic carriers from Argentinean cohort showed reactivity to Tax (while for the Brazilian cohort this number was 36.17% and world data shown 25-60%). The asymptomatic individuals from Argentinean and Brazilian cohorts presented, respectively, 20 and 18.75% of samples showing anti-Tax O.D higher than the mean of HAM/TSP patients. In the Argentinean cohort, of the total number of reactives, 66.6% (10) were female, while 33.3% (5) were male. In the Sym-Arg group, female represented 87.5% of reactive individuals despite being a group with few samples, this tendency has been observed in other studies. Although the anti-Tax response per se is not assumed as a biomarker to predict an infected patient to be at risk to develop HAM/TSP and other neurological disorders, the asymptomatic individuals from both cohorts with high levels of antibodies seen in this study should be followed at shorter time intervals once they could be in risk for worst outcome.

Palavras-chave: HTLV-1, Tax, HAM/TSP, ELISA
Introduction. Human Herpesvirus 7 (HHV-7) is one of the causative agents of exanthema subitum, belongs to the Betaherpesvirinae, along with Cytomegalovirus (CMV) and human herpesvirus 6 (HHV6), one of the three subfamilies of the human herpesviridae. HHV-7 has a specific tropism for CD4+ lymphocytes and neurons. Primary HHV-7 infection delayed into adolescence can cause serious neurologic disease. During acute primary infection, viral DNA can be found in cell free body fluids by PCR. After the viremic phase, the virus is able to establish latency in several body sites and cell types, including CD4+, PBMC, CNS, skin and salivary gland epithelial cells. Immunocompetent hosts with CNS HHV-7 infection are usually young children that develop convulsions and rarely encephalopathy or adults presenting with encephalitis and flaccid paraplegia. The aim of this work is to study the link of HHV-7 infection in patients with HHV-7 DNA in CSF and symptoms of neurological disorders. Methodology. The study included patients with who had neurologic disease seen at the Clinics Hospital, Campinas/SP, Brazil, whose cerebrospinal fluid (CSF) was collected and examined by polymerase chain reaction by all 8 human herpesvirus: HSV-1 and 2, Varicella-zoster, Epstein-Barr, cytomegalovirus, HHV-6 (A and B) HHV-7 and Herpesvirus 8 (HHV-8). PCR for viral genomes was run on cerebral spinal fluid (CSF) after performing lumbar puncture, resulting positive only for HHV-7 DNA. Results. HHV-7 DNA was detected in the CSF in 7 (3.1%) of the 223 patients with symptoms of neurological disorders tested. Four patients had symptoms of meningitis, two had multiple sclerosis and one had myelitis as cause of neurological syndromes. Conclusion. Our work is important because it describes cases of HHV-7 CNS infection due to reactivation in immunocompetent and with autoimmune diseases hosts, like as multiple sclerosis, suggesting the utility to expand investigation on CSF via PCR for Viral DNA including HHV-7, especially in immunocompetent patients with meningitis and myelitis presenting focal neurological signs. Financial Support: FAPESP

Palavras-chave: HHV-7, Infection, Neurological, Disorders, Trigger Agent
HUMAN TONSILLAR TISSUES HARBOR INFECTION AND RELEASE OF VIABLE INFLUENZA A VIRUS

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Resumo

Influenza viruses cause more than two million annual episodes of seasonal acute respiratory infections (ARI) and approximately 500 thousand deaths worldwide. Evidence from several models indicate that depending on virus strain and host immune status, acute infections by seasonal influenza A virus may reach sites other than the respiratory tract, such as kidneys, intestines, mucosal lymphoid tissues and lymph nodes. Infection of lymphoid cells by influenza virus raises the possibility that these cells could represent potential persisting sites of infection. In the present study, influenza A virus nucleic acids and antigens were searched for in tissues of palatine tonsils and adenoids removed from patients who underwent surgery for tonsillar hypertrophy, in the absence of ARI symptoms. A qRT-PCR screening revealed that tonsillar tissues from 6 patients were positive for Influenza A virus. Flow cytometry analysis of dispersed tissue fragments, naturally infected, indicated that expression of FLUA nucleoprotein in CD8+ lymphocytes and CD19+ cells was substantially higher than control samples. We next sought to investigate whether these lymphoid tissues could be sites of viral replication, and so, sources of viable viral particles. 24-well plates were seeded with MDCK and inoculated with tissue lysates. After 4 d.p.i., cytopathic effect was strong for 2 samples. Infected cells were collected and IF approaches showed strong staining of FLU A nucleoprotein. These data indicates that lymphoid tissues not only harbor expression of viral proteins, but also releasing of infectious virus. To further understand the impact of FLU A infection of T and B lymphocytes we performed in vitro infection of lympho-monoenuclear cells enriched from FLU A negative tissues. Infection with three different FLU A subtypes was performed at different MOI and supernatant were collected at 12, 24, 36 and 48 h.p.i. Release of TNF-a, IFN-b and IL-10 was measured by ELISA according to kit manufacturer’s guidelines. Interestingly, infection of lympho-monoenuclear cells from tonsillar tissues induced release of IL-10 in high concentrations. These results suggests that FLU infection in lymphoid tissues leads to anti-inflammatory cytokine production, which can promote viral maintenance in the tissue. Also, further investigation is currently underway to understand the spreading pattern and pathogenesis of FLUA in human tonsils. Financial support: CAPES, CNPq, FAPESP.

Palavras-chave: Influenza A, Asymptomatic Infection, Lymphoid tissue, viral maintenance
IDENTIFICATION AND CHARACTERIZATION OF GENOTYPE III OF THE HEPATITIS DELTA VIRUS IN INDIGENOUS POPULATION IN THE WESTERN AMAZON REGION OF BRAZIL.

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Resumo

Introduction: Delta hepatitis virus (HDV) infection is endemic in the legal Amazon. Viral genetic diversity is related to the geography calorig in of the isolates. HDV has eight genotypes distributed globally. Genotype 3 (HDV-3) is prevalent in hepatitis B and HDV infection in Amazonia and widely known to develop a more severe infection with rapid progression of liver disease. The state of Rondonia (RO), located in the North country in western Amazonia, presents characteristics of a region of high endemicity for viral hepatitis. Few studies involving genotype analyzes and genetic variations of HDV were performed in the region, mainly in indigenous ethnicities. Objective: the present study aims to identify the presence and genotypic characteristics of individuals with the HDV-3 genotype in the indigenous population of the western Amazon region. Methodology: The study was approved by CEP / CONEP. The population participating in this study are 129 indigenous people belong in to the ethnic group Wari polo of Guajará Mirim. Until now 23 serum sample shave been collected In this population. Results: Molecular tests were performed on the 23 samples for detection of HDV RNA by RT-PCR/Nested. All positive samples were submitted to sequencing. The genotypes identified were correlated with the clinical characteristics presented. Until now has been observed the presence of the HDV-3 genotype in the analyzed samples. Conclusion: Therefore it was possible to confirm the presence of genotype 3 related to the indigenous population. To elucidate the dynamics of the virus, specifically in this population and region philodynamic and phylogenetic analyzes are being carried out. Key Words: HDV, HBV, Infection, genotypes. financing: FAPERO, CAPES.

Palavras-chave: HDV, CHARACTERIZATION, INDIGENOUS
Identification of the Main Respiratory Viral Infections in Samples from Emergency Pediatric ICU

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Resumo

Annually, countless young children are infected by some type of virus, mainly because their immune system isn’t fully developed. With the aim of fast diagnoses and reduce the unnecessary use of antibiotics, besides helping the medical team on medical decisions about hospitalizations, potential and contention of infections, the main goal of this work is to identify the main viruses responsible for causing severe acute respiratory diseases in children under 5 years old, using Real Time PCR method, the results can help the hospital team dealing with possible risks, avoid unnecessary treatment and obtain faster results. 121 nasopharyngeal aspirate (ASN) samples from patients younger than 5 years old who had signs and symptoms of acute respiratory infection (ARI) were analysed. The symptoms considered severe were bronchiolitis, pneumonia, mechanical ventilation, wheezing or/and bronchopneumonia. All samples came from patients admitted to the Intensive Care Units (ICUs) of HU-USP and the Santa Casa de Misericórdia Brotherhood of São Paulo, from March 2016 to March 2017. The genetic material analysis of the samples was extracted by automated method in NUCLISENS® easyMag® platform (BioMerieux). The real-time RT-PCR reaction was performed for 16 species of viruses: Respiratory Syncytial Virus (RSV); Human Metapneumovirus (HMPV); Rhinovirus (HRV); Influenza virus A (IA); Influenza virus B (IB); Adenovirus (AdV); Human Coronavirus OC43 (OC43); Human Coronavirus NL63 (NL63); Human Coronavirus HKU (HKU); Human Coronavirus 229E (229E); Parainfluenza virus 1 (P1); Parainfluenza virus 2 (P2); Parainfluenza virus 3 (P3); Parainfluenza virus 4 (P4); Human Enterovirus (HEV) and Human Bocavirus (HBOV), with primers and probes previously described in the literature. Among all the analyzed samples, 103 samples (85%) were positive for at least one of the studied species. The most frequent viruses were RSV in 37% of the positive samples, followed by HRV (14.5%), AdV (12.6%) and HBOV (8%). It was concluded that most of the respiratory viruses detected had a higher incidence in the fall and that RSV was the most prevalent. Financial Support: CNPq/FAPESP

Palavras-chave: virus, respiratory, pediatric, emergency
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IN SILICO ANALYSIS OF ANTIVIRAL PEPTIDES FROM THE FUSION PROTEIN OF HUMAN PARAINFLUENZAVIRUS 3

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Resumo

Acute respiratory infections (ARIs) are a substantial cause of global morbidity and mortality. The Paramyxoviridae family has three important viral species that cause this kind of infection: respiratory syncytial virus (RSV), human metapneumovirus (hMPV) and human parainfluenza virus 3 (PIV 3). However, there is no preventive vaccine against these pathogens and the available treatments are expensive and liable to have adverse effects. The development of antiviral peptides (AVPs) derived from fusion proteins is an attractive option for the treatment of ARIs and bioinformatic tools are important for prediction of new AVPs, saving time and costs. This study aimed to predict AVPs derived from the fusion protein of RSV, hMPV and PIV 3. Nucleotide sequences of the fusion genes from different strains of these species identified worldwide in different years were obtained from GenBank. Multiple progressive alignments (Muscle and ClustalOmega) were performed to identify conserved regions in the nucleotide sequence of each viral species. The CAP3 Sequence Assembly Program and Weblogo tools (version 2.8.2) were used to obtain the consensus sequences for the fusion gene of each viral species and later translated with the Translate tool (Expsay). The conserved regions of the consensus sequence of the fusion protein were used in the AVPpred and AVP-IC50pred servers for the prediction of AVPs and determination of the IC50, respectively. Peptides that presented results >50% in the physical-chemical characteristics and amino acid composition models were considered a possible AVP and selected to determine the 3D structure with the PEP-FOLD software. In silico analyzes predicted 9 specific unpublished AVPs against RSV, 8 against hMPV and 5 against PIV3. In addition, the IC50 values for these AVPs ranged from 6.31μM to 0.3μM, being classified as effective or highly effective, respectively. The use of bioinformatic tools may be an alternative for the screening of AVPs against respiratory paramyxovirus, reducing costs and time of experimentation. Financial support: FAPEMIG, UFU

Palavras-chave: respiratory syncytial virus, human metapneumovirus, parainfluenzavirus 3, bioinformatic tools, AVPs
IN VIVO EVALUATION OF SMALL INTERFERING RNA AGAINST MEASLES VIRUS

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Resumo

RNA interference (RNAi) mechanism has been studied as a potential strategy to inhibit virus replication. The study of small interfering RNA (siRNA) as therapeutic and prophylactic tools against morbillivirus infections is a goal of our group, which already obtained good advancements by identifying siRNAs against the nucleoprotein gene of measles virus (MV) efficiently silencing the virus replication in vitro. To evaluate the in vivo efficacy of three siRNAs against MV, a human CD46 mouse model in combination with a recombinant MV strain that expresses the luciferase protein (rMV-luc) were used. Before any manipulation, mice were anesthetized and placed on the bioimaging block of IVIS Lumina LT In Vivo Imaging System (PerkinElmer). Two experiments were perform to access the antiviral siRNA effect locally. In the first experiment the left tibial muscle of 9 mice were injected with 10.6 μg of a mixture of MV siRNAs (NMV1, 1NMV and NMV1-1). As a negative control, the same mice were injected with an irrelevant siRNA in the right tibial muscle. The following day, all mice were inoculated with 106 TCID50/60 μl of rMV-luc in each tibial muscle. In the second experiment, only one siRNA was injected (NMV1), and a reduction was performed in the rMV-luc titer of infection (5.104 TCID50/60 μl). After each siRNA injection, tibial muscles were immediately electroporated to deliver the siRNA. The bioluminescence generated by rMV-luc was detected by the IVIS Imaging System through the isometric regions of interest (ROIs) using Living Image software (Xenogen Corporation). As no antiviral effect in vivo of MV siRNAs were observed it was performed a proof of concept test in vivo. For that, the rMV-luc was replaced by a double reporter plasmid containing the target sequence of MV siRNAs and the experimentation was performed as already optimized in our laboratory to other morbillivirus. Nonetheless, after statistical analyses we concluded that the MV siRNAs tested did not demonstrated to be effective as a prophylactic tool against MV in vivo. The reasons for the in vitro versus in vivo discrepancy results are not yet clear but experiments about different delivery systems and/or more stable RNA interference molecules are been performed to improve our methodology.

Palavras-chave: MV, siRNA, RNA interference, antiviral strategy, bioimaging
IN VIVO STUDY OF THE ACTIVITY OF ANTIVIRAL SOFOSBUVIR AGAINST THE ZIKA VIRUS.

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Resumo

On February 2016, the World Health Organization (WHO) declared state of emergency on public health at international awareness levels due to the increasing of zika virus (ZIKV) confirmed infection cases and its relation to congenital malformations, such as microcephaly and neurological syndromes, including Guillain-Barré syndrome. For that reason, it’s vital to search for new pharmacological strategies to inhibit ZIKV replication. Initially used for Hepatitis C treatment, the Sofosbuvir-triphosphate - a nucleotide analogue of uridine that acts as a viral polymerase inhibitor – has shown effective results in inhibiting ZIKV propagation in vitro. The aim of this work is to establish a model of ZIKV infection in vivo and to evaluate the effects of the treatment with sofosbuvir. For that, we used 4 experimental groups: uninfected control, uninfected control treated with sofosbuvir, animals infected with ZIKV and animals infected with ZIKV and treated with sofosbuvir. Newborn swiss mice were infected on postnatal day 3 (P3) with intraperitoneal injection of 2x107 PFU of ZIKV diluted on DMEM high glucose. Control animals received vehicle injections on the same date. Animals were treated with sofosbuvir at 20mg/kg/day through intraperitoneal injection starting 1 day before infection and kept for 7 days. The weight of mice, length of body and tale, encephalitis indicators (letargy and mobility problems) and surface righting reflexes were registered daily. We demonstrated that infection of P3 mice with ZIKV generated weight loss, decreasing in body length and signs of encephalitis, followed by death. We also noticed microhemorrhagic spots on the brain 3 days after infection. After treatment with sofosbuvir, there was a decrease in weight loss and increase in survival. Infected animals treated with sofosbuvir also had a better performance on the surface righting test 5 days after infection when compared to untreated animals. These results indicate that although further investigations on the mechanisms of action are still necessary, sofosbuvir has great therapeutical potential on ZIKV treatment. Financial Support: CNPq, FAPERJ.

Palavras-chave: antiviral, sofosbuvir, zika, virus
The Mayaro Fever, caused by Mayaro virus (MAYV), is a sublethal disease with symptoms confused to those of Dengue with the exception of the symptoms of polyarthalgias that can cause a disabling disease. To date, there is no therapy available and studies have shown that vaccines appears to be an ineffective option to protect against chronic arthralgia. Antiviral drugs are the most convenient for treatment of infections by Alphavirus arthritogenic. Obtaining antiviral targets is of great importance, since the 3D structures of the proteins of MAYV are not yet available in databases. Previously, the protein nsP3 of MAYV model was constructed using the nsP3 of Sindbis virus (VSV) and Chikungunya (CHIKV) and Venezuelan Equine Encephalitis viruses (VEEV). The case was defined as a cube with dimensions of 20x20x20 Å and coordinates X, Y and Z 88.21, 82.62, and 68.96, respectively. The value obtained for ADP-ribose binding energy was 8.7 kcal/mol. ADP-ribose bound to nsP3 of MAYV mainly by hydrogen bonds. The analysis of the binding site of the ADP-ribose in MAYV, CHIKV and VEEV showed that 53% of the aminoacid residues are conserved. These results suggest that ADP-ribose has functional significance in study site and that this ligand may be apromising starting compound for the development of antivirals drugs against these viruses. Further, molecular dynamics simulations will be performed to validate the interaction of the ADP-ribose to the studied site of the protein nsP3 of the MAYV. Financial Support: FAPEMIG

Palavras-chave: Mayaro Virus, Alphavirus, Homology modeling, Molecular Docking
Oropouche virus (OROV) is an arthropod-borne emerging virus with segmented single-stranded negative RNA genome of the family Bunyaviridae. OROV is frequently found in Amazon region in Brazil, but dissemination of arthropod vectors resulted from anthropologic and climate changes have increased the chance of OROV emerge in new areas. OROV infection induces a febrile illness in humans usually associated with myalgia, skin rash, arthralgia and photophobia. OROV can also reach the central nervous system in humans, leading the development of meningitis and encephalitis. However, the molecular mechanisms associated with OROV neuropathogenesis is poorly understood. In a previous study of our lab was demonstrated that IRF-5, a transcription factor activated by pattern recognition receptors (PRR), is essential to control OROV neuroinvasion. How IRF-5 is essential for B cell maturation, we hypothesized that this factor can be necessary for adaptive response against OROV. Thus, to test the importance of B and T cells response during OROV infection, we measured the morbidity and mortality in μMT (deficient in B cells), TCRbd−/− (deficient in T cells), and Rag1−/− (deficient in both cells) KO mice after subcutaneous viral inoculation. While μMT and Rag1−/− mice were vulnerable to OROV infection, TCRbd−/− mice were resistance to lethal infection. In addition, we demonstrated that Irf-5−/− mice had slightly lower neutralizing titers at day 8 and 12 post infection than WT mice, suggesting that others mechanisms dependent of B cells but independent of neutralizing antibodies production are essential for OROV restriction. Financial support: FAPESP and CNPq.

Palavras-chave: B cells, IRF-5, Neuroinvasion, Oropouche virus
Arthropod borne viruses cause mild to severe illnesses in vertebrates, representing a public health challenge in countries where their arthropod vectors are found. Isolates of Chikungunya virus (CHIKV) and Zika virus (ZIKV), which were absent in Brazil some years ago, have emerged and, together with dengue viruses (DENV), are the most important arboviruses transmitted by Aedes aegypti circulating in tropical countries nowadays. In this work, arboviruses circulating in the Federal District (DF) have been isolated from patient sera supplied by the Central Laboratory of Public Health (LACEN) at DF, Brazil. In order to isolate the viruses, sera positive for CHIKV, DENV and ZIKV by PCR were added into C6/36 (Aedes albopictus), AaG2 (Aedes aegypti), and Vero (Monkey) cell cultures. The cytopathic effect (CPE) was observed over 4 days. Then, supernatant and the remaining cells were collected for further infections and RNA extraction. To make sure that the CPE was due to virus infection instead of serum toxicity, RT-PCR was performed. RNAs were first extracted from cells using Trizol, following the manufacturer’s instructions. Then, M-MLV reverse transcriptase was used for viral cDNA synthesis, followed by PCR with specific primers designed for the envelope of CHIKV, DENV and ZIKV. Amplicons were just obtained for cells infected with CHIKV and DENV. More precisely, we detected six out of nine CHIKV isolates, three out of ten DENV isolates and none out of ten ZIKV samples. A second infection with the supernatants from the first infections was performed and the viruses were detected again as described above. The complete envelope gene of the isolated viruses was then amplified and phylogenetic analysis was performed. The isolated viruses will then be used for competence and persistence studies of arbovirus transmission by mosquitoes. These virus isolates will be also sequenced following a high-throughput methodology. Financial support: UnB, CNPq, CAPES, FAP-DF.

**Palavras-chave:** arboviruses, Aedes, DF, isolation
The first cases of Chikungunya virus (CHIKV) infection in Brazil were reported between August and September 2014. In 2015, 101 samples of patients with suspected CHIKV infection were analyzed by the Central Public Health Laboratory of Minas Gerais (LACEN-MG), with 10.9% being positive for anti-CHIKV IgM. In 2016, 1439 samples were analyzed, with a positivity of 10.6%. In 2017, from January to May, 3439 samples were analyzed and 65.8% positive. In this period of 2017, it was identified that the majority of the investigated cases came from the east of Minas Gerais, mainly from the Health Regionals of Governador Valadares and Teófilo Otoni. This study aims to evaluate the laboratory and clinical profile of patients with suspected Chikungunya (CHIKV) virus infection in these regions. We evaluated 1964 epidemiological records sent along with the suspected samples. All the samples were submitted to serological tests for anti-CHIKV and anti-Dengue antibodies. Samples collected from the sixth day after the onset of symptoms were tested for anti-CHIKV IgM. Samples collected from the twentieth day after symptoms onset were tested also for anti-CHIKV IgG. A positivity of 75% was observed in the 1964 samples tested for anti-CHIKV IgM. 326 samples were also tested for Chikungunya IgG being 82% positive. Among the 1476 samples positive for anti-CHIKV IgM, 12.6% also were positive for anti-Dengue IgM, suggesting cross reaction or co-infection. Of the 380 samples that presented a negative result for anti-CHIKV IgM, 12.4% were positive to anti-Dengue IgM. Regarding the main symptoms presented by the individuals evaluated, it was possible to observe that 82.5% had fever, 74.6% myalgia, 74.0% severe arthralgia, 72.5% headache, and 36.7% exanthema. The positive results for CHIKV since 2015 show an expressive increase of CHIKV infection cases in Minas Gerais. The wide distribution of Aedes aegypti and A. albopictus and the low previous exposition of the population to CHIKV make the State susceptible to the viral propagation. Factors such as climatic conditions, infrastructure problems in cities and the intense displacement of people favor the spread of this disease and other arboviruses. The differential diagnosis for dengue and a better filling of the epidemiological data considering the similarity of the symptoms are of fundamental importance. Continuing education actions regarding the completion of the epidemiological record are an important role for LACEN-MG.

**Palavras-chave**: CHIKUNGUNYA, EPIDEMIOLOGY, ARBOVIRUSES, DIAGNOSIS, SEROLOGY
LARGE-SCALE ANALYSIS OF IMMUNE MEDIATORS IN SERUM OF ZIKV-INFECTED PATIENTS FROM CAMPINAS-SP

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Resumo

Zika virus (ZIKV) infections have been linked to different levels of clinical outcomes ranging from mild rash and fever, to severe neurological complications and congenital malformations. We aimed to investigate the clinical and immunological response, focusing on the immune mediators profile in 95 acute ZIKV-infected adult patients from Campinas, Brazil. Among the 95 patients, there were 6 pregnant women who later delivered during the course of this study. Additional serum samples from 4 other babies from ZIKV-infected mothers were also included. Samples were collected at median 3 days post-illness onset. In addition, samples from the 6 pregnant women were also provided during the convalescent phase. All samples were processed and tested for ZIKV on real-time RT-PCR, and ELISA for presence of ZIKV-specific antibodies. Healthy samples from 13 donors were included and pre-screened for presence of ZIKV viral RNA and ZIKV specific antibodies. The profile of 45 immune mediators in ZIKV-infected patients with/without neurological complications was determined by multiplex microbead-based immunoassays. We observed that 11.6% of patients had neurological complications, 88.4% displayed mild disease of rash and fever. Furthermore, out of the total 10 babies born from ZIKV-infected mothers in this study, 2 (20%) had intrauterine growth restrictions, 1 (10%) had paraplegia, 1 (10%) had microcephaly, and 1 (10%) had meningoencephalitis. Only one out of the 6 pregnant women was later found to carry a baby with fetal growth associated malformations. Several immune mediators were specifically higher in ZIKV infected patients, while levels of IL-10, IP-10, and HGF could differentiate between patients with and without neurological complications. Interestingly, higher levels of IL-22, MCP-1, TNF-α and IP-10 were observed in ZIKV-infected pregnant women carrying babies with fetal growth associated malformations. Notably, babies with congenital CNS deformities had significantly higher levels of IL-18 and IP-10 but lower HGF compared to normal babies from ZIKV-infected mothers. Therefore, this is the first observation showing a differential up- and down-regulation of immune mediators in babies born from ZIKV-infected mothers. This study identified several key markers for the control of ZIKV pathogenesis. Thus, it will allow a better understanding of the molecular mechanisms of ZIKV infection. Financial Support: FAPESP and CNPq.

Palavras-chave: Biomarkers, congenital CNS deformities, immune mediators, Zika virus
Ligand-based virtual screening uses computer based methods to discover new ligands for biological targets using actives compounds as a template. Zika virus (ZIKV) is an arthropod-borne virus member of the Flaviviridae family, which is transmitted to humans by the bite of infected female mosquitoes of Aedes genus. In Brazil, ZIKV infections have been associated with neurological complications such as microcephaly and Guillain-Barre syndrome. There is no vaccine, vector control has failed, so specific antiviral drugs able to inhibit ZIKV replication are necessary. ZIKV NS3 protease plays an important role during viral replication and been implicated as potential targets for rational antiviral drugs discovery. Therefore, the present work aimed to perform a ligand-based virtual screening using 3D models of NS3 protease for virtual screening and development of new antiviral drugs. Crystal structure of NS3 protease was selected from PDB and then the prediction of protein binding pockets druggability around crystallographic ligand was carried out using PockDrug-Server. Two high-resolution crystal structures PDB 5H4I and 5LC0, bound to their ligands were selected among available models. The druggability of PDB 5H4I and PDB 5LC0 were 0.75 and 0.80, respectively, indicating promising affinity of these pockets to bind drug-like molecules. Then, ligand-based virtual screening of several libraries of drug-like molecules using pharmacophore features of crystallographic ligands was performed by SwissSimilarity and ZINCPharmer. As a preliminary result, 3700 compounds available in a purchasable subset of ZINC database. Molecular docking methodology has been standardized to further studies to predict protein-ligand interaction geometries and binding affinities. The most promising compounds will be purchased and in vitro evaluation of antiviral against ZIKV will be performed aiming to rational drug discovery.

Palavras-chave: zika, antiviral, protease, bioinformatic, screening
LIPID DROPLETS ARE INCREASED IN MONOCYTES CD16+ AND CD16- FROM DENGUE VIRUS-INFECTED INDIVIDUALS

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Resumo

Dengue fever is an acute febrile illness caused by Dengue virus and transmitted by the bite of a female Aedes mosquito. It is a disease with a wide spectrum of symptoms, varying from a self-limited fever to severe bleeding and shock. The infection begins in the skin site of virus inoculation. Subsequently, the virus spreads to the lymphatic system causing a systemic disease. The same immune response that generates protection, when exacerbated may cause severe cases of dengue. However, the role of eicosanoids in the immunopathogeny of the Dengue virus infection is not completely known. These compounds are bioactive lipids that regulate several inflammatory mechanisms involved in numerous diseases. Their generation is an enzyme-mediated process that can develop in different cell compartments, including in lipid droplets. Thus, for a better understanding of the role of these organelles in the Dengue virus infection, this study evaluated, by flow cytometry, the lipid droplets content in the peripheral blood leukocytes from Dengue virus-infected individuals stained with Bodipy 493/503. Data from infected individuals (n=28), subdivided into IgM- and IgM+ groups, were compared to non-infected controls (n=13). The frequency of each leukocyte subpopulation was analyzed and an increase in CD16+ monocytes and a decrease in CD16- monocytes were detected in both IgM- and IgM+ groups when compared to the non-infected group. However, the lipid droplets content was higher in both monocytes subpopulations from the IgM- patients, but not from IgM+ individuals. In addition, there was no modification on the frequency, as well as, in the lipid droplets content in neutrophils, eosinophils, T and B lymphocytes, NK and NKT cells. These results suggested that increased activation of eicosanoid-synthesis pathway could be occurring in peripheral blood monocytes during Dengue virus infection. To verify whether eicosanoid-synthesis pathway is activated in peripheral blood leukocytes during dengue, analysis of gene expression of eicosanoid-forming enzymes cyclooxygenase-2, 5-lipoxygenase, prostaglandin E2 synthase, thromboxane A2 synthase and leukotriene A4 hydrolase will be performed in peripheral blood leukocytes from Dengue virus-infected individuals by real time PCR. In addition, plasma levels of prostaglandin E2, thromboxane A2 and leukotriene B4 will be measured by ELISA. These data will help to understand the relationship between eicosanoids and immunopathogeny of the dengue.

Palavras-chave: corpúsculo lipídico,, dengue, eicosanoides
LOW FREQUENCY OF HPV AND HPV16 INFECTION IN QUILOMBOLA WOMEN LIVING IN A SEMI-ISOLATED GEOGRAPHIC AREA

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Resumo

Infection with high-risk HPV types (HR-HPV) can lead to cervical cancer (CC). HR-HPV16 and 18 are the most common types, responsible for about 70% of CC cases. In addition to the HPV type, subtypes, variants, persistence and viral load also play a key role as adjuvants in cancer pathogenesis. Socio-environmental inequalities are common in Brazilian society and are evident in Quilombola communities, whose population lives in a situation of social vulnerability and difficult to access to health services. Thus, the aim of this study is to describe HPV types, HPV16 variants and viral load of HPV16 and 18 in cervical specimens from women descendant of African-Brazilian slave living in Quilombola communities of Espírito Santo, Brazil. Cervical samples were obtained with cytobrush and cervical scraping from 128 women, from March 2016 to June 2017. Viral DNA was extracted using the QIAamp DNA Mini Kit™ (QIAGEN). HPV was screened with the sets of PGMY09/11 primers and genotyped by Reverse Line Blot (RLB). HPV16 variants were described by sequencing using BigDye® (Thermo Fisher) reagent. Real-time PCR, using TaqMan® protocol, was used to determine the viral load of HPV16 and 18. We detected HPV in 15.6% (20/128) of women, 30% of them with mixed infection (up to seven different types). In all we found 20 different HPV types; HR-HPV was present in 65% (13/20) of them, mostly represented by HPV31 and 39. Cytological abnormalities were observed in 7.5% (14/187) of samples with significant association with positivity for HPV (66%; p<0.001). The only two HPV16 strains were European variants and a high viral load was detected, 1527.54 and 456.05 copies/cell present in low-grade and high-grade intraepithelial lesions cases, respectively. The HPV18 was detected in only one sample with low viral load and normal cytological finding. This is the first study conducted with Quilombola women in Southeastern Brazil and reveals a low frequency of HPV and HPV16. Moreover, the most frequent HR-HPV genotypes detected in this population are not exactly the most common in the general Brazilian women, which may reflect inherent characteristics of this population that lives in semi-isolated geographic area. Financial support: UFES, Fundação de Amparo à Pesquisa e Inovação do Espírito Santo (FAPES)

Palavras-chave: HPV, Genotypes, Sequencing, Viral load
Dengue is a disease of great impact on the world public health and its clinical symptoms may decline or develop into severe hemorrhagic fever. Therefore, the search for biomarkers associated with prognosis and morbidity is crucial for clinical healthcare of patients and evaluation of effective interventions in order to reduce the mortality rate. Thus, the aim of this study was the prospection of cytokines and chemokines that could be associated with dengue severity in DENV-4-infected patients. For that reason, 20 DENV-4 positive serum samples (confirmed by RT-PCR) from patients attended at the Hospital Escola Hélvio Auto (Maceió city) were used for cytokines and chemokines measurement (IL-6, IL-10, TNF, IFN-γ, IL-12p70, CXCL8 / IL-8, CXCL9 / MIG, CCL2 / MCP-1 and CXCL10 / IP-10) by cytometric bead array (BD Biosciences). As a negative control group, we used 25 serum samples from healthy blood donors. The cytometry data were analyzed in the FCAP Array program, 3.0 (BD Biosciences) and the statistical analysis was performed by software Prism 6.0 (GraphPad Prism). DENV-4 infected patients had an average age of 25.15 ± 9.55 and was in the fourth-day ± 1.11 of infection. Interestingly, we detected high levels of all quantified serum analytes in DENV-4 infected patients compared to control group including pro-inflammatory cytokines as IFN-γ and TNF and IL-12p70 as well as chemokines such as MIG. Importantly, infected patients showed high levels of IL-10 and IP-10, which have been associated with severe dengue symptoms such as hepatic injury and plasma extravasation in previous studies. Additionally, an increase in IL-6, IL-8 and MCP-1 levels, previously related with antiplatelet and anti-endothelial antibodies (IL-6), neutrophil recruitment and degranulation (IL-8), and platelet activation and apoptosis (MCP-1) were detected. In conclusion, these findings highlight the importance of the quantification of these biomarkers in DENV-infected patients for dengue prognosis and morbidity. Financial Support: Ministério da Saúde/CNPq/SESAU-AL/FAPEAL

Palavras-chave: cytokines, chemokines, severe dengue
Measles virus (MeV) belongs to Paramyxoviridae family, its genome is composed of SS RNA. It is classified into 24 genotypes based on nucleoprotein (N) gene. MeV is a single serotype, it is highly contagious and since 1960s, a vaccine has been available. In 2016, the Americas region achieved MeV elimination, although MeV continues to circulate worldwide and the risk of imported cases remains. In Brazil, imported cases and small outbreaks have occurred since 2000. However, in 2013 a large outbreak started in northeast Brazil and circulation of virus continued until 2015. The aim of this study is to characterize the sequence of the MeV strains detected from 2013 to 2015, to compare with epidemiological data and to describe the origin and spread of outbreak in the Northeast region. To conduct this study we sequenced the N gene to characterize the sequence of the strains and the hypervariable region (MF-UTR) to classify lineages inside the genotypes. Phylogenetic and evolutionary reconstruction were conducted with the genetic data obtained and compared to epidemiological data. The genetic characterization identified three genotypes B3, D4 and D8 in Brazil during 2013 to 2015. Strains B3 and D4 were found as isolated imported cases, however the D8 genotype was introduced in southeast and northeast regions in 2013 and spread in the northeast States of Pernambuco and Ceara. Using MF-UTR Bayesian analyses, it was possible to observe that at least two lineages were introduced during the D8 outbreak in northeast Brazilian region. However, further analyses are being conducted to understand better the spread of D8 outbreak. This study reinforce the value of molecular and epidemiological surveillance of MeV and highlight the importance of sequencing the MF-UTR region to elucidate the evolutionary dynamics of genotypes during the course of an outbreak.

Palavras-chave: Bayesian analyses, Measles, MF-UTR region, Outbreak
The Zika virus (ZIKV) was first identified by viral isolation in Rhesus monkey serum. Zika is a disease that has similar symptoms with other flavivirus such as Dengue and Chikungunya. The usual symptoms of the clinical cases include fever, myalgia, headache, retro-orbital pain, rash, as its main infection signs. In some cases, there are reports of neurological manifestations such as Guillain-Barré Syndrome. It is already known that there is a relationship between ZIKV infection in pregnant women and its potential teratogenic effect, as it can be associated with the increased number of microcephaly cases. The aim of this study was to describe the microcephaly cases of patients whose samples were evaluated by Fundação Ezequiel Dias (Funed) to investigate ZIKV infection. The procedure used in this study was real time RT-PCR (RT-qPCR) for the detection of viral RNA found in biological samples. Furthermore, immunological tests by ELISA in serum and liquor of patients were performed. In 2016, year of the occurrence of the first ZIKV epidemics in Minas Gerais, Funed has received 6290 samples of patients with suspicion of ZIKV infection. In 2712 samples, the virus or its specific antibodies, were detected. Among the received samples, 353 were from microcephalic newborns (from 138 different patients) in which only 7 were positive for ZIKV infection (6 IgG and 1 RT-qPCR positive). Not all of their mothers performed molecular and serological tests for the disease. Among those who searched for ZIKV infection, it was found that 49 had a positive result (IgM, IgG or RT-qPCR). Also it was observed that, among microcephalic newborns with negative results, 43 had their mothers with positive results, and 85 with negative results. In addition, 4 children positive for ZIKV infection had their mothers also with positive tests. The collection of samples from mothers and their microcephalic children in a timely manner and the correct register of clinical information is fundamental for the establishment of the relationship between infection by ZIKV and the risk of developing microcephaly.

Palavras-chave: ZIKA, MICROCEPHALY, OUTBREAK
MOLECULAR CHARACTERIZATION AND EPIDEMIOLOGIC PROFILE OF HUMAN ADENOVIRUS IN PEDIATRIC PATIENTS WITH ACUTE RESPIRATORY INFECTION

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Resumo

Human Adenovirus (HAdV) belongs to the Adenoviridae family, genus Mastadenovirus, and has a genome of double-stranded DNA. HAdV is an important etiologic agent responsible for various diseases in adults and children, particularly respiratory tract infections, eye infections, gastroenteritis and hemorrhagic cystitis. Currently there are 79 known types of HAdV divided into seven species (HAdV-A to HAdV-G). In the case of acute respiratory infections, they are associated with HAdV B, C and E species. Acute respiratory infection in the lower tract is the fourth leading cause of death worldwide. In children under two years of age, Human Adenovirus is responsible for 5 to 15% of viruses that cause acute respiratory infection and 1 to 5% of all respiratory infections, which shows the importance of surveillance and monitoring of HAdV. In this context, 1141 nasopharyngeal aspirate samples from children under five who presented acute respiratory infection framework were collected in the University of São Paulo’s University Hospital (HU-USP), in 2015. Samples were extracted by automated method and analyzed by real-time polymerase chain reaction for detection of HAdV and positive samples were sequenced by Sanger method. Out of 1141 samples, 262 (23%) were positive for HAdV by qPCR, against 28 samples considered positive by immunofluorescence, showing that this assay is not a good diagnostic method for this virus, once qPCR can almost ten-fold the detection comparing both. We achieved 99 sequences out of the 262 samples. The C species was the most abundant with 59 sequences, followed by species B with 29, E with 8, D with 2 and F with 1. Within the species HAdV is divided in types, we found 29 samples of type 3 (B3), 19 of type 2 (C2), 7 of type 5 (C5), 1 of type 6 (C6), 8 of type 4 (E4), 2 of species D (types to be defined by other region) and 1 of enteric-type 41 (F41) found in nasopharyngeal aspirate. Therefore, we demonstrated that Adenovirus circulates the whole year, immunofluorescence is not a good method to detect HAdV and C species and types 1, 2 and 3 are the most present in Brazil these days. Financial Support: Research funded by: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) - Process: 2015/25643-7

Palavras-chave: Adenovirus, Respiratory, Pediatrics, Molecular characterization, Epidemiology
MOLECULAR DETECTION OF RESPIRATORY VIRUSES IN PATIENTS WITH ACUTE RESPIRATORY INFECTION IN THE STATE OF CEARÁ, BRAZIL

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Resumo

The Acute Respiratory Infection (ARI) is one of the major diseases of the respiratory tract, mainly in children under five years of age, exhibiting considerable impact on public health due to its high morbimortality. The ARI in most cases is triggered by viral agents, such as Influenza Virus (FLU), types A and B; Respiratory Syncytial Virus (RSV); Parainfluenza Viruses (PIV), types 1, 2 and 3; Human Metapneumovirus (HmPV) and; Adenoviruses (AdV). These viruses are transmitted by direct contact and aerosols, and may cause pharyngitis, acute otitis media, bronchiolitis and pneumonia. As the symptomatology of ARI caused by different pathogens is similar, the laboratory diagnosis to detect these agents is essential. Therefore, the purpose of this study was to verify the occurrence of FLU, RSV, HmPV, AdV and PIV in samples collected from ARI patients treated at health units in the state of Ceará, Brazil, from January to April 2017. The samples were collected by combined swab, being subsequently submitted to extraction of the viral genome by using a commercial kit and detection by Polymerase Chain Reaction, preceded by Real-Time Reverse Transcription (rRT-PCR), with the use of specific primers. Of all 142 samples analyzed, 82 (57.7%) were positive for one or more viruses investigated, being 46 (32.3%) positive for RSV; 14 (9.8%) for FLU, of which 7% was Influenza A (H3N2) and 2.8% Influenza B; 6 (4.2%) for PIV 3. There were also 16 (11.3%) cases of co-detection, of which 93.7% were with RSV, and the majority of cases observed was in children under four years of age. All samples tested for AdV, HmPV, PIV 1 and 2 were negative. Thus, it’s possible to emphasize the importance of viral agents as inducers of ARI, especially in children, corroborating with the literature data; and also the molecular diagnosis in the detection of these pathogens. Financial Support: FAPESPA/ IEC

Palavras-chave: Respiratory Viruses, Molecular Biology, Epidemiology, Ceará
The Influenza virus is the etiologic agent of influenza, a highly contagious disease that affects the respiratory tract. It belongs to the *Orthomyxoviridae* family and influenza A virus currently presents two distinct subtypes circulating in the human population: H1N1pdm09 and H3N2. The influenza B virus has the Victoria and Yamagata lines circulating among humans. This pathogen is subject to intense epidemiological surveillance due to its ability to cause epidemics and pandemics. This is due to the segmented nature of their genome and the constant antigenic variation that these viruses present in their surface proteins - haemagglutinin (HA) and neuraminidase (NA) -, thus impacting in the vaccine formulation and the emergence of resistance to the drugs used in the clinical management of patients. Therefore, the objective of this study was to genetically characterize the genes encoding the HA and NA surface proteins of influenza virus strains collected from patients treated with complaints suggestive of acute respiratory infection in a health unit in the city of Belém, during the period of January to June 2017. The genetic characterization of HA and NA occurred in four main stages: a) extraction of viral RNA; b) complementary DNA synthesis (cDNA); c) cDNA amplification by PCR and d) sequencing. During the study period, 18 samples were positive for Influenza A (H3N2) and 13 for Influenza B. The phylogenetic analysis of the HA gene of influenza A (H3N2) virus demonstrated that these strains belong to two phylogenetic groups, the 3c2a and 171k groups. In addition, the analysis did not indicate disagreement between the circulating strains and those contained in the vaccine. For the influenza B virus, the sequenced samples were pooled in the B/Yamagata/16/88 lineage and the strain contemplated in the vaccine B/Victoria/2/87, demonstrating disagreement with the vaccine composition. The analysis of the NA, for both viruses did not identify any amino acid change associated with antiviral resistance. The disagreement between the circulating strains and those contained in the vaccine demonstrates the need for constant monitoring of this pathogen, since the data obtained may support the adequate evaluation of the vaccine composition with the same genetic characteristics of the circulating strains, for a more precise immunization and also promote the better clinical management of patients infected with influenza viruses.

**Palavras-chave:** Epidemiological surveillance, Respiratory infection human, Amazon
Severe Acute Respiratory Infection (SARI) is leading cause of hospitalization, morbidity and mortality in children worldwide. SARI is caused by a heterogeneous group of viral pathogens with similar clinical manifestation, which etiology depends on virological assays that identify the most important viruses. We aimed to identify viral pathogens in children hospitalized in an intensive care unit Southeast Brazil with SARI diagnosis. From 2017 February until now, nasopharyngeal aspirates were obtained from 47 children of both genders. Viral RNA was extracted by reverse transcription kit Qiagen® and two Multiplex Real-Time PCR assay, diagnosis of respiratory infection, respiratory virus infection was observed (RSV and hMPV). The use of a fast, accurate and sensitive detection of respiratory viruses allow to promptly identify the respiratory viral infection, and revealed the high rates of RSV in the hospitalized children with SARI. Moreover, the continuous collection of the respiratory specimens would also increase our understanding of the epidemiology of these viruses. Taking into account the development of vaccines and antiviral drugs, successful measures of prevention and control of the disease can be warranted as soon as the etiology of the disease is revealed. Financial Support: CNPq and FAPES.

**Palavras-chave:** multiplex RT-PCR assay, diagnosis of respiratory infection, respiratory virus infection
NEW IMPROVED RTPCR PROTOCOL FOR EFFICIENT ZIKA VIRUS DETECTION IN PATIENT’S BLOOD FROM THE 2016 OUTBREAK IN CAMPINAS.

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Resumo

Zika virus (ZIKV), is an emerging arthropod-borne flavivirus that has been casually linked to neurological disorders, like Guillain-Barre syndrome, and congenital defects. Highly sensitive and specific tools to precise diagnose of this infection is crucial for cases identification and mapping for infection control measures. In this way, we developed a ZIKV NS5 qRT-PCR assay by combining primers described by Balm et al. and a new Taqman probe. The assay was evaluated and compared with another assay described by Lanciotti et al. (ZIKV 1107) using 51 blood and 42 urine samples from 54 suspected ZIKV patients. ZIKV NS5 performed better in terms of sensitivity with more samples detected as ZIKV-positive (n=37) than ZIKV 1107 (n=34) for urine, and ZIKV-positive (n=29) than ZIKV 1107 (n=26) for blood. It was observed that quantification by the ZIKV NS5 qRT-PCR reflected lower Ct values and therefore higher copy numbers than that from ZIKV 1107 qRT-PCR. This results suggests that our new probe-based assay could act as a good target for molecular diagnostics for improved sensitivity.

Palavras-chave: Zika Virus, Diagnostics, rtPCR, NS5
Norovirus (NoV) and Group A rotavirus (RVA) are the leading cause of acute gastroenteritis (AGE) in children worldwide. Host susceptibility according to human histo-blood group antigens (HBGAs) polymorphism is widely known for NoV, but only recently described for RVA and still poorly defined. In order to evaluate the association between HBGAs phenotypes (ABH, Lewis and secretor status) and susceptibility to RVA and NoV infection, a cross-sectional study was conducted from October 2014 to July 2016 with 202 children presenting AGE, from Vitória, ES. Buccal specimens were collected from 172 children. RVA and NoV were detected by qPCR and genotyped by sequencing of VP4 and VP7 encoding genes and ORF1-ORF2 junctions, respectively. HBGAs phenotypes were determined by immunoenzymatic assay from buccal specimens. RVA was detected in 27 (13.3%) and NoV in 53 (26.2%) samples. RVA occurred equally in all age groups (4 m - 10 yr old) and the following genotypes were detected: 18 G12P[8], 7 G7P[8] and 2 G2P[4]. All the NoV belonged to genogroup II (GI) and the majority were recombinants (GI.P17-GII.17, GI.P16-GII.4, GI.PNA-GII.4 Sydney2012, GI.Pg-GII.1, GI.Pe-GII.4 Sydney 2012, GI.P16-GII.3, GI.P7-GII.6); the newly GI.P16-GII.4 recombinant was the predominant strain. Among 68 children infected with either RVA or NoV, 97% were HBGAs secretors and 3% were non-secretors. Of note, one secretor RVA-infected child had a Lewis negative phenotype, differing from studies done hitherto in which P[8] and P[4] infection occurred only in Lewis positive individuals. Diverse ethnic population with distinct HBGAs polymorphisms and a higher frequency of Lewis negative phenotype in Brazil may explain this result, although warranting confirmation with a larger sample size. Nine NoV-infected children (21.4%) had a Lewis negative phenotype and one B group and one non-secretor were NoV-infected. Among 104 non-infected children, 80 (77%) were secretors and 24 (23%) were non-secretors. Fifteen (14.4%) and 89 (85.6%) were classified as Lewis negative and Lewis positive, respectively. We observed a correlation between secretor status and the risk of RVA and NoV infection (P < 0.001). Our findings provide the first evidences of the association between HBGAs phenotype and RVA infection in Brazil which will particularly help further understanding RVA host susceptibility and epidemiology. Financial Support: Fundação de Amparo à Pesquisa e Inovação do Espírito Santo (FAPES/CNPq/DECIT/MS/SESA).
Noroviruses (NoV) are considered an important cause of outbreaks of acute viral gastroenteritis in all age groups worldwide. They belong to the Caliciviridae family and have a single-stranded RNA of positive sense as genetic material. Currently, the NoV classification is divided into seven genogroups (GI - GVII), of which GI, GII and GIV are the most important in human public health. Infection occurs primarily through the fecal-oral route, person-to-person contact and ingestion of contaminated food/water, although the contagion by aerosolized particles from vomiting episodes is also admitted. In addition to its already known role in gastrointestinal tract infections, the presence of NoV has recently been described in samples of respiratory secretion, suggesting that this pathogen can also be disseminated through this route, making the upper respiratory tract a probable other NoV replication site, and suggesting a possible association of the NoV with respiratory conditions. Thus, this study aims to investigate NoV in samples of respiratory secretion of individuals attended at a health clinic in the city of Belém-PA, with respiratory symptoms, and sometimes with diarrhea. To date, 314 samples of respiratory secretion were collected through oropharynx swab or nasal aspirate of individuals of both genders, varied age range and with symptoms associated with some respiratory disease. The samples were submitted to the quantitative Polimerase Chain Reaction (qPCR) (Nº=314), Reverse Transcription-Polimerase Chain Reaction (RT-PCR) (Nº=35) and One-Step RT-PCR (Nº=3) tests for NoV detection and/or confirmation. Of those 314 samples, 25 (8.0%) were from diarrheic patients. Of the total of collected samples, 3 (0.9%) showed positive results for NoV when tested by qPCR. All the other tests presented negative results, so far. Still in relation to the positive samples, one was of a patient also presenting diarrhea at the time of collection (4%). This percentage is lower than that found in a study conducted with hospitalized children in the city of Goiânia-GO, Brazilian Central-West region, where the percentage observed among nasal swab samples from diarrheic patients was 11.4%. It is worth mentioning that this is the first study of this kind in the Brazilian Northern region, and that further analysis is needed to elucidate the real role of NoV in respiratory secretions and its relation with acute gastroenteritis. Financial Support: Instituto Evandro Chagas/SVS/MS

Palavras-chave: norovirus, respiratory secretion, gastroenteritis, Belém
Introduction. Human herpesvirus 6 (HHV-6) is a virus of the Hesperviridae family which belongs to Betaherpesvirus subfamily and its infection occurs mainly in childhood. Acute infection in young children may cause Roseola infantum and fever. The HHV-6 variants, A and B, infect many cells in vivo: T cells (especially CD4+ T cells, but also CD8+ T cells), macrophage cells, bone marrow hematopoietic cells, kidney epithelial cells, salivary glands, endothelial cells, microglial cells, oligodendrocytes and astrocytes. In adults, it is associated with the development of various neurological disorders, including meningitis, encephalitis, epilepsy and multiple sclerosis. Objective. Detect the HHV-6 genome in DNA from cerebrospinal fluid (CSF) specimens from patients with signs and symptoms of neurological disorders attended in the Emergency units of Clinics Hospital between 2016 and 2017, using Nested-PCR (N-PCR) and SYBR Green Real-Time PCR (qPCR) techniques; to correlate the positivity between these two techniques and analyze the clinical impact with the viral load. Methodology. One hundred and forty-eight patients were included in the study. CSF-DNA was extracted from 200 ul of CSF samples using the Biopur Mini Spin Plus extraction kit, following the specifications. After DNA extraction, the quality of the extracted DNA was verified by amplification of the Beta-2-Microglobulin gene by the PCR technique. Detection of both strains of HHV-6 (A and B) in the samples were performed using the N-PCR technique with internal primers, producing an amplicon with 195 base pairs (bp) and 423 bp for viruses A and B, respectively. The qPCR reactions were performed on StepOne equipment using SYBRTM Green. Results and discussion: Seventeen out of 148 (11.5%) samples were positive for HHV-6 type B using N-PCR. Fifteen of these samples were positive by qPCR (agreement in 88.2%) with values ranging from 39 to 1,326 copies/µL. These patients had symptoms of meningitis, myelitis, meningoencephalitis, epilepsy, encephalitis and headache. Two patients with coinfection has died, one of bacterial meningitis and another fungal meningitis. Conclusion: The detection and quantification of HHV-6 DNA in CSF is crucial to verify a primary infection, especially in children, and a possible viral reactivation (more usual in adults) and to guide the treatment of neurological syndromes. Financial Support: FAPESP

**Palavras-chave:** sybr green real time pcr, human herpesvirus 6, cerebrospinal fluid, neurological syndrome, encephalitis
PCR was then completed as previously described (Bernardes et al., 2009); RT-PCR and sequencing of amplicons was performed using S1Oro and C2Oro primers and BigDye Terminators version 3.1 (ABI, USA) in ABI377 automated sequencer. 1% agarose gel electrophoresis was performed with N-PCR products. Sequences were aligned using BLAST (NIH, USA). Two of the five patients were confirmed positive for OROV via sequencing from both the forward (S1Oro) and reverse (C2Oro) primers, however all five samples had similar patterns on gel electrophoresis. There are four different genotypes, I-IV, of OROV and we have aligned both of the sequences here within genotype I. The first strain isolated in Rondônia was from genotype II (H. B. Vasconcelos et al., 2011), and it was later discovered that genotype III, a second genotype, was also in circulation in Rondônia. Now, for the first time, a third genotype has been detected in Rondônia, suggesting continued migration of OROV around Brazil. The likely migration route for this strain is from Acre; the strain most closely related to those found in this study was detected in 2004 (EU561644); Acre is directly west of Rondônia. Financial support: Ministério da Saúde, PADC (FCF/UNESP).

**Resumo**

Oropouche virus (OROV), an Orthobunyavirus residing within the Bunyaviridae family and first identified in 1955 on Trinidad and Tobago (Anderson, Spence, Downs, & Aitken, 1961), has been responsible for over 30 outbreaks with approximately 500,000 cases in Central and South America (P. F. Vasconcelos & Nunes, 2014). Usually OROV is detected in rapid, isolated outbreaks, however, due to serological studies conducted in non-epidemic areas, it is suggested that OROV circulates endemically in Brazil (Pinheiro et al., 1981). We are reporting two cases of OROV arising in the city of Ji-Paraná (10°53′07″S and 61°57′06″W) in Rondônia state, Brazil. In January 2014, serum samples were collected from 5 humans who had presented with fever, headache, and myalgia to Hospital Cândido Rondon and had tested negative for dengue (via NS1) and malaria. Using the methods outlined by Bronzioni et al. (2005) we first tested for dengue and other Flaviviruses and Alphaviruses and confirmed the negative dengue result before proceeding to test for OROV. Viral RNA was extracted using the QIAmp Viral RNA Mini Kit (QIAGEN Inc., Germany) following manufacturer guidelines. RT-PCR was then completed as previously described (Bernardes-Terzian et al., 2009); RT-PCR used C2Oro primers and PCR used S1Oro. N-PCR and sequencing of amplicons was completed using S1Oro and C1Oro primers and BigDye Terminators version 3.1 (ABI, USA) in ABI377 automated sequencer. 1% agarose gel electrophoresis was performed with N-PCR products. Sequences were aligned using BLAST (NIH, USA). Two of the five patients were confirmed positive for OROV via sequencing from both the forward (S1Oro) and reverse (C2Oro) primers, however all five samples had similar patterns on gel electrophoresis. There are four different genotypes, I-IV, of OROV and we have aligned both of the sequences here within genotype I. The first strain isolated in Rondônia was from genotype II (H. B. Vasconcelos et al., 2011), and it was later discovered that genotype III, a second genotype, was also in circulation in Rondônia. Now, for the first time, a third genotype has been detected in Rondônia, suggesting continued migration of OROV around Brazil. The likely migration route for this strain is from Acre; the strain most closely related to those found in this study was detected in 2004 (EU561644); Acre is directly west of Rondônia. Financial support: Ministério da Saúde, PADC (FCF/UNESP).

**Palavras-chave:** Brazil, Genotype I, Oropouche, Rondônia
Chikungunya virus (CHIKV) is a re-emerging arbovirus that is causing outbreaks in several countries of the Americas. The virus was introduced in Brazil in 2014, and since then, several Brazilian states have notified autochthonous cases. Here, the main objective consisted in providing epidemiological evidence on a CHIKV outbreak and an outline of the laboratory and clinical profile of symptomatic patients in Sergipe, Brazil. In February 2016, we collected 142 serum samples from symptomatic patients for arboviruses in Sergipe, Brazil. All samples were submitted to qRT-PCR for the emerging arboviruses circulating in Brazil - DENV, CHIKV and ZIKV - and later submitted to the immunoenzymatic assay. RNA positive samples were randomly selected and sequenced for characterization of the genotype involved in the outbreak. Our study had 75.35 % (107/142) positive for CHIKV infection, with all age groups and genera being equally infected. The virus being identified in 11 of the 13 cities studied in that state included the ECSA genotype. Importantly, fever was the only statistically significant symptoms for CHIKV infection (p < 0.05), while asthenia was significantly associated with symptomatic patients that were CHIKV-negative (p < 0.05). No DENV or ZIKV genomic RNA was detected in all sera samples. Our findings support the importance of fever as a clinical marker and contribute molecular and serological surveillance data, which may help in the understanding of CHIKV circulation, emergence and clinical description. Financial Support: This work was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (Process No. 2014/17766-9). MPC and DFLN received a FAPESP fellowship: 2016/08204-2 and 2016/03605-9, respectively.

Palavras-chave: Chikungunya virus, re-emerging arbovirus, infection
The objective of this study was to optimize the PRNT using a non-CO2 system for a wildtype strain of DENV-2. The DENV-2 strain isolated in the C6/36 HT cell line and confirmed by PCR was propagated through 5 passages in the Vero-76 cell line. Three cell concentrations: 1.5 x 10^5 Vero cells/ml, 2.0 x 10^5 Vero cells/ml and 2.5 x 10^5 Vero cells/ml were evaluated in 24-well plates. Thus, 3 different pH adjustments of growth medium were performed (pH: 6.5, pH: 6.9 and pH: 7.2) using hepes to avoid alkalization of the medium. Then, the plates were sealed and incubated at 37 °C without CO2 for 9 days. Subsequently, the plaque assay was performed inoculating 10-fold serial dilution of viral stock on 24-well plates with Vero-76 cells applying the semi-solid method with pre-formed monolayer (with 3 hours of viral adherence and addition of overlayer with 3% of carboxymethyl cellulose (CMC) and applying the semi-solid method with cells in suspension (with 4 hours of viral adherence and addition of 3%, 4%, and 6% CMC), the plates were sealed and incubated at 37 °C without CO2 until the staining day with Naphthol blue black. Once the optimal concentration of cells, pH and staining day were obtained, the PRNT was performed using 5 sera seronegative to yellow fever virus (YFV) and without reports of dengue diluted in 1: 5, 1:10, 1:20; 5 sera seropositive to YFV by PRNT and without reports of dengue diluted in 1: 5, 1:10, 1:20 and 5 sera with reports of dengue diluted in 1: 160; 1: 320 and 1: 640. As results, the plaque assay with monolayer cells produced undefined and non-countable plaques. Using Vero-76 cells in suspension at 2,5 x 10^5 cells/ml, with the growth medium adjusted to pH: 6.9, overlayer with 6% CMC as results defined plaques of diameter 2 mm approximately on the eighth day and a viral titer of 1.2 x 10^6 PFU/ml were obtained. Sera with reports of dengue were positive for DENV-2 (plaque reduction greater than 70%) at dilutions: 1: 160 to 1: 640. YFV positive sera did not show cross-reaction at any dilution. In conclusion, the PRNT, applying the semisolid method with cells in suspension and using an incubator without CO2 injector, allows to detect neutralizing antibodies against the wild type strain of DENV-2 and does not show cross-react with YFV at a dilution of 1: 5 to more. Financial support: FINCyT-INNOVATE PERÚ

**Palavras-chave:** DENV-2, Non-CO2 system, VERO-76, YFV, PRNT
Diarrheal disease is a major cause of death in children under age 5, worldwide. The causes of diarrhea in children vary with the location, time of year, and population studied. Bacterial and parasitic gastrointestinal infections have decreased in frequency as a result of the provision of safe drinking water and disposal of sewage. However, viral gastroenteritis has not declined in a comparable fashion. The improvement of laboratory methodologies for the detection and identification of pathogens have allowed the intensification of the search for new infectious agents that may be associated with diarrhea illness. In this context, the newly described polyomaviruses Malawi (MWPyV) and Saint Louis (StLPyV) were detected in fecal material and tentatively associated with the diarrhea disease. However, because they were detected in feces of children with and without diarrhea, its pathogenic role is not yet clear. In this study, we tested stool specimens from children with acute diarrhea to investigate the occurrence and frequency of MWPyV and StLPyV infections. Stool specimens (n = 460) obtained from children <10 years of age who presented with acute diarrhea of unknown etiology were retrospectively analyzed; 108 stool specimens obtained from healthy children (control group) were analyzed for MWPyV and StLPyV using PCR. The stool specimens were collected from 1999 to 2012 and from 2016 to 2017 in the city of Rio de Janeiro and surrounding areas. The diarrheic specimens included in the present study were also previously shown to be negative for other enteric viruses and bacteria, including rotavirus, norovirus, astrovirus, adenovirus, salivirus, Aichivirus A, Escherichia coli, Salmonella spp, Yersinia enterocolitica, Campylobacter spp, and Shigellasp. HPyV were detected in thirty-three (7.2%) samples from diarrheic children whereas no positive sample was detected among the healthy children. StLPyV genome was detected in 20 (4.3%) samples whereas MWPyV was detected in 11 (2.4%) samples. Coinfection was observed in 2 (0.5%) samples. The results demonstrate that these viruses continuously circulate in the country for, at least two decades. The association of StLPyV and MWPyV with diarrhoea has been suggested in previous reports, using paired diseased vs healthy controls. In this study, because no other potential pathogens were identified in the samples collected, an association between the viruses detected and diarrhoea could be suggested. Financial Support: CNPq, CAPES and FAPERJ.

Palavras-chave: Gastroenterite, Malawi, Poliomavirus, Saint Louis
PRELIMINARY EVALUATION OF ANTIADENOVIRAL ACTIVITY OF EXTRACTS FROM CYMBOPOGON SPP.

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Resumo

Human adenoviruses (HAdV) have been implicated as infectious agents, which are responsible for numerous diseases, including gastroenteritis, hepatitis, myocarditis, conjunctivitis and respiratory infections. These last ones are a frequent and potentially serious cause of infection after solid organ transplantation, being adenoviruses one of the agents involved in this kind of complication. Despite the global distribution of HAdV, there are no currently approved drugs to treat such infections. Natural products are a rich source of compounds with potential pharmacological activities, including antiviral effects. Studies have demonstrated antiviral activity of Cymbopogon species against measles, herpes, dengue and canine norovirus. Thereby, the present study aimed to evaluate the potential antiviral effect of the C. nardus and C. citratus extracts against HAdV-5. The extracts were obtained by dynamic maceration (8 hours; room temperature) with ethanol (60%), which was removed in rotatory evaporator. Posteriorly, the aqueous residue was lyophilized. The cytotoxicity and anti-HAdV-5 activity were evaluated by MTT and plaque reduction assays, respectively, using A549 (human lung cancer) cells. Cytotoxicity was not observed in the concentration ranges of 39.06 - 625µg/mL to both extracts. Based on these results, a preliminary screening of the antiviral activity was performed using three different concentrations of each extract (600, 150 and 37.5µg/mL). The preliminary results showed a decrease on the number of plaques in the presence of both extracts, suggesting an antiviral effect. Financial support: CAPES, CNPq, Universidade Feevale.

Palavras-chave: Antiviral, Cytotoxicity, Human Adenovirus, Plaque assay
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PRESENCE OF WU AND KI POLYOMAVIRUS IN RENAL TRANSPLANT PATIENTS

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Resumo

Introduction: Some studies point to the occurrence of polyomavirus KIPyV and WUPyV in patients who underwent renal transplantation and immunosuppressive therapy. Objectives: To evaluate the presence of WU and KI polyomaviruses in renal transplanted patients with respiratory symptoms Methodology: A retrospective study was carried out on stored samples obtained of renal transplanted patients with respiratory infections from 2002 to 2003. RT-PCR reaction was performed for both types of Polyomavirus and for the most common respiratory viruses as well. Results: A total of 64 adult patients (mean age 38 years) were analyzed. Respiratory viral infections were detected in 78.1% of the samples. WU / KI detection (2/64) occurred in 3.2%, one sample with isolated KI infection and WU in another sample with triple virus infection. (FluA, HBoV and WU). Other monoinfections were detected HAdV 1.6%, Flu A 26.5%, Flu B 1.6%, HRV 1.6%, HCoV 6.2%, PIV 3.1%. In these patients two viral etiologies were detected in 31.2% and in 7.8% three different viral etiologies were detected. Conclusion: There was circulation of WU / KI polyomavirus among kidney transplant recipients with respiratory symptoms. These preliminary data point to the hypothesis that immunosuppression due to transplantation procedures may result in reactivation of these viruses or increase susceptibility to KIPyV and WUPyV. Financial Support: FAPESP (2016/09279-6), Capes.

Palavras-chave: Respiratory viral infection, Polyomavirus, Renal transplant patients
Resumo

Hepatitis C is an infectious disease caused by hepatitis C virus (HCV), that is a RNA virus of the Flaviviridae family. The infection may develop as symptomatic and asymptomatic way and can be transmitted through parenteral, sexual, by sharing contaminated objects or vertical transmission. On average, 80% of the infected are unable to eliminate the virus, evolving into chronic form, the 20% manage to eliminate it within a period of six months from the start of the infection. Chronic forms can evolve to cirrhosis or even hepatocarcinoma. The diagnosis is made through serological tests and examinations with molecular biology techniques. There is no vaccine for hepatitis C, the treatment is of great complexity and need to be done in specialized services and, when indicated, can be done through the association of antivirals. The objective of this study is to identify the prevalence of HCV infection in users of illicit drugs in the State of Amapá, serological tests were carried out to identify HCV antibodies in 49 illegal drug users residing in the State of Amapá, the results obtained were compared with the prevalence found in the general population. Data on detection rates in the general population were acquired from the Ministry of Health data for the years 2015 and 2016. Of the 49 users of illicit drugs investigated, only one tested positive for anti-HCV test, representing a prevalence of approximately 2%, much larger than the results found in official records between 2014 and 2015 for the population of Amapá, where only 3 cases form identified in a universe of 450,000 inhabitants, representing a prevalence of approximately 0.7%. Negative results indicate that these individuals have not been in contact with HCV or that immunological window time was not respected for a possible detection of the virus. The detection of viral RNA can be considered a more sensitive diagnostic methodology that the serology, seen that the prevalence of infection identified in the population of Amapá in this same period was 0.11% when this methodology was employed, methodology which will be subsequently employed in this study. Serological analyses show that, proportionally, the prevalence of HCV infection is approximately 4 times greater than the prevalence in the general population, indicating the need for public health policies specific to this population. Financial support: CNPq

Palavras-chave: anti-HCV, Flaviviridae, HCV, Hepatitis C, prevalence
PREVALENCE OF VIRAL HEPATITIS B, C AND DELTA, SYPHILIS AND HIV IN PRISONERS IN THE WESTERN AMAZON, BRAZIL

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Resumo

Introduction: Sexually transmitted Infections such as viral hepatitis and HIV are a serious global public health problem. Prevalence studies show that in the northern region of Brazil there are areas of high endemity, mainly for hepatitis B and Delta, although studies in this respect are still scarce. Entering the population deprived of freedom in this context, specific studies have been developed to determine the contribution of this population in the maintenance of the chain of transmission of these infections due to the conditions of confinement, social marginalization, drug addiction, low socioeconomic status and access to health services, which contributes to the sexual and parenteral transmission. In this scenario, this study aimed to determine the prevalence of viral hepatitis B, C and Delta, HIV and syphilis in the male population deprived of freedom in closed prison system in the Western Amazon. Materials and methods: The screening was performed by filling in an epidemiological investigation and venous blood collection for immunoassays and molecular tests, as flowcharts recommended by the Ministry of health, followed by statistical analysis. We included reeducating aged between 18 and 70 years old who have agreed to participate in the study, through the signature of informed consent-FICS. Results: 650 apprentices participated in the study, 345 (53.1%/650) had between 18-30 years; 339 (52.2/650) have not completed elementary school. Were diagnosed 79 (12.2%/650) apprentices, 10 (1.5%/650) for HBV, 9 (1.4%/650) for HCV, 10 (1.5%/650) for HIV and 55 (8.5%/650) for Syphilis; 3 (0.3%/650) were carriers of Coinfection, 1 (0.1%/650) for HIV/HCV and 2 (0.2%/650) HBV/Syphilis. Among the positives 51 (64.6%/79) do not receive intimate visits; 33 (41.8%/79) did not use a condom during sexual intercourse; 42 (53.2%/79) reported not having sex in the last year; 13 (2%/79) related with disabled partners IST; and, 31 (39%/79) are drug users. The samples were submitted for genotyping and genetic variability assessment, including sample 1 (0.1/650) reagent for HIV type 2 in triage. Conclusion: The data shows high prevalence of STI in the prison population, which reinforces the possibility of intramural infection, demonstrating the need for nurturing.

Palavras-chave: Epidemiology, Sexually Transmitted infections, Prisoners
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PROFILE OF BALB/C LUNG INVOLVEMENT IN SECONDARY INFECTION WITH DENGUE VIRUS: HISTOPHATOLOGICAL AND ULTRASTRUCTURAL ASPECTS

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Resumo

Dengue virus (DENV), member of the Flaviviridae family, occurs as four antigenically distinct serotypes (DENV-1, -2, -3, -4). Dengue fever (DEN) is a leading cause of illness and death in the tropics and subtropics. One of the major challenges regarding DEN studies in humans and the development of vaccines and drugs is the lack of an appropriate experimental animal model that reproduces an infection similar to DEN human cases, including Dengue Hemorrhagic Fever (DHF). Patients with secondary DEN infection of a heterologous serotype present higher probability that the disease may evolve to the hemorrhagic form. The susceptibility of DENV murine models has been reported in many studies. However, these models use neonatal or immunosuppressed mice, or resort to intracerebral inoculation, an extremely invasive route of infection and neuroadapted virus strains. The present study demonstrates morphological alterations in lung of immunocompetent mice with DENV secondary infection. The BALB/c mice were infected by the intravenous route with non-neuroadapted DENV samples (DENV-1: primary infection and DENV-2: secondary infection) with doses of 10000 TCID50/0.1 mL. The titers of DENV-1: 106.23TCID50/0.1mL and DENV-2: 106.66TCID50/0.1mL were calculated by the Reed & Muench method. Euthanasia occurred 72 hours after reinfection. For histological studies, the lung samples were fixed in Millonig solution, embedded in paraffin and stained with hematoxylin and eosin. For ultrastructural studies, the specimen were fixed by perfusion and immersion, embedded in epoxy resin and contrasted with uranyl acetate and lead citrate. Analysis by phase contrast light and transmission electron microscopy revealed interalveolar septum thickening due to presence of inflammatory cells, vascular congestion, presence of red blood cells inside the alveolar spaces, inflammatory cells, platelets and edema of capillaries. Morphometric analyzes evaluating interalveolar septum thickening resulted in an average of 6.74 μm interalveolar septum thickness for negative control mice (non-infected mice) and 16.03 μm thickness for infected mice. Morphological alterations observed in lung were similar to those observed in DEN human cases and in murine model studies for primary DENV infection and confirm the susceptibility of BALB/c mice to DENV-1 infection and DENV-2 reinfection with non-neuroadapted virus strains. Support: CNPq, IOC

Palavras-chave: dengue, secondary infection, mouse model, morphology
Yellow fever (YF) is an endemic zoonotic disease in tropical regions of South America and Africa caused by the *Yellow Fever Virus* (YFV), an arbovirus. An effective vaccine is available since the 1930s, consisting of attenuated viruses derived from the African *Asibi* strain. However, adverse vaccine reactions have been reported. As clinical symptoms and signs presented by vaccine reaction may not be distinct from those caused by natural infection with the wild-type virus, we developed a rapid molecular diagnostic protocol for YFV based on a duplex Taqman RT-qPCR assay able to differentiate between wild-type infections caused by American genotype from vaccine reactions and other African genotypes infections. Primers and probe targeting the vaccine strains were designed based on the alignment of available vaccine strains sequences, using Geneious software version 4.8.3 and were named 17DD. Yfall primers and probe which target both wild type and vaccine strains were also used in this study. 17DD probe was labeled with a different fluorescent reporter to allow for specific detection of vaccine and other African strains in a duplex reaction. YFV RNA was in vitro transcribed from plasmids containing the target sequence of both primer/probe sets. All RT-qPCR reactions were performed using Quantitec Probe RT-qPCR Master Mix kit (Quiagen) on ABI 7500 fast system (Applied Biosystem). First, the ideal reaction conditions were determined and then a standard curve was constructed to evaluate the limit of detection (LOD), limit of quantification (LOQ) and efficiency of the assay. Optimal concentration of Yfall and 17DD primers was 0.4 μM whereas the optimal probes concentration was 0.2 μM. The LOD was 100 copies/reaction and the LOQ was 1000 copies/reaction. The assay showed values close to 100% efficiency. The duplex RT-qPCR assay developed in this study was able to quantify YFV viral genome load with satisfactory efficiency and reproducibility. In addition, the assay was capable of differential detection of American genotype wild type virus from vaccine 17DD strain used in Brazil in a single reaction. All together, these data indicate the assays potential to be a valuable tool for the diagnostics of YFV suspected cases by not only providing fast results but also by helping in the investigation of vaccine reactions and by surveillance of the possible introduction of African YFV strains in Brazil. Financial Support: CNPq, Evandro Chagas Institute.

**Palavras-chave:** Detection, RT-qPCR, Standardization, Yellow fever, Yellow fever virus
Noroviruses are an important cause of acute gastroenteritis. The high incidence of norovirus, associated to its great genomic and antigenic variability, reflects mechanisms of viral evolution, such as mutations and recombination. Recombinants cause both outbreaks and sporadic cases; however, there are few studies on norovirus recombination in Brazil. The objective of this study was to investigate the occurrence of recombinant strains of norovirus in children with or without acute gastroenteritis symptoms in two different periods of sample collection in Goiânia, Goiás. The first sample collection period (P1) was from October 2009 to October 2011 in a day-care center and the second sample collection period (P2) was from May 2014 to May 2015 in a children’s referral hospital. Norovirus positive samples from, both periods were amplified and sequenced at ORF1/ORF2 junction target region. Genetic recombination was evaluated by phylogenetic analysis of the partial sequence of the polymerase and capsid gene. Phylogenetic trees were constructed using the MEGA program (Molecular Evolutionary Genetics Analysis) version 7.0. The analyzes were performed by the Neighbor-Joining method, Kimura nucleotide substitution model 2 parameters and 1000 replicates and bootstraps values were considered above 80%. Seven sequences from the P1 and nine sequences from the P2 were obtained and characterized as: one GI.P7/GI.7, six GIIP.P7/GII.6 (P1); one GI.P5/GI.5, one GII.P16/GII.3, two GII.P7/GII.6 and four GIIP.Pe/GII.4 (P2). Genotypes GII.P7/GII.6, GII.P16/GII.3 and GIIP.Pe/GII.4 were identified as having the potential to be norovirus recombinants. This is the first study conducted in the Central West region of Brazil to characterize recombinant norovirus strains. Characterization of recombinants from different parts of the world is of great importance, since variability is one of the factors that contributes to the burden of norovirus gastroenteritis. Financial Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); Fundação de Amparo à Pesquisa do Estado de Goiás (FAPEG); Universidade Federal de Goiás (UFG).

Palavras-chave: Norovirus, Recombination, Genetic diversity, Norovirus recombinants
The Zika virus (ZIKV) is an arbovirus belonging to the family Flaviviridae, genus Flavivirus. The disease is characterized as mild and self-limiting, usually without serious complications. In 2015, Brazil confirmed the first autochthonous case of Zika resulting in a serious public health problem due to the dramatic increase in the number of newborns with microcephaly associated with ZIKV. Based on phylogenetic studies there are hypotheses suggesting that ZIKV was introduced in the country in 2014 during the World Cup or Canoeing Championship or in 2013 in the Confederations Cup. However, these studies did not include samples from Rio de Janeiro (RJ), considered the largest tourist center in the country, which hosted several mass events between 2012 and 2014. Therefore, it is important to carry out a retrospective study in negative sera for dengue and rubella from patients who presented a clinical picture compatible with Zika infection from the period of 2012 to 2014. The real-time RT-PCR method applied to look for RNA of ZIKV in dengue and rubella negative sera patient’s from different municipalities of the state of RJ. To date, 680 sera were analyzed and ZIKV has not been detected, suggesting that the virus has not circulated in the period 2012-2014, which reinforces that the introduction of the virus into the state may have occurred in January 2015, when the first autochthonous cases were detected. However, 550 sera comprising the period from August to December 2014 are being tested. After the completion of this study, we hope to be able to estimate the year the ZIKV was introduced in the State of Rio de Janeiro.
Sensitivity Assessment of qPCR for Zika Virus

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Resumo

The Zika (ZIKV) virus is an Arbovirus, Flaviviridae family, Flavivirus genus, transmitted by Aedes mosquitoes, associated with severe cases of viral infection with increase of congenital neurological anomalies and neuropathic disorders. Virus detection is performed by propagation in cell cultures and Real-Time PCR. The molecular method is considered appropriate for detection and quantification of the viral genome rapidly and accurately. In this context, the Laboratório de Tecnologia Virológica is committed in establishing a sensitive method for quantification of Zika to characterize the replicative profile of a vaccine strain candidate and the viral load of animal models in preclinical trials. Then, it were tested two sequences previously described by Lanciotti (2008) and the sequence described by Faye (2013) that encodes part of Envelope and NS5 proteins respectively. In order to quantify copy numbers we designed a synthetic gene (273 base pairs) inserted in a commercial plasmid that contains three regions of virus genome. Plasmid DNA was transformed in E. coli Top 10, followed by DNA isolation and fluorometry quantification (268 ng/μL) and copy numbers were determined by Avogadro formula (10e10 copies/μL). The standard curve was serially diluted in the following range: 10e7 – 10e1 copies/μL. Sensitivity was evaluated by serial dilution of Zika Virus PB (BeH815744) in negative monkey sera (10e7 – 1 copies/reaction). Amplification was performed using TaqMan Fast Virus 1-Master Mix (Life Technologies) with specific oligonucleotides for each target. The oligonucleotides described by Lanciotti (genome position 1086-1162), was evaluated using fluorescent probes labeled with FAM or VIC. The results indicated better performance by the oligonucleotides described by Lanciotti (835-911) using the FAM probe to quantify low copy numbers. This system was sensitive and reproducible to quantify up to 10 copies/reaction (LOD). Besides that, it was compared de Threshold Cycle (CT) of each viral concentration using different oligonucleotides sets. A difference of 3 CTs was observed in the same concentration, depending on the set that was used. This finding is highly important since most techniques use only this parameter as result. Then, we developed a sensitive method that precisely quantify low copies of Zika virus genome to evaluate viremia of animal models and may also be used in diagnostic support. This technique is in validation process according to RDC27 – ANVISA.

Palavras-chave: qPCR, ZIKA, VACCINE, SENSITIVITY
**Introduction**: The Brazil’s Influenza Surveillance is composed by sentinel surveillance for Influenza-like Illness (ILI) and Severe Acute Respiratory Infection (SARI) for the Intensive Care Unit (ICU) patients and universal surveillance of SARI. The Sentinel Surveillance relies on a network of units distributed in all geographic regions of the country and its main objective is to identify circulating respiratory viruses and to monitor the demand for care for this disease.

**Materials and Method**: The data were collected from the “Sistema de Informação da Vigilância Epidemiológica da Gripe” (SIVEP Gripe v.1.28.11) and analyzed by Business Influenza-Influenza; EpilInfo v.7 and Excel 2010. **Results**: In 2016, all the 137 ILI sentinel units collected 20,397 samples. Of these, 16,957 (82.1%) were processed and 26.1% (4,368/16,757) positive for respiratory viruses, of which 2,510 (57.4%) were positive for influenza and 1,854 (42.6%) for Influenza Respiratory Viruses. Furthermore, 3,193 samples were collected by the 115 SARI sentinel units at ICU, 3,193 were made, of which 2,822 (88.4%) were processed. Among these, 939 (33.2%) had a positive result for respiratory viruses, of which 447 (47.6%) for influenza viruses and 504 (52.4%) for other respiratory viruses. **Conclusion**: The sensitive and rapid diagnosis identifying circulating respiratory viruses, improved by the RT-qPCR implementation, encourages the sentinel units’ work and incorporates surveillance strengthening actions. This work enabled the co-infection detection cases and demonstrates the opportunity to study these viruses co-circulation. **Financial Support**: Ministry of Health of Brazil

**Keywords**: influenza, surveillance, epidemiological, profile, virus
Arbovirus (Arthropod-bone virus) often cause diseases in humans, with a severity of clinical evolution varying from asymptomatic cases to fatal cases. Arboviruses are commonly associated with epidemics in urban centers such as Dengue virus (DENV), Mayaro (MAYV) and Chikungunya (CHIKV). In the municipality of Jataí-Goiás, several dengue epidemics have occurred, but there is a small number of studies directed at the epidemiological investigation of arboviral circulation. Therefore, the present study had the objective of investigating the arboviral seropositivity of Alphavirus (MAYV) and Flavivirus (DENV) from human samples presenting acute febrile disease and suspected Dengue fever from Jataí-GO. Consequently, a total of 293 samples, suspected of Dengue fever, stored in the hospital of the Municipal Medical Center of Elzevir Ferreira Lima Laboratory were selected for laboratory analysis. First, the samples were screened by IgM/IgG, and NS1 DENV ELISA. Then, for the DENV-negative samples, the EIA-ICC (enzyme immunoassay on infected cultured cells) method was used, which uses cells of Aedes albopictus (C6/36) mosquitoes that were fixed in the ELISA plates and infected with the MAYV viral line, aiming the serological detection of anti-MAYV IgM. Thus, 43.8% (130/293) and 57% (163/293) of the samples belonged to male and female, respectively. Regarding the results of the ELISA assays, 52.2% (150/293) were considered DENV reagents. For the DENV-negative samples (139/293) screened by the EIA-ICC, stands out 2.2% (3/139) of IgM seropositivity against MAYV. With the accomplishment of this study, it was possible to verify a high rate of DENV-negative samples, since 47.8% (143/293) of the samples were negative by laboratory methods. In summary, our results of MAYV seropositivity probably suggest that cases of Mayaro fever are occurring and going unnoticed in the study region, a fact that reinforces the need for the adoption of epidemiological surveillance measures aimed at the MAYV.

**Palavras-chave:** Dengue virus, Mayaro virus, EIA-ICC, Arbovirus, ELISA
Resumo

Hepatitis B virus (HBV) infection is a serious public health problem worldwide, as it can cause severe chronic liver disease and hepatocarcinoma. HBV can be transmitted by percutaneous or mucosal exposure to infected blood or body fluids. Graduation students in the health area carry out part of their academic activities in situations similar to professional practice, placing them at risk of exposure to biological material. Determine the seroprevalence and associated factors for HBV in students of the Health area of a University Center in Caruaru-PE. The study was of the descriptive cross-sectional type. The students answered a questionnaire containing questions related to the vaccination schedule for HBV; previous serological tests for anti-HBs; injected drug usage; use of condoms; blood transfusion/blood products. The Elisa test was performed to investigate anti-HBc Total and anti-HBs serological markers. Biokit manufacturer kits were used for both determinations. The entire technical procedure was performed according to the package inserts and the results were analyzed according to the manufacturer's validation criteria. The results were stored and evaluated in Excel®. 181 students from biomedicine and pharmacy courses at the University Center were evaluated. The prevalence for total anti-HBc was 18.8% and the anti-HBs was 63%. Of the participants evaluated, 78% were female; 75% had an age group of 19 to 25 years; 1.7% had already received blood transfusion/blood products; 19% did not use a condom in sexual intercourse; only 10.3% had previously been tested for anti-HBs; 88% had already taken HBV vaccine, although only 50% had received the 3 doses. We found students who had been in contact with HBV at some time in their lives, however, most of the students were immunized due to the vaccine, corroborating with other works in the literature. Epidemiological studies to know the immunological status of HBV in the population are relevant, since in this way data related to infection can be known, such as the number of people who have already become infected, immunized and susceptible to the virus and thus to think about vaccination strategies.

Palavras-chave: HEPATITIS B, PREVALENCE, SEROLOGY, STUDENTS, VACCINE
Among the 272 samples tested for measles, 195 (71.69%) were positive in the 10-20 year-old group, and 38 (84%) in the 21-30 year-old group. For measles, this rate was 26 (58%) positive between the 10-20 yr-old group, and 24 (51%) positive in the 21-30 yr-old group. For measles, this rate was 26 (58%) positive between the 10-20 yr-old group, and 24 (51%) positive in the 21-30 yr-old group. The MMIR vaccine is recommended to all ages, however, youngers are showing low antibody rates, probably due to the lack of a booster dose. Therefore, our study will perform the active search of these patients to verify their vaccination status. Currently, anti-vaccine movements have worried countries worldwide, as it may contribute to the increase of measles and rubella cases, which will lead to higher mortality rates, especially in children, thus making it difficult to eradicate it. Financial support: FAPESP, CAPES.
The Orthohantavirus genus, are medically important rodent-borne pathogens responsible for two severe diseases being a burden on public health worldwide. These viruses are maintained in natural cycles in rodents’ reservoirs and humans are accidentally infected through inhalation of aerosolized rodent excreta, causing the Hantavirus Cardiopulmonary Syndrome (HCPS). In this context, understanding the ecology of Hantavirus disease in wildlife and rural areas has become a crucial theme. The aim of this work was assess the circulation of Hantavirus in small mammals and rural human population belonging of the Serra municipally, Central Minas Gerais State, Brazil through a One Step qPCR platform and serological tests for hantavirus. Small mammals were trapped in several environments (forest, pasture and domiciliary areas) and biomes (Transition between Cerrado and Atlantic Forest). Small mammals’ organs and blood samples of human population were collected for further lab processing. A total of 240 individuals and 50 small mammals were sampled during 2012–2013. The seroprevalence rates of IgG and IgM antibodies in humans were 7,1% (17) and 1,6% (4) respectively and an Oligoryzomys nigripes, captured in peridomestic area close to the farm, tested positive for IgG antibodies. Molecular screening of small mammals revealed the same rodent (O. nigripes) positive in tissue samples tested. The partial fragment of S segment (213 pb) amplified was sequenced and the orthohantavirus identified here was clustered in monophyletic group with JQTV strains, shared the common ancient ancestor with strains reported in O. nigripes captured in Rio de Janeiro and Espírito Santo State. In this study, we found evidences of JQTV circulation in Central Minas Gerais State, Brazil, an area poorly examined to the present date. Interesting to note, that 4 individuals were positive for IgM antibodies, probably representing a recent infection, coinciding with a current orthohantavirus circulation, although the seropositive individuals were apparently healthy because not even prodromal symptoms. Our findings suggest a silent circulation of hantavirus in a region of intense livestock and mining activities, without previous report of HCPS, highlighting the need for surveillance programs to improve the understanding of circulating hantaviruses ecology in poorly studied regions in Brazil and their associated public health burden.

**Palavras-chave:** Hantavirus, Emerging Infectious Diseases, Environment, wild rodents, Minas Gerais
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**SMALLPOX BIODEFENSE IN BRAZIL: CLINICAL AND MOLECULAR DIAGNOSIS**

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**Resumo**

Smallpox was eradicated in 1980, but there is still concern about the potential use of the orthopoxvirus variola virus (VARV) as a bioweapon. A successful biodefense response is based on the rapid clinical diagnosis of potential smallpox cases. Therefore, we have started a cross sectional study to evaluate the diagnostic capacity of Brazilian physicians from different regions and health institutions. By using confidential two-step questionnaires, we have already surveyed 172 voluntary physicians who are expected to recognize a standard clinical case of smallpox based on color photographs and prioritize two diagnostic hypothesis. Smallpox was considered as a possible diagnosis by 69.5% of 59 evaluations (from 26 to 60 years-old) that have been fully analyzed. Of those, 80.5% opted for smallpox as the first diagnosis choice. The second multiple-choice questionnaire investigates physicians’ specific knowledge to handle smallpox patients. Chickenpox was correctly chosen as a differential diagnosis by 78% and, although 72.9% assured to have studied smallpox in Medical school, only 25.4% were correct about the time frame of smallpox infectiousness period. In addition to the clinical diagnosis, laboratory confirmation is also necessary. Therefore, we are testing a Taqman duplex real-time PCR assay associating the specific detection of VARV A4L gene (Kondas et al, 2015) with the OPV F4L gene (Maksyutov et al, 2015). Cantagalo virus (CTGV) is an emergent OPV that circulates in Brazil and causes pustular disease in dairy cattle and milkers. Infection of immunocompromised patients could be clinically misdiagnosed as a mild smallpox case. Therefore, a specific diagnostic test is needed. Assays were performed using the reporter dye FAM (VARV) and/or VIC (OPV) measured against the passive reference dye (CXR) signal for normalization. The analytical LOD was established using a standard curve of serial 10-fold dilutions of a synthetic 330-bp DNA fragment, containing 154bp and 176bp of the target regions of VARV A4L and OPV F4L genes, respectively. The LOD for both VARV and OPV were 10 molecules at Ct of 36.98 and 37.01, respectively. DNA samples at 5 and 10 ng/μl of purified CTGV were OPV positive with Ct of 16.74 and 15.25, respectively with no cross-detection by the VARV-specific primers. DNA samples from approximately 30 clinical isolates of CTGV are currently being processed for similar analysis. This study aims to contribute to smallpox biodefense in Brazil.

**Palavras-chave:** smallpox, biodefense, clinical diagnosis, molecular diagnosis, Brazil
Chikungunya fever is an arbovirus transmitted by the Aedes aegypti and Aedes albopictus mosquitoes infected by the Chikungunya virus (CHIKV). Clinical manifestations can range from the mild form of the disease to more severe forms, such as arthralgia that can last from days to years, leaving some people incapacitating to perform their services. In recent years, methods for a rapid and early diagnosis of CHIKV infection have been widely researched. The objective of this study is to standardize the xMAP Luminex methodology for the detection of CHIKV RNA in acute phase of infection in clinical samples of patients assisted at a University Hospital in the city of Rio de Janeiro. For this study, 5 cell culture supernatants infected with CHIKV were used to standardize amplicon hybridization temperature with activated beads, where temperatures of 42°C, 45°C, 48°C were used. Then, 18 samples with CHIKV cDNA previously amplified by real-time PCR were tested by Luminex assay at the standardized temperature. In addition, 10 samples of individuals without symptoms of arbovirus infection and no cDNA amplification in real-time RT-PCR were tested to evaluate the specificity of the method. RNA extraction from the samples was performed, followed by cDNA acquisition steps, RT-PCR amplification and RT-PCR/ Luminex detection. Detection by the Luminex system relies on hybridization of amplified products in RT-PCR and a capture probe that has been linked to a respective set of fluorescent carboxylated magnetic microspheres. When we analyzed cell culture supernatants infected with CHIKV we observed that the amplicons when hybridized at 42°C with the active magnetic microspheres obtained satisfactory results. From the 18 samples tested using the hybridization temperature of 42°C, all reacted in the Luminex system, being possible to distinguish from the negative samples used in this work. The RT-PCR/ Luminex system designed in the present study could be an efficient tool to aid in the detection of CHIKV. However, a larger number of samples should be tested in the validation step and the standardization for simultaneous detection with the four dengue virus serotypes and with the zika virus detection is in progress. Financial support: FAPERJ, CAPES, CNPq

**Palavras-chave:** Chikungunya virus, early diagnosis, molecular diagnosis, Luminex system
RESUMO

Hepatitis C Virus (HCV) replicates at high rate and the viral polymerase (NS5B) does not have corrective activity, which provides wide genetic variability. In 2015, the new direct acting antivirals were approved in Brazil, Simeprevir (protease inhibitor), Daclatasvir (DAC; NS5A inhibitor) and Sofosbuvir (SOF; NS5B polymerase inhibitor), which resulted in an increase of sustained virological response (SVR). SOF and DAC or SOF associated with Interferon (IFN) and Ribavirin (RBV) is the current treatment of genotype 3 infected patients in Brazil. However, genotype 3 infected patients, the second most prevalent in the country, particularly cirrhotic, present minor SVR when compared to the other genotypes. The emergency of resistance associated variants constitutes the major challenge in antiviral therapy success, and studies involving these mutations in genotype 3 are still scarce. This work aims to investigate resistance mutations in NS5A and/or NS5B regions before treatment in patients infected by HCV genotype 3 submitted to the treatment regimens with SOF or SOF/DAC. Until now blood samples from 42 patients infected with HCV genotype 3, treated for 12 weeks with IFN, RBV and SOF or SOF and DAC were collected before the beginning of the treatment. Viral RNA was extracted and cDNA was synthesized. Samples were amplified by Nested-PCR for NS5A and NS5B regions and sequenced. So far, NS5A and NS5B regions were sequenced in 42 and 11 patients, respectively. For NS5A, from the resistance mutations described in the literature we found substitutions at amino acids 30 (A/K – 4.7%; A/T – 2.4%) and 62 (A/S – 76.2%; A/T – 16.6%; A/L, A/N e A/F – 2.4%). Undescribed mutations were observed in 12 different positions. For NS5B, no described mutations were identified. However, 32 mutations were found when compared to the reference sequence in different sites. Up to now, mutations at position 62 of NS5A protein showed to be at high frequency, such as A62S (76.2%) which is 30.1% higher than what is observed in literature. The pursuit of resistance mutations already known in literature and the description of possible new mutations, before antivirals administration, is fundamental to understand the process of treatment evasion, as well as understand, in a precise way, the ideal medication regimen for each patient.

Palavras-chave: Direct-acting antivirals, HCV, Resistance mutations, Treatment evasion
Following the establishment of universal rotavirus (RVA) vaccination in Brazil in 2006, a significant decrease were observed in under-5-years diarrhea-related mortality and hospital admissions for diarrhea in the country. In this context, other enteric viruses could emerge; including enteric Adenovirus types 40 and 41 (AdV 40/41). The aim of this study was to determine the frequency of AdV in children <5 years with acute gastroenteritis in Brazil in 2015, and conduct it's the molecular characterization. A total of 456 fecal samples were obtained between January and December 2015, and first screened for RVA and norovirus (NoV). A total of 250 RVA and NoV negative samples were tested further for the presence of AdV by PCR. Positive AdV samples were sequenced to genotype characterization. AdV was detected in 29 cases (11.6%); median age of 11.3 months. AdV infection was observed throughout the year, with an increased incidence occurring from February to April. AdV F 41 was the most frequent genotype (51.7%, 15/29), followed by AdV F 40 (10.3%, 3/29), AdV C 1 (10.3%, 3/29) and AdV C 2 (10.3%, 3/29). Together, AdV F 41 and 40 were responsible for more than half (62%) of the AdV positive cases obtained here. Other genotypes, including AdV C 5 (6.9%, 2/29), AdV A 12 (3.5%, 1/29), AdV A 31 (3.5%, 1/29) and AdV D 56 (3.5%, 1/29), were also detected.

The nucleotide analysis showed that the Brazilian AdV samples clustered together with strains from distinct continents, indicating that AdV strains circulating in Brazil were closely related to those strains circulating worldwide. Although AdV species F was the most frequent detected, the role of non-enteric AdV species A and C cannot be ignored in diarrheal children. AdV D 56 was described for the first time in a fecal sample from patients with acute gastroenteritis, although a clear association between genotype and acute diarrhea could not be established. The results presented contribute to the knowledge of the AdV genotypes circulating in Brazil, and suggest an increase in the AdV detection rate after RVA vaccine introduction. AdV screening should be considered as differential diagnosis in Public Health Laboratories, and continuous studies are essential to verify the impact of others enteric viruses in the community after RVA vaccination.

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**Palavras-chave:** Gastroenteritis, diarrhea, enteric adenovirus, molecular epidemiology
Chikungunya virus (CHIKV) has been responsible for important emerging and reemerging epidemics of a disease characterized by intense and incapacitating polyarthralgia in several tropical and temperate regions of the world. In Brazil, there is a currently triple arbovirus epidemic caused by the concomitant circulation of CHIKV, dengue virus (DENV) and zika virus (ZIKV), which makes the differential diagnosis extremely difficult for health professionals. Thus, the main objective of the present study was to investigate CHIKV suspected cases and the possible occurrence of coinfections among the three circulating arboviruses, realize the comparative analysis of different clinical outcomes and perform the molecular characterization and phylogenetic analysis of CHIKV strains from an epidemic that have occurred in Rio de Janeiro, Brazil during 2016. For that, 91 suspected cases were collected and submitted to serological and/or molecular diagnosis of the three arboviruses and the results demonstrate that 69/91 (75.82%) cases were confirmed for CHIKV and/or DENV and/or ZIKV, being 40/69 (57.97%) CHIKV monoinfection cases, 9/69 (13.04%) ZIKV monoinfection cases, 16/69 (23.18%) CHIKV and ZIKV coinfection cases, 3/69 (4.34%) CHIKV and DENV-4 coinfection cases and 1/69 (1.44%) ZIKV and DENV-4 coinfection cases. In all those clinical outcomes, we observed a predominance of female patients, individuals aged 26 to 30, followed by 41 to 45, and signs or symptoms such as arthralgia (89.85%), myalgia (91.30%), prostration (82.60%), headache (81.15%), fever (78.26%), anorexia (66.66%), chills (66.66%) and low back pain (65.21%). We also demonstrate for the first time in the literature the E1-K211T mutation was revealed in all analyzed samples. Other studies will be needed to clarify the consequences of these changes in mosquito fitness and in the human immune system, being of fundamental importance the monitoring of the CHIKV suspected cases and the dispersion of circulating genotypes, besides the identification of possible mutations that facilitate mosquito vectors transmission, especially in regions where there is a vast territory, high density of the vector, presence of susceptible individuals and intense movement of tourists.
Viral hepatitis is a of the most important public health problems in the world, affecting several segments of the population and causing great impact of morbidity and mortality. The main etiological agents of viral hepatitis are classified in alphabetic order of A-E. Chronic viral hepatitis, mainly B and C, present high endemicity in the Western Amazon region. Therefore, the present study aimed to identify the prevalence of chronic viral hepatitis in the indigenous population belonging to the DSEI Porto Velho / RO located in the Amazon Region. The study was subsidized in a documentary survey of information regarding the quantitative immune chromatographic tests for HBV and HCV carried out in the indigenous area covered by the DSEI Porto Velho / RO, from January 2014 to June 2016. According to criteria of Research Ethics With Human Beings with approval n° 304/2000. It was observed the performance of these tests in 11729 indigenous people, where in 2014 were performed 10,147, in 2015 an average of 11,702 and 3,321 tests in the first half of 2016. Based on the results, the prevalence of anti-HCV was 2664 (26%). Cases and 2621 (25.8%) for HBsAg in 2014, 2979 (25%) for anti-HCV and 2511 (21.4%) of reactive HBsAg, and 852 (25.6%) anti-HCV And 837 (25%) HBsAg in the first half of 2016. The incidence of viral hepatitis B and C in the DSEI pole of Porto-Velho / RO was 20.73 / 1000 in inhabitants in the year 2016. We conclude that the presence of the virus HCV has been increasing in the indigenous population. Lack of vaccine has contributed to this growth, and despite the efforts to expand vaccine coverage for hepatitis B throughout the state of Rondônia, the endemicity of B virus remains high in the region. Support: PAP–PRÓ RONDÔNIA FAPERO/CAPES Nº. 012/2016, PPSUS –FAPERO/MS DECIT/CNPq/SESAU-RO – Nº. 003/2016.

Palavras-chave: Prevalence, viral hepatitis, indigenous
In 2017, Brazil faced the largest epidemics and epizootics of yellow fever (YF) in recent years. When we analyzed the epidemics in Minas Gerais state (MG), the pattern fits the expected one during YF epidemics, apart from the huge number of cases and deaths: 1,147 cases (446 confirmed) and 209 deaths (159 confirmed), and the great affected geographical area. Most cases (64%) concentrated in four regions of MG, in small municipalities (up to 20,000 residents) with low-medium vaccination coverage. Confirmed cases/deaths (77%/72%) concentrated in rural areas, mostly observed in men, rural workers (72%). In contrast, the epizootics had some uniqueness, being widespread in MG (confirmed in 121 and suspected in 351 municipalities) in rural and urban areas. We received, 301 NHP carcasses to be identified, processed, analyzed and deposited in the Collection of Mammals-ICB/UFMG. Total viral RNA was extracted from liver (RNAeasy Mini Kit - Qiagen) and used for qPCR with primers and probe for YFV (GoTaq®Probe 1-StepRT-qPCR System-Promeega). From 94 sample, 39.3% were positive. YFV was detected in Alouatta sp., A. fusca, A. guariba (10.8%), Callicebus sp. (10.8%), and Callithrix geoffroyi and C. penicillata (78.3%), in rural (35.1%) and urban (32.4%) areas. Phylogenetic analysis (based on envelope gene) indicated that the YFV from detected here in NHP clustered together with other ones from the current outbreak. The analysis reinforced the importance of vaccination to prevent YF in humans, especially in rural areas. YFV was detected in NHP 8 macroregions of MG, confirming new epizootics. The data showed the extensive occurrence of YFV in MG and worryingly, the presence of YFV in NHP in urban areas, including big urban centers, as Belo Horizonte. The role of the infected NHP inserted in urban matrixes in virus cycle should be carefully addressed, since it may be a source of virus in urban areas. In fact, during the epidemics in MG, four human cases had origins in urban areas. Further spatial, temporal and phylogeographical analysis will be done, and other studies are vital to measuring the impact of YF epizootics in NHP population. YELLOW FEVER GROUP(other members): UFMG: F.Perini, F.Santos, A.Paglia, A.I.A.Prado, R.G.A.V.Stump, R.L.Massara, A.M.O.Pascoal, E.G.Kroon; IBAMA/CETAS-BH/MG: E.P.Teixeira, C.Barreto, D.A.R.Vilela, L.S.S.Matos; IRR-FIOCRUZ-MG: P.A.Alves. FINANCIAL SUPPORT: FAPEMIG, CNPq, CAPES, ERDF from European Commission, and Institut Pasteur de la Guyane

**Palavras-chave:** Yellow fever virus, Yellow fever, Zoonosis, Non-Human Primates, Epizootics
THE POST EPIDEMIC SOROEPIDEMIOLOGY STUDY OF MOTHERS AND NEWBORNS IN SALVADOR BY ELISA- NS1 (ΔNS1)- ZIKV- IgG SPECIFIC ANTIBODY.

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Resumo

Zika virus (ZIKV) is a constituent of the Flaviviridae family, genus Flavivirus, and is transmitted by Aedes species mosquitoes. The spread of ZIKV throughout the Americas and its association on the increasing incidence of fetal neurological abnormalities has led to an unprecedented interest in this scarcely known pathogen until the outbreak of Yap/Micronesia’s Island, in 2007. Since 2015 the country committed to responding several questions about the viral pathogenesis and its relationships with neonatal microcephaly. The serological diagnosis are gradually available being implemented in the Brazilian health system and there is no serological test produced in the country to detect IgM and IgG against ZIKV. In this context, our goals is to evaluate serum sample from mothers who had newborns with and without microcephaly in 2015 and 2016 from the city of Salvador (epicenter of the cases of microcephaly in Brazil). In this study we are being test 885 serum from Mothers for specific IgG antibodies ELISA using a recombinant antigen, encompassing the C-terminal portion of the NS1 (ΔNS1) that allowed the detection of ZIKV-specific antibody. Preliminary results showed that among 273 samples tested until now only 136 (49,8%) were positive for specific ZIKV IgG antibody. In conclusion the detection of specific IgG antibodies is an important tool to determine if there was any exposure to ZIKV during the period of pregnancy and to establish the prevalence of specific antibodies in both groups of mothers of study. Financial Support by Fundação de Apoio à Pesquisa e à Extensão, Conselho Nacional de Desenvolvimento Científico e Tecnológico and Fundação de Amparo para Pesquisa do Estado de São Paulo processo 08727-5 OLIVEIRA, D.B.L

Palavras-chave: ZIKV, IgG, ELISA, Mothers, Newborns
Ever since its introduction in the Brazilian territory, in 1981, dengue virus serotype 4 (DENV-4) has remained absent from the national epidemiological scene for almost 25 years, until its reintroduction in 2010. This event marks the cocirculation of the 4 known serotypes in Brazil, and although the mechanisms behind the virus’ pathogenesis are not yet fully understood, the interaction between antibodies originated from heterotypical infections is an essential factor for the evolution of mild dengue (DEN) cases into hemorrhagic and shock cases. The difficulty in studying the interaction between virus and host is mainly due to the absence of an experimental animal model that adequately replicates the DENV infection as observed in humans. Current models utilize immunodeficient animals, invasive inoculation routes, and neuroadapted viral inocula, conditions that not only hamper the comprehension of the pathogenesis but also prevent the use of such models for testing vaccines and drug candidates. The present study aims to analyze the potential tropism of DENV-4 for hepatic, pulmonary and cardiac tissue and evaluate the morphological and ultrastructural alterations caused by the virus in said tissues. To achieve this goal, immunocompetent mice of the BALB/c line were inoculated via intravenous route with non neuroadapted doses of DENV-4 isolated from human case. Alterations befitting those observed in human cases of DEN, such as the presence of inflammatory infiltrate, hemorrhage and vascular congestion were observed in infected mice tissues via phase contrast light, and further analysis via transmission electron microscopy revealed a widespread presence of inflammatory cells, areas of necrosis, uncontained platelets, and DENV-like particles. The virus’ RNA was detected in multiple tissue samples in very high titters. Such alterations presented a similar profile to those shown in human cases of DEN, and the detection of the virus within the tissue show the susceptibility of the model to the serotype. Financial Support: CNPq, IOC

**Palavras-chave:** DENV-4, BALB/C MICE, VIREMIA, TROPISM, ELECTRON TRANSMISSION MICROSCOPY
Resumo

After Zika virus circulation in Brazil, it was observed a correlation of the infection of pregnant women with this virus and poor fetal development, leading to severe conditions such as microcephaly and other neurological diseases. Regarding the Zika association with serious health conditions, researchers began to look for different ways of prevention and treatment of Zika virus infection. Berberine, an isoquinoline alkaloid present in many plants including B. vulgaris, has several pharmacological properties, and has showed to be particularly effective against the entry and replication steps of many viruses. This study was conducted in order to test the virucidal activity of Berberine in ZIKVBR and analyze the possible interaction between the natural compound and the viral proteins in silico. For virus preparation, Zika virus strain (ZIKABR) was inoculated into Aedes albopictus mosquito cells (clone C6/36). Different concentrations of Berberine (0–600 µM) were incubated on Vero E6 cells to perform a cytotoxicity assay. To confirm the possible interaction of Berberine with the ZIKVBR particles, this compound was mixed with exact amounts of the virus and used to infect cells of the Vero E6 lineage. Subsequently, the drug-containing supernatant was titrated by plaque reduction neutralization test (PRNT). It was observed that concentrations above 160 µM of Berberine were toxic (cell viability less than 80%). Moreover, when the virus was incubated with 160 µM it was noticed a reduction of 87.7% in the number of foci. Values of CC50 (221 µM) and EC50 (55.43 µM) for Berberine were calculated for obtain the Selectivity Index (SI) of 4.1, where the higher the value, the more promising are the drugs. Vero E6 cells pre-treated with Berberine was inoculated with ZIKVBR to test if Berberine could influence cellular receptors used by the virus on the entry step. However, treatment with Berberine had no effect on blockage of virus entry. Furthermore, silico analysis were performed in order to analyze the possible regions of interaction between E protein and EGCG. Our results infer that Berberine interact with αβ helix of this virus, a region of E protein, suggesting that this interaction could explain the virucidal activity of Berberine. For the first time it has been shown that a natural compound, Berberine, extracted from plants widely used in oriental medicine presents virucidal effect in ZIKVBR into Vero E6 cells. Financial Support: FAPESP

Palavras-chave: Berberine, Virucidal, Zika virus, ZIKV
Whole genome sequencing and phylogenetic analysis of Zika virus in the DF, MT and to regions in Brazil.

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Introduction: Zika virus (ZIKV) is an emergent Flavivirus transmitted by mosquitoes of the genus Aedes, first isolated in the Zika forest in Africa at the year of 1947. Human infections usually are asymptomatic or self-limited but the associations with neurological syndromes like Guillain-Barré and microcephalia were the cause of its emergence as a public health problem, mostly in the American continent, specifically in Brazil. Considering that the emergence of ZIKV in Brazil is still obscure and that there is a lot to understand about this virus, this work has as main aim to generate the completed sequence of ZIKV in the states of Mato Grosso (MT), Tocantins (TO) and Distrito Federal (DF) of Brazil. In addition, we want to generate the phylogeny and phylogeography of the virus in these regions. Methods: For this study, we have collected 100 samples from individuals with positive diagnostics for ZIKV at the Laboratory LACEN/SES/DF. The RNA of the samples was extracted, cDNA was generated and amplification for each sample was performed with two pools of primers that combined will cover the whole genome of ZIKV. Agarose gel and quantification reactions were performed after PCR amplifications. Sequencing of the whole genomes will be done using NGS methods. Bioinformatics analyses will assemble the whole genome of ZIKV and this sequence will be used to do phylogenetic trees that will be compared with all completed sequences present to date in the public databases. Results: Our preliminary results were generated in a pilot study performed with 68 samples where 15 showed amplification for the whole genome of ZIKV. Conclusion: We believe that the ZIKV whole genome sequence will show the genetic diversity of the virus and with this information it will be possible to build phylogenetic trees, find possible evolution traces and also better understand the patterns of dissemination of ZIKV in these regions. Financial Support: FAPDF and CAPES.

Palavras-chave: Zika virus, Whole Genome Sequencing, Genome Assembling, Epidemiology, Phylogenetics
YELLOW FEVER VIRUS IN NON-HUMAN PRIMATES: PRELIMINARY DATA AND VIRAL DISSEMINATION TO NEW AREAS IN SÃO PAULO STATE, BRAZIL (2016-2017)

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Resumo

Yellow Fever virus (YFV) is a single positive RNA virus that belongs to family Flaviviridae, genus Flavivirus. In humans, yellow fever may vary from inapparent to a fatal disease, with patients presenting fever, prostration, hepatic, renal and myocardial injury, hemorrhage and shock. In Brazil, the virus is maintained by a sylvatic transmission cycle involving non-human primates (NHP) and forest canopy-dwelling mosquito, mainly Haemagogus spp. Urban yellow fever, transmitted by Aedes aegypti, was eradicated in 1942 after years of vector control programs. This is a preliminary descriptive study encompassing epizootic events between July 2016 and March 2017, in São Paulo State, Brazil. Fresh tissues and tissues fixed in 10% neutral buffered formalin from NHP found dead or serum from live animals were sent to Adolfo Lutz Institute, São Paulo, for YFV molecular detection and immunohistochemistry. A total of 429 NHP from different counties of São Paulo State were received by Núcleo de Doenças de Transmissão Vetorial, and 541 by Centro de Patologia. A total of 67 NHP were positive for YFV during the time studied. However, 9 animals were positive only by molecular detection, being 3 due to autolysis and six belonging to genus Callitrix spp. with Ct values in liver ranging from 17 to 38, and in brain ranging from 26 to 36. Until December 2016, only cities within the vaccine recommendation area reported epizootic events due to YFV. However, in the begging of 2017, YFV was detected in areas not deemed to be at risk for yellow fever. During the time studied, positive NHP were detected in 25 cities. Three complete genomes from Ribeirão Preto, Tabapuã and Catanduva were obtained. Samples from this study clustered within YFV group 1E of South American genotype I. We found a nucleotide similarity range between 66.8% and 86.9% across the entire genome. The identification of the epizootics events prompted vaccination in local susceptible population. Therefore, this report highlights the importance of Adolfo Lutz Institute for YFV epizootics identification, an important tool for the prevention of human cases of sylvatic yellow fever

Palavras-chave: Yellow fever virus, Non-human primates, São Paulo
ADJUVANT-MEDIATED EPITOPE SPECIFICITY OF ANTIBODIES TARGETING DENGUE VIRUS ENVELOPE PROTEIN

DENICAR LINA NASCIMENTO FABRIS MAEDA, MILENE TAVARES BATISTA, LENNON RAMOS PEREIRA, JAIME HENRIQUE AMORIM, SARA ARAÚJO PEREIRA, SÍLVIA BEATRIZ BOSCARDIN, SANDRIANA RAMOS DA SILVA, STEPHEN ALBERT JOHNSTON, LUIS CARLOS DE SOUZA FERREIRA, JULIANA FALCÃO RODRIGUES

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Resumo

Introduction: The heat-labile toxins (LT) produced by enterotoxigenic Escherichia coli (ETEC) strains have been intensively investigated as vaccine adjuvants. LT toxins augment antibody responses to co-administered antigens and modulate IgG subclasses in mice immunized via mucosal or parenteral routes. Despite several studies of the adjuvant properties of LT and its non-toxic derivatives, little is known about the properties of these adjuvants in modulating the epitope specificity of antibodies antigen-specific. The present study investigated the property of LT and a non-toxic derivative, the B subunit (LTB), to modulate the antibody responses mounted to an antigen co-administered to mice via a parenteral route. For that purposes we used as a target antigen a recombinant protein corresponding to the domain III of the type 2 dengue virus (DENV2) envelope glycoprotein (EDIII). Methods and Results: The administration of LT and LTB induced higher EDIII-specific serum IgG responses in comparison with vaccine formulations containing the antigen alone or co-administered with alum as adjuvant. Antibodies induced with LT or LTB showed higher viral neutralization activity, despite similar serum IgG subclass responses and antigen affinity. Immunosignature analyses carried out with synthetic peptides revealed that mice immunized with LTs elicited serum EDIII-specific IgG antibodies with different epitope-binding patterns with regard to animals immunized with EDIII alone or alum-adjuvanted protein. Based on this analysis, it was possible to identify of an EDIII-specific epitope located in the EF to FG loop. In vitro assays demonstrated that a synthetic peptide corresponding to the identified epitope inhibited the infection of Vero 2 cells by a DENV2 strain. Conclusion: The results demonstrate that administration LT or LTB modulates the epitope specificity of antibodies generated in mice immunized with a recombinant protein. Such property may particularly relevant for the development of anti-dengue subunit vaccines. Financial support: FAPESP, CNPq.

Palavras-chave: Adjuvant, Dengue, Envelope glycoprotein (EDIII), Epitope specificy, Heat-labile toxins (LT).
384 - ÁREA: IMUNOBiológicos

Cloning, Expression and Purification of Recombinant Antigens Based on the Zika Virus Non-Structural Protein 1 (NS1)

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Resumo

The Zika virus belongs to the family Flaviviridae and to the flavivirus genus, having, therefore, evolutionary links with other emerging pathogenic viruses like Dengue and Yellow Fever viruses. Currently, ZIKV infection represents a major public health problem mainly due to neurological complications of neonates born from infected mothers. There aren’t specific vaccines or treatments available so far and differential laboratory diagnoses are performed by molecular biology techniques. ZIKV nonstructural protein 1 (NS1) is employed in serological assays but the specificity of the assay regarding other flavivirus, particularly Dengue virus, remains uncertain. One alternative to circumvent such limitations would be the generation of fragments derived from this protein that may confer better serological specificity without compromising the sensitivity of the assay. Thus, the objective of the present study was to express and purify a fragment of the non-structural protein 1 (Delta NS1) of ZIKV and evaluate its use as an antigen in serological tests. To selected sequence was cloned and expressed in Escherichia coli ArticExpress and BL21 (DE3) strains using the pET28a expression plasmid. Transformants were selected and cultured in LB medium and expression of the recombinant protein was achieved after overnight incubation with IPTG under constant aeration. The soluble protein extract was subjected to affinity purification and gel filtration using an automated AKTA system. The procedure led to the expression of ZIKA Delta NS1 in both E. coli strains but expression of a soluble form was improved with the ArticExpress strain. Aliquots of the purified protein, either native or heat denatured, were used as a solid phase antigen in an ELISA assays, using sera positive for ZIKV infection. As a conclusion the present results demonstrated that the recombinant protein was successfully obtained at high purity, rather good recovery yields and preserved antigenic structure. Collectively, these results indicate that the recombinant Delta NS1 represents a potential antigen candidate for specific ZIKV serological assays. Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) e Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Palavras-chave: Diagnoses, NS1 protein, Zika virus
385 - ÁREA: IMUNOBIOLOGICOS

DETECTION OF FLAVIVIRUS BY PLASMON RESSONANCE

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Resumo

Dengue virus is transmitted by Aedes aegypti which also transmits Yellow fever virus and Zika virus. The absence of effective vaccines and specific treatments for these viruses makes the elimination of the vector insect the most effective action to control these diseases. In this context, the detection of these viruses in mosquitoes prior to their transmission to the population may be very important to improve efforts to eliminate mosquitoes, preferably in areas where the circulation of these viruses is observed. The gold nanoparticles (AuNPs) have unique physicochemical properties, such as collective oscillation of electrons, classified as a surface plasmon resonance (SPR), that can be easily adjusted to detection of biomolecules such as proteins and antibodies bound to the AuNPs. Therefore, AuNPs appear as an excellent nanomaterial option for the development of an innovative and effective method to detect the circulation of flaviviruses in mosquitoes. The objective of this study was to develop and analyze the effectiveness of the nanoparticle functionalization methodology with specific anti-DENV and anti-flavivirus antibodies as well as its ability to detect antigens. For this, these IgGs were connected to AuNPs and used to detect different virus concentrations by analyzing the plasmon resonance in a UV-Vis scanning spectrometer. Our results showed that when AuNPs were incubated in solution containing dengue virus, a strong change was observed in the first minutes of incubation. When these nanoparticles were incubated with the Mayaro virus (Alphavirus), displacement was observed only in the presence of a very high concentration of virus, which is not found in natural samples, demonstrating the specificity of the system. Thus, it is suggested that gold nanoparticles can be used for a low cost diagnosis of production and are faster, more accurate and convenient than existing techniques. Financial Support: CNPq and Fapemig

Palavras-chave: flavivirus, diagnosis, nanomaterial, gold nanorods, plasmon resonance
DEVELOPMENT AND EVALUATION OF INDIRECT ENZYME-LINKED IMMUNOSORBENT ASSAYS FOR SEROLOGIC DIAGNOSIS OF CHIKUNGUNYA AND MAYARO VIRUSES

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Resumo

Chikungunya (CHIKV) and Mayaro (MAYV) are emerging arboviruses transmitted by the bite of infected mosquitoes from Aedes genus and Haemagogus genus, respectively. These viruses are single-stranded positive RNA genome with 13 kilobases that encodes to non-structural (nsP1 - nsP4) and structural (Capsid, E3, E2, 6K and E1) proteins, they are classified into Alphavirus genus from Togaviridae family. Both viruses are responsible to cause human disease, which includes symptoms as a sudden fever and joint involvement that can persist for long periods of time. The objective of this study was to express and purify a recombinant envelope (E2) protein of CHIKV and MAYV without transmembrane domain, as well as to apply it to develop and standardize serologic indirect ELISA’s. The E2 gene targets sequences were cloned in an expression plasmid vector, expressed using a prokaryote expression system (Escherichia coli) and purified using a chromatography affinity system with nickel columns. The proteins were used as antigen to develop the indirect ELISA’s to detect specific IgG or IgM antibodies against MAYV and CHIKV. In order to standardized both ELISA’s, a panel of polyclonal antibodies against MAYV and CHIKV, and human sera samples suspected of CHIKV or MAYV infection were used to evaluated the sensibility and specificity of both assays. In addition, the indirect ELISA’s developed in our study was compared with plaque reduction neutralization assay (PRNT50) and other serologic ELISA’s based on infected C6/36 cells or total viral particle as the capture antigen and also to a commercial rapid diagnostic test. We have observed a higher sensibility, specificity and detection limits of both ELISA’s based on E2 of MAYV and CHIKV when compared to PRNT50, ELISA based total viral particle and rapid diagnostic test. In addition, we have performed a seroepidemiology survey with 2,000 human serum samples from human blood donor bank from São Carlos City, São Paulo State, Brazil, collected during 2016 and 2017. Overall, we developed a sensitive, specific, simple, low-cost, able to can detect recent and past MAYV and CHIKV infections for surveillance studies, and epidemiological surveys. Financial Support: Fundação de Amparo à pesquisa do Estado de São Paulo (FAPESP)

Palavras-chave: ELISA, E2 Protein, Chikungunya virus, Mayaro virus
DNA VACCINES BASED ON OPTIMIZED GENES OF THE DENGUE 2 ENVELOPE AND NON-STRUCTURAL 1 PROTEINS: EXPRESSION IN VITRO AND IMMUNE RESPONSE IN MICE

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Resumo

Dengue virus (DENV) comprises four antigenic serotypes (DENV 1-4) and the viral genome encodes three structural proteins (capsid, premembrane/membrane and envelope) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5). The envelope (E) protein binds to receptors on the host cell surface and mediates entry of the virus into those cells. Hence, the immune response against the E protein may be protective by eliciting neutralizing antibodies. The NS1, which plays an important role in viral RNA replication and viability, may also induce protective immune responses. Therefore, our group constructed two DNA vaccines against DENV2 (pE1D2 and pcTPANS1) that were able to induce protection in mice. Based on these original DNA vaccines, new plasmids were constructed containing synthetic sequences of E and NS1 genes from DENV2 optimized for expression in mouse and human cells. The optimized E and NS1 genes were cloned into a pcTPAotm expression vector and were respectively named pEotmD2 and pNS1otmD2. Transfections were carried out in cell lines from human monocyte-like (THP-1), human hepatocarcinome (HuH7) and mouse neuroblast (Neuro2A), in order to evaluate the expression of recombinant proteins by immunofluorescence and flow cytometry. BALB/c mice were immunized with plasmids containing original or optimized genes and challenged with DENV2. Results revealed that THP-1, HuH7 and Neuro2A transfected with optimized genes were able to promote expression of E and NS1. Flow cytometry analysis of Neo2A and HuH7 cells revealed a higher number of cells expressing E protein after transfection with the pE1D2 plasmid when compared to pE1otmD2 transfected cells. There was no variation in the number of cells expressing NS1 after transfection with both original and optimized plasmids. As for the mouse immunization, plasmids pcTPANS1 and pNS1otmD2 induced similar levels of protection in terms of mortality and antibody titers. However, mice immunized with pNS1otmD2 presented higher morbidity rates after virus challenge when compared to pcTPANS1-inoculated animals. Plasmid pE1otmD2 elicited a poorer humoral and protective immune response when compared to pE1D2. Overall, the superior performance of the original plasmids was evidenced, although further experiments will be performed in order to confirm these results. Financial Support: PAPES-Fiocruz, IOC-Fiocruz, CNPq, INCTV, PPSUS-FAPERJ.

Palavras-chave: Dengue virus, DNA vaccines, Envelope, NS1, codon optimization
EVALUATE THE TIMING OF MEMORY STATUS AFTER 17DD-YF-PRIMARY VACCINATION INVESTIGATED BY SYSTEMS BIOLOGY-BASED APPROACHES

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Resumo

In this study, machine-enhanced techniques were applied to bring about scientific insights to identify a minimum set of phenotypic and functional (P&F) memory-related biomarkers for post-vaccination follow-up upon yellow fever (YF) vaccination. For this purpose, memory status of circulating T-cells (Naïve/early-effector/Central-Memory/Effector-Memory) and B-cells (Naïve/non-Classical-Memory/Classical-Memory) along with the cytokine profile (IFN/TNF/IL-5/IL-10) were monitored before-NV(day0) and at distinct time-points after 17DD-YF primary vaccination - PV(day30-45); PV(year1-9) and PV(year10-11). A set of P&F biomarkers (eEfCD4; EMCD4; CMCD19; EMCD8; IFNCD4; IL-5CD8; TNFCD4; IFNCD8; TNFCD8; IL-5CD19; IL-5CD4) were observed in PV(day30-45), but not in NV(day0), with most of them still observed in PV(year1-9). Deficiencies of P&F biomarkers were observed in NV(day0), while total lack of memory-related attributes was observed in PV(year10-11), regardless of the age at primary vaccination. Venn-diagram analysis pre-selected 10 attributes (eEfCD4, EMCD4, CMCD19, EMCD8, IFNCD4, IL-5CD8, TNFCD4, IFNCD8, TNFCD8, IL-5CD19, IL-5CD4), of which the overall mean presented moderate accuracy to discriminate PV(day30-45) & PV(year1-9) from NV(day0) & PV(year10-11). High-dimensional data handling, starting from comprehensive decision-tree algorithms, defined EMCD8 and IL-5CD4 as the top-two predictors with moderated performance. Together with the PRNT titers, the top-two biomarkers led to a resultant memory status observed in 80% and 51% of volunteers in PV(day30-45) and PV(year1-9), contrasting with 0% and 29% found in NV(day0) and PV(year10-11), respectively. The deficiency of memory-related attributes observed at PV(year10-11) underscores the conspicuous time-dependent decrease of resultant memory following 17DD-YF primary vaccination that could be useful to monitor correlates of protection in areas under risk of YF transmission.

Palavras-chave: Biomarkers, Cell immunology, PRNT, Vaccinology, Yellow Fever
Patients present increased expression of activation markers and production of cytokines, which contributes to an enhanced inflammatory status that characterizes AIDS progression. Patients under ART usually fail to restore their immunological status, even when achieving a virological response. Therefore, new therapeutic strategies able to control the viremia and modulate immune activation may be beneficial to prolong patient’s life, and reduce the use of ART. Beta-cyclodextrin (BCD) is a cholesterol-sequestering drug, which is safe for human use, and that has been shown to inactivate HIV in vitro and to control the infectivity of SIV in a macaque model. Recent studies demonstrated that alteration in cholesterol content or metabolism affect macrophage immune activation. Therefore, we evaluated whether BCD treatment would modulate the activation of HIV+ derived monocytes in response to inflammatory stimuli. Monocytes were isolated from HIV+ and HIV- donors, treated with BCD and, then, stimulated with LPS. Our data showed the BCD treatment induced a decreased expression of CD36 and intracellular TNF-alpha after LPS stimulation. Accordingly, BCD-treated monocytes showed significant reduction of TNF-alpha and IL-10 secretion, but not of several other cytokines analyzed, demonstrating that BCD did not induce an overall unspecific inhibition of monocytes. Also, this response was not due to lipid rafts disruption, since membrane cholesterol levels and the expression of surface proteins CD45 and CD59 (raft and non-raft associated proteins) were already recovered at the time of LPS stimulation. In fact, BCD treatment downregulated the expression of TNF-a and IL-10 mRNA, indicating its action at transcriptional levels. Also, LPS-induced PI3K activation was diminished in BCD-pretreated cells, suggesting that the drug may affect PI3K/akt signaling pathway. We also observed that BCD treatments impaired the expression of MHCII and costimulatory molecules in monocytes and dendritic cells stimulated by LPS, what might contribute to the control of T cell activation. Our data suggest that, besides its well-known antiviral activity, BCD have immune-modulatory role, leading to a decreased inflammatory response mediated by antigen presenting cells. Therefore, BCD treatment, may contribute not only to restore the virological status of HIV patients, but also to modulate chronic immune activation in AIDS.

Palavras-chave: HIV, BCD, Monocytes, Inflammation
The development of a safe and efficient HIV vaccine is considered the best measure/strategy to control the worldwide HIV epidemic. This project aims to construct and evaluate the immunogenicity of recombinant 17D vaccine yellow fever viruses that express viral infectivity factor (Vif) antigens of simian immunodeficiency virus (SIV). The yellow fever 17D vaccine virus has been used as a vector for vaccine prototypes for being a robust and safe immunogen. Our goal is to test yellow fever 17D recombinant viruses bearing fragments of VIF (HIV/SIV) genes that were constructed with the infectious clone technology and the gene insertion in the E/NS1 intergenic region. We verified previously that the recombinant 17D YF/Vif 1-110 virus is genetically unstable, completely losing the Vif 1-110 fragment until the fifth serial passage in Vero cells. Considering that the Vif N-terminal region, which is an RNA binding domain, could interfere with the 17D YF vaccine virus replication, we constructed a new recombinant 17D YF/Vif 42-110 (Vif 2) virus, that is genetically stable. We hypothesized if the viral genetic stability could increase viral immunogenicity. Hence, we proceeded with C57BL/6 mouse immunization with vaccine and recombinant 17D/Vif viruses. The immunogenicity of recombinant 17D viruses has been evaluated regarding induction of neutralizing antibodies and memory T cells. Cytokine production will also be assessed. In preliminary analyses, we observed that recombinant viruses recombinant YF/SIV- Vif 2 induced higher YFV-neutralizing antibody titers as well as activated significantly more T CD8⁺ cells and T CD4⁺ effector memory cells in comparison with the original one expressing Vif 1-110. Our results indicate that the viral genetic stability increases the immunogenicity perhaps due to an increased viral fitness and proliferation in the vaccinees. Financial support: Grant P01 AI094420-01, funded by HIV Vaccine Research and Design Program (HIVRAD) of the National Institute of Allergy and Infectious Diseases and the Oswaldo Cruz Institute/Fiocruz.

Palavras-chave: Human Immunodeficiency Virus (HIV), Simian Immunodeficiency Virus (SIV), Vaccine, Viral infectivity factor (Vif), Yellow fever virus
Introduction: Seasonal Influenza viruses can undergo high mutation rates by antigenic drift, leading the vaccine manufacturers to change the vaccine formulations frequently. Annual vaccination is the most effective method to prevent seasonal influenza infection. The IB seasonal influenza vaccine contains 15ug of hemagglutinin (HA) from H1N1, H3N2 and B strains. The limited capacity and complications in the production of influenza vaccine made WHO suggests the production of adjuvanted vaccines. Studies demonstrated an oil-in-water (o/w) emulsion effectiveness; therefore we are interested in the mechanisms involved in the immune response induced by o/w used as the IB flu vaccine adjuvant. Methods and Results: We investigated the differences between subcutaneous (SC) and intramuscular (IM) administrations of the chosen formulations in animals. Groups used: I (Saline), II (w/o), III (0.4ug HA), IV (0.4ug HA+ w/o). We analyzed immune cells subsets in animal's Peripheral Blood Mononuclear Cells (PBMC) and those ones recruited to quadriceps muscle (in IM) by flow cytometry (2 days after immunization); performed sera hemagglutination inhibition assay (HI) and ELISA for the total IgG from animal's samples (21 days after immunization). Results: We observed significant differences in peripheral macrophages and neutrophils percentage between groups IV SC and IV IM. ELISA and HI assays confirmed the differences between SC and IM. IM administration showed significantly higher production of specific and neutralizing antibodies in comparison to the SC. Conclusion: The analysis of the immune cells populations recruited into the muscle (IM) reinforced that w/o possibly creates a favorable microenvironment for the uptake of the antigen, increasing the amounts of APCs and neutrophils combined with the production of specific antibodies against Influenza. Our next perspectives are to use w/o as adjuvant of pandemic influenza strains vaccines and understanding how the antigens are presented in the lymph node due to the use of this so efficient adjuvant. Supported by: Fapesp and CNPq. Affiliation: All authors are from Laboratory of Bacteriology II except Ho, P. L. that is from Special Laboratory of Innovation and Industrial Development.

Palavras-chave: Influenza, Vaccine, Adjuvants
Bovine papillomatosis is an infectious disease caused by bovine papillomavirus, a virus of circular double strand DNA, non-enveloped and icosahedral conformation. This infectious agent provokes skin and mucosal lesions known as papillomas. This disease in bovines leads to a higher susceptibility to weight loss, reduction of meat and milk production and devaluation of leather. One possible tool to support prevention and therapy are the DNA vaccines, which have been documented as steady and low cost. The selected genes for study were wild E5, which is the main oncoprotein of BPV, being a good therapeutic target. Also, the N-terminal region (amino acids 11-200) of the L2 structural protein which induces antibodies production, being a target for prophylaxis. We propose the vectors construction based on E5 and L2 (11-200) fusion, to use them as candidates for DNA vaccines against BPV with prophylactic-therapeutic activity. With that aim, we amplify by conventional PCR using specifics primers of genes wild E5 and the N-terminal region containing the amino acids from 11-200 of gene L2, both from BPV-2 genome. In the first step, gene L2 (11-200) was inserted into the cloning vector pGEM T easy to use for appropriate restriction sites for subcloning into expression vector. The gene L2 (11-200) was then excised using the appropriated restricted enzymes and connected to the expression vector pCI neo, generating the pCIL2 (11-200) construct. The confirmation of this step were executed by DNA sequencing. The wild E5 gene was then attached to the pCIL2 (11-200) expression cassette generating the pCIESwtL2 (11-200) fusion. The preliminary result from the pCIESwtL2 (11-200) construction was confirmed by digestion. In the future, we will evaluate the expression and production of pCIESwtL2 (11-200) fusion protein in vitro.

FINNANCIAL SUPPORT: PIBIC-FACEPE

Palavras-chave: BPV, DNA Vaccines, Papillomatosis
PRODUCTION OF RECOMBINANT ZIKA VIRUS NS3 PROTEIN FOR ASSESSMENT OF DIAGNOSTIC AND IMMUNOGENIC POTENTIAL

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Resumo

The Zika virus (ZIKV) is recognized as a neurotropic virus and its infection has been related to several neurological disorders such as Guillain-Barré syndrome in adults and cases of microcephaly in fetuses. ZIKV infection has been documented in 66 countries and, despite the rapid spread and severity of clinical manifestations that may be related to the virus, there are difficulties in establishing diagnostic methods and vaccine strategies. Among the main difficulties encountered in the diagnosis, it can be highlighted the co-circulation of other antigenically similar arboviruses that may lead to cross-reactivity. Several viral proteins have been studied for their potential use in the diagnosis, prevention and anti-viral therapy. Besides an interesting target for anti-flavivirus drugs, some studies report the possible use of NS3 protein in the diagnosis of Dengue virus (DENV) infection exhibiting good levels of sensitivity and specificity. Furthermore, some studies report the induction of humoral and cellular immunity after immunization with recombinant DENV NS3 protein. Transposing these findings to possible applications for ZIKV, this work proposes the production of recombinant ZIKV NS3 protein in a bacterial system that is an economical, easy to handle and high yield biotechnology platform. The NS3 gene was cloned into the bacterial expression vector pGEX4T-3 and the obtained clones were used to transform Escherichia coli BL21 (DE3). Subsequently, the transformants were cultivated and the heterologous gene expression was induced by addition of IPTG (isopropyl-β-D-galactoside). Confirmation of the protein production was by Western blot with Anti-polyHistidine antibody. The obtained results demonstrate the production of the recombinant NS3 protein which, after purification, can be used in immunological assays to evaluate the diagnostic potential as well as to assess the ability to induce immune responses in challenged animals. Financial Support: FACEPE

Palavras-chave: Diagnosis, E. coli, NS3, ZIKV
Resumo

The gastroenteritis is the second cause of morbidity/mortality in children ≤ 5 years, being the rotavirus A (RVA) causing up to 215,000/year deaths mostly in developing countries. Since 2006, Brazil has introduced the rotavirus vaccine G1P[8] (RV1). Recent studies have shown that RVA recognize the histo-blood group antigens (HBGA) in particular Lewis and secretor antigens, on host cell surface through the VP8* subdomain of the spike protein VP4, which could explain the susceptibility to RVA. The present study aimed to evaluating the homogeneity of the RV1 and the relationship of detection of RVA and the susceptibility profile of the host in a prospective cohort of 153 newborns/infants in Manguinhos/RJ. The detection of viral genomes in feces (15 days before/after the 1st/2nd doses of the RV1) was performed by RT-PCR (quantitative/qualitative) followed by sequencing of the VP7 and VP4 (VP8*) genes. The Lewis and secretor phenotype was determined in saliva by enzyme-linked immunosorbtent assay (ELISA). Up to the present were determined 30 profiles of susceptibility and analyzed together the detection (or not) of RVA. Twelve children were classified as Lewis-secretors without RVA (or RVA-). Eighteen children were RVA+, G1P[8], and in 17 of them it was detected viral shedding (15 samples/1st dose; 3 samples/2nd dose; from 4 to 9 days) and 1 of them was also RVA+ before of the 1st dose. Of these, 15 were classified as Lewis-secretors, and in four of them was found the F167L mutation of VP8* protein. Two children were Lewis-nonsecretors and one of them occurred the shedding for both doses, showing the sample of the 2nd dose had two mutations in VP7 (I20N and N126D) and a silent mutation in VP8*; and one Lewis-nonsecretor child was RVA+ before of the 1st dose. The preliminary results demonstrate that 90% of the children evaluated belongs to the Lewis-secretor status and that there is a pronounced viral shedding G1P[8] (57%) in this cohort. Mutations in the proteins of the viral capsid were detected, and the L167F mutation of VP8* was too identified during vaccine attenuation and it may interfere with the effectiveness of the vaccine; however, are needed more studies to evaluate the possible impact of these mutations and the association with host HBGA susceptibility profile. CNPq founds this study.

QUANTIFICATION OF INFECTIOUS BRONCHITIS VIRUS IN VACCINE BY REAL-TIME QUANTITATIVE REVERSE TRANSCRIPTASE-PCR AND THE RELATIONSHIP WITH TITRATION IN EMBRYONATED EGGS

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Resumo

The prophylaxis of Infectious Bronchitis Disease (IB) in Brazil is based on the active immunization using live vaccines. To a live vaccine be used in the field, it may be evaluate by official quality control. The titration of vaccine is used to verify the quantity minimum of the infectious virus required in a dose of vaccine. The Brazil legislation contemplate the classic titration conducted in embryonated eggs. In this study, the qRT-PCR data of IB vaccine were examined and compared those data with titration in embryonated eggs, to verify if these two methodologies of evaluation are comparable. The QuantiTect Probe RT-PCR Kit (Qiagen) was used to perform qRT-PCR assays. Forward primer IBV5_GU391, reverse primer IBV5_GL533 and probe IBV5'G probe set for IBV were used to amplify and detect a 143-bp fragment of the 5'untranslated region (UTR gene). For this, four types of IB vaccines with the different strains (H120-effervescent, B48, Brazil variant and H120) were tested. After resuspension of the three batches of vaccines, according the manufacturer instruction, the vaccine was titrated, and an aliquot was harvested and kept at -80°C until extraction of viral RNA into quantitative qPCR. The statistical analyses of the titer by dose of vaccines were obtained by three titration of each vaccine strains (a, b, c) and two runs were made by the fresh air (FA) method, was compared with the results obtained with qPCR (3 repetition of 3 titration each vaccine strain) in two runs. The estimation of the relation between the titers obtained in embryonated eggs with the respective number of viral particles/dose obtained by qPCR showed a linear relation with (R2=93,12%), being that it is influenced by the vaccine strain limited to the 4 strains studied. The estimation model from qPCR for titration of each vaccine strain is A = H120 effervescent: TIT = 2.78 + 0.00479 X exponent of log10. qPCR + 1.48752; B = B48: TIT = 2.78 + 0.00479 X exponent of log10.qPCR + 0.59327; C = Brazil variant: TIT = 2.78 + 0.00479 X exponent of log10. qPCR + 1.65762; D = H120: TIT = 2.78 + 0.00479 X exponent of log10. qPCR. It is suggested to add new studies with other strains to reinforce the robustness of the model found. These results indicate that qPCR assays there were one relationship with embryonated eggs to quantify virus in IB vaccines. Financial support: MAPA and VLA

Palavras-chave: Infectious Bronchitis virus, quantification, vaccine, qPCR, titration
Viruses that infect bacteria are the most abundant organisms on the planet and they play important roles in maintaining the ecosystem. In recent years, the use of viruses as a biological control tool has gained a lot of attention, mainly due to the difficulty of isolating new antimicrobial drugs and the selection of bacteria that is resistant to existing drugs. Because of this potential as biological control agents, the studies on these viruses have increased expanding the perspectives of the ecological management of diseases in plants. Bacterial wilt caused by Ralstonia spp., a soil-borne Gram-negative bacteria, is considered one of the most important plant diseases in tropical and subtropical regions of the world. A large number of bacteriophages are capable of lysing or physiologically reprogramming cells of Ralstonia spp. Despite the potential use of these organisms in the management of bacterial wilt, information on viruses that infect Ralstonia spp. is nonexistent in the Americas. The objective of this study is to test and analyze the potential use of bacteriophage in the control of bacterial wilt disease caused by Ralstonia solanacearum in tomato. To perform the biological control experiment, we used the R. solanacearum GM11000 and the bacteriophage PhiAp1, previously characterized by our group, and 5 new isolated phages that have not been characterized yet. To evaluate the intensity of the disease we used the Disease Progress Curve (AUDPC). The delineation used was completely randomized with 5 repetitions for each treatment. Plants inoculated only with GMI 1000 died 3 days after inoculation. Plants inoculated with GMI 1000 + phiAP1 showed a delay in bacterial infection, and the better results were observed in plants inoculated with GMI1000 + phiAP1 + viral mix. The results demonstrate the great potential of phage therapy for future biological control. Financial support: fapemig, capes and cnpq

**Palavras-chave:** control biologic, bacteriophages, Ralstonia, tomato, virus
CHARACTERIZATION OF A NEW COMMON BEAN-INFECTING CYTORHABDOVIRUS TRANSMITTED BY BEMISIA TABACI MEAM 1

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Resumo

Common bean (Phaseolus vulgaris) is an important crop in Latin America and Africa. The bean yield may be affected by the incidence of different viruses. As part of a virus survey in beans, we have used high-throughput sequencing approach to identify viruses with RNA genome in bean plants collected in Goiás (GO) state. Bioinformatic analysis of the de novo assembled contigs identified a putative cytorhabdovirus (family Rhabdoviridae) with low similarities with Northern cereal mosaic virus (NCMV). This new cytorhabdovirus was denominated Bean associated cytorhabdovirus (BaC). Cytorhabdoviruses have enveloped particles, negative ssRNA of 11-14 kb genome and are usually transmitted by aphids or leafhoppers. An isolate from Luziânia, GO, (BaC_Luz) was used for the virus characterization. The 3’ and 5’ ends of BaC were identified by RACE (Rapid Amplification cDNAs Ends). The complete BaC_Luz genome was recovered by PCR of six overlapping fragments and is 13.449 nt in length. The genome presents five essential genes common to rhabdoviruses (N: nucleoprotein, P: phosphoprotein, M: matrix protein, G: glycoprotein and L: polymerase) flanked by two non-transcribed leader and trailer regions. In addition, a gene encoding a likely movement protein is located between P and M. Phylogenetic analyzes conducted with the amino acid sequence of the nucleoprotein showed that BaC clustered together with members of the genus Cytorhabdovirus. BaC grouped in the same clade as NCMV and Barley yellow striate mosaic virus that infect monocotyledons and are transmitted by leafhoppers, but in a different branch of the clade. BaC_Luz was transmitted by whiteflies from a field-infected plant to ‘Jalo’, ‘Pérola’ and ‘BRS FC 401 RMD’ beans with an inoculation access period (IAP) of 14 days, resulting in 100% of transmission efficiency. Under more controlled conditions, aviruliferous adult whiteflies were given a seven-day acquisition access period in infected bean plants and subsequent seven-day IAP in healthy plantlets were tested. Under these conditions, BaC could be transmitted to soybean (Glycine max) ‘BR16’, cowpea (Vigna unguiculata) and common bean ‘BRS FC 401 RMD’, with 25, 50 and 75% transmission rate, respectively. This study presents novel data reporting the identification of the first rhabdovirus infecting beans and for the first time a virus member of the family Rhabdoviridae vectored by Bemisia tabaci MEAM1. Financial Support: Embrapa, CNPq, CAPES and FapDF.

Palavras-chave: Phaseolus vulgaris, Cytorhabdovirus, whitefly, Bemisia tabaci
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COMPLETE SEQUENCE OF TOMATO CHLOROTIC SPOT ORTHOTOSPOVIRUS (TCSV) AND CO-INFECTION BY DIFFERENT VIRUS-DERIVED GENOMES FROM CULTIVATED VEGETABLES IN DOMINICAN REPUBLIC

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Resumo

The plant virus Tomato chlorotic spot orthotospovirus (TCSV) was recently reclassified within genus Orthotospovirus, family Tospoviridae. TCSV was first report in the 1980s, having its occurrence limited to Brazil and Argentina. Recently, TCSV has spread over the USA and Caribbean countries. In Dominican Republic (DR), for example, TCSV has been recently reported in important cultivated crops such as pepper, beans and tomato. Due to an apparent lack of importance in the past, molecular studies concerning TCSV were neglected. In this work, we provide the first complete genome of a TCSV isolate from symptomatic plants in DR, which was obtained by high-throughput sequencing and confirmed by specific PCR assay. In addition, the orthotospovirus Tomato spotted wilt orthotospovirus (TSWV) was identified, as well as other three viruses from different virus families, the endogenous viruses Bell pepper endornavirus (BPEV), Southern tomato virus (STV) and Pepper cryptic virus 2 (PCV-2).

Phylogenetic analysis showed that the Dominican Republic TCSV isolate has a close relationship with other TCSV isolate and a reassortant variant between TCSV and Groundnut ringspotorthotospovirus (GRSV) from USA. BPEV, STV and PCV-2 isolates from DR are close related to other American isolates. The recent DR scenario represents an important model to study plant virus interaction and evolution, since multiple plant virus infections seem to be a common feature and not the exception. The NGS and PCR data showed viruses, which have not been described yet in DR coexisting in the same host with important plant viruses recently reported in the country, as the orthotospoviruses TCSV and TSWV. However, the nature and the consequences of such interactions are still unknown.

Palavras-chave: Dominican Republic, Orthotospovirus, Endogenous viruses, Long beans, chili pepper
The Loreto virus (LORV) strains PeAR 2612/77 and PeAR 2617/77 were first isolated from pools of *Culex sp.* mosquitoes and phlebotomine sandflies collected in Iquitos, Peru, in 1977. It is a virus restricted to infect insect hosts and although the transmission path remains unknown, it has been suggested that the infection occurs via vertical and sexual. LORV is a member of *Negevirus* genus, a new taxon of insect-only viruses closely related to plant viruses. The goal of this study was to describe the first occurrence of LORV in Brazil. From 2006 to 2014, samples from hematophagous insects were collected using CDC traps, hand net and Castro catcher tubes during an entomological surveillance in the Atlantic Forest, Bahia, Brazil. At the Division of Arbovirology and Hemorrhagic Fevers at Evandro Chagas Institute, insects were identified and grouped on 304 pools, based on taxon, site and methods of capture. The arthropod samples were inoculated in C6/36 cell cultures and 63 of them presented cytopathic effect. From these samples the total RNA extraction were performed, followed by the cDNA library construction; 21 samples showed enough quality for the nucleotide sequencing. Next generation sequencing were performed in Illumina platform using MiSeq® and the results analyzed in Geneious v.9.1 program. Among 21 samples, three mosquitoes pools presented the complete sequence of LORV: *Trichoprosopon digitatum, Aedes Ochlerotatus fulvus* and *Limatus durhamii*. The genomes were compared to the references already deposited in NCBI by BLASTx. The average of identity was 94%. The sequences correspondent to hypothetical protein-1 of related viruses included in data set were aligned and analyses by JModelTest 2.1.10 program were performed to find the best fit model. The phylogenetic tree was generated using RAxML v 8.2.9 program. This is the first description of LORV in Brazil and the first notification of natural infection of the referred insect species. Furthermore, the present study contributed to amplify and increase the scarce information about insect-only viruses circulating in Brazil. Financial Support: Saint Louis Zoo WildCare Institute (USA), The Wild Animal Fund, from the American Association of Zoological Veterinarians (AAZV, USA), CNPq (Brasil), the Centre for Research and Conservation of the Royal Zoological Society of Antwerp (Belgium), National Lottery of Belgium, Department of the Health of Bahia State (6ª DIRES), Evandro Chagas Institute and ICMBIO.

Palavras-chave: Negevirus, entomological surveillance, insect-only viruses
The wild potato (Solanum commersonii Dan.) is native to Central and South America. It is of great research interest due to resistance characteristics that are not commonly found in cultivated potatoes. During 2014-2015, wild potato plants exhibiting geminivirus-like symptoms such as yellow mosaic and deformation on the upper leaves and plant stunting were observed at the Experimental field of Embrapa Vegetables, Brasília-DF, 45-50 days after planting of tubers. Whitefly populations were observed on potato plants during the time course of the field experiment. Symptomatic plants were estimated on nearly 5% in the field assay. This work aimed to report begomovirus infection on wild potato S. commersonii, in Brazil. Thirty-one leaf samples were collected from symptomatic wild potato plants and subjected to total DNA extraction using CTAB method. Total DNA was tested by PCR-based methods using a pair of universal primers for begomovirus detection, which amplifies a fragment of ca. 1.1 kbp of DNA-A component. In addition, the viral genome was amplified by Rolling Circle Amplification (RCA) using Phi-29 DNA polymerase and afterwards, RCA products were digested with a panel of restriction enzymes for cloning purposes. Digested RCA products were analyzed on 1.2% agarose gel and Hind III restriction enzyme was selected for cloning virus genome. A DNA fragment of ca. 2.6 kb purified from agarose gel was cloned into pBluescript vector and sequenced. Leaf samples were also tested for presence of Potato virus Y (PVY), Potato virus X (PVX), Potato virus S (PVS), and Potato leafroll virus (PLRV) by DAS-ELISA, using polyclonal antibodies against the coat protein produced at Embrapa Vegetables. From a total of 31 potato plants tested by PCR, 20 were positive for begomovirus, producing 1.1 kbp amplicon, confirming that a begomovirus was constantly detected in the wild potato symptomatic samples. Analysis of virus sequence indicated nucleotide identity of 95% with Tomato severe rugose virus (ToSRV), a predominant begomovirus infecting tomato in the Central region of Brazil. No samples were positive for PVY, PVX, PVS and PLRV. From our knowledge, this is the first report of a begomovirus infecting wild potato in Brazil. Financial support: Embrapa and FAPESP.

Palavras-chave: Begomovirus, Wild potato, Detection
Molecular and Biological Characterization of New Potyviruses Infecting Forage Legumes of the Stylosanthes Guianensis.

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Resumo

In Brazil, forage crops represent a large and significant area, with more than 190 million hectares of tropical pastures, both natural and planted serving as a food source for the Brazilian cowherds that has about 214 million animals. These pastures are largely composed of grasses, forage legumes and are essential for the production and quality of bovine meat and milk. The use of Stylosanthes (legume) has been increasing in Brazil its nutritional value; in addition, it presents great potential in the recovery of pastures and degraded areas due to the nitrogen fixation. It being able to grow in very sandy regions and adapted to poor soil areas. Besides the problems, recently, it has observed in experimental and natural fields of Embrapa, typical symptoms caused by agents of viral etiology. In view of the above, the present work had the objective to characterize molecularly and biologically the viruses that are infecting forage legumes of the species Stylosanthes guianensis in Brazil. Preliminary results obtained through new generation sequencing (NGS) in symptomatic samples of Stylosanthes guianensis collected at the Embrapa Gado de Corte, showed the presence of three new members in the Potyviridae family. The contigs were assembled and RT-PCR performed using specific primers designed in a genomic region corresponding to 3’UTR and CP. The amplified segments were sequenced by the Sanger method. From the consensus of the NGS sequences, comparative phylogenetic analyzes of nucleotides and of amino acids of the viral proteins were performed. Phylogenetic analysis showed the presence of three new potyviruses infecting Stylosanthes guianensis, denoted: Stylosanthes mosaic-associated virus 1 (StyMaV-1) and Stylosanthes mosaic-associated virus 2 (StyMaV-2) closely related to Blackberry virus Y (genus Brambyvirus) with capsid protein (CP) amino acid (aa) identity of only 40% and 41%, respectively. In contrast, Stylosanthes mosaic-associated virus 3 (StyMaV-3) was related to the Rose yellow mosaic virus (genus unassigned) with aa CP identity of 59%. Based on the taxonomic rules for species demarcation, these viruses can be considered putative members of two new genera within the family Potyviridae. Currently, our studies involve the production of infectious clones that will allow a better understanding of the interaction between the new members of the Potyviridae and their hosts, as well as, it will make possible a search of genetic resistance in forage plants.

Palavras-chave: Potyviridae, legumes, livestock, Stylosanthes
Cytoplasmic type of citrus leprosis disease (CL-C) is the main viral disease affecting Brazilian citrus orchards. The disease causes localized chlorotic and/or necrotic lesions and induces premature leaf and fruit drop. Citrus leprosis virus C (CiLV-C, genus Cilevirus, ss(+)-RNA) is the prevalent virus producing CL-C from Mexico to Argentina and from which two viral clades are known: CRD and SJP. Extant of members of the clade SJP were firstly discovered in 2015 and bioinformatic tools indicated that viral strains from the two clades were involved in putative natural recombination processes. To shed some light about the molecular epidemiology of CL-C, viruses of the two clades were surveyed in citrus orchards where the disease is widespread. Samples kept in -80°C freezer were also included in the study. A total of 84 samples [Brazil (66), Paraguay (3), Bolivia (1) and Argentina (4)] of sweet orange plants showing typical symptoms of CL and collected during the period 2003-2017 were tested by RT-PCR for the confirmation of CiLV-C presence and the specific identification of the occurring CiLV-C strains. Generated amplicons were independently sequenced by the Sanger method. Results indicated the unequivocally presence of CiLV-C in all samples, confirming that it is the prevalent virus associated to CL in Southern South America. Strains of the clade SJP were detected in samples from Argentina (1), Paraguay (1) and Brazil (45) collected since 2010. Forty-three of the Brazilian samples were from orchards of the state of SP and two from MG. Moreover, CRD strains were detected in SP (26), MG (2), PR (1), ES (1), RS (1), DF (1), and in Argentina (4), Paraguay (4) and Bolivia (1). Remarkably, viruses from the two clades were simultaneously detected in 19 samples from SP, indicating the occurrence of mixed infections in isolated fruit lesions. These results give a natural contextual support for the recombinant processes previously predicted, alert for the epidemiology consequences resultant from the interaction of viral mixed infection with their mite vectors and, additionally, offer insights into the evolutionary forces driving the distribution and diversity of CiLV-C population along the region harboring the largest extension of sweet orange orchards of the world. Financial Support: FAPESP 2016/01960-6 and 2014/08458-9.

**Palavras-chave:** Cilevirus, CiLV-C, Brevipalpus mites
MOLECULAR, ULTRASTRUCTURAL, AND PATHOLOGICAL CHARACTERIZATION OF SPODOPTERA COSMIOIDES MULTIPLE NUCLEOPOLYHEDROVIRUS, THE FIRST BACULOVIRUS HARBORING A NAD-GLUTAMATE DEHYDROGENASE

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Resumo

Baculoviruses are orally infectious to the larval stage of insects. They are used in biological control of agricultural pests due to their specificity, feasibility to be applied, and safety. The virus has circular dsDNA genome and two infectious virions phenotypes; one of them is trapped into a protective occlusion body (OB) and responsible for the spread between hosts. Currently, baculoviruses are divided into four genera, Alphabaculovirus in special infects moths and butterflies. Spodoptera cosmioides is a lepidopteran pest of great economic importance during its larval stage that has been detected attacking soybean. In this work, we characterized a novel virus isolated from a S. cosmioides extract with baculovirus infection symptoms. To do this, we performed the sequencing of the virus genome using the 454 method and de novo assembly with the Geneious-R9 program. Genome annotation was performed using BLASTX. The virus genome contained 148 kbp (G+C of 44.8%) with 141 ORFs. 23 ORFs were unique, including an NAD-glutamate dehydrogenase (glud) never related before in baculovirus. Preliminary analyses revealed that the glud was closely related to ascoviruses. GLUD is ubiquitous in eukaryotes converting reversely glutamate to α-ketoglutarate and playing a key role to link catabolic and anabolic pathways in cells. The presence of glud in insect viruses must be investigated. The virus phylogeny was generated from the alignment of the 38 core genes (shared by all baculovirus), confirming that the virus belongs to the group II of Alphabaculovirus, genetically similar to other Spodoptera-isolated viruses. The virus fulfills all the criteria for new species demarcation. Electron microscopy analyses have demonstrated the uniform shape of the OBs and the presence of more than one nucleocapsid per envelope within the occluded virions. Thus, we proposed as a tentative name Spodoptera cosmioides multiple nucleopolyhedrovirus (SpcoMNPV). A bioassay was performed with third instar host larvae. Insects were fed on artificial diet contaminated by virus in six different OB concentrations. Mortality was assessed after 12 days post-infection. The concentration that generated LC50 was about 3000 OBs/mL (n=710). It is of great importance the characterization of new baculoviruses so that the virus evolutionary history could be better understood. Moreover, this study allows for the development of SpcoMNPV as a biological controller. Financial support: CNPq, FAP-DF.

Palavras-chave: Baculovirus, Viruses, Biological control, SpcoMNPV, Alphabaculovirus
The Baculoviridae family is a vast group of infective viruses of Diptera, Lepidoptera, and Hymenoptera. Baculoviruses have a circular dsDNA genome with 80-180 kbp and have been used as vectors for heterologous protein expression in insect cells and as biopesticides. Previous work have shown that the introduction of the NSs gene into the genome of a baculovirus under the control of a very late phase promoter, increase viral replication, expression of recombinant protein expression in insect cells and virulence towards permissive and semi-permissive insects. The NSs from Tomato spotted wilt virus (TSWV) is a known suppressor of gene silence in plants. In order to analyse the role of the NSs expressed in insect cells at earlier times post-infection together with the presence or absence of the antiapoptotic baculoviral P35 protein, different recombinant viruses were constructed. Besides NSs, the enhanced green fluorescent protein gene (egfpflu) were also inserted into the genome of the recombinant viruses as reporter genes for microscopic and expression analysis of infected cells. Since the flu gene is under control of a very late gene promoter, these viruses were used to analyse the effect of NSs and P35 in very late gene expression. Non permissive mosquito cells did not show viral cytopathic effects in the presence of any recombinant virus. In lepidopteran cells, virus without p35 induced apoptosis in semi permissive and permissive cell lines. The production of occlusion bodies, a sign of viral replication, was only detected in permissive cells infected with virus containing p35. These results indicate that P35 function is not substituted by the NSs protein. The highest expression of luciferase in infected insect cells was obtained with recombinants containing only p35. On the other hand, the weakest expression was found in cells infected with virus containing both genes, p35 and NSs. Our results suggest a possible interaction between P35 and NSs in gene silence pathways when expressed at the same time during viral infection, which results in lack of suppression of gene silence.

**Palavras-chave:** NSs, BACULOVIRUS, TSWV, RNA SILENCE, APOPTOSIS
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SURVEY OF COWPEA APHID-BORNE MOSAIC VIRUS IN PASSIFLORA SPP. PLANTS FROM THE EMBRAPA CERRADOS GERMPLASM BANK “FLOR DA PAIXÃO”

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Resumo

Passion fruit woodiness is a widespread virus disease that causes severe yield losses due to hardening, reduction in size, and deformation of fruits, which devalues the fruits commercially. The disease also diminishes the useful life of the passion fruit orchards and growers have to renew the plants yearly. In Brazil, the disease is caused by the potyvirus *Cowpea aphid-borne mosaic virus* (CABMV) and is prevalent in passion fruit fields. Embrapa Cerrados holds a *Passiflora* germplasm collection of wild species (BAG “Flor da Paixão”) which are used in breeding programs as source of traits of interest to commercial passion fruit cultivars. As part of a virus survey in the plants of the BAG “Flor da Paixão” this study aimed to identify the accessions infected by CABMV. The evaluation was carried out in 58 accessions belonging to 45 different *Passiflora* species. Leaves from both symptomless plants and plants with virus-like symptoms such as mosaic, blistering, yellow spots, and deformation were collected, and total RNA was isolated using TRIzol reagent. Three micrograms of RNA were spotted in a nylon charged membrane and hybridized with a CABMV-derived probe labelled with α32PdCTP. CABMV was detected in 77.6% of the tested plants indicating a high incidence of the virus in the *Passiflora* plants from the Embrapa Cerrados germplasm bank. From the thirteen plants negative for CABMV, 12 exhibited mosaic symptoms implying that they are probably infected by other virus. Financial Support: Embrapa, CNPq, FAP-DF, CAPES.

Palavras-chave: Potyvirus, CABMV, Passiflora spp.
B. yothersi tenuipalpid mites are the main vectors of Citrus leprosis virus C (CiLV-C), the causal agent of citrus leprosis disease, currently the main viral disease of citrus in Brazil. The disease is characterized by symptoms that include local chlorotic and necrotic lesions restricted to the mite feeding sites, leading to leaf and fruit drop, and demanding high costs with control measures. However, there are very few studies on the biology of B. yothersi mites and their interaction with CiLV-C. Thus, through the study of the mite vector transcriptome, we investigated the differential gene expression (DGE) profiles between aviruliferous and viruliferous populations using the RNA-Seq technique. For this, mite populations were reared onto sweet orange fruits (healthy and symptomatic for CiLV-C), and a pool of 500 mites were collected with four replicates/treatment for the extraction of total RNA. Then, molecular analyses were performed by RT-PCR to certify the status of each treatment, and the samples were sequenced by a HiSeq 2500 Sequencing System – Illumina. The data were analyzed with R language and Bioconductor software packages. Reads were mapped in the B. yothersi genome and analyses of DGE were performed. We identified 841 differentially expressed genes in B. yothersi viruliferous population, with 711 transcripts being up-regulated and 130 down-regulated. Functional analyses were performed using Blast2GO and the NCBI database, targeting similar proteins in Tetranychus urticae mites and others arthropods. Detoxification-related genes, such as acetylcholinesterase and cytochrome P450 monooxygenase were found, as well as possible genes involved in virus-vector interaction. The most expressed genes and those putatively important for the leprosis pathosystem were selected and are being validated by qPCR, aiming to elaborate a model of the virus-mite vector interaction. Financial Support: CAPES, FAPESP (Process number 2014/08458-9)

Palavras-chave: Brevipalpus mites, Cilevirus, virus-vector interaction, RNAseq
A NEW VARIANT OF CANIS FAMILIARIS PAPILLOMAVIRUS 1 ASSOCIATED WITH ORAL SQUAMOUS CELL CARCINOMA

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Resumo

Canis familiaris papillomavirus (CPV) is the causative agent of viral papillomatosis in dogs. There are 20 different CPV types distributed worldwide. Each CPV type is associated with either oral or cutaneous lesions, which can be classified as benign exophytic or endophytic papillomas, or malignant squamous cell carcinoma (SCC). Although CPV types present different pathological aspects, it is known that genetic variants in human viruses could have increased pathogenicity. However, knowledge on CPV variants and their association with clinical aspects is still incipient. Therefore, the aim of this study was to assess the CPV genetic variability in canine lesions from Northeastern Brazil and their association with clinical aspects. Then, 18 samples were collected from mix breed dogs with oral and cutaneous warts. Histopathological analysis were performed by using hematoxylin-eosin method. CPV isolates were molecularly identified by PCR and sequencing. Staden package was used to assess sequencing quality and contig assembly. Blast tool was used for typing based on genetic identity. A multiple sequence alignment was created and a distance matrix was determined based on L1 gene sequences. Phylogenetic analysis was carried out with maximum likelihood method. Histopathologic analysis has confirmed the presence of oral exophytic papillomas, cutaneous papillomas, and oral SCC. The most prevalent CPV type in this study was CPV1. Nine isolates were characterized as CPV1 variants. Eight CPV1 isolates has 100% of identity with other Brazilian isolates. However, one isolate was identified as a new CPV1 variant, with a genetic distance of 0.8% when compared with the reference CPV1. The detected mutations were non-synonymous, and was predicted to disturb the L1 protein structure and function, which could be associated with a more aggressive infection development. This new variant was associated with oral SCC, which demonstrates the possibility of this novel CPV1 variant to be responsible for a more aggressive lesion development, leading to cancer. Although novel studies that focus on a wider epidemiological approach is important to confirm this association, this study has demonstrated the diversity of CPV isolates from Brazil, and a new CPV1 variant with novel protein structure changing mutations associated with oral SCC, which is important for the understanding of the papillomavirus pathogenesis, and for the development of more effective diagnostic and treatment methods.

Palavras-chave: canine papillomavirus, CPV1 variants, oral squamous cell carcinoma
Pacheco’s Disease (PD) is caused by an avian herpesvirus belonging to Alphaherpesvirus group, denominated Psittacid herpesvirus (PsHV). Actually, the causative agent have been detected around world, include in Brazil, causing almost exclusively parrots diseases and others birds belong to psittacid family. The PsHV can even infect large populations of birds, especially captive birds, leading to a high mortality rate among these birds. The main goals of this study was to detect the presence of herpesvirus associated with Pacheco’s Disease (PD) using Nested PCR in tissue samples from captive psittacids in an outbreak that occur in the Mairiporã city, São Paulo. For the study we collected liver and trachea fragments of 37 dead birds with died and presented clinical symptoms of Pacheco’s Disease (PD). The samples were crushed and the total nucleic acid was extracted by protocol automated NUCLISENS® easyMag® (BioMerieux, Lyon, France). The Nested PCR method was performed using degenerate primers by detection universal herpesvirus, followed by sequencing of positive samples. The Psittacid herpesvirus type 1 (PsHV-1) was detected in 75%(27/37) of the samples by Nested PCR method. The sequencing of fragments obtained showed that 28 positives samples, 96% (27/28) were characterized with PsHV-1 by Sanger method. Our study showed that psitacidiforms birds, mainly of captive are highly susceptible to PsHV-1, demonstrated the growing evidence that Psittacid herpesvirus type 1 (PsHV-1) are important causes of outbreak illness and death in birds.

Palavras-chave: Disease, herpesvirus, outbreak, birds, Psittacid
ANIMAL WELFARE IN DAIRY GOAT AFFECTED BY ARTHRITIS ENCEPHALITE CAPRINA VIRUS

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Resumo

Caprine arthritis encephalitis virus (CAEV) belongs to the lentivirus genus of the Retroviridea family. Caprine arthritis encephalitis (CAE) disease is a chronic, degenerative viral with progressive and irreversible symptomatology. Clinically the expression of CAEV infection is found in the nervous system, lungs, the mammary gland, the arthritis joints, and gradual lose weight. These symptoms will cause discomfort and pain in animals, difficulties in locomotion, including the search for food, which confronts two pillars of animal welfare (free of pain, injury and disease, and free of hunger and thirst). The objective of this study was to evaluate the clinical conditions that may impair animal welfare in dairy goats infected with CAEV.

The body score of 482 dairy goats, clinical data of respiratory, locomotor and the mammary gland system were collected from saanen and anglo nubian breeds. At the same time, blood samples collected for immunodiffusion agarose gel (IDGA) and western blot (WB) tests. Of the animals, 77% was serologically positive for CAEV, being 76.8% positive by WB and 50.2% by IDGA. From the negative animals in the IDGA, 36.5% were positive by the WB. In this study, we considered a positive animal when it reacted to at least one of the diagnostic tests. Of the all animals evaluated, 52.1% presented clinical symptoms of the CAE, and of these symptomatic animals 81.7% were positive and 18.3% were negative (P<0.05). Considering each symptomatology as hardened chronic mastitis, respiratory problems such as cough and nasal secretion, low body escorte and joint problems with or without the presence of pain, we found that were more presented in seropositive animals than seronegative (P<0.05). We concluded that the CAEV presence in the dairy goat herd interferes negatively on animal welfare, and it is important that the welfare evaluation protocols enhance the merit of the CAE control programs. Despite the obvious link between disease and suffering, this aspect is rarely taken into account in the evaluation of disease control programmers. The World Organization for Animal Health (OIE) has recommendations and guidelines covering animal welfare practices, reaffirming that animal health is a key component of animal welfare. In this way, CAE control is important for the welfare of the animals, with repercussions on financial benefits and the ethical production of animals, which has been a growing demand of society. Financial Support - FUNCAP

Palavras-chave: Health, Ethical production, Symptomatology, CAE, Goat
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Resumo

The proventricular dilatation disease (PDD) is caused by the avian Bornavirus (ABV), a member of Bornaviridae. PDD may be clinically characterized by gastrointestinal and or neurologic signs, due to lesion to the autonomous inervation of the digestive system (DS), including oesophagus, crop, proventriculus, ventriculus (gizzard) and duodenum. A natural episode of PDD involving the central nervous system (CNS) of one African grey parrot (Psittacus erithacus), one yellow-crowned amazon (Amazona ochrocephala) and one green-thighed parrot (Pionites leucogaster) is described. Birds were originated from the same conservation facility, with psittacines previously diagnosed with PDD by the detection of a fragment of the gene encoding the matrix protein of ABV (RT-PCR). The neurologic signs were associated to the CNS and involved motor incoordination, lateralization of the head, inability to stand, tremors, convulsions, and exacerbated aggressivity and reaction to contact. In the advanced stage, signs included undigested seeds in the excreta, weight loss and death. The in vivo diagnosis included the X-ray (without contrast) evaluation, showing the enlargement of the proventriculus. The histopathology evaluation of the DS revealed lymphoplasmacytic infiltrations and neural loss in nerve plexus. All three psittacines were positive for avian ABV in brain by RT-PCR, and two (A. ochrocephala and P. leucogaster) were positive in cloacal swab and feces. The differential diagnosis was implemented for Alphavirus and Paramyxovirus (RT-PCR) and for the psittacine herpesvirus 1 (Pacheco disease) (PCR) with negative results. The phylogenetic analysis of the M gene partial sequences revealed an identity with genotype ABV4, previously described associated with a CNS form of PDD in Germany and Canada. The infection with ABV might be associated with CNS disease in the early stages of virus-host and immune response interactions.

Palavras-chave: ABV, Bornavirus, parrot, PDD
Infectious bronchitis virus (IBV) is a member of the Coronaviridae family, and it is currently one of the most important pathogens in the poultry industry. The H120 and Ma5 viral strains, which are from the Massachusetts serotype, are the only ones approved by the Brazilian Ministry of Agriculture and Supply (MAPA) as the constituent of vaccines. Despite the systematic vaccination in Brazil, IBV has not yet been controlled efficiently and diseases associated with this virus have also been reported in vaccinated chickens. Here, we investigated the genetic variability and stability of H120 and Ma5 strains present in the IBV vaccines from different Brazilian manufacturers. We performed DNA sequencing of the spike glycoprotein gene (S1) to investigate genetic differences and presence of viral subpopulations among all vaccines and also between batches of the same vaccine. Additionally, we investigated the presence of viral subpopulations in each vaccine after a single passage in chicken embryonated eggs. We predicted the three-dimensional (3D) structures of the vaccines to investigate the impact of the polymorphisms found on the S1 protein in each vaccine. Our results revealed up to 13 amino acid substitutions among vaccines and some of those substitutions are localized in regions of the S1 protein that play a role in the virus-host cell interaction and in epitope regions for B and T lymphocytes. Secondary nucleotide peaks identified in the chromatogram for the S1 gene sequence were further investigated, and our results revealed that all original vaccines (H120 and Ma5) were composed by different subpopulations of IBV. Moreover, viral subpopulations, other than those found in the original vaccines, were found in vaccines after a single passage in chicken embryonated eggs. These findings suggest that the occurrence of outbreaks in vaccinated farms in Brazil may be caused by the lack of immunological protection due to these genetic variations in the commercial vaccines. Moreover, the viral strains in these vaccines could possibly revert to virulent strains in the field. Financial support: FAPEMIG, CNpQ and CAPES

Palavras-chave: control, IBV, subpopulation, vaccines
ORF or contagious ecthyma is a disease of sheep and goats caused by a virus (ORFV), belonging to genus *Parapoxvirus*, family *Poxviridae*. Generally, young animals are more affected and crusty lesions are observed in the lips, labial commissure and nostril region. The present study aimed to describe the main clinical and epidemiological aspects of ORF outbreaks identified in the western region of Rio Grande do Sul (RS), Brazil, during the years 2014 to 2017. Clinical and epidemiological information were collected during visits to properties. Nine outbreaks were identified in the municipalities of Uruguaiana (5), Alegrete (1), Itaqui (1), Barra do Quaraí (1) and Unistalda (1). The clinical diagnosis was done based in the presentation of the lesions and crusts were collected for laboratory diagnosis and molecular characterization of the ORFV. Sheep flock from Texel, Ideal, Corriedale, Crioula, Ile de France, Romney Marsh or Australian Merino breeds were affected. Lesion were present in the lips and labial commissure, nostrils and/or the distal region of the limbs and interdigital space. The severity of the illness ranged from focal and mild to disseminated and severe lesions. The size of affected herds ranged from 50 to 1,750 animals and the prevalence ranged from 2.3 to 100% (average 27.3%). In five outbreaks, only animals younger than 90 days were sick. The ORFV was considered endemic in five outbreaks, as the first report in three and was undefined in one case. The disease was identified throughout the year and no seasonality was observed. In some animals, it was possible to observe the presence of secondary contamination. Thus, it can be concluded that infection by the ORFV is endemic in the ovine herds of the western region of RS and produces clinical manifestations. Also, the molecular analysis of the collected samples could generate information about the genetic characteristics of the circulating virus.

**Palavras-chave:** Orf, poxvirus, contagious pustular dermatitis, clinical and epidemiological characterization
The frequency of most common enteropathogens in dogs in Brazil are cloudy by the absence of studies. The aim of this study was to detect coronavirus, parvovirus and rotavirus in diarrheic and non-diarrheic dogs. Stool samples were collected from 154 dogs, of which 92 were diarrheic and 62 were apparently healthy. To balance further analysis, the animals were categorized in four groups based on their age: younger than 6 months (n = 45, 29.2%), from 7 to 12 months (n = 28, 18.1%), from 1 to 5 years (n = 36, 23.3%) and older than 5 years (n = 44, 28.5%). In each age group, at least one apparently healthy dog sample (control) was included for each two diarrheic dog samples. The presence of parvovirus (CPV), rotavirus and coronavirus was evaluated by commercial lateral flow tests (chromatographic immunoassay) (Ecodiagnostica, Brazil). In addition, aliquots of stool samples positive for CPV were further submitted to sequencing (typing). Categorical variables were examined using Chi-square test or Fisher’s exact test, as appropriate. For the variable age, Mann-Whitney’s or Kruskal-Wallis’ tests were used. Logistic regression was used to identify possible bivariate associations between different pathogens in the same samples. Coronavirus and rotavirus were detected in 4 (2.6%) and 2 (1.3%) animals, respectively. CPV was the only enteropathogen associated with diarrhea in the present study (p = 0.006), being detected in ten (10.8%) dogs, nine of which under one year old. Parvovirus infection in repeatedly vaccinated adult dogs, like the present one, is commonly associated with CPV-2c type. Thus, it has been speculated that commercial vaccines, which is mostly based on CPV-2a and CPV-2b, might confer limited protection against this CPV type. On the other hand, the sequencing of all ten CPV-positive samples in the present study revealed the involvement of CPV-2b, including in this adult dog. The present work suggest that parvovirus is strongly associated with diarrhea in dogs, mostly in puppies, while coronavirus and rotavirus seem to be less frequent.

Palavras-chave: Coronavirus, Diarrhea, Dog, Parvovirus, Rotavirus
Resumo

Birds can be recognized as reservoirs of viruses and are of concern to humans. Picobirnavirus (PBV) was detected in humans and several animals worldwide, such as cattle, dogs, monkeys, snakes, swine, rats and birds, with or without diarrhea. Several studies have described PBV in samples from different species of mammals and reptiles; however, in relation to the molecular detection of genogroup II (PBVII) from broiler birds, there are so far no reports in the literature. The objective of this study was to detect PBV II in fecal samples of Gallus gallus fowl from 37 farms located in Pará state, northern Brazilian Amazon, using RT-PCR and sequencing techniques with the primers PicoB23 and PicoB24 for PBVII. 40 samples were analyzed by RT-PCR, of which, PBVII was detected in one sample (1.17%), which was also positive for genogroup I, in another study using the same samples. This sample was then sequenced and confirmed as positive for PBVII. PBV is a virus of vertebrate hosts, but its occurrence as causal agent of diarrhea is quite contradictory. Although it is detected in the feces of different hosts, it has not yet been possible to attribute the causality of diarrhea to the PBV. The coexistence of two or more PBV populations in a single host may suggest a possible reassortment. However, further studies need to be carried out. There are no studies to date with detection of PBVII in birds restricting the discussion about the frequency of this genogroup in the species studied. This is the first study of detection of PBVII in birds in Brazil, showing that there is circulation of this genogroup in this species.

Palavras-chave: Picobirnavirus, Broiler chickens, Molecular analysis, RT-PCR
DETENTION OF ROTAVIRUS A BY RT-PCR IN BAT AND RODENTS OF THE METROPOLITAN MESOREGIONS OF BELÉM AND NORTHEASTERN PARÁ STATE

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Resumo

The rotavirus a infections (RVA) have been shown with zoonotic character, due to their ability to undergo reassortment and its extensive genetic diversity. The identification of RVA in wild animals in areas of environmental changes provides the acquisition of information about the distribution of the agent in wild animals of our region, making it possible to assess which genotypes of RVA are present in the affected regions in the metropolitan city of Belém and northeastern Pará. In the period of September of 2014 to March of 2016, were collected fecal samples from wild mammal flying and non flying (bats n=50) and (rodents n=50) in the municipalities of Santa Bárbara, Viseu and Peixe-Boi of the state of Pará. All the fecal samples were subjected to suspension in Tris Ca++ and screening by immunochromatographic test for the detection of RVA, throught of the kit ridacquik rotavirusR. The fecal specimens were extracted by the method Boom in the laboratory NB-3 and all samples have subjected to Polycrylamide Gel Electrophoresis (PAGE) and to Reverse Transcription Polymerase Chain reaction (RT-PCR) for RVA, using primers designed. With a employed of a positive and a negative controls for all tests. The products obtained of the RT-PCR were subjected to electrophoresis in agarose gel 1.5%, and photodocumented in image processor (GEL DOC 1000 Vilber Lourmat). The amplicons of 600bp were purified by EasyPrep kit PCR Cleanup Mini, followed to the guidelines of manufacturers and sequenced by Sanger method with the kit Big Dye Terminator. The nucleotide sequences obtained were aligned and edited with the software Geneious. The phylogenetic trees were built with the Mega Program Version 7. To compare the positivity between the studied populations (bats and rodents), was applied to the test Chi-square (x²), using the program BioEstat 5, applied with the value of α = 0.05. Among the 100 fecal samples tested by RT-PCR, 3% (3/100) were positive for RVA, and 1.5% (3/50) of the positives isolated were detected in bats. Tested by immunochromatographic and PAGE tests. All samples and were negative for RVA. The present study show prevalence of 3% of RVA and circulation of genotype I2, in bats, two belonging to the municipality of Viseu (Carollia perspicillata, adult one male and one adult female) and one to the municipality of Santa Bárrbara (Desmodus rotundus adult femal).

Palavras-chave: Rotavirus, RT-PCR, Wild.
DETECTION OF ROTAVIRUS A IN PERIDOMESTIC ANIMALS IN THE MUNICIPALITY OF PEIXE-BOI, PARÁ

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Resumo

The acute gastroenteritis is one of the main causes of morbidity and mortality in humans and young animals. Studies demonstrated that domesticated animals of different species including horses, pigs, dogs, cats and birds have been affected from infections by rotavirus (RV), symptomatically or asymptotically. The RV belong to the Reoviridae family, genus Rotavirus and their segmented genome consist of 11 segments of RNA (dsRNA). Each segment encodes a protein, except the 11º which encodes two in the open reading frame. Six of these proteins are structural (VP1-VP4, VP6 and VP7) found in viral particles and six non-structural (NSP1-NSP6) found in infected cells. The RV are classified into eight groups (RVA-RVD, RVF-RVI) in accordance with the sequence nucleotide polymorphism of VP6. The species RVA, RVB, RVC and RVH infect humans and animals, while RVD, RVE, RVF and RVG were detected only in poultry. The RVA is considered as potential zoonotic agent, thus contributing to the genetic diversity of RV infection for humans. In the period from February to April 2016 were collected 120 samples of dogs, cats, horses, cattle, pigs and poultry. All samples were performed fecal suspensions prepared in buffer Tris-Ca++ 0,01M pH 7.2. The screening of stool samples was performed using the Immunochromatographic test (IT), enzyme-linked immunosorbent assay (ELISA) and extraction of nucleic acid, followed by the polyacrylamide gel electrophoresis (PAGE) and the reverse transcriptase polymerase chain reaction (RT-PCR) for the gene VP6. Among the fecal samples tested it was obtained 1,6% (2/120) were positive for RVA by RT-PCR in samples of canines. The samples of horses, cattle and poultry were negative for RVA by RT-PCR. As for the tests to PAGE, RT-PCR, ELISA and IC all samples were negative. The amplicons were sequenced from the product of the RT-PCR of the gene VP6 and the phylogenetic analysis showed that these samples had genotype I2 of lineage human. The results presented showed the circulation of group A rotavirus among these animals from this municipality of Pará state and indicated the necessity for genotypic characterization using other initiators, aiming to assess the degree of similarity with lineage human. Financial support: Instituto Evandro Chagas (IEC/SVS/MS)

Palavras-chave: ROTAVIRUS, RT-PCR, PERIDOMESTIC ANIMALS
In the last decades, the world witnessed the emergence and reemergence of arboviruses. In Brazil, mosquito-borne diseases such as dengue, yellow fever and, more recently, Chikungunya fever and zika disease have a great impact on the public health. Previous studies reported the presence Zika virus (ZIKV) in NHP from northeast Brazil and the antibody presence against several arboviruses in Brazilian Capuchin monkeys. Contrariwise, few studies have focused on the impact of the presence of such viruses on wild animal population and their possible role zoonotic reservoir. Our objective was to detect viruses threatening human and animal health potentially harbored in two capuchin monkey species. A total of 49 free-ranging and captive black-striped capuchin (Sapajus libidinosus) and blond capuchin (S. flavius) monkeys were sampled between May 2015 and January 2016. Captive monkeys were collected from the Zoo and the wildlife conservation unit (CETAS-IBAMA) in Recife. The free-living animals were collected in the border of Pernambuco and Paraíba states, the epicenter of the current ZIKV outbreak. We searched for neutralizing antibodies against ZIKV, dengue virus (DENV-1, DENV-2, DENV-3, DENV-4), Yellow Fever Virus (YFV), West Nile Virus (WNV) Saint Louis virus (SLEV), Ilheus Virus (ILHV), Rocio Virus (ROCV) using the plaque reduction neutralization test (PRNT). For WNV, SLEV, ILHV and ROCV viral chimeras were used in the PRNT. The Project was authorized by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio/SISBIO) (process number 47672-1/2015) and by the ethic committee from the Faculty of Medical Sciences in Campina Grande (FACISA) (number: 0048/18032015). We have found neutralizing antibodies for ZIKV, DENV-1, DENV-2, DENV-4, YFV, WNV, ILHV, SLEV in samples from both species. Our results suggest that these viruses are circulating among capuchin monkey population in the studied region. Even though we employed the gold standard technique, it is important to note that the Flaviviruses are serologically related and it is challenging to get rid of cross-reactivity. The presence of these viruses in capuchin monkeys raised public and animal health concerns in addition to conservational importance, since S. flavius is a critically endangered species and is facing high risk of extinction. Suported by FACEPE APQ: 2112 - 5.05/12 and 1380-5.05/15 / CNPq DCR: 0032-05.05/12 and 0003-5.05/16.

Palavras-chave: Flavivirus, Non-human-primates, PRNT, Zoonosis
**Resumo**

The equine infectious anemia (EIA) is caused by Equine infectious anemia virus (EIAV), a Lentivirus of the Retroviridae family, which infects equids, being one of the 11 equine diseases of compulsory notification by World Organisation for Animal Health (OIE). EIAV presents a worldwide distribution, and is also widespread in Brazilian territory. In Pantanal the impact is high on horse’s performance and indirectly on the livestock activity of this region, since the horses are used for cattle management. Currently, EIA diagnosis is made using the AGID test (agar gel immunodiffusion), however there is a need for the development of more sensitive and specific tests, since serological tests have some limitations. In the present study we developed a semi-nested PCR (sn-PCR) in the 5’ LTR to tat gene region and a PCR in the region of tat to gag gene, for the detection of proviral DNA of EIAV which amplify a fragment of 185 bp and 311 bp, respectively. We tested 59 peripheral blood mononuclear cells (PBMCs) samples of naturally infected horses from Corumbá city, Mato Grosso do Sul, Brazil. The PCR products were submitted to electrophoresis on a polyacrylamide gel, and DNA was purified, sequenced and a phylogenetic analysis was made. Serological tests AGID, ELISA for p26 and gp90 protein were performed for these same samples. The sn-PCR for LTR allowed the amplification of proviral DNA from 30 samples, and sn-PCR for gag amplified 18 samples. The 23 nucleotide sequences obtained for 5’ LTR to tat gene presented until 91% nucleotide identity with EIAV reference sequences available in GenBank. The 18 nucleotide sequences obtained for the tat to gag gene region presented until 87% nucleotide identity with EIAV reference sequences. The two phylogenetic trees showed that Brazilian sequences form a separate clade as compared the other world sequences, supported by a high value bootstrap. Considering the limited number of EIAV sequences available in public databases and the great variability of the EIAV genome, EIAV molecular characterization become important studies. This work provides tools for molecular characterization of the viruses, besides the Brazilian Pantanal sequences contributes to the knowledge of the circulating EIAV in Brazilian territory. It contributes significantly with new informations about EIAV genetic diversity and provides also data for epidemiological studies of EIA incidence in Pantanal and Brazil.

**Palavras-chave:** Equine infectious anemia virus, gag gene, LTR, PCR, tat gene
Resumo

Nucleocapsid protein (NP) is one of the most abundant and the most conserved protein expressed by canine distemper virus (CDV) during the infection. Due these characteristics, NP has been used as viral antigen for diagnosis tests of morbillivirus infections. This study evaluates the use of a recombinant NP expressed in *Escherichia coli* (rNPCDV) to detect antibodies against CDV. A consensus and conserved region of NPCDV was expressed in *E. coli* and purified by affinity chromatography. Once confirmed rNPCDV expression by polyacrylamide gel electrophoresis and western blot, microplates were sensitized with rNPCDV at a concentration of 249 µg/ml in carbonate-bicarbonate buffer and left at 4°C overnight. The plates were blocked with PBS solution containing 5% of skim-powdered milk for 1h at room temperature. Serum samples of 35 domestic dogs infected or vaccinated against CDV were analyzed. Positive controls were dogs recently vaccinated and negative controls were samples from puppies without antibodies, as demonstrated by serum-neutralization test. Serum samples were diluted at 1:20 in PBS containing 0,05% of Tween 20 (PBS-T) and added in a volume of 50 µl per well during 1 h at 37°C. After each step, plates were washed using the PBS-T solution. After incubation with rabbit immunoglobulin anti-dog IgG conjugated to peroxidase diluted 1:2000 in PBS-T for 1h at 37°C, it was added 100 µl per well of hydrogen peroxide 0,15% in ortho-phenylenediamine in citrate-phosphate buffer. Within 15 minutes, results were recorded in the spectrophotometer by measuring absorbance at 450 nm. Of 35 serum samples, 34 (97,15%) had antibodies able to recognize rNPCDV. Serological negativity of only one of the animals (2,85%) can be effect of a lack of serological conversion or a false negative result. rNPCDV evaluated in this study by indirect ELISA demonstrated antigenic characteristics to react with IgG from dogs which were immunized against CDV, indicating that rNPCDV can be applied in CDV immune diagnosis assays. Financial support: Capes

Palavras-chave: antibodies, canine distemper virus, enzyme-linked immunosorbent assay, recombinant protein
EQUINE INFECTIOUS ANAEMIA IN THE WEST OF RIO GRANDE DO SUL, BRAZIL

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Resumo

The equine infectious anaemia (EIA) is a retroviral equine disease worldly spread. The aim of this study was to characterize the outbreaks of EIA identified, between the years 2009 and 2015, in the municipalities of Barra do Quaraí, Itaquí, Maçambará, São Borja and Uruguaiana, in the state of Rio Grande do Sul, Brazil. Primarily were identified 26 positives animals in 24 properties, all were horses and none had any clinical sign of infection besides each property being considered a site of disease. The diagnosis were made by Coggins test in the occasion of transport or as sanitary measures in cases where there were some link with infected animals or just to certify the sanitary status. An outbreak was identified in animals being illegally transported from Argentina to Brazil. The positive properties were farms or temporary stabling and the infected animals were used for work, sport or reproduction. Fifteen outbreaks happened in properties that weren’t registered with the SVO. Eleven outbreaks were located in the urban area and thirteen in the rural area. In 2015 only 12 of the 24 outbreaks were diagnosed, nine of these being occurred in São Borja. In two properties the initial result was not confirmed in the second test (retest), causing these outbreaks to be closed immediately. In three properties, during the sanitation, 12 positive animals were identified among a population of 1,108 susceptible individuals. Therefore, it can be concluded that the infection is present in that area, it occurs in a subclinical form and it is linked to properties that are not registered in SVO and animals that are illegally transported, including international traffic.

Palavras-chave: EIAV, retrovirus, horse, AIE, illegally transported
FIRST COMPLETE GENOME CHARACTERIZATION OF A PRIMATE PAPILLOMAVIRUS DETECTED IN ORAL MUCOSA OF A HOWLER MONKEY IN THE AMRECICAS ALOUATTA GUARIBA PAPILLOMAVIRUS 1 (AGPV1).

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Resumo

Papillomaviridae family is currently classified in 51 genera and 388 virus types that infect humans and other animal hosts. Papillomavirus genomes are composed by circular dsDNA ranging from 6953 to 8607 bp. Primate papillomaviruses have been described in old world primates (Colobus guereza, Macaca fascicularis, M. mulatta, M. fuscata, Papio hamadryas, and Pan paniscus), but until now the only description in new world primates was based on molecular detection of papillomavirus infection in genital mucosa of squirrel monkeys (Saimiri sciureus papillomavirus types 1, 2, and 3). Here, we describe for the first time the complete genome sequencing of a novel papillomavirus species detected in the oral mucosa of two free-ranging howler monkeys (Alouatta guariba clamitans) from São Paulo city, Brazil. Genomic DNA was obtained from frozen samples of oral mucosal using organic solvents extraction and precipitation was made with ethanol. The DNA mass used for sequencing was 1µg in the Ion Torrent platform. The sequencing procedures generate 281.859 reads that were compared to a public data bank of annotated sequences containing 316 Papillomavirus complete genomes. Of the total reads analyzed 1049 presented similarity to these genomes and were separated, assembled and ordered by de novo strategy. The scaffold generated was about 7.04 Kb and was used as reference mapping in the final genome assembling. The genome have 7.722 bp in length and is double strand arranged, with 7 genes and absence of the E5 open reading frame, and GC content of 59.2%. The new genome identified was covered about 340 times forming a round unitig containing the complete genome. The final result was corresponding to a typical Papillomavirus genome that differs from the closest species belonging to Saimiri sciureus Papillomavirus type 1 in 30%. This is the first characterization of a Papillomavirus genome detected in oral mucosa of a howler monkey and designed as Alouatta guariba papillomavirus 1 (AgPV1) belonging to Dyoomikronpapillomavirus species and represent an important finding that can be used as reference to further comparative studies of Papillomavirus evolution.

Palavras-chave: Alouatta guariba, Brown Howler, Complete genome, Papillomavirus, Primate
Torque teno sus virus (TTVSuV) is a non-enveloped, single-stranded DNA circular virus, classified in the *Anelloviridae* family. Two genetically distinct species, TTSuV 1a and TTSuV 1b, are members of the *lotatoquevirus* genus, while TTSuV k2a and TTVSuV k2b belong to the genus *Kappatorquevirus*. Such viruses seem worldwide distributed in domestic pigs. Here, twelve complete genome sequences of TTSuV are reported. The genomes were recovered from sera of sows with and without stillbirths from six farms in Southern Brazil. After filtered (0.22 µm) and ultracentrifuged, the samples were treated with nucleases and viral DNA was extracted using phenol protocol. The DNA obtained was prepared with Nextera and sequenced with the Illumina MiSeq platform. Paired-end reads generated were trimmed by Prinseq and de novo assembled by *metaSPAdes* (v 3.9.1). The recovered contigs were compared with the viral reference database and the GenBank non-redundant protein database using BLASTX. The full genomes consisted of 2,640 to 2,910 nucleotides and contained four major open reading frames (ORFs): ORF 1, ORF 2, ORF 1/1 and ORF 2/2. ORF1/1 and 2/2 suffer splicing before translation. ORF 1 encodes a putative capsid protein and associated replicase. Untranslated region (UTR) is CG-rich and included a potential stem-loop structure (stem-loop motif TAGTATTAC) and TATA box (ATATAA) domains. A total of 45 sequences of TTSuV 1a and TTSuV 1b were aligned using MUSCLE version 3.5. The nucleotide identity between genome sequences ranged from 62% to 98% for 1a, and from 68.9% to 97.8% for 1b. The phylogenetic tree was reconstructed with the full genomes by maximum-likelihood (GTR+I+G model; bootstrap of 1,000 interactions). All genomes (except for two sequences of TTSuV 1b, AY823990-KY742733) clustered with the sequences according to the species which they belong, forming two mainly clades (1a and 1b). The sequences of TTSuV 1a identified grouped into different subclades. The TTSuV 1b sequences identified clustered distant from each other, but close to sequences from China (HM633226, HM633236). These findings provide useful information for further studies on TTSuVs, once this is the first complete genome sequences of TTSuV 1a recovered from sera of sows in Brazil. Financial Support: CNPq, CAPES, FINEP.

**Palavras-chave:** NGS, swine, TTSuV, virome
Porcine circovirus 2 (PCV2) belong to the family Circoviridae and is associated with several diseases collectively referred to as porcine circovirus diseases (PCVD). For PCV2, five genotypes - PCV2a to PCV2e - have been described, and recently the genotype shift from PCV2b to PCV2d has been associated with cases of suspected vaccine failure in several countries. There are few studies about the detection of PCV2 genotypes in Brazil. Therefore, this study aimed to perform the genetic characterization of the PCV2 by deep sequencing in selected samples that had high viral load (>106 copies DNA/ml) of pigs from commercial farms in Brazil from 2013, 2015 and 2016. Then, previously DNA was extracted from whole blood (n=10) and serum (n=3) samples, and quantified by quantitative PCR (qPCR). The pigs were 50 to 140 days-old. The animals were from Distrito Federal (DF; n=1), Mato Grosso (MT; n=3), Paraná (PR; n=2), Santa Catarina (SC; n=1), Rio Grande do Sul (RS; n=3), and Minas Gerais (MG; n=3) states. The complete genome of PCV was amplified by long PCR. The library was prepared by Nextera XT kit (Illumina) according to the manufacturer’s instructions. The purified products were sequenced using Illumina NextSeq platform. A consensus tree based on nucleotide sequences from complete genome was generated by the Maximum Likelihood method (MEGA6), using Tamura-Nei model, and 1,000 bootstrap replicates. The phylogenetic tree showed that eight whole blood samples were grouped with PCV2b genotype. These samples were from DF, MG, PR, SC, and RS from 2013 and 2015. While three serum and two whole blood samples were grouped with PCV2d genotype. These samples were from MG and RS from 2016. Therefore, detection of PCV2d in Brazil highlights the importance of this study, since the detection of this genotype in other countries was associated with cases of vaccine failure. The results may assist in the prevention, control, and diagnosis of PCVD in Brazil, especially in relation to the emergence of PCV2 mutants.

Palavras-chave: Porcine circovirus, PCV2, Genotype, Deep sequencing, Genetic characterization
GENOMIC ANALYSES OF CHICKEN PARVOVIRUS IDENTIFIED IN BROILERS WITH MALABSORPTION SYNDROME

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Resumo

Chicken parvovirus (ChPV, Galliform aveparovirus 1) is currently classified in the Aveparovirus genus of the family Parvoviridae. It is an icosahedral non-enveloped virus with a linear, single-stranded DNA genome. Various studies have been pointing ChPV as a potential etiological agent of malabsorption syndrome (MAS) in broiler chickens. Such syndrome is characterized by the occurrence of diarrhea, growth delay and batch unevenness, resulting in important losses in poultry production. However, the exact role of ChPv in the etiology of MS requires further investigation, since the virus can be detected in both affected and healthy broilers. In the present study, the complete genome of ChPV, as recovered from broilers affected by MAS. Samples of feces (n=20) were collected in four poultry farms in the state of Rio Grande do Sul, Brazil. These were diluted in PBS, filtered and centrifuged at 100,000 x g for 2 hours. The pellet was submitted to DNA extraction, enriched by multiple displacement amplification and submitted to high-throughput sequencing. A total of 1,030,046 reads were obtained and assembled in contigs with aid of the SPAdes 3.5 program. The full ChPV genome (named ChPV RS/BR/2015/2) is 5,265 nucleotides (nt) long, with three open reading frames (ORF), typical of members of the Aveparovirus genus. The first ORF (694 amino acids, 78 kDa) codes a putative nonstructural protein, NS1, which is 99% similar to the equivalent region of a previously reported ChPV genome (ChPV1-IPV; GenBank AMZ04136). The second ORF (101 aa, 11.5kDa), which codes a nucleoprotein (NP1) has an identity of 100% at the amino acid level with the equivalent region in ChPV1-IPV. The third ORF codes two putative structural proteins, VP1 (675 aa, 76,6kDa) and VP2 (536 aa, 60,4 kDa), both with 99% similarity to the equivalent region in ChPV1-IPV’s genome. The phylogenetic analysis, based on the sequence of nucleotides in the complete codifying region, reveals that ChPV RS/BR/2015/2 is intimately related with other two members of Aveparovirus genus detected in South Brazil. Together, these three genomes (ChPV RS/BR/2015/2, RS/BR/2015/1 and ChPV1-IPV) make a distinct group of other fowl parvovirus previously identified in different countries. Future studies will be carried out in attempting to investigate the pathogenic potential of ChPV in the infected host. This research had financial support from Capes, CNPq and FINEP funds.

Palavras-chave: high-throughput genomic Sequences, parvovirus, poultry
Smacoviruses are small, rep-encoding, ssDNA (CRESS-DNA) genomes with ~2.5kb in length. Genomes of smacoviruses were detected in fecal matter from humans exhibiting unexplained cases of diarrhea in France. Also, smacoviruses have been identified in stools of non-human primates, cattle, sheep, pigs, chickens and other sources. Here, four novel, complete smacovirus genomes detected in feces of diseased chickens are reported. The sampled chickens showed clinical signs suggestive of malabsorption syndrome (MAS), such as dehydration, decreased food intake and growth retardation. Samples (n=20) were collected from four high density poultry farms in the state of Rio Grande do Sul, Brazil. These were pooled, diluted to 10% in PBS, filtered (0.45 µm) and ultracentrifuged at 100,000 x g for two hours. Viral nucleic acids were extracted, enriched by multiple displacement amplification using Φ29 DNA polymerase and sequenced in a high throughput sequencing platform (Miseq, Illumina®). A total of 1,030,898 reads were obtained and assembled in contigs using SPAdes 3.5 software. Four circular contigs showed 79% to 100% aa similarity to the Rep protein of smacoviruses recently reported in feces from healthy chickens. These were provisionally named "chicken-associated smacoviruses" (ChSmCV RS/BR/2015 from 5 to 8). The four smacovirus genomes revealed a small variation in length (2343 to 2497 nt). These show two ORFs arranged in opposite orientations and encoding two putative proteins: a replicase (248–258 aa) and a capsid protein (424–427 aa). Conserved nonamers (NANTATTAC) at the top of predicted stem-loop structures were present in all four genomes. Phylogenetic analysis based on Bayesian Markov Chain Monte Carlo (MCMC), revealed ChSmCV RS/BR/2015 6, 7 and 8 clustering along with other smacoviruses previously detected in chickens feces. However, ChSmCV RS/BR/2015 5 remained in a separated branch and appears to be unique in comparison to the other smacoviruses reported. Further studies shall be performed to better evaluate the role of such viruses in MAS-affected broilers’ gastroenteric tract. Financial Support: Capes, CNPq and Finep.

**Palavras-chave:** broilers, gut, single-strand DNA circular virus
IN VITRO FACTORS ASSOCIATED WITH ADAPTATION OF HUMAN SEASONAL H3 INFLUENZA A VIRUSES TO SWINE

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Resumo

The current diversity of influenza A viruses (IAV) circulating in swine is largely a consequence of human-to-swine transmission events and subsequent evolution in pigs. However, little is known about the requirements for human IAVs to transmit to and subsequently adapt in pigs. Novel human-like H3 viruses were detected in swine herds in the USA in 2012 and have continued to circulate and evolve in swine. Reverse genetics (rg)-generated reassortants between a human-like H3N1 isolated from swine and a seasonal human H3N2 virus with common HA ancestry were evaluated by in vitro models to understand the contributions of individual gene segments on the ability of these viruses to infect pigs. Swine-adapted hu-H3 demonstrated abundant attachment to epithelial cells from upper-swine respiratory tract tissues by virus histochemistry, while the seasonal human virus bound to fewer cells. Growth kinetics in porcine intestinal epithelial cells (SD-PJEC) and in ex-vivo porcine trachea explants was significantly reduced by replacing the swine hu-H3 with the human seasonal H3, indicating the swine-adapted hu-H3 HA was important for binding and entry in swine cells. The human seasonal internal genes improved replication of the swine-adapted HA at 33°C, but decreased replication at 40°C. Although the HA was crucial for the infectivity in pigs and swine tissue, these results suggest that the adaptation of these novel H3 viruses to swine was multigenic and that the swine-adapted HA alone was not sufficient to confer the full phenotype of the wild-type H3N1 parental virus.

Palavras-chave: influenza, swine, H3, host, adaptation
INDIRECT ELISA USING A RECOMBINANT NUCLEOPROTEIN FOR INFLUENZA A VIRUS ANTIBODY DETECTION IN SWINE

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Resumo

Influenza A virus (IAV) causes an important respiratory disease in pigs leading to significant economic losses in the swine industry. The major subtypes that circulate endemically in the swine population worldwide are H1N1, H1N2 and H3N2. Although surveillance of IAVs in Brazilian swine population is not systematically conducted, it is relevant due to public health concerns. Nucleoprotein (NP) is highly conserved between IAV subtypes and broadly used for diagnostics. Considering the absence of a cost competitive serologic assay available in Brazil for IAV screening, an indirect ELISA based on the NP was developed for use in serologic surveys. The NP gene was amplified from an IAV (A/swine/Brazil/12A/2010) strain, cloned into pET23d plasmid and transformed into E. coli. The recombinant protein (rNP) was expressed and purified by Ni-based affinity chromatography followed by anion exchange chromatography. The protein mass (55 kDa) and identity were confirmed by SDS-PAGE and western blot using anti-histidine antibody and anti-NP monoclonal antibody. In order to produce antisera as a positive control, two 3-week-old piglets were immunized with 200 µg rNP. The rNP-ELISA plates were coated with 200 ng/well of rNP along with negative and positive controls. Plates were blocked using 3% non-fat dry milk. Swine serum samples were diluted (1:100) in 3% non-fat dry milk containing E. coli extract (1:10). Secondary antibody (anti-swine IgG peroxidase-conjugated antibody) was diluted to 1:5000. After color development using OPD in citrate phosphate solution, the enzymatic reaction was stopped with 12% H2SO4 solution and the optical density (OD) was read. The test sensitivity (SE) and specificity (SP) of rNP-ELISA was estimated by evaluating 151 pig sera (92 positive and 59 negative), previously tested by a commercial ELISA (AI Multi-Screen Ab test®, IDEXX). The ROC curve analysis resulted in a cut-off (0.542) derived from the maximum SE (84.8%) and SP (89.8%) values. Test SE and SP estimated by a Bayesian analysis were 84.7% (95% CI: 76-91%) and 93.4% (95% CI: 93-98%), using the same cut-off (0.542). Based on the test performance analysis, the rNP-ELISA was considered suitable for serologic detection of IAV in pigs. The test developed here is also a cost-effective, safe, and a rapid tool to detect influenza-specific antibodies in swine herds.

Palavras-chave: Diagnosis, ELISA, Influenza A virus, Nucleoprotein, Swine
Equine Infectious Anemia (EIA) is a disease that affects members of the Family Equidae, caused by Equine Infectious Anemia Virus (EIAV), which belongs to genus Lentivirus, subfamily Orthoretrovirinae and family Retroviridae. Although it is endemic worldwide, the diversity and distribution of global isolates is poorly characterized, and little is known about the origin and pandemic spread of the virus. Therefore, the objective of the work was to construct a phylogenetic tree allowing temporal and geographical inference of different EIAV strains emergency, using genomic gag region sequences available from GenBank. The field strain sequences were selected and previously aligned using as reference the gag region of the genome of endogenous retrovirus Lagomorph, a member of the genus Lentivirus described as the closest to the EIAV, after the alignment sequences that had degree of correspondence above 50% (423bp) were maintained, totaling 87 strains from Europe, Asia, and America. Sequences from each continent were submitted molecular clock substitution rate inference using the TempEST software. A phylogeographic analysis was done through the software Beast v1.8.4, the estimated substitution rate was utilized in a strict clock with a normal truncated distribution, SRD06 model was utilized to predict nucleotide substitutions considering 4 gamma categories, population was assumed as constant size in the tree coalescent model, the Markov Chain (MC) Length was set as 1E8, and parameters log was set to every 1E5. Tracer v1.6 software was utilized to assess the parameters of the MC analysis, and FigTree v1.4.3 enabled the visualization of the resulting condensed tree, also, SPREAD v1.0.6 was utilized to allow better comprehension of the pandemic spread scenario. The nucleotide substitution rate was calculated as 4E-4 (substitutions/site/year) based on 33 American strains. The clades division excepting RELIK outgroup comprehended a time span ranging from 1911 to 2014. The strains got strongly clustered according to they’re geographic origins and three major clades can be identified: an American, an Asian, and an European. The recent common origin of all strains indicate a relation to the dynamic and intense equids traffic on the world.

**Palavras-chave:** EIAV, Philogeographic, Epidemiology
The seropositivity of BVDV in cattle were found in buffaloes. This is the first appears in buffaloes. Moreover, the absence of BVDV eradication programs in Brazil is prevalent in Europe which is an important exporter of semen and animals to Brazil, therefore the emergence of this viral strain may be associated with the transboundary/transatlantic BVDV spread via infected animals or contaminated bio-products. We conclude that the results of BVDV diagnostics dedicated to bovines should be interpreted with caution in buffaloes. Moreover, the absence of BVDV eradication programs in Brazil seems to be favoring the uncontrolled spread of different BVDV-1 subgenotypes among cattle and buffaloes.

**Palavras-chave:** BVDV, Bovine, Diagnostic, ELISA, RT-PCR
OCCURRENCE OF EQUINE INFECTIOUS ANEMIA VIRUS IN SANTA INÊS - BAHIA

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Resumo

Equine Infectious Anemia (EIA) is considered the main infectious disease of the Brazilian equideoculture, which there is no vaccine and no effective treatment. The disease is caused by the virus of Equine Infectious Anemia (EIAV), belonging to the family Retroviridae, genus Lentivirus, which provokes a persistent infection with a long period of latency, which makes the diagnosis difficult by serological techniques. Considering the aggravated situation of the EIA in Bahia and the continuous socioeconomic impact caused by the virus, this work aimed to detect the occurrence of EIAV and to carry out a study of the epidemiological and clinical situation in the city of Santa Inês-Ba. To this end, visits were made to 23 properties of the municipality, totaling blood samples of 121 equids from distinct races, ages and sex. Samples were tested for antibodies to the EIAV p26 protein using an Agarose Gel Immunodiffusion Kit (IDGA) (Brush). At the time, questionnaires were applied to the breeders and caretakers about the EIA and a form filled out on the clinical condition of the animals at the time of collect. Of the sera tested by IDGA, 3.31 % had a positive result. Among the animals that had a positive reaction to EIAV, 80% had an age group greater than or equal to 36 months, which could be justified due to the longer time of exposure to the virus; 100% were asymptomatic; 86% are raised in extensive system and are used for work. The survey also showed misinformation of breeders and caregivers in relation to EIA, considering that 24% had not even heard about the disease, 75% did not know that the disease is caused by a virus, 81% did not require the examination to buy animals, 100% have the habit of sharing the same needle with several animals and 40% of the producers think that the prevention of the disease can be done with the administration of vaccines. This misinformation may be an aggravating factor in the epidemiological situation of the EIA in the state, since it may hinder prevention and control, since management has a determinant role in the transmission of the disease. Financial Support: FAPESB / IFBaiano

Palavras-chave: EIAV, Equine Infectious Anemia, OCCURRENCE, Prevention
ORIGIN OF BOVINE ALPHAHERPESVIRUS 5: POTENTIAL RECOMBINATION BETWEEN BUBALINE ALPHAHERPESVIRUS 1 AND BOVINE ALPHAHERPESVIRUS 1

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Whereas bovine alphaherpesvirus type 1 (BoHV-1) is prevalent in cattle throughout the world, bovine alphaherpesvirus type 5 (BoHV-5) infections seem to occur more often in particular regions of the world, such as Australia and Latin America. Bubaline alphaherpesvirus 1 (BuHV1), a virus hosted by water buffaloes (Bubalus bubalis), usually causes asymptomatic infections in the host species. Here, evidence is presented to highlight the close relationship between BuHV-1 and BoHV-5. Phylogenetic and recombination analyses were performed to examine the evolutionary relationship between all complete genome sequences of BoHV-1 (n=8), BoHV-5 (n=3) and BuHV-1 (n=1) available to date. It is proposed here that the most recent common ancestor of all BoHV-5 sequences most likely resulted from multiple recombination events between BuHV-1-like and BoHV-1-like viruses. The BoHV-5 whole unique short (US) region and most of the unique long (UL) genomic regions seem to have been derived from a BuHV-1-like parental genome, whereas five small segments of the UL (corresponding to nucleotides 8,510 to 8,848; 10,883 to 14,721; 71,638 to 72,186; 81,774 to 85,356 and 94,359 to 97,195 of the BoHV-5 genome) most likely derived from a BoHV-1-like genome. Thus, BoHV-5 may have originated from a series of recombination events between two or more BuHV-1 and BoHV-1 variants. Such hypothesis is consistent with the geographical distribution of BoHV-5, which seems to be most prevalent in regions where the geographical distribution of cattle and water buffalo farming overlap.

Palavras-chave: Bubaline alphaherpesvirus, BuHV-1, BoHV-5, BoHV-1, phylogenetic analy
HYLOGENETIC AND SEQUENCE DIVERGENCE OF THE HEPACIVIRUS A (EQUINE HEPACIVIRUS) IN RIO DE JANEIRO STATE, BRAZIL

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Resumo

The Hepacivirus A (Equine hepacivirus) is a hepatotropic virus recently classified into the genus Hepacivirus, family Flaviviridae. It is the closest relative to the recently renamed Hepatitis C virus (HCV, Hepacivirus C). Hepacivirus A has been found around the globe, including Brazil. Two viral variants are suggested to circulate worldwide based on phylogenetics of portions of the NS5B gene. The aim of this work was to analyze the phylogenetic and sequence divergence of the complete NS5B gene of the Hepacivirus A in Rio de Janeiro state. Six degenerate primers were designed for the NS5B region and a total of 231 serum samples from 6 mesoregions of Rio de Janeiro were screened by Nested RT-PCR. Hepacivirus A prevalence in Rio de Janeiro was of 13.4% (31/231). Fourteen RNA-positive samples were sequenced and analyzed with other 23 international isolates from the Hepacivirus A and 4 HCV isolates (1a, 1b, 3a and 3b). Sequences were analyzed with the Molecular Evolutionary Genetics Analysis (MEGA) software the Sequence Editor Database and Analysis Platform (SSE) and Synonymous Non-synonymous Analysis Program (SNAP). Phylogenetic analysis was conducted through the Maximum Likelihood (MEGA) and Bayesian Inference (Bayesian Evolutionary Analysis Sampling Trees - BEAST) algorithms with a total extension of 1740 base pairs (bp). Both trees had similar topologies, showing two distinct statistically robust groups. Nucleotide divergence between the two groups was of 17%. Divergence calculated for all the Hepacivirus A sequences was of 48% in synonymous sites and 2.7% in non-synonymous sites. Ratios for the synonymous/non-synonymous (ds/dn) sites was of 33.6 for the Hepacivirus A and 9.7 for HCV. For the inverse ratio (dn/ds) values were of 0.0298 and 0.103, respectively. Divergence between the two Hepacivirus A groups formed is compatible with subtype differentiation described for the HCV, which is above 15%. Also, these data show a more conserved genomic structure for the Hepacivirus A than for the HCV as well as a negative selection profile for the NS5B region of the Hepacivirus genus. This work strengthens the hypothesis of two circulating subtypes of the Hepacivirus A in Brazil and worldwide, it also furthers the divergence information between the Hepacivirus A and the HCV and could represent the first steps towards a better comprehension of the genetic variability of the Hepacivirus A in Brazil and its relation with HCV.

Palavras-chave: Equine hepacivirus, Hepacivirus A, NS5B, Phylogeny, Sequence divergence
DETECTION OF ANTIBODIES TO BLUETONGUE VIRUS IN TWO FEMALES DOGS FROM SOUTH OF BRAZIL

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Resumo

Bluetongue virus (BTV) is dsRNA viruses within the genus Orbivirus within the Reoviridae Family. Bluetongue is a viral disease of ruminants transmitted by midges in the genus Culicoides. BTV is very diverse, there are at least 27 serotypes circulating based on the specific neutralizing antibodies raised against VP2, and at least three putative new serotypes. In the past, BTV endemic areas were found worldwide within tropical and subtropical climates from approximately 40°N and 35°S; however, bluetongue has recently spread far beyond this traditional range. BTV infects and replicate in many species of ruminants, often asymptotically or causing an acute disease in certain breeds of sheep with high morbidity and mortality. The susceptibility of carnivores affected by BTV has been documented by competitive enzyme-linked immunosorbent assay (cELISA) and virus neutralization in enzootic areas and by the identification of clinical disease in dogs. dogs can occasionally acquire BTV by eating virus-contaminated meat. The objective was evaluated the seroprevalencia for BTVexposure in dogs, from 2 separate cases of canine abortion. Three serum samples were collected from apparently healthy farm dogs at two females and one male. serum samples were tested for BTV-specific antibodies using a cELISA and AGID (Veterinary Medical Research and Diagnostics, Bluetongue Virus Antibody Test Kit, Inc., Pullman, U.S.A), test kits as described by the manufacturer. Two females developed antibodies against BTV infection. The positive cELISA sera were also positive by AGID. The study population was fed canned commercial feed and had access to raw meat products. Dogs also are susceptible to African horsesickness viruses (AHSV), another serogroup of orbiviruses. Female sera were tested for Brucella abortions and B. canis with negative results. All dogs had access to an outdoor area that was adjacent to a pasture with sheep. Studies indicate that antibody titers to BTV can persist in a dog for a period of at least 5 years. This is the first report describing in Brazil the domestic dogs with antibodies specific to BTV.

Palavras-chave: Abortion, AGID, Arbovirus, BTV, Sheep
Currently, the use of molecular techniques in the diagnoses routine in veterinary medicine has gained prominence in comparison to conventional diagnostic techniques, due to its high sensitivity, specificity and accessibility. The aim of this study was to detect canine distemper virus (CDV) and canine parvovirus (CPV) in suspected cases attended from November 2015 to July 2017 at the veterinary hospital of veterinary school in UFRRJ. Urine and fecal swab samples were collected for the CDV and CPV molecular screening, respectively. Data of collect day, identification, age, symptoms and vaccine information from animal were obtained by the fulfillment of an epidemiological form. CDV RNA and CPV DNA were extracted using QiAamp viral kit (Qiagen™) according to the manufacturer. After extraction, RT-PCR and PCR techniques were performed for partial genome amplification of CDV (nucleoprotein, 287bp) and CPV (helicase, 583bp), according to previous standardized protocol. Amplicons were visualized an agarose gel electrophoresis (1.5%) stained with SYBR Green (100x) using an UV light transilluminator. During the period of the study, 83 samples were collected being 57 suspected cases of CDV infection and 33 suspected cases of CPV infection. Of these, 24/57 (42.11%) for CDV and 15/33 (45.5%) were positive for CPV. In the months of March to August, which correspond to the mildest time of the year in the state of Rio de Janeiro, a higher positivity in suspected cases of distemper was observed. In the case of CPV infection, the distribution of cases per year did not show significant difference, except in the year 2016, from March to August, when 10 of 20 suspected cases, were confirmed. Of the animals positive for either disease, 28 (71.79%) had no vaccine history and 11 (28.21%) were vaccinated with at least a dose of the polyvalent vaccine. All positive animals for CPV were aged under 12 months old. For CDV infection, 41.67% were under and 58.33% were over 12 months old. As a consequence of the development of infection of one or both of the diseases in vaccinated animals, an evaluation is underway through the sequencing of 14 CPV samples, to determine genotypes and compare to the standard strain of the vaccine, in order to identify possible vaccine leaks. Vaccinated animals have been reported to develop both CDV and CPV infection, which reinforces the necessity of prevalence and surveillance studies in veterinary hospitals. FINANCIAL SUPPORT: FAPERJ and PROIC/DPPG-UFRRJ.

Palavras-chave: Canine distemper, canine parvovirus, RT-PCR, Epidemiology
PRODUCTION OF ANTIGEN OF BOVINE LEUKEMIA VIRUS (BLV) FROM BRAZILIAN ISOLATES FOR USE IN LABORATORIAL DIAGNOSIS

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Resumo

Bovine leukemia virus (BLV) is the etiological agent of enzootic bovine leukosis (EBL), a disease that affects cattle, present all over the world. The serological prevalence of BLV is high in Brazil, mainly in dairy herds. Serological tests are largely used as one of the tools to disease control. However, only one diagnostic test for BLV is commercially available in Brazil, the agar gel immunodiffusion (AGID) test, based on the detection of anti-gp51 antibodies. The antigen used in AGID test is produced in persistently BLV infected fetal lamb kidney (FLK) cells, co-cultivated with infected lymphocytes of American cattle. Phylogenetic studies of strains isolated from several countries demonstrate that BLV can be classified into at least nine distinct genotypes, based on variations of gp51, specifically at antibody neutralization domains. These changes may influence the antigenic characteristics of the virus, reducing the sensitivity of serological tests. In addition, FLK cells are susceptible to bovine viral diarrhea virus (BVDV) infection, leading to non-specific reactions or misdiagnosis. The aim of this work was to produce an antigen from the BLV Brazilian isolate, in Tadarida brasiliensis lung cells (Tb1Lu), which are highly permissive to BLV and resistant to BVDV infection. Virus isolation was done co-culturing Tb1Lu cells with leukocytes obtained from PBMC of a BLV naturally infected cow. Tb1Lu cells infected with BLV field isolate were cultured in propylene culture flasks for subsequent purification of the antigen, by precipitation of supernatant in ammonium sulfate and then concentrated in polyethylene glycol. The antigen produced was subjected to polyacrylamide gel electrophoresis (SDS-PAGE) and compared to the commercial antigen in AGID. In SDS-PAGE, a single band of approximately 52kDa was seen, indicating the presence of a pure gp51 antigen. A pronounced precipitation line was observed in the AGID when tested with a BLV serum positive control and no non-specific lines were found. In conclusion, the antigen from BLV Brazilian isolate produced in a BVDV-free cell line showed promising results and is a good alternative to be used in serological tests.

Palavras-chave: EBL, Serological diagnosis, gp51
436 - ÁREA: VETERINÁRIA

RABIES IN HERBIVORES IN MUNICIPALITIES IN THE CENTER-WEST OF SÃO PAULO: A CONCERN FOR PUBLIC HEALTH

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Resumo

The rabies in herbivores in Brazil is considered endemic in some regions and causes the death of 40,000 head of cattle and a loss of 15 million dollars annually. In Brazil, the main transmitter of the disease to herbivores is the hematophagous bat Desmodus rotundus. Since the 1980s, there has been an increase in the population of this bat species and in the occurrence of rabies foci in herbivores in São Paulo (SP) State. The city of Botucatu, SP, and its neighboring municipalities (Conchas, Anhembi, Itatinga, Bofete, Pardinho, São Manuel) have steep relief on one side with a slope on the other side, being surrounded by mountain remains, which allows the occurrence of D. rotundus shelters. The laboratory of the Zoonoses Diagnosis Service, Faculty of Veterinary Medicine and Zootchny, UNESP Botucatu, carries out the diagnosis of animal rabies by direct immunofluorescence and biological tests in mice. The historical series of positivity in herbivores in previous years is an average of 20 animals/year and 02/month. However, in 2017, until July, 43 herbivores (30 bovine, 11 horses and 02 sheep) were diagnosed positive, evidencing the occurrence of an outbreak of rabies. Most cases (26) were diagnosed in May and June. The cases involve herbivores of six municipalities of the region of Botucatu, with this distribution: Botucatu, 14 bovine and equine cases; São Manuel, 03 bovine cases; Bofete, 03 bovine and equine cases; Pardinho, 03 bovine; Itatinga, 02 bovine; and Conchas, 1 equine case. Based on the records that accompany the biological sample sent to the laboratory, all animals were from rural and periurban properties and had at least one clinical sign of rabies, with death between 4 and 8 days after the onset of symptoms. It was also verified that no rabies vaccination was carried out at these properties and that in the majority of cases, the manipulators of these animals were in direct contact with their secretions, put themselves at risk of contracting the disease. In these cases, they were referred to the public health service of their municipalities, to receive the pertinent guidelines and procedures. In view of the costs incurred by herbivorous rabies, animal losses and post-exposure treatment of their contact persons, besides the risk of human infection, competent authorities should review non-mandatory rabies vaccination of animals of zootechnical interest, especially in endemic areas, in order to prevent the occurrence of outbreaks.

Palavras-chave: Outbreak, Rabies, Veterinary
RecBoHV was obtained, molecularly characterized, and its immunogenicity was assessed in rabbit model. The rabbit is a useful and well-established animal model to study both pathogenesis of BoHV-1 and 5 and the efficacy of vaccines against these viruses. In order to obtain the experimental vaccine, we constructed a recombinant MVA containing the coding sequence for the multiepitope protein of BoHV-1 and 5. After screening and amplification of the MVA-RecBoHV, the presence of the foreign gene was confirmed by PCR using specific primers and by sequencing. Six New Zealand rabbits were intramuscular immunized with 1x107 PFU of MVA-RecBoHV in a prime-boost protocol. After the boost, all the animals were able to recognize BoHV-1 and 5 viral proteins in an indirect ELISA. Two doses of MVA-RecBoHV administered without any adjuvant were effective in inducing a systemic humoral specific response as evidenced by the presence of IgG anti-BoHV-1 and 5 antibodies in serum. These results suggest that the recombinant MVA could be used as a potential vaccine against the herpesviruses. In the future, a challenge test will be performed administering MVA-RecBoHV in the rabbit model in order to test the protection conferred by that experimental vaccine. Financial support: CNPq.

Palavras-chave: Bovine Herpesvirus, MVA, Immune Response, Vaccine
Paraguay has registered no human cases of rabies since 2004 and the last case in dogs, reported in 2009, was due to a variant maintained in the common vampire bat “Desmodus rotundus”. In 2014, a dog from the “Chaco” region was diagnosed as positive for rabies with aggression towards a boy and all required measures of control were successfully adopted. Epidemiological investigation revealed that the dog was not vaccinated and was likely attacked by a crab-eating fox, “zorro” (Cerdocyon thous). In 2015, a second rabies-positive case from the same district was diagnosed in a dog, without reports of aggression by a crab-eating fox. These samples, and eleven samples from a rabies outbreak that occurred in Assuncion in 1996, were submitted to antigenic and genetic characterization. Antigenic characterization was carried out using a panel of monoclonal antibodies against rabies virus nucleoprotein and the antigenic profile of the samples, AgV2, was compatible with one of the variants maintained by dogs in Latin America. For the genetic characterization, the phylogenetic tree was reconstructed with sequences of samples from this study and representative sequences isolated the Americas listed in GenBank. The samples from 2014 and 2015 segregated in the canine (domestic and wild species) related group in an independent subgroup that also included samples from a dog and a crab-eating fox from the Chaco region of Argentina. The samples from the 1996 outbreak in Paraguay segregated in the canine group in a subgroup with four samples from other regions of Argentina. These results are indicative that rabies virus is circulating in canid populations between these two countries, particularly in the “Chaco” region. The epidemiology of the 2014 case indicates that despite being under control in domestic dogs, rabies virus can be circulating in the crab-eating fox population and may be transmitted to domestic animals and humans; as observed in northeast Brazil, where wild dogs maintain a virus lineage derived from the virus of domestic dogs. These results demonstrate the importance of maintaining anti-rabies vaccinations in domestic animals, the importance of collaboration between countries for rabies control and the continuous surveillance aimed at controlling the disease in wildlife.

**Palavras-chave:** Rabies virus, Dogs, Cerdocyon thous, Paraguay, Rabies
SEARCHING FOR VIRUSES IN BATS

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Viruses from different families have been identified in bats however only rabies virus is currently investigated in these animals as part of Health Surveillance Programs in Brazil determined by Government Authorities. The aim of this study was to identify viruses in different species of free bats. Oral and anal swab samples were collected from bats in the city of Maringá, state of Paraná, southern Brazil. The species of bats included at this work were Sturnira lilium, Artibeus lituratus, Molossus rufus, Myotis nigricans, Noctilio leporinus and Phyllostomus hastatus. Samples were submitted to nucleic acids extraction and Polymerase Chain reaction (PCR) or Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) according to investigated virus. By PCR or RT-PCR were searched viruses from families Rhabdoviridae, Herpesviridae, Adenoviridae and Paramyxoviridae. In addition a pool of samples was submitted to metagenomic to analyze the data of virome. Although all negative results by PCR and RT-PCR by metagenomic analysis, sequences of morbillivirus-like (588 bp), adenovirus (385bp), parvovirus (512 bp), papilomavirus (366 bp) endogenous retroviruses (510 bp) and many phages could be detected in bats of species S. lilium and A. lituratus. This work highlight the importance to investigate viruses in bats and indicates that metagenomic can be more suitable than PCR or RT-PCR to explore the viral diversity in bats. Financial support: FAPESP-Fundação de Amparo à Pesquisa do Estado de São Paulo

Palavras-chave: Bats, metagenomic, PCR/RT-PCR , viruses
Resumo

Bluetongue (BT) is a disease caused by bluetongue virus (BTV), RNA virus, belonging to the genus Orbivirus, of the Reoviridae family, which are Arbovirus. It is transmitted by blood-sucking vectors of the Culicoides genus, the infection is endemic and occurs in temperate and tropical areas. The Culicoides become infected by ingesting blood of domestic ruminant or savage in the viraemia period, among them cattle and goats serve as reservoir. The infection occurs in a way asymptomatic form, except in sheep and deer, whose clinical signs are: hyperthermia and your consequences, nasal secretion serous to mucous, crusts on the muzzle, facial and lip edema, mucous erosion and ulcers, tongue cyanosis, lameness, hoof coronary hyperemia. The objective of this study was to estimate the prevalence of antibodies anti-BTV and determine the epidemiology in goats and sheep herd from Parana and Rio Grande do Sul (RS). Were tested using the technique of Agar Gel Immunodiffusion a total of 2626 blood serum samples, collected in the year 2015, considering that 1634 are from sheep and 797 are form goats from the RS. From de 129 properties considering that 120 properties are from Parana and 9 from RS. The samples of sheep and goats are from 35 municipalities and those of goats ate from 3 county of RS. The subtropical climate Cfa occurs in Parana and in the RS is the subtropical Cfb in one municipality and the Cfb in two. The statistical analysis was found for goats and sheep of the State of Parana, 57.84% and 52.32% from reagents animals, respectively, and in goats from RS, 56.92%. In the 38 municipalities studied there was the detection of antibodies anti-BTV. Among the 129 properties studied 117/129 (90.7%) reported reactive animals anti-BTV. On properties of sheep, 71/81 (87.65%) reported reactive animals. All the properties in creation of puddles, presence of dogs and cats in the property. These risks factors have been described in previous studies, which demonstrated that the humidity degree and domestics animals are important to develop of BTV vector. Thus, now the present study allows concluding that the BTV is widespread in the sheep and goats herd from states Parana and Rio Grande do Sul, therefore is necessary to implant programs for your control.

Palavras-chave: Agar Gel Immunodiffusion, BTV, Culicoides, Reoviridae, Small Ruminants
VALIDATION OF A REAL-TIME PCR FOR THE DETECTION OF COCAL VESICULOVIRUS IN BOVINE EPITHELIUM

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Resumo

Vesicular stomatitis (VS) is a disease that affects a range of domestic animals, including cattle, swine and horses. The economic losses generated are related to the reduction of animal weight, decrease in milk production and losses generated by the quarantine period. The etiological agents are rhabdovirus, vesicular stomatitis virus (VSV), which are divided into four species. In Brazil, outbreaks of Alagoas vesiculovirus (VSAV) and Cocal vesiculovirus (COCV) has been detected. The validation of a real-time PCR (qPCR) for the detection of COCV in clinical samples requires procedures that ensure repeatability and reproducibility of the test. The objective of the study was to validate an RT-qPCR for the COCV. The repeatability test was performed by two operators in three replicates, in which each RNA was extracted seven samples of negative bovine tongue epithelium and seven samples of bovine tongue epithelium inoculated with different concentrations of the virus. The comparison of the operators' data consisted of the reproducibility test. RT-qPCR were performed in triplicate using a taqman probe in the CFX96 Touch™ Real-Time PCR Detection System (BioRad). RT-qPCR showed an efficiency of 97.2% ($r^2 = 0.999$, slope = -3.392), and a detection limit of 3.71 infecting doses of the virus. The repeatability and reproducibility tests showed no significant difference (0.48% and 2.82% [$p \leq 0.05$], respectively). The performance evaluation presented an adequate and acceptable system of measures regarding reproducibility and repeatability. Financial support: MAPA, CNPq, FAPEMIG

Palavras-chave: DIAGNOSIS, RT-QPCR, VALIDATION, VESICULAR DISEASE