EVALUATION OF TEMPORAL VARIATION OF FECAL CONTAMINATION INDICATORS AND PERSISTENCE OF HAdV IN RECREATIONAL WATERS USED TO SPORTS AQUATIC ACTIVITIES AREAS IN A PRE-OLYMPIC PERIOD, RIO 2016.

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Resumo

The recreational water's quality can be significantly affected by the presence of the enteric pathogens from wastewater released in these environments. Recreational and sports activities, by primary and secondary contacts, may represent a human health risk of infection by waterborne pathogens. This study aimed to evaluate daily variability concentration of water quality indicators as total coliforms, Enterococci and Escherichia coli as well to assess persistence of HAdV, a potential candidate to be used as viral indicator of human fecal. From June to July 2016 (pre-Olympic period) 100 seawater samples were collected every 30 minutes (from 8:30 a.m. to 1:00 p.m.), during in the morning, in Copacabana Beach located in Rio de Janeiro, Brazil. The point of collection was the Copacabana Fort from which was the starting point of the Triathlon competitions. All samples were concentrated by using the Skimmed Milk Flocculation Method and HAdV was quantified by using quantitative polymerase chain reaction TaqMan® assays. All samples were inoculated with PP7 bacteriophage as internal process control. Physicochemical parameters such as temperature, pH, turbidity, salinity and conductivity were performed at the time of collection presenting mean values of 22.4°C, 8.1, 6 ntu, 49.8 mS/cm, respectively. The bacteriological parameters were measured by the Collilert©-18 Quanti-Tray/2000 kit. Preliminary results showed high concentrations of total coliforms, Escherichia coli and Enterococcus, where counts were 6.56E+02-1.84E+03MNP/100mL, 2.79E+02-7.75E+02MNP/100mL and 1.00E+00-2.42E+03 MNP/100mL, respectively. Up to now, HAdV was detected in 13/20 samples (65%): viral loads ranging from 5.58E-0.4 to 1.82E+03 cg L-1. The maximum viral load was detected at 8:30 a.m. and a minimum at 12 p.m. but there are limited studies that evaluate the representativeness of the samples used. Our results show the continuous presence of human enteric, viruses in the analyzed area during the study period. Financial Support: CNPq-Universal-01/2016; Fiocruz. This research works within the scope of the activities of FIOCRUZ as a collaborating center of PAHO/WHO of Public and Environmental Health.

Palavras-chaves: Enteric pathogens, Fecal indicators, HAdV, Variability
The presence of adenoviruses (AdVs) is described in many species of vertebrate animals, including mammals, fish, amphibians, birds and reptiles. Birds are known to be common hosts for AdVs and this is due to the large number of bird AdVs described several decades ago. In addition, the birds are among the few hosts that can be infected by highly divergent AdVs classified into three different genus such as Aviadenovirus, Siadenovirus and Atadenovirus. *Megascops choliba* of the family Strigidae, *Guira guira* e *Crotophaga ani* of the family Cuculidae are widely distributed in South America. Based on the ecology of the species and the great fragmentation of the habitats, the pathogens, hosts and the environment have a wide interaction favoring the transmission of diseases, which can result in the dispersion and adaptation of pathogens to new hosts. We sought to investigate the occurrence and to understand the interaction between possible AdV and the species *Megascops choliba*, *Guira guira* and *Crotophaga ani*. The ES 060 highway is monitored at 67 km, 24 hours a day, to investigate the occurrence of incidents, accidents and trampling of fauna. A total of 19 stools of birds (5 *Megascops choliba*, 4 *Guira Guira* and 10 *Crotophaga anion*) killed by trampling were identified and subjected to autopsy and stools collected directly from the rectum. Nucleic acid extracted, a PCR reaction was performed followed by nested-PCR using the primer pairs described by Wellehan et al. (2004) with expected fragments of 300bp. The DNA fragments were purified and routed to DNA polymerase gene sequencing. In total, 21% (4/19) of stools were positive for adenovirus. The samples presented 30% (3/10) of adenovirus positivity in *Crotophaga ani* and 20% (1/5) for the *Megascops choliba*. All the *Guira guira* samples were negative. Evaluated and compared using the BLAST program the *Crotophaga ani* confirmed 78% identity for AdV described in gull and 78% identity for AdV of pigeon-2b. The *Megascops choliba* sequence obtained 77% identity to AdV-1 described in lizard. Whereas there is no previous description of these viruses for the species investigated here in addition the possibility of gene rearrangement, evidence of similarity of viruses of the other hosts of birds and found reptiles, indicates that *Megascops choliba* and *Crotophaga ani* can act as dispersers of adenovirus.

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EVALUATION OF HUMAN MASTADENOVIRUS INFECTIVITY IN WATERS FROM BEACHES OF RIO GRANDE DO SUL NORTHERN COAST

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Resumo
Regions with poor sanitation system the recreational water is an excellent way of transmission and dissemination of pathogenic microorganisms since they can reach the environment through the discharge or sewage. Moreover, beach sand is an important reservoir of pathogenic microorganisms because it concentrates fecal material and the contact with contaminated sand can facilitate the onset of enteric disease. *Human mastadenovirus* (HAdV) is transmitted by fecal- oral route and causes a variety of infections, such as respiratory, gastrointestinal, ocular and urinary tract diseases. The objective of this study was to evaluate the infectivity of HAdV-C in samples of marine water and sediment collected on the beaches of the northern coast of Rio Grande do Sul. A collection of five-hundred mL of water and sediment was carried out in summer season, december (2017), january and february (2018), on the four most inhabited beaches of the region: Torres (P1), Capão da Canoa (P2), Imbé (P3) and Tramandaí (P4). Samples were concentrated by ultracentrifugation method, followed with inoculation in cell culture with the A549 cell line and incubated for 72 hours at 37ºC, followed by treatment with DNase. Genetic material was extracted using a commercial kit (Promega®) and finally the Integrate cell culture real-time Polymerase Chain Reaction (ICC-qPCR) was performed using primers for the Hexon gene of type C HAdV. From the total of 24 samples, seven were positive for infectious HAdV. In December, 12,5% (3/24) of samples were positive, all of them from beach sand, with viral loads ranging from 3.13x10^5 to 4.46x10^5 genomic copies (gc)/ g. In January, 16,66% (4/24) were positive, one sample of seawater, with viral load of 1,55x10^7 (gc)/liter, and the other three of beach sand with 2,63x10^5 to 2,92x10^5 (gc)/ g. In February, all samples were negative for infections HAdV. With a prevalence of positivity in the beach sand samples, without relation of positivity between beach sand and seawater, this study becomes relevant and, evaluations allow to inform the quality of the sand and the utility in the measures of protection to the public health. Also, the presence of HAdV-C in the water of the analyzed beaches also demonstrates the poor sanitation of the area and can pose a risk to the population. Financial Support: FEEVALE UNIVESITY, CNPq Edital Universal/2016.

Palavras-chaves: Beach sand, Human Mastadenovirus, ICC-PCR, seawater

RISK ASSESSMENT OF MASTADENOVIRUS INFECTION IN SEA SURFACE MICROLAYER SAMPLES

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Sea Surface Microlayer (SML) is an interface between the water and the air, which may store and allow the release of pathogens and as aerosols from water to the atmosphere. This may be a link for waterborne transmission of respiratory infections. Another important characteristic these waters are richer in suspended solids than subsurface waters. Nevertheless, there are few studies related to the detection of enteric viruses, such as Human mastadenovirus (HAdV), in SML. The Quantitative Microbial Risk Assessment (QMRA) estimate the probability of an individual to be infected for a specific pathogen when entering in contact with some sort of contaminated matrix. The main objective of this study was to detect HAdV in SML samples and to estimate the risk of infection from QMRA analysis. Sampling was conducted in 8 beaches located in the North Coast of Rio Grande do Sul, Brazil, during four months, namely October (2016), January, April and July (2017). For the detection of HAdV real-time polymerase chain reactions (qPCR) were performed, using specific primers VTB2 (HAdV-C) and VTB1 (HAdV-F). Viral isolation was conducted to detector infectious particles. After, daily and annual QMRA were estimated for each point, using the minimum genomic copies/L (GC/L) concentration between direct detection and viral infectivity values. To estimate the infection risk, an exposition volume of 1 mL was considered throughout. HAdV-C was detected in all months and every beach showed contamination at least one time. Genomic copies ranging between 5.21E+05 and 2.04E+10 GC/L. HAdV-F was detected only in the sites 1 and 2 in October, with 1.31E+04 and 2.92E+04 GC/L, respectively. Daily and annual risk for HAdV-C and HAdV-F in throughout samples were 9.99E-01. Results indicate the anthropic interference and sewage discharge into beaches of the North Coast of RS. We highlight the significant results of QMRA in SML samples, especially for HAdV-C, since this species is mainly associated with respiratory infections and has as one of its main transmission routes the aerosols. Probably occur in the sites an important exchange of viral particles from the water to the air layer, this conclusion is reinforced since we have found HAdV infectious particles in Air sentinel samples distributed along the beach, being a potential health risk for those bathing in these beaches.


Palavras-chaves: Aerosols, Beaches, HAdV, QMRA, SML

PRESENCE OF HUMAN ADENOVIRUS IN THE WATERS OF CATURETÊ RIVER, SARANDI, RIO GRANDE DO SUL

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Resumo

The increase in urbanization near the rivers is accompanied by a large flow of untreated domestic sewage, making water a means of transporting pathogenic microorganisms. The Caturetê River belongs to the Várzea River Basin, being formed by a group of streams that bathe the city of Sarandi/RS. Human adenovirus (HAdV) are enteric viruses that cause gastroenteritis, and present great resistance in the environment and the gastrointestinal tract, being used as bioindicators of environmental quality. The objective of this work is to evaluate fecal water contamination in samples of waters of the Caturetê River by means of molecular detection. Five water samples were collected in 500 ml sterile flasks at five points along the Caturetê River. The collections were carried out in April 2018. The ultracentrifugation method was used to obtain the viral particles. Viral genomes were
extracted using the Mini Spin Plus extraction kit (BIOPUR), and then the quantification of HAdV was obtained using the qPCR (real time quantitative PCR) technique using the SYBR Green kit. The presence of HAdV was identified in 80% of the samples, and point three was the only site where no positive samples were detected. Points one and four presented lower and higher quantification of genomic material, respectively. The Caturetê River supplies the resident population of the municipality of Sarandi, receiving several contaminants, due to the agricultural activities of the local population and the urban concentration. The present study revealed a deficit in the quality of the basic sanitation of the region, besides the importance of the monitoring of the rivers so that public policies can be properly implemented and consequently better the quality of life of the residents who use these waters.

**Palavras-chaves:** environmental monitoring, qPCR, water quality

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**VIRAL VIABILITY ANALYSIS BY ICC-QPCR IN SEDIMENT SAMPLES**

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**Resumo**

Quantitative Polymerase Chain Reaction (qPCR) is a method with high viral detection capacity in environmental samples. The integration of molecular biology into a cell line not only detects the presence of viral DNA, but also samples with infectious potential. Enteric viruses, such as Human Mastadenovirus (HAdV), are mainly from domestic effluents that affect soil/sediment quality. The process of environmental contamination is constant due to the anthropic actions, being the lack of sewage treatment the main responsible for this scenario. The Paranhana River, located in the southern Brazil, continually receives human waste along its course. Four bimonthly sediment samples were collected (May/2015 to March/2016), totaling 72 samples (12 points) along the Paranhana River. The desorption of the viral particles was performed using the EMEM eluate with basic pH (11.5), then the ICC-qPCR process was started to evaluate viral infectivity. The samples were inoculated into A549 cells, and after 3 passes, the extraction of the viral DNA was performed. Genomic detection by qPCR was made using primers specific for HAdV. Regarding the results, 66.7% of infectious positive samples (5.82x10² to 4.25x10³ genomic copies per reaction) were detected in May; 58.3% (6.00x10² to 1.46x10⁵) in July; 8.3% (9.32x10⁴) in September; 25% (2.55x10⁵ to 1.06x10⁶) in November; 75% (1.47x10⁷ to 9.42x10⁸) in January; 41.7% (4.48x10² to 1.46x10⁴) in March. All samples tested had been previously analyzed by qPCR, and it is important to note that 8 negative samples in the previous genomic detection in May and July (both 3/12, 10² to 10³) and in March (2/12, 10² to 10³) showed become infectious after cell culture. The sediment is a reservoir of viral particles with infectious potential, through the adhesion-desorption phenomenon, such particles can reach the water body of the Paranhana River, which is used as a supply in the region. The present study demonstrated the importance of the interaction of the molecular technique and the ICC test due to the relevant growth of genomic loads and the identification of a potential contamination risk for the population, mainly children, elderly and immunocompromised.

Financial Support: CAPES, CNPQ, FEEVALE UNIVERSITY.

**Palavras-chaves:** Human mastadenovirus, Molecular biology, Paranhana River, Sediment, Viral viability

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**OCCURRENCE OF HEPATITIS A VIRUS IN AQUATIC ENVIRONMENTS FOR OSTREICULTURE IN**
Oyster farming is an important alternative source of household income in the Northeastern Pará, and the occurrence of viruses in the aquatic environment is often related to outbreaks associated with the consumption of these animals, such as gastroenteritis and hepatitis, including hepatitis A. The aim of this study was to evaluate the occurrence of hepatitis A virus (HAV) using two different methods for sample concentration. From January to December 2017, 84 water samples were collected in areas of ostreiculture, located in 5 municipalities in the northeast of the State of Pará: Curuçá, Maracanã, São Caetano de Odivelas, Salinópolis and Augusto Corrêa. Organic flocculation and membrane adsorption-elution methods were used to concentrate water samples. Viral RNA extraction was performed with the Viral QIAamp RNA kit, followed by reverse transcription with SSIII-RT. HAV detection was performed by nested-PCR with amplification of the VP1/2A region of the genome, positive samples were purified with the BigDye XTerminator Purification kit and submitted to BigDye Terminator Cycle Sequencing v3.1 kit sequencing on the 3130xl Genetic Analyzer. The quantification of thermotolerant coliforms was performed by the chromogenic substrate method. HAV was detected in 31% (26/84) of the collected samples: Maracanã (3/11), Augusto Correia (5/15), Salinópolis (5/18), São Caetano de Odivelas (6/22) and Curuçá (7/18). The 26 sequenced samples belong to genotype IB. Of the 26 samples positive for HAV, 34.6% (9/26) were concentrated only by the membrane adsorption-elution method, 53.8% (14/26) only by the organic flocculation method with skim milk and 11.6% (3/26) by both methods. Considering the concentration of thermotolerant coliforms, only 25% (21/84) of the samples were in agreement with the legislation in force in the country for waters destined for oyster farming, however, HAV was also detected in these samples. The data from this study demonstrate the occurrence of HAV, genotype IB, in the aquatic environment of the five municipalities studied. Different types of viral concentration methods are suggested to reduce the occurrence of false negative results. Therefore, it is necessary to adopt hygienic-sanitary measures in the communities dedicated to the cultivation of this food so that it meets the minimum standards established in the current legislation, aiming at the interest of the expansion of oyster farming in the region and greater sanitary security for consumers.

Palavras-chaves: Hepatitis A virus, Water, Ostreiculture

Iron as accelerator of Adenovirus and Hepatitis A virulence in contaminated water by mine dam mud (Bento Rodrigues, Mariana-Brazil)

Interference of metals in microorganisms virulence and in their infection ability is target of many studies, considering bacteria as model. However, the metals’ interference in viral infectivity/virulence is a new line of studies, considering enteric viruses as model. In the present study we evaluated infectivity/virulence of human adenoviruses (HAdV) and hepatitis A (HAV) in contact with contaminated waters by mud from Fundão (Mariana City, Minas Gerais State, Brazil). The studied area was affected...
on November 5th, 2015 by 60 million m$^3$ of mud from a mining reservoir (Fundão), reaching the Gualaxo do Norte River (sites evaluated in this study), “Rio Doce” River and after that, the Atlantic Ocean, with iron as the main component of the Fundão mud. In this context, iron interference in viral infection processes was evaluated considering infectious viral particle of HAdV-2 and HAV-175, as DNA and RNA enteric virus model, respectively, exposed in natural contaminated water. The results showed the high natural prevalence of infectious HAdV and HAV (by qPCR) in all sampled sites from Gualaxo do Norte River, indicating the low basic sanitation in this area. Experiments conducted in real and laboratorial scale showed that HAdV and HAV from contaminated water with high iron concentrations, presented plaque forming units (PFU) significantly larger than those formed by viruses from waters with low iron concentration (vs viral virulence increased in contaminated water with metals and metalloids. PNPD-CAPES.

Palavras-chaves: PFU, metal, virulence, interaction

Environmental contamination by coliforms, Parvovirus and Adenovirus in a municipal kennel shelter

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Resumo

Water contamination and sewage disposal bring negative impacts in public and animals health. The present study research of: Mastadenovirus (AdV), canine AdV (CAV) and canine protoparvovirus (CPV) in canine feces, water and vegetable cultivated in one Centro de Educação Ambiental, which harbour one municipal kennel shelter of animal with left dogs from region, Sinos Vale. Survey samples were collected in Oktober 2017 and February 2018, totalizing: 33 canine feces samples, 8 vegetables (Lactuca sativa species), which were analyzed in three diferentes parts - root, stem and leaves - resulting in 24 processed samples, 10 water samples coming from sources like, dam and ditch, named P1 at P5. All samples were submitted the DNA extraction protocols and analyzed by PCR technical. Nested-PCR was used at AdVs anlyzes, using DNA polimerase (DNA Pol) gene target, and conventional PCR at CAV and CPV detection, which hexon and capside protein was the target gene respectively. Positive samples at all PCR were purified and submitted at sequencing at characterization of species at AdVs and types or serotypes at CAV and CPV. AdV was present in 9 samples: five of water, three of feces and in one Lactuca sativa stem, which were characterized CAV-1, HAdV-C and E insequencing proceeds (water samples); HAdV-E in Lactuca sativa and CAV-1 in feces which in one sample it not characterized by sequencing. By conventional PCR, CAV not have seen detected; CPV was identified in all feces samples and in one water sample. CPV-a was the subtype characterized in feces samples, one probable hypothesis at CPV-2a presence in dogs was due asymptomatic infection, since symptomatic dogs was not observe or even so virus what resist envirommental pressures, because the positive samples were collected in kennel floor. One amazing result in water sample was the detection of one virus originated from wild carnivore like protoparvovirus.
recently reported in wild animals in Canada and China, RPV (*raccoon protoparvovirus*). Likewise, virus present in vegetable, bring to us an alert of importance and water controls used at to cultivate as well as in irrigation systems. The need for both urban and rural sanitation are solutions to be rethought as well as reformulation of actual vaccines at dogs.

Financial support: Feevale University, CNPq.

Palavras-chaves: vegetables, HAdVs, CAV, CPV-2a, RPV

MICROBIOLOGICAL ASSESSMENT OF HUMAN ENTEROPATHOGENES IN AQUATIC ECOSYSTEMS OF THE JACAREPAGUÁ WATERSHED, RIO DE JANEIRO.

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Resumo

The dissemination of waterborne enteropathogens contributes to the increase of diseases, affecting Public and Environmental Health. The search for the standardization of microbiological indicators, associated to bacterial routinely used, is one of the greatest challenges of environmental microbiology. Human adenovirus (HAdV) and human polyomavirus JC (JCPyV) are promising indicators of the human fecal contamination in the environment, due to its high host specificity, resistance to heat and the water treatments. The objective of this study was to evaluate the bacterial and viral indicators, besides other human enteropathogens in aquatic ecosystems of the Jacarepaguá Watershed (Rio de Janeiro, Brazil) using three methodological approaches. Forty-six samples were collected in two ecosystems of the region (Lagoon Complex and Untreated Sewage of Health Units and Olympic Village Housing Complex), from June to November 2016. IDEXX Quant-Tray/2000 Colilert®-18 method was used for bacteriological parameters, required by legislation, while for viral indicators the samples were concentrated by organic flocculation and quantified using protocols previously described for TaqMan® qPCR System. Others enteropathogens were investigated by using TaqMan Array Card (TAC) and ViiA™ 7 Real-Time PCR System in seven representative samples. The PP7 bacteriophage was used as an internal control process. All samples showed high concentrations of total coliforms and *Escherichia coli*, with averages of 1.0x10^7 MPN/100mL and 4.7x10^6 MPN/100mL, respectively. The HAdV and JCPyV indicators were detected in 84.4% (37/46) and 78.3% (36/46) of the samples, with mean values of 1.3x10^6 genomic copies/Liter (cg/L) at 8.7x10^5 gc/L, respectively. PP7 was detected in 100% of samples. Six or more gastroenteric pathogens were detected in samples analyzed by TAC, including different groups of viruses, bacteria and protozoa. E. coli results showed that 100% of the samples studied were unsuitable for bathing and fishing activities. Regarding human viral indicators, similar concentrations were observed in the sewage and lagoon samples, evidencing high anthropogenic contamination in the ecosystems of this coastal urban by domestic sewage disposal. The impact of the dissemination of these pathogens on seawater along the coast of the tourist area of Barra da Tijuca will be evaluated in future studies. Financial Support: CNPq-Universal-01/2016 and CAPES.

Palavras-chaves: HAdV, JCPyV, Enteropathogenes, Watershed
ANALYSIS OF VIRAL INFECTIBILITY BY HUMAN MASTADENOVIRUS (HADV) IN SEDIMENT SAMPLES FROM RIO CAÍ, RS.

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Resumo

The Caí River Watershed (BHRC) has 42 municipalities. Its main hydrography is the Rio Caí, which provides water for the public supply of the cities. Due to the disorderly growth of the urban population, the monitoring of the quality of natural resources has become increasingly relevant. The use of bioindicators is an important tool in assessing the impact and conservation of the ecosystem. Enteric viruses are known for infections in the gastrointestinal tract of humans and animals, transmission occurs via fecal-oral route, and can cause diverse pathologies in susceptible individuals. Human Mastadenovirus (HAdV) is excreted in large amounts in the feces of infected mammals and is highly resistant in the environment. Waterborne sedimentation can make the riverbank sediment a final reservoir of these viruses, due to high water retention capacity, high mobility and high particulate matter. The objective of this work is the analysis of viral infectivity of HAdV detected in different locations along the course of the Caí River. Samples were obtained from 10 collection points held quarterly, between July 2016 and June 2017. The evaluation of human viral viability was performed through the passage of A549 cells (human lung carcinoma) by ICC-qPCR (integrated cell culture - quantitative PCR). This assay consists of quantifying the number of infectious viral particles present in samples by means of a cell culture integrated with the real-time polymerase chain reaction (qPCR) molecular technique. From the total of 40 samples collected, 25% presented infectious particles by HAdV. Throughout the collection period, there was absence of HAdV in points eight (part of the municipality of Montenegro - urban region) and ten (municipality of Nova Santa Rita - mixed region). At the point seven (municipality of Montenegro) 4.20x10^4 cg / reaction was detected and at point four (Harmonia municipality) the quantification was 4.51x10^2 cg / reaction, the highest and lowest values found, respectively. Both points are located in a mixed region (urban and rural). The results obtained in the present study demonstrate the infectious potential of HAdV in different locations of the Caí River, generating an important warning of health risk to the local population, that uses the waters of Rio for consumption and irrigation and shows the anthropic impact in the BHRC.

Palavras-chaves: basic sanitation, environmental quality, ICC-qPCR

SPREAD OF MASTADENOVIRUS FROM CONTAMINATED SEA WATERS TO THE AIR ABOVE SHORELINE

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Resumo
In coastline regions, changes in the population due to seasonal tourism may be accompanied by challenges in what concerns to environmental impacts, since these places are normally not prepared in terms of infrastructure with the extra amount of sewage that comes with these people. Consequently, through the incorrect release of effluents in coastline areas, many pathogens, such as enteric viruses, are released into water bodies, compromising the quality of the water, posing a threat to human and animal health. Among enteric viruses, Human mastadenovirus (HAdV) from different species are reliable markers of human fecal contamination of environmental matrices route. Different environmental matrices can assist in the analysis of viral coastline regions, in addition to the samples of water, sediment and sea surface microlayer (SML) may act as viral deposit, as well as bivalves, since they are recognized as bio-accumulators, filtering and retaining the viral particles in water. The main objective of this study was to detect the presence of diversity in the various samples HAdV. Sampling was performed in four different times in eight beaches along the northern coast, Rio Grande do Sul, Brazil, during October 2016, January, April and July 2017, a total of 160 samples were collected. The samples were processed in a manner specific to each type of array. Nested polymerase chain reaction (nested-PCR) was performed to amplify the partial sequence of DNA polymerase gene, where the positive samples were purified and sent for sequencing. Phylogenetic analysis was performed by comparing the genomic sequences obtained and already characterized sequences available from GenBank. Of the total samples, 14% (22/160) were positive, identified to the specie HAdV-C. One of the positive, 23% (5/22) was represented by samples of water, sediment and sea surface microlayer, 18% (4/22) corresponded to bivalves and 13% (3/22) Air Sentinel. Therefore, based on the results obtained by phylogenetic analysis, was only found HAdV-C in higher number in water, sediment and SML samples, showing that there is a noticeable spread through the air from viruses that are originally contaminating water. It is important to highlight the relevance of evaluate different environmental matrices and components as they may have different capacities for viral retention.

Financial Support: CAPES, CNPq.

Palavras-chaves: Coastline Areas, HAdV-C, Spread, Diversity, Phylogenetic Analysis

MICROBIOLOGICAL CHARACTERIZATION OF THE DIGESTIVE TRACT OF CRASSOSTREA BRASILIANA OYSTERS FROM CULTIVATION IN COMMUNITIES IN THE NORTHEAST OF THE PARÁ STATE, BRAZIL.

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The consumption of bivalve organisms from contaminated water can lead to the onset of foodborne diseases, for these molluscs are efficient filters and accumulate in their tissues an enormous amount of microorganisms and therefore, they can be transmitters of diverse human pathogens like the Hepatitis A and E viruses. This study aimed at the microbiological characterization of oysters by quantifying the Most Likely Number (MLN) of Total Coliforms (Ct) and Thermotolerant...
Coliforms (CT) in oyster samples of Crassostrea brasiliana, from the Multiple Tubes Technique and to evaluate the occurrence of hepatitis A virus (HAV) and hepatitis E (HEV) in the analyzed samples. The samples were collected in areas of oystericulture, in the municipalities of Curucá, São Caetano de Odivelas, Salinópolis and Augusto Corrêa located in the northeast region of the state of Pará. Viral RNA extraction was performed with the QIAamp RNA Viral kit. The detection of hepatitis A virus will be performed by nested-PCR with amplification of the VP1/2A region of the genome using Taq platinum DNA polymerase, then sequenced, in addition to viral quantification by the RT-qPCR technique. The analysis of the presence of hepatitis E virus will be performed by the RT-qPCR technique. The NMP from CT/g ranged from 26-110 NMP/g and a mean of 51.5 NMP/g only in São Caetano de Odivelas and Salinópolis, where it was possible to recover E. coli isolates that will be characterized from the point of view of their pathogenicity. The international standards that determine the limits of microbiological contamination such as the Codex Alimentarius defined a limit of 230 NMP g-1 of E. coli, however the European Union Shellfish Quality Assurance Program defined that these values should not exceed 6,000 NMP 100 g-1. The National Program for Sanitary Control of Bivalve Molluscs that establishes criteria for the quality of bivalve molluscs in Brazil defined numbers up to 6,000 NMP 100 g-1. According to both international and Brazilian standards, oysters analyzed were classified as satisfactory for consumption. The results are preliminary, however, they call attention to the need to monitor the quality of this food because of the risks associated with its consumption with the levels of contamination found. Financial support: Instituto Evandro Chagas/Secretaria de Vigilância em Saúde/Ministério da Saúde.

Palavras-chave: CRASSOSTREA BRASILIANA, MICROBIOLOGICAL CHARACTERIZATION, OYSTERS

**ENTEROVIRUS A71 IN SOUTH AMERICA: A RETROSPECTIVE STUDY OF SEWAGE SAMPLES REPORTS THE CIRCULATION OF GENOGROUP C2 IN ARGENTINA AND URUGUAY**

**Autores**

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**Resumo**

Enterovirus A71 (EV-A71) belong to Human Enterovirus species A and is a causative agent of epidemics of hand-foot-and-mouth disease (HFMD) as well is associated with severe neurological complications. Based on genetic diversity at VP1 level, EV-A71 is classified into 7 genogroups (A to G), being B and C the most frequently detected. These, are further grouped into different subgenogroups. While the circulation of different variants of EV-A71 is well documented in other parts of the world, the genetic diversity of EV-A71 in the Latin American region has been barely considered. Past studies reported the presence of Genogroup B circulating in Colombia and Brazil in 1994 and 1999, respectively, and subgenogroup C1 in Peru between 2006 and 2009. In 2012, subgenogroup C2 was reported in Cuba. As part of an environmental surveillance of Human Enterovirus in the South American region, sewage samples collected monthly between January 2011 and December 2012 in Córdoba city (Argentina) and in Salto, Paysandú, Fray Bentos and Bella Unión (Uruguay) between March 2011 and February 2012, were retrospectively studied. Sewage samples were concentrated by precipitation with PEG-6000 (Córdoba city) or by an adsorption-elution to a negatively charged membrane method (Uruguayan cities) and viral concentrates were subject to RNA extraction and RT-PCR towards VP1 segment. PCR products were sequenced with Illumina MySeq 300 bp paired-end. Raw reads were subject to different bioinformatics tools, and single sequences were mapped against reference sequences for all the Enterovirus types. Sequences mapped with EV-A71 reference sequences were extracted and studied in depth. Neighbor joining method and Kimura 2-parameters model were used for phylogenetical analysis. Two samples from Córdoba (January 2011 and December 2012), presented reads of EV-A71, as well as a sample from Paysandú (February 2012). Our results indicate that EV-A71 circulated in both countries as subgenogroup C2. Uruguayan reads of EV-A71 were between 2.3% and 6.8% divergent at nt level with Argentinians reads. The reads from samples collected in January 2011 and February 2012 in Argentina and Uruguay, respectively, clustered together with the C2 Cuban strain (8% divergent at nt level). The close genetic relation among Cuban, Argentinian and Uruguayan C2 strains
Circulating contemporary (2011-2012) would indicate a wide circulation of this variant among the population of Latin American and Caribbean regions.

**Palavras-chaves**: ARGENTINA, ENTEROVIRUS, ENVIRONMENT, SEWAGE, URUGUAY

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**COMPARISON OF MICROBIOLOGICAL INDICES OF WATER QUALITY IN THE NORTH COASTLINE OF RIO GRANDE DO SUL**

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**Resumo**

Balneability of the North coastline of Rio Grande do Sul is evaluated through the presence of total coliforms (TC) and *Escherichia coli* (FC) by the government agency (FEPAM) during summer season. However, these methods are inefficient to correlate with the presence of enteric virus (EV) and other pathogens. The goal of this work was to evaluate the efficiency of TC and FC compared to *Enterococcus spp* (ENT) and EV, represented by *Human Mastadenovirus* – group C (HAdV-C). Samples were collected in December (2017), January and February (2018), on the beaches of Torres (P1), Capão da Canoa (P2), Imbé (P3) e Tramandaí (P4). Levels of contamination of TC, FC and ENT in the waters were evaluated by the Colilert® and Enterolert® methods. For viral analysis, samples were concentrated using ultracentrifugation and the genetic material was extracted using Promega® kit, followed by the amplification with the polymerase chain reaction (qPCR). From the microbiological analysis, December was the month with the highest values of FC, ranging from 2809 to 333 NPM/100mL and TC ranging from 2851 to 984 NMP/100mL. February presented the highest ENT values, ranging from $U = 52.00, p>0.05$ or TC and ENT ($U = 42.0, p>0.05$). There were no statistically significant differences between the methods when compared between the months and the different places of FC ($H = 0.039, p>0.05$); ENT ($H = 1.318, p>0.05$). For HAdV-C, 97% of the samples were positive with quantification ranging from $5.43 \times 10^6$ to $2.47 \times 10^7$ genomic copies/(cg)/L. In this preliminary study, although the methods have not differed significantly, the indices of contamination were high in all parameters. In the months where the CT indices were lower, the ENT levels were above of limit for Resolution CONAMA 274/00. It is important to highlight that in the points where levels of bacteria were acceptable by the Resolution, genome of HAdV-C was present. The lack of correlation between the presence of HAdV-C, FC and ENT, indicate that the use of only bacterial indices as pattern of water quality is not enough to guarantee the virological quality of water. Moreover the inclusion of ENT analysis is also important the evaluate the seawater balneability since they can be present in higher levels than FC. Financial Support: CNPq edital universal/2016, Feevale University

**Palavras-chaves**: *Escherichia coli*, Enteric virus, *Human Mastadenovirus*, North coastline, qPCR

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**ENVIRONMENTAL RESISTANCE OF ZIKA VIRUS (ZIKV) IN POROUS SURFACES AND SOILS**

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**Resumo**

Zika virus (ZIKV), an emergent *Flavivirus* is transmitted mainly by arthropod vectors, but also by blood...
transfusion, organs transplants, sexual contact and vertically during pregnancy. Of course, it is not expected that the virus could be transmitted through environmental exposure, but since ZIKV has been already found in semen and urine, it is interesting to know more about its environmental resistance to enhance laboratory and hospital biosafety. Temperature may contribute to virus genomic stability, but when inadequate, it tends to affect its infectious potential. In the present study, ZIKV resistance and viability were evaluated on porous surfaces (wood slabs) and soil, simulating the approximate climate of southern Brazil (6°C, 21°C e 37°C). Soil samples were prepared with a mixture of a commercial organic fertilizer and gardening soil. For porous surface analysis, 5x5cm size wood boards were used, both in duplicates. To perform the experiments, a known concentration of viruses (10^3 genomic copies) was artificially inoculated into samples and arranged at defined temperatures. Samples were collected from 1 to 6 hours after inoculation, eluted in E-MEM to promote viral elution and then concentrated by standard methods. Resulting solution was used in extraction of viral RNA using TRI Reagent® Solution Kit (Ambion®). After, synthesis of cDNA and real-time polymerase chain reaction (qPCR) were performed. Analysis of porous surface and soil samples showed that ZIKV recovery was possible in all collection periods (1 to 6 hours after viral inoculation). Viral viability test by ICC-qPCR showed that samples kept under refrigeration were found viable throughout the tested period, specially the soil samples. It was noticed that the temperature significantly influenced the damage of infectious particles, especially when arranged at 37°C because presented viability only in the collection (1 hour in soil), with a quantification of 9.70x10^0 genomic copies/5uL. This preliminary study shows that ZIKV might remain viable in the environment and this may vary in time depending on temperature and surface factors.


Palavras-chaves: Zika viruses, Resistance, Temperature, Surface, Soil

MOLECULAR DETECTION OF WATER BORNE ENTERIC VIRUSES IN STREAMS OF ASUNCION, PARAGUAY AND RISK ESTIMATION OF ROTAVIRUS INFECTION

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Resumo

Enteric viruses are excreted to the environment in high concentrations; they remain stable and maintain its infectivity even after the exposure to disinfection procedures, such as the ones used by the national water supply systems. In Paraguay, the circulation of group A rotavirus (RV-A), norovirus (NoV), human astrovirus (HAstV) and human adenovirus (HAdV) in superficial water remains unknown. Twelve samples were collected from two streams, Antequera “CA” and Las Mercedes “CLM”, which cross through the most vulnerable neighborhoods in Asuncion, during 2015 and 2016. Viral particles were concentrated by skim milk flocculation and were detected by RT-PCR. RV-A was quantified by qPCR of NSP3 gene and the Quantitative Microbial Risk Assessment (QMRA) was estimated by online calculator.
Water quality parameters such as microbiologic and chemical indicators were also monitored. Enteric HAdV was detected in more than 50% of the CA and CLM water samples, 7/12 (58%) and 9/12 (75%), respectively; NoVs (CA: 50%, CLM: 42%) were predominantly from genogroup I. HAstV was detected with the same frequency in both streams (CA and CLM: 33%) and were typed as HAstV-4, HAstV-6 and HAstV-8. RV-A was detected only in CA (42%), where genotype G3 was the predominant (45%), followed by genotypes G1 (22%), G9 (22%) and G2 (11%). The risk of infection with rotavirus was 23%. This is the first study on the subject in Paraguay and reveal that the waters studied are potential sources of infection with RVA, NoV, AstV and AdV to the exposed population.

Financial support: CONACYT, Prociencia, IICS-UNA.

Palavras-chaves: enteric viruses, environment, qmra, streams

**EVIDENCE OF FLAVIVIRUS CIRCULATION AMONG NON-HUMAN PRIMATES IN SÃO PAULO STATE-BRAZIL**

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**Resumo**

In the past decades, arboviruses reappeared as major public health concern since outbreaks of viruses such as zika, yellow fever and chikungunya spread throughout the world and hit Brazilian population. Non-human primates have an essential role in the maintenance and diffusion of many arboviruses in a zoonotic and epizootic scenario were the mosquitos reservoir-vectors are present. However, our lack of knowledge on the how dynamics of those arboviruses sylvatic cycle and host interaction are sustained in nature hinder public efforts to understand, better prevent and control arbovirus outbreaks before it happens. In our study, from January 2017 to July 2018, we collected 213 biological samples (whole blood, saliva, and excreta from living animals, and brain, liver and salivary glands from deceased animals) of captive and wild non-human primates from the northeastern and northwestern region of São Paulo State, Brazil. Samples were collected from an overall of 173 Callithrix penicillata, 1 Callithrix jacchus, 9 Allouattacaraya, 1 Allouatta guariba, 2 Saguinus midas, 7 Sapajus apella, 2 Sapajus libidinosus and 2 Sapajus spp.. From the 213 non-human primates samples 24 were tested by a Semi-nested multiplex RT-PCR for viruses from the Flaviridae family, including Dengue, Zika, Yellow fever, West Nile virus, Rocio and Saint Louis encephalitis virus, and the Alphaviridae family, including Easter equine encephalitis, Wester equine encephalitis virus, Mayaro and Chickungunya virus. Two of the 24 non-human primates samples (19 Callithrix penicillata, 1 Callithrix jacchus, 2Saguinus midas and 2 Allouattacaraya) presented positive results for a Flavivirus currently on investigation for species . These results suggested there is an evidence of Flavivirus circulation among non-human primates from the northeastern and northwestern regions of São Paulo State, Brazil. This is an ongoing study and the remaining samples will be analyzed and with the results we intend to generate a risk map for arbovirus outbreak in...
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 November 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 November 2017 in an ecosystem located in the city of Castanhal, totaling 24 and 12 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: Evandro Chagas Institute / SUS / MS and CNPq.

Palavras-chaves: Hepatitis E virus, Suinoculture, Ecosystems impacted
ISOLATION OF BACTERIOPHAGES IN SEWAGE SAMPLES

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Resumo

Bacteriophages are viruses that infect bacteria and are the most abundant biological entities on Earth. Phages and their properties have been constantly studied because of their lytic capacity over bacteria. In this context, it is possible to emphasize its use in the therapy against multidrug resistant bacteria, as agents of biological control in the formation of biofilms and in the control of undesirable microorganisms in foods. It is known that phages are found where its host is present, and it is estimated that $10^{32}$ bacteriophages exist in the planet. Thus, they are detected in sewage and soil samples, natural waters, among other sources. Therefore, the aim of this study was to identify the presence of bacteriophages in sewage samples through the plaque assay. Two samples of sewage were collected, the first in the city of Teutônia and a second in the city of São Leopoldo, both in the state of Rio Grande do Sul. To eliminate bacteria and suspended solids the samples were concentrated and filtrated. An additional step of ultraconcentration was performed in the first sample due to its low viral concentration. Afterwards, the plaque assay was performed by mixing the viral concentrate with 100µL of *Escherichia coli* ATCC 13706 in semi-solid medium. The mixture was deposited on a petri dish containing solid medium and after solidification was incubated for up to 48 hours at 35°C. Both sewage samples showed lysis plaques and translucent and rounded areas, indicating the presence of bacteriophages. The first sample (Teutônia) had a lower number of bacteriophages due to lower contamination, requiring an additional stage of concentration and a longer incubation time (48 hours). The second location (São Leopoldo) is characterized for receiving a large part of the sewage produced in the city, constituting a sample with high contamination. In this case, it was possible to observe the formation of several lysis plaques in reduced incubation time (20 hours). The presence of large amounts of these viruses in the sewage makes it an important source for the isolation of bacteriophages with lytic potential on bacteria of interest, such as antibiotic resistant bacteria. The isolation of bacteriophages is the initial step for the follow up of researches in the different scopes of the use of bacteriophages.

Financial Support: Universidade Feevale

Palavras-chaves: bacteriophages, sewage, plaque assay, ultraconcentration, *Escherichia coli*

ENVIRONMENTAL SURVEILLANCE OF ENTEROVIRUS AND POLIOVIRUSES IN THE CITY OF RIO DE JANEIRO (RJ), BRAZIL, DURING THE OPV SWITCH (tOPV – bOPV) AND BEGINNING OF TRANSITION OPV-IPV

Autores
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Human enteroviruses (EV’s) are single strand RNA viruses belong to the Enterovirus genus of Picornaviridae family. Infections can result in a wide variety of symptoms, however, infections with these viruses are often asymptomatic. EV’s are transmitted by the fecal-oral route, multiply in the gastrointestinal tract, and are excreted in large numbers in the faeces. Thus, sewage is a rich source of enteroviruses, which are circulating in the community. Polioviruses (PV’s) are members of the specie C Enterovirus and are the etiological agent of poliomyelitis. The disease mainly affects children under five years and is caused by one of the three poliovirus serotypes (PV1, PV2 and PV3). During 2012 the trivalent Oral Poliovaccine was replaced by the bivalent Sabin vaccine. That was also the beginning of transition from OPV to IPV. The aim of this study was to apply environmental surveillance to evaluate circulation of enteroviruses and polioviruses in sewage in the city of Rio de Janeiro (RJ), Brazil. A total of 37 sewage samples collected from May to July, 2013 at the sewage treatment plant of Alegria, RJ, were tested to evaluate the presence of EV’s and PV’s. Viral RNA were extracted from 140µl of clarified materials using spin columns (QIAgen®), following the manufacturer’s instructions. Real-time RT-PCR was carried out using pan-enteroviruses and pan-polioviruses real-time RT-PCR systems. Out of the 37 samples analysed, 35 (94.6%) were positive for Non-polio EV’s but negative for PV’s. The results of this study point to a high rates of EV’s in sewage samples, compatible with others studies, and absence of PV’s, which may be related to the chance in the poliomyelitis immunization schedule (trivalent to bivalent OPV and the introduction of IPV) since 2012. Wild polioviruses has been eliminated from Brazil (last case in 1989). Environmental surveillance is an excellent method for monitoring the circulation of EV’s and PV’s, to evaluate the effectiveness of immunization campaigns against poliomyelitis and to detect possible mutations of the Sabin vaccine strains still used in the country.

Palavras-chaves: enterovirus, poliovirus, environmental surveillance, real-time RT PCR

**Teschovirus and other emerging swine and human enteric viruses in Brazilian watersheds impacted by swine husbandry**

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**Resumo**

Several emerging viral agents related to gastroenteritis are distributed in human and animal populations, and may contaminate the environment due to anthropic activities. The dissemination of enteric viruses is facilitated by mismanagement of domestic sewage and animal manure due to inappropriate structure for agricultural activities, household septic systems and bad practices when handling animal wastes. Swine teschovirus (TV-A), Sapelovirus A SV-A and Enterovirus G (EV-G) are non-enveloped viruses belonging respectively to the genus Teschovirus, Sapelovirus and Enterovirus of the family Picornaviridae, and can be used as markers of fecal contamination due to inadequate management of animal waste in rural areas. Due to the lack of proper hygiene and sanitation in rural areas, other important human and zoonotic enteric viruses such as Human mastadenovirus C and F (HAdV), Rotavirus (RV) and Hepatitis E virus (HEV) are commonly found in the environment and are the main cause of waterborne diseases such as gastroenteritis, conjunctivitis, and hepatitis. The
present work aims to analyze the contamination by enteric virus in water from streams of Vale do Taquari, Rio Grande do Sul, from September 2016 to June 2017. Water samples were collected in eight points bimonthly. Samples were concentrated by ultracentrifugation, and RNA/DNA extraction were performed with TRIZOL protocol and commercial kit Viral Mini Spin Plus extraction kit (Biopur®, Brazil) respectively. For RNA genomes, cDNA synthesis was performed with the commercial kit High Capacity cDNA synthesis (Applied Biosystems) and then conventional PCR and qPCR were performed. HEV, EV-G and SV-A genomes were not detected. On the other hand, RV was detected in 3% (1/32) of the samples referring to genogroup A, whereas TV-A was detected in 6% (2/32) corresponding to genotypes TV-A7 and TV-A3. HAdV- C was the most frequent detected viral agent in 9.3% with values of 2.54X10⁵, 7.13X10⁴ and 3.09X10⁵ genome copies /liter (gc/L). Thus, with the results obtained, it is possible to observe the circulation of these viral agents derived from pig farming, and the impact generated by this activity on the water quality of the water bodies of the Vale do Taquari region. In this way, enteric viruses can assist in monitoring the quality of watersheds, tracking sources of contamination and establishment of profiles of the circulating genotypes enabling strategic planning of control and prevention.

Fomento: CNPq, CAPES, FAPERGS

Palavras-chaves: Teschovirus, gastroenteritis, environment, contamination, water

Genomic characterization of lytic Escherichia coli bacteriophage, a promising candidate for phage therapy

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Resumo

Bacteriophages are viruses that infect bacteria and are used in diverse biotechnological applications. The ability to lyse bacterial cells after infection is one of the most explored characteristics of these organisms, once that phage can be used to control the growth of their host in an approach called phage therapy. Some phage enzymes, capable of breaking down the bacterial biofilm, such as depolymerases, are also promising tools to be explored. Thus, the isolation of new lytic phage and the analysis of their genome, make this new perspectives and applications possible. The objective of this work was to analyze the genome of a lytic phage isolated from environmental samples using Escherichia coli as host, called vB_EcoM-UFV09. Genome was extracted following the protocol described by Sambrook and Russel, 2001, and sequenced using the Illumina MiSeq platform. Results shown that phage belongs to the order Caudovirales, family Myoviridae, with genome has about 165 kb, encoding 267 genes, among them 6 tRNAs. Searches in silico, using “ResFinder” and “VirulenceFinder” programs indicate that there are no genes that confer resistance to antibiotics and any virulence genes, an essential characteristic in phage therapy. The “Hostphinder” program presented as potential hosts the bacterial species Escherichia coli, Shigella flexneri, Yersinia pestis and Shigella sonnei. A comparative analysis between the phage and the database indicated 97% similarity between the genome of vB_EcoM-UFV09 and Escherichia coli O157 typing phage 7, both showing an interruption in the gene encoding the rIIA protein. Phylogenetics analyzes between UFV09 and type species of the Myoviridae family, grouped UFV09 with classical T4 phages and Enterobacteria phage AR1. These results indicate that vB_EcoM-UFV09 can be classified as a T4-like and has great potential for use in phage therapy.

Palavras-chaves: Bacteriophages, Phage therapy, Escherichia coli, Genomic characterization
ENVIRONMENTAL RESISTANCE OF HUMAN MASTADENOVIRUS (HAdV) IN SOILS AND POROUS SURFACES UNDER CONTROLLED CONDITIONS

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Resumo

Human mastadenovirus (HAdV) is naturally transmitted through environmental exposure, either via the fecal-oral route, aerosol particles, contaminated waters or fomites. HAdVs are considered worldwide as important indicators of contamination due to their association with diseases in populations, high prevalence in the environment and potential environmental resistance. Typically, low temperatures contribute to the virus genomic stability, while higher temperatures tend to compromise its infectious potential. In the present study, resistance and viability of HAdV-5 were evaluated on porous surfaces (wood slabs) and soil, simulating southern Brazil typical temperature conditions (6°C, 21°C and 37°C). Soil samples were prepared with a mixture of commercial organic fertilizer and gardening soil. Porous surface samples were prepared on wooden boards of 5x5cm, in duplicates. In order to perform experiments, a known concentration of viruses (10^3 genomic copies) was artificially inoculated into samples and then exposed to preset temperatures. Samples were collected monthly from October (2017) to February (2018), eluted in E-MEM to promote viral elution and then concentrated by standard methods. Resulting solution was used in viral DNA extraction using the Promega® kit, and then real-time polymerase chain reaction amplification (qPCR) was realized. Porous surface and soil samples analysis showed that viral recovery was possible in all collection periods and that after 150 days at 37°C there was a decline of 2 log10 in soil samples. Viral viability assay by ICC-qPCR showed that the samples kept under refrigeration remained predominantly viable, especially soil samples. It is important to distinguish infectious (viable) viruses stability versus viral genome presence in environmental matrices that have not yet been studied, such as soil and wood, since these have different adsorption profile and porosities.


Palavras-chaves: Contamination, Environmental, Soil, Surface, Temperature.

Detection, quantification and genotyping of Rotavirus A detected in water samples from Lagoa Maior de Três Lagoas - MS

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Resumo
Detection, quantification and genotyping of Rotavirus A detected in water samples from Lagoa Maior de Três Lagoas - MS

Rotavirus (RV) are considered to be important surface water contaminants and although they are not included in the bathing parameters, they are the main cause of gastroenteritis in humans, especially those of serogroup A (RVA), which are subdivided into ten G serotypes. The city of Três Lagoas - MS has 3 bodies of water, where Lagoa Maior, stands out for being a tourist attraction. Previous studies, analyzing physicochemical and microbiological parameters of these waters, have shown an acceptable presence of *E. coli*, which is an important microbiological indicator of fecal contamination. The objective of this study was to detect the presence of RVA in water samples from Lagoa Maior of the city of Três Lagoas - MS, collected at 8 points previously analyzed by microbiological tests and that had acceptable detection levels of *E. coli*. One liter of surface water was collected at each point, which were concentrated 1000X using the negatively charged membrane and adsorption-elution technique, being concentrated again using Centricon P-70 to a final volume of 1 mL. Detection was performed by conventional PCR, preceded by reverse transcription (RT), and the samples considered positive were submitted to quantification through qPCR. All positive samples were submitted to G (VP7) genotyping through new RT-PCR and Nested PCR reactions. The genetic material of RVA was detected in 37.5% (3/8) of the analyzed samples, showing no direct relation with the previous detection of *E. coli* in these samples. Additionally, the quantification of RVA showed low levels of detection, ranging from $2.3 \times 10^1$ to $3.7 \times 10^2$ copies of the viral genome / liter. The identification of G genotypes was possible in the 3 positive samples, with G1 genotype prevalence (66.6%) followed by G4 genotype (33.4%). These data, obtained in this study, reinforce the need for constant monitoring of the quality of these waters since Lagoa Maior is an important tourist spot in the municipality. In addition, these results emphasize the need to establish parameters for the evaluation of viral quality of water.

**Palavras-chaves:** Gastroenteritis, Rotavirus, RVA, Três Lagoas

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**ANALYSIS OF THREE ISOLATES OF PANDORAVIRUSES REVEALS IMPORTANT STEPS IN THE REPLICATION CYCLE THESE GIANT VIRUSES**

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**Resumo**
Giant viruses are the most complex members of the virosphere, exhibiting structural and genomic features that blur the classic concept of viruses. Among these viruses, the pandoraviruses are one of the most intriguing members, exhibiting giant particles and genomes of up to 2.5 Mb, with many genes having no known function. The current knowledge about these viruses is limited due to only a few reported isolates and investigations focused on their genomic features. In this work we describe the isolation of three new pandoraviruses from samples collected in different regions of Brazil and shed light on different steps of their replication cycle by using different biological and microscopy approaches. The new viruses exhibit typical morphological characteristics, with amphora-shaped particles of ~1.0 µm in size and an ostiole-like apex at one extremity of the particle, from where the genome is released. These viruses enter the host cells through phagocytosis and establish large electron-lucent viral factories, where the viral morphogenesis occurs. No pattern was observed in the initiation of the capsid formation and the assembly of the giant viral particles seemed to be started either by the apex or by its opposite side. Finally, these viruses are released by exocytosis in early times of infection and by cellular lysis, later in the cycle. This study provides new information about the diversity of pandoraviruses and shed light on unexplored aspects of the biology of these viruses.

Palavras-chaves: PANDORAVIRUS, GIANT VIRUS, VIRUS DIVERSITY, REPLICATION CYCLE, MORPHOGENESIS

CLIMACTIC FACTORS AND THE FREQUENCY OF ARBOVIRUSES' VECTOR MOSQUITOES IN URBAN AREAS FROM RIO GRANDE DO SUL

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Resumo

Mosquitoes of the genus Aedes belong to the family of Culicidae are considered of great epidemiological importance, being the main vectors of at least three important diseases caused by arboviruses: Dengue, Zika and Chikungunya. Although Dengue has been present in Brazil for decades, in Rio Grande do Sul it has been present in autochthonous cases for approximately only 10 years, in alarming isolated outbreaks, but they do not yet reach the reality of other states. Despite this, the detection rates of Aedes sp. are high. In addition to other issues, possible climatological variables such as temperature and precipitation may be influencing the mosquito's permanence in the region. Thus, the data on temperature, rainfall and air humidity for the state of Rio Grande do Sul in the period from 2007 to 2017 were carried out. Likewise, we searched the number of municipalities infested by the mosquito and the amount of cases of Dengue, Zika and Chikungunya throughout this period. This study showed that, considering all 18 meteorological stations in the state, average temperatures ranged from 14 to 24°C, with minimum annual averages of 10°C and annual mean maximums of 29°C. The relative humidity of the air has turned around 77% and it rains in the state around 1700mm annually. Comparing with previously published data, it has been observed that in the last years the average temperatures are getting higher and the seasonal thermal amplitude, smaller. Almost half of the municipalities of Rio Grande do Sul state have Aedes aegypti infestation, although this increase is not proportional to the number of autochthonous cases of these diseases. This suggests that the variation in average temperatures and the irregular distribution of precipitation may be related to the facilitation of the mosquito stay in its breeding grounds.

Financial Support: Capes/CNPq/DCIT

Palavras-chaves: Aedes, Autochthonous, Humidity, Precipitation, Temperature
Isolation of a non-cytopathic Human mastadenovirus C from environmental sample

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Resumo

Human mastadenovirus C (HAdV-C) belongs to the Mastadenovirus genus within Adenoviridae family. It is a nonenveloped virus with an icosahedral nucleocapsid containing dsDNA genome. This species is one of the main etiological agents of upper respiratory tract infections in children. HAdV-C is widespread distributed in the environment and it has been frequently found in rivers, groundwater, water for public supply, recreation, wastewaters and sewage. Moreover, most recent studies demonstrate that these virus particles found from environment can often be infectious and they present a risk to human health. However, there are no studies yet that have demonstrated the interactions between an isolate from the environment and a live organism in order to attest about the virulence of these viruses from aquatic environments. That way the study aims to isolate HAdV from water samples, so that can be used to conducts studies about the host-pathogen interaction dynamics. For this purpose, 124 samples have been selected (86 groundwater and 38 surface water). All samples were concentrated by ultracentrifugation after they were filtered with a membrane (0.2 µm) and 200 µL of each sample were inoculated in the 24-well plates containing A549 cells. The first and third samples passage in cell were treated with DNAse and were submitted to qPCR for detection and quantification of HAdV-C genome. The positives samples from the third passage were passed again until they were isolated. Just 12.7% (11/86) of the groundwater were infectious, being five samples were at first passage and six at the third, among these one sample were positive in both passage. In the surface water 18.4% (07/38) of the samples were infectious, however just two samples at the first and five at the third. Of 18 infectious samples, only one was possible to be isolated (5.5%), namely isolate LMM 2863. The sample isolated (HAdV-C) at the first passage probably was below the detection limit of qPCR and only be detected after at third passage with 3,81E+02 gc/5 µL and at tenth passage the quantification was 1,35E+04. The isolate LMM 2863 did not provide any cytopathic effect despite of being detected by molecular techniques. This study showed how much laborious is isolate environmental viruses because less than 1% was isolated (1/124). The virulence of this isolate will soon be tested in a murine model.


Palavras-chaves: HAdV-C, isolated viruses, environment

MOLECULAR EPIDEMIOLOGY AND FREQUENCY OF TRANSMITTED DRUG RESISTANCE AMONG HIV-1 INDIVIDUALS ART-NAIVE IN BELÉM, PARÁ, BRAZIL

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Resumo

The introduction of antiretroviral therapy (ART) has led to a significant decrease in mortality and improvement of life for people living with HIV/AIDS (PLHA), but the emergence of antiretroviral drug resistance is a threat risk of transmission of resistant strains. The present study describes the frequency of mutations in transmitted resistance and genetic diversity of HIV-1 in PLHA in the city of Belém, Pará. A cross-sectional study was conducted between May and September 2016, all
patients answered a questionnaire containing socio-demographic and epidemiological questions. Blood collection was performed, followed by DNA extraction, amplification and nucleotide sequencing of regions corresponding to protease (PR) and reverse transcriptase (TR) in HIV-1 pol gene. Identification of transmitted drug resistance mutations (TDRM) was performed using Calibrated Population Resistance (CPR) 6.0 tool, while genetic variability was assessed using the REGA HIV-1 Automated Subtyping Tool 3.0 tool and the results were confirmed by phylogenetic analyzes. TDRM were found in four patients (4/41; 9.76%), where two presented mutations associated with nucleoside reverse transcriptase inhibitors (2/39; 5.13%), one to non-nucleoside reverse transcriptase inhibitors (1/39; 2.56%) and one to protease inhibitors (1/40; 2.50%), however, none of the patients presented mutations to more than one drug class simultaneously. Intermediate or moderate levels of transmitted drug resistance were observed, similar to previous studies done in Brazil. The subtype B was the most prevalent among the patients (32/40; 80.0%), followed by the sub-type F1 (5/40; 12.5%) and recombinants of both (BF1; 3/40; 7.5%). The results suggest the need for constant monitoring of antiretroviral drug resistance levels and HIV-1 genetic diversity in the State of Pará, as well as the consideration of the implementation of pre-treatment genotyping tests in Brazil.

Financial Support: CAPES

Palavras-chaves: ART, HIV-1, molecular epidemiology, Transmitted drug resistance

In vitro formation of nucleocapsid-like particles of Dengue Virus

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Resumo

Dengue virus (DENV), a member of the Flavivirus genus of the Flaviviridae family, is responsible for causing one of the most important human arboviruses. The DENV genome consists in a single-stranded, positive sense RNA, which is translated in a polypeptides that is processed in three structural and seven non-structural proteins. Among the structural proteins, the capsid protein plays an important role in the encapsidation of the viral genome to form the nucleocapsid. However, this process is not well known and there are many questions about the structural organization of the flavivirus’ capsid. Since it is not possible to isolate the capsid from infected cells, to study the formation of these particles, we chose to establish a protocol for obtaining nucleocapsid-like particles after the interaction of the DENV capsid protein with oligonucleotides of different sizes. After choosing oligonucleotides of interest for interaction using bioinformatics tools, the following techniques were used to analyze and characterize these particles; determination of fluorescence intensity and light scattering during protein interaction with small oligonucleotides; small-angle X-ray scattering (SAXS); transmission electron microscopy (TEM); atomic force microscopy (AFM); and turbidity analysis to follow the kinetics of nucleocapsid-like particle formation. During the development of the protocol, the technique that provided the most relevant information about the organization of particles was the AFM. This technique allowed the visualization of homogeneous and organized structures, especially when the protein interacts with larger oligonucleotides, which makes the particles more stable when compared to those assembled with smaller oligonucleotides. Thus, the established protocol proved to be effective for the study of nucleocapsid-like particles. From the information obtained in this work, it will be possible to
study structural characteristics and the process of assembly of the DENV capsid.

Agência de fomento: CAPES

Palavras-chaves: Atomic Force Microscopy, Capsid, Dengue

EXPERIMENTAL INFECTION MODEL FOR HUMAN ROTAVIRUS USING CYNOMOLGUS MONKEYS (Macaca fascicularis)

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Resumo

Group A rotaviruses (RVA) are one of the most common causes of severe acute gastroenteritis, and a major cause of morbidity and mortality in children worldwide, especially in developing countries. Rotaviruses spread from person to person, mainly by faecal–oral transmission. Almost all unvaccinated children may become infected with RVA in the first two years of life. The establishment of an experimental monkey model with RVA is important to evaluate new therapeutic approaches. In this study, we demonstrated viral shedding and viraemia in juvenile–adult Macaca fascicularis orally inoculated with Wa RVA prototype. Nine monkeys were inoculated orally once by an appropriated gavage tube: seven animals with human RVA suspension (3.1x10^6 FFU/mL) and two control animals with saline solution. During the study, the monkeys were clinically monitored during a period of eleven days, and faeces and blood samples were tested for RVA infection. Serum electrolyte and haematological evaluation were also performed. In general, the inoculated animals developed an oligosymptomatic infection pattern. The main clinical symptoms observed were diarrhoea in two monkeys for three days, associated with a reduction in plasmatic potassium content. Viral RNA was detected in seven faecal and five sera samples from inoculated animals, suggesting virus replication. In order to confirm that the virus detected in faeces was not merely free RNA, but the complete infectious particles, the infection of MA-104 cells with faecal samples was performed. The presence of infectious particles in faeces was confirmed in seven inoculated animals, independently if monkeys presented diarrhoeal episodes or not. No evidence of infectious particles was detected in the faeces of the two control animals. Cynomolgus monkeys are susceptible hosts for human Wa RVA infection. When inoculated orally, they presented self-limited diarrhoea associated with presence of RVA infectious particles in faeces. Thus, cynomolgus monkeys may be useful as animal models to evaluate the efficacy of new antiviral approaches.

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Cotton and Brazilian-pepper tree aqueous extracts: antiherpesvirus drug possibility?

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Resumo

The immunocompromising conditions are predisposing factors for the establishment of infections by the Herpesviridae family. In addition, these conditions are favorable for the establishment of infections caused by other opportunistic microorganisms that cause diseases such as periodontitis. The search for bioactive compounds from ethnobotanical survey data constitutes a promising possibility for the treatment of infections of multiple etiology including concomitant viral infection. The objective of this work was to evaluate the antiviral action of two medicinal plants indicated for the treatment of oral diseases on equide alphaherpesvirus type 1 (EHV-1) in vitro, as herpesvirus model. Based on popular indications, two medicinal plants were elicited in the region of Ilhéus, Bahia: cotton (Gossypium barbadense) and Brazilian-pepper tree (Schinus terebinthifolius). From the leaves, 10% infusion was prepared which was lyophilized and subsequently solubilized in 0.9% NaCl solution at an initial concentration of 10 mg.mL⁻¹. From the concentrations ranging from 1000 to 15.6µg.mL⁻¹, the maximum non-cytotoxic concentration (MNCC) was performed in Vero cells and percentage of inhibition (PI) of EHV-1 A4/72 strain was determined. The results showed 46%PI at a MNCC of 100µg.mL⁻¹ for cotton and 81.8%PI at a 50µg.mL⁻¹ MNCC for Brazilian-pepper tree. The results indicated that under conditions of this study and criteria stablished in the literature to consider a good antiviral substance, aqueous extract of cotton did not have antiviral activity on equide herpesvirus while one of Brazilian-pepper presented a moderate action. From both plants indicated by traditional knowledge, Brazilian-pepper is the most promising plant to treat multiple oral infections.

Palavras-chaves: antiherpesvirus, antiviral activity, oral diseases, medicinal plants

ESTABLISHMENT OF STABLE REPLICON CELL LINES FOR CHIKUNGUNYA AND ZIKA VIRUSES

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Arboviruses infections have been a constant concern of public health in tropical and subtropical regions. More recently, infections by chikungunya (CHIKV) and Zika (ZIKV) viruses have gained attention mainly due to the severe polyarthralgias and congenital alterations, respectively. In addition, there are no vaccines or drugs licensed against these viruses. In this context, viral reverse genetics platforms have been demanded especially for the development of vaccines and high-throughput assay for screening of antiviral compounds. The reverse genetics systems available for CHIKV and ZIKV, however, use costly and laborious cloning protocols in Escherichia coli. Here, we have developed two different recombinant cell lines expressing CHIKV and ZIKV reporter replicon. For the development of the CHIKV replicon, the Gaussia luciferase (GLuc) and neomycin phosphotransferase genes were cloned with the CHIKV (strain 99659) subgenome into the pBSC-HDR vector. In the ZIKV replicon, the Firefly luciferase (FLuc) and neomycin phosphotransferase genes were cloned with the ZIKV PE243/2015 strain subgenome into the pSVJS01 vector. Both replicons plasmids were assembly in yeast by homologous recombination technique. Subgenomic replicons cDNA clones were then in vitro transcribed and the RNAs were individually transfected into BHK-21 cells. Three days after transfection, the cells were selected with geneticin (600 μg/mL). After ten days of selection, the obtained cell clones, BHK-GLuc-nsP-CHIKV-99659 and BHK-rZIKV-FLucNeoIres, were amplified and the reporter genes were measured. For BHK-21-GLuc-nsP-CHIKV-99659, GLuc expression was of about 100-fold higher than the negative control (in 10⁵ cells, 18 hours after seeding) and the result remained constant during the analyzed passages, from passage 3 to 13. On BHK-21-rZIKV-FLucNeoIres, in 3 x 10⁵ cells, 18 hours after seeding, FLuc expression was approximately 27-fold increase over the negative control. This expression also remained constant from passage 4 to 21. Finally, the results confirm the stability and efficiency of our replicon cell lines and suggest its future use for large-scale screening of antiviral candidates and replication machinery studies of CHIKV and ZIKV.

Financial Support: Capes, CNPq, FIOCRUZ, FACEPE

Palavras-chaves: ARBOVIRUS, CHIKV, ZIKV, REPLICONS

Herpes simplex virus type I (HSV-1) is an enveloped virus, with double-stranded DNA, widely distributed around the world. HSV-1 causes orofacial lesions, which may aggravate due to the immunological state of the host, such as central nervous system (CNS) infections, culminating in herpetic meningitis and encephalitis. These more severe symptoms of infection are associated with high mortality/morbidity. The standard treatment is the administration of nucleoside analogs, which are highly specific for cells infected with a viral genetic material. However, they have shown some limitations such as low
bioavailability and selection of drug-resistant strains. The limitations encountered by current anti-herpetic agents, in addition to the more severe conditions of the infection justify the search and development of new and better anti-HSV-1 therapies. Drug repositioning proves to be a promising approach in the search for new drugs, mainly because it reduces the time and costs associated with drug development. The aim of this study was to perform the screening of drugs clinically approved for other diseases as potential drugs to combat HSV-1 infections. In this study, 7 drugs were tested for anti-HSV-1 activity, they are: quinidine, nitazoxanide, isoxicam, naproxen, diclofenac potassium, nimesulide, and quinine. The antitherpetic activity and cytotoxicity were determined by the MTT colorimetric cell viability assay. Therefore, CC50 (cytotoxic concentration for 50% of cells) and EC50 (effective concentration of 50%) and IS (selectivity index) were determined. The drug with the best antiviral activity was diclofenac potassium with IS = 7.9. In order to understand the mechanism of action of diclofenac potassium, a series of assays were conducted to determine in which viral multiplication step the drug could be acting. Diclofenac showed higher activity in the post-penetration phase, with EC50 of 152 ± 11 µM. It is suggested that diclofenac may act on intracellular pathways that could inhibit viral multiplication. However, such pathways are still under investigation for a better understanding and elucidation. With this work, using drug-repositioning approach, we hope to contribute to new drugs for the treatment of infections caused by HSV-1, mainly in its most severe forms. FINANCIAL SUPPORT: CNPq.

Palavras-chaves: Antiviral, Diclofenac, Herpes simplex Type 1, Repositioning, Screening

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DENGUE SEROTYPE-SPECIFIC CROSS-REEACTIVE ANTIBODIES ENHANCE ZIKA VIRUS INFECTION IN FcγR BEARING CELLS.

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Resumo

Introduction. Dengue virus (DENV) serotype-specific antibodies cross-react with Zika virus (ZIKV) structural proteins. These antibodies facilitate viral uptake into cells expressing Fc-gamma receptors (FcγR) (monocytes/macrophages) through the mechanism of antibody-dependent enhancement (ADE). We assessed the cross-reactivity between ZIKV and DENV serotype-specific antibodies (anti-DENV1-4) as well as the ability of these antibodies to induce ADE of ZIKV infection. Moreover, we characterized immune-complexes (IC) in sera with either enhancing or neutralizing properties against ZIKV.

Materials and methods. The in vitro assays were performed in well-characterized sera of DENV primary infected individuals (n=35), Flavivirus-naive individuals (n=18) and YFV vaccine recipients/DENV naive (n=10). Cross-reactive antibodies for ZIKV E protein and the whole virus particle (WVP) were determined through indirect ELISA. ADE of ZIKV infection by cross-reactive antibodies was measured on FcγR-II expressing K562 cells through flow cytometry. Antibody titers were estimated by non-linear regression, log transformed and compared between groups using non-parametric tests. ICs formed in vitro were characterized by the presence of antibodies binding to E protein and WVP through a simple IC dissociation ELISA assay. Proportion of associated (aELISA) and dissociated (dELISA) ICs were estimated using the formula IC= (dELISA-aELISA)/dELISA). Results. DENV-3 (median 2.7; range 1.7-4.4) and DENV-4 (median 2.8; range 1.7-3.1) specific antibodies exhibited the highest levels of cross-reactivity to both ZIKV E protein (p=0.0013) and WVP (p=0.0023). DENV-3 (median 14.4; range 1.1-23.7) antibodies mediated higher levels of ADE of ZIKV infection (p=0.007). The proportion of ICs binding to both ZIKV E protein (p=0.0012) and WVP (p<Conclusions. DENV serotype-specific antibodies exhibited distinct levels of ZIKV cross-reactivity and ADE. The higher levels of ZIKV ADE by DENV-3 antibodies suggest a possible role of this antibody in ZIKV pathogenesis. Sera with ZIKV enhancing properties showed higher levels of ICs containing antibodies binding to ZIKV E protein and WVP. Serological diagnostic tools based on ZIKV E protein...
detection might not discriminate ZIKV and DENV infections due to the high degree of cross-reactivity.

**Financial support:** PIBIC/FACEPE; CNPQ/FIOCRUZ; PROEP/FIOCRUZ.

**Palavras-chaves:** Zika virus, Dengue antibodies, Antibody-dependent enhancement

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**ANTIHERPETIC ACTIVITY OF DIBENZYLIDENEACETONES**

**Autores**
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**Instituição**

**Resumo**
Herpes simplex virus type 1 (HSV-1) is an enveloped virus, double-stranded DNA and one of the most prevalent infections worldwide. After direct contact between susceptible and infected individuals, primary infection occurs in epithelial cells and the virus can establish latency in nerve cells. Therefore, HSV-1 infections are associated with oral, ocular, cutaneous and central nervous system manifestations and may result in mild to severe morbidities depending on the host's immune status. In addition, current contexts indicate the increasing presence of HSV-1 as a sexually transmitted infection. The treatment consists in the administration of nucleoside analogues such as Acyclovir. However, there has been a selection of Acyclovir-resistant HSV-1 strains, especially among immunodepressed patients. Dibenzylideneacetones (DBA) forms a class of compounds containing an acyclic dienone linked to aryl groups in both β-positions, whose biological activity against Leishmania amazonensis and Trypanosoma cruzi had been shown by our research group. The objective of this work was to evaluate the cytotoxicity and anti-HSV-1 KOS strain in vitro of DBA structures by the MTT colorimetric method in VERO cells. After obtaining the values of CC50 (cytotoxic concentration 50%) and EC50 (effective concentration 50%), the SI (selectivity index) was determined by the ratio CC50/EC50. We analysed 17 substances which results are presented as following: Chemical formula (SI). C21H21NO (>40); C19H15NO3 (20H18O3 (55.3); C22H18O4 (21); C21H18O5 (18H12N2O4 (>18.8); C19H16O4 (22H20F3NO3 (13H12O2 (20H17BrO2 (20H18NO5 (21H22NO6 (14H17NO3 (21H20N2O5 (2775.2). Considering that the substance C20H14ClO presented greater selectivity, it will be employed in subsequent analyses, such as elucidation of the mechanism of action and anti-HSV-1 activity to Acyclovir-resistant strain. It is hoped to contribute to the development of alternative and more effective drug against the strains of HSV-1 resistant to the current treatments. Financial Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior e Conselho Nacional de Desenvolvimento Científico e Tecnológico.

**Palavras-chaves:** Antiviral activity, Dibenzylideneacetones, Herpes simplex virus type 1, Screening, Synthetic compound

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**BIOLOGICAL AND GENOTYPIC CHARACTERIZATION OF CLINICAL ISOLATES OF CANTAGALO VIRUS**

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Resumo

Orthopoxviruses (Poxviridae) are important disease agents, highlighting variola virus and vaccinia virus (VACV). VACV is a rare zoonotic pathogen in the world, but frequently found in Brazil and India. In Brazil, VACV strain Cantagalo (CTGV) was isolated in 1999 in farms of Rio de Janeiro state, and since then, outbreaks have been reported throughout the country. CTGV causes a pustular disease in dairy cattle and milkers, leading to significant economic losses. The rapid spread of CTGV in Brazil may be influenced by different genomic and biological features of the clinical specimens. Therefore, the study of the genetic diversity of different clinical isolates of CTGV is a valuable tool to investigate virus dissemination in the country. However, there is limited knowledge on CTGV genomes and its association with biological properties. Thus, our aim was to characterize the biological and genetic diversity of five clinical isolates of CTGV obtained from pustular lesions in milkers and dairy cattle from different states of Brazil. Full-genome sequencing was performed with Illumina NextSeq500. Reads were mapped against CTGV reference strain with 70-80% of properly paired reads. De novo assembly generated genomes with lengths that ranged from approximately 181 kb to 184 kb with 33% of GC content and 7023 to 7393bp ITR regions. Phylogenetic analysis grouped all isolates within the CTGV cluster, which shares a most recent common ancestor with VACV-IOC, the main Brazilian smallpox vaccine strain. Genomes have more than 200 ORFs, with few single nucleotide polymorphisms (SNPs) among them. Highly variant regions were mainly found in C9L and A51R genes. Three clinical strains have a 3.7 kb deletion in C9L, similarly to CTGV, while two others have the full gene. A51R gene is the major polymorphic region with an AT-rich repeat of variable extension. Plaque areas ranged from 0.14 to 0.37 mm² and all isolates but MU-07 produced a 3-log increase in virus production at 24 hpi. MU-07, which has the smallest viral plaques and lowest titers, presents SNPs in 6 (A27L, A36R, B5R, F5L, F12L and H2R) out of the 27 genes analyzed in this study because of their role in viral spread. Altogether, our results bring new information on the diversity of CTGV genomes and possible associations with viral dissemination, reinforcing the importance of a continuous investigation of CTGV diversity. Funding: CAPES, CNPQ and FAPERJ.

Palavras-chave: Cantagalo strain, Biological features, Genetic diversity, Poxvirus, Vaccinia Virus

CCR5 AND CXCR4 CELL RECEPTOR MODULATION OF HOST ANTIVIRAL RESTRICTION FACTORS

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Resumo

Recent data have shown that exposure of A(H1N1)pdm09-infected epithelial cells to HIV-1 viral particles, or its gp120, enhanced interferon-induced transmembrane protein (IFITM3) content, resulting in a decrease in influenza replication. The gp120 binds to CCR5 (R5) or CXCR4 (X4) cell receptors during HIV infection. Thus, we have studied

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the role of cellular receptors R5 and X4 in modulating virus restriction factors. For this purpose, MDCK cells (main cellular model for human influenza virus propagation) were treated with 2x effective dose (ED50) of endogenous R5 receptor agonists, CCL3 (20 ng/ml), CCL4 (10 ng/ml) and CCL5 (10 ng/ml) for 24h. Then, the cells were infected with the virus A(H1N1)pdm09 at MOI (multiplicity of infection) 0.05 and 0.5 for 1h. 24 hours post infection (hpi), the supernatant was harvested and the viral titre assessed by quantifying neuraminidase activity (NA). We found that R5 agonists inhibited influenza virus replication by 70% in a MOI-dependent fashion. To evaluate the kinetics of IFITM3 protein levels, MDCK cells were treated with 2x ED50 of CCL3, CCL4, CCL5, CXCL12 (3 ng/ml, X4 agonist) and the exogenous agonists of R5 and X4 receptors, gp120-Bal and gp120-IIIB (5 µg/mL), respectively. The cell monolayer was lysed with Laemmli buffer at different times after agonists exposure. The result showed that IFITM3 protein levels were increased 18h after exposure. Next, MDCK and A549 cells (adenocarcinomic human alveolar basal epithelial cells) were exposed to R5 and X4 endogenous and exogenous agonists, interferon α (10 ng/mL) was used as positive control. After 18h, the cells were infected with influenza virus A(H1N1)pdm09 and A/H3N2, respectively. 24 hpi, the supernatant was harvested and the viral titre measured by NA activity. We observed that R5 and X4 agonists inhibited the replication of influenza A viruses by approximately 80% in MDCK and 70% in A549 cells. Next, we analyzed the virus restriction factors modulation in A549 after agonists exposure by customized RT 2 Profiler PCR Array (Qiagen), according to manufacturer instruction. Interestingly, the result showed an upregulation of SAMHD1 in cells exposed to agonists, but not increased IFITM3 expression. Future studies should provide further insights about the role of SAMDH1 in influenza replication inhibition. The aim of this work is to find new strategies of the innate immune system that control important viral infections.

Financial Support: FAPERJ, CNPq, POM-IOC

Palavras-chaves: IFITM, Influenza, Interferon stimulated genes, Restriction factors, SAMHD1

MOLECULAR CHARACTERIZATION OF THE FIRST COMPLETE GENOME OF HEPATITIS C VIRUS SUBTYPE 2B FROM THE LATIN AMERICA

Autores
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Resumo
Hepatitis C virus (HCV) is an important human pathogen affecting nearly 3% of the world’s population, and is a leading cause of chronic liver diseases including cirrhosis and hepatocellular carcinoma. HCV is a rapidly evolving RNA virus that has been classified into seven genotypes and numerous subtypes. In the last years, HCV subtype 2b has been detected in different geographic regions of Brazil. However, no complete genome of this subtype from the Latin America was obtained until now, limiting studies on the diversity and molecular epidemiology of the virus. Furthermore, amplification of large HCV genomic fragments is challenging, since reverse transcription polymerase chain reaction (RT-
PCR) must overcome low template concentrations and high target sequence diversity. The aim of this study is to perform the molecular characterization of the first HCV subtype 2b full-length genome from the Latin America. This was done by optimization of HCV RNA isolation, cDNA synthesis, and nested PCR steps to concentrate HCV RNA after extraction from serum. Sequencing was performed using the Sanger method. A complete genome, with two overlapping fragments of 3,388 and 4,541 base pairs (bp) in length, was successfully amplified. Phylogenetic analysis confirmed that both PCR fragments belonged to subtype 2b. Surprisingly, the full-length genome presented a total size of 7,298 bp, showing a deletion of 2,022 bp (genome position 965 - 2986) covering most of the E1, E2, p7 and the 5’ end of NS2 genomic regions. Molecular cloning assays to investigate the quasispecies population of this HCV isolate are in progress. In conclusion, the strategy used here was effective to amplify a complete genome of HCV subtype 2b, and could be adapted for full-genome amplification of other HCV subtypes.

**Financial support:** CAPES, CNPq, FIOCRUZ

**Palavras-chaves:** Complete genome, HCV, Subtype 2b

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**THE ZIKA VIRUS ENHANCE THE ROS PRODUCTION IN ASTROCYTE AND OLIGODENDROTIC PRECURSOR CELLS PRIMARY CULTURE**

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**Resumo**

During the major Zika virus (ZIKV) epidemic in 2013 in French Polynesia and Brazil in 2015, neurological complications such as microcephaly and Guilian-Barré syndrome were reported and related with the virus. According to the Brazilian Ministry of Health, until October 29, 2016, 10,039 cases of microcephaly were reported and also related with the ZIKV. It is known that one of the main causes of cellular damage in the Central Nervous System (CNS) is due to the increase and consequent accumulation of Reactive Oxygen Species (ROS), which can be caused by viral infections, as described for Dengue, Japanese Encephalitis, Hepatitis C, among other viral diseases. Based on its susceptibility to ZIKV infection and its role in CNS homeostasis, astrocytes and oligodendrocytes precursor cells (OPC) provide a good cellular model for investigating the mechanisms of the viral entry into cells and its effects. The primary culture of these cells is a tool used in this work in view of its greater similarity with the functions in vivo. For the primary culture, neonate Swiss mice (1-4 days old) were used, and at this stage, mouse brain developmental is equivalent to the early second trimester-stage of human CNS development. The animals were euthanized, the brain removed and the cells enzymatically dissociated. After plating, the glial cells were isolated into astrocytes and OPC precursor cells by shaking. For quantification of ROS production, Sigma DCFDA reagent was used in microplate assay, the cells were then analyzed on a SpectraMax 4M fluorescence reader. The readings were taken at post-infection time intervals. The data shows a gradual increase in ROS production over time, where there is a significant accumulation in the interval between 12 and 24 hours post infection. OPC show an even higher ROS production and accumulation compared to the other two analyzed cell lines (astrocytes and VERO). Analyzes made on BD FACS flow cytometer show that the number of cells with high production of ROS also increases from 3 hours post infection, where once again OPCs have a higher index than the other cells. These data suggest that cell types react differently to ZIKV infections and that a large accumulation of ROS occurs over the course of infection time.
IDENTIFICATION OF TYPE 2 AND 4 DENGUE VIRUS CO-CIRCULATION IN THE PIAUÍ STATE IN 2017


Instituição: UFPI-CMRV - Universidade Federal do Piauí - Campus Ministro Reis Velloso (Av. São Sebastião 2891)

Resumo

Dengue virus (DENV) is an arbovirus which belongs to Flavivirus genus and affects millions of people. It represents a major public health problem worldwide because of its high incidence and morbimortality rates. DENV presents four serotypes (DENV 1-4) which are widely distributed throughout Brazil, and they have started circulating as endemic in Piauí since the 1990’s. Ever since, several outbreaks and epidemics have occurred due to its four serotypes co-circulation in different regions of this State, characterizing a hyper-endemicity situation in the region. The most of the virus infections are asymptomatic and, when symptomatic, can evolve to cure or hemorrhagic clinical conditions and neurological impairment. In this context, the present study objective to detect circulating serotypes of Dengue virus in samples with suggestive symptoms in Parnaíba, Piauí during 2017. Serum samples of 347 were collected from suspected patients infected by DENV with public and private health institution. From the total, viral RNA from 50 samples was extracted and analyzed by Reverse Transcription - Polymerase Chain Reaction (RT-PCR) method, using primers that amplify the NS5 gene region for DENV. Molecular analyzes detected positive samples for DENV, and DENV-2 and DENV-4 were identified as circulating serotypes during 2017 in the Piauí, suggesting a hyperendemic state for Dengue virus and the possibility of the disease's forms to occur. Previous studies in our laboratory have confirmed co-circulation of viral serotypes in the State. In addition, the patients infected by these two viral serotypes presented mild clinical manifestations with no evolution to its severe forms. These results are relevant to support epidemiological surveillance in the monitoring of DENV viral serotypes circulation in the State, aiming to reduce incidence rates and emergence of new epidemics, as well as, to help in the adequate clinical management of the patient.

Financial Support: CNPq, FAPEPI, UFPI and Prefeitura Minicipal de Parnaíba.

Palavras-chaves: Dengue, Arbovirus, Molecular detection, Circulation, Piauí

NEF REGULATES THE HIV PROTEASE ACTIVITY AND IS REQUIRED TO MIR-718 EXPRESSION
Human Immunodeficiency Virus (HIV) is the etiologic agent of AIDS. HIV-1 infections represent a public health problem, reaching 37 million people worldwide. Our group studies aspects of HIV-1 replication, including the role of the viral protein Nef. Although Nef is an accessory protein, it is an important factor to the evolution and establishment of infection. Nef promotes viral infectivity by a still not completely established mechanism. As described by our studies, Nef regulates viral protease activity, thus interfering with viral particle assembly and maturation. By using a infectious clone expressing Nef together with the GFP reporter gene on a bicistronic transcript (HIVbi) we have found a 2-fold increase in levels of Nef protein when compared to the wild type infectious clone (HIV). As the structural poliprotein Gag is the substrate of the viral protease, we investigate whether Gag processing is altered by higher levels of Nef. We transfected each of these clones using cationic liposomes into HEK293-T cells and collected virus in cell supernatant and the cellular lysates. The viral supernatant was further precipitated through a 20% sucrose cushion and lysed. Cellular lysates were processed for Western Blot and we observed a 2-fold decrease in Gag processing rate, calculated by the relationship p24 levels/Gag levels, and lower levels of the structural protein p24 in the HIVbi. Virus lysate also had lower levels of p24 for HIVbi. These results may also explain the 2.5-fold reduction of HIVbi infectivity. As described previously, Nef regulates PTEN/AKT/mTOR signaling pathway by inducing miR-718. miR-718 inhibits PTEN expression leading to AKT activation. To understand the mechanism by which Nef interferes with Gag processing we then conduce a RT-qPCR with the RNA extracted from cell lysates to measure levels of miR-718. We included two conditions with Nef-deleted versions of the clones described above (HIVΔNef and HIVbiΔNef). We did not observe any alteration in miR-718 levels when compared to the control cells to Nef-expressing clones. However, HIVΔNef has shown a clear reduction in miR-718 levels. We also analyse mTOR phosphorylation but there was no difference. Our data show that, although Nef is important for viral infectivity, higher levels of this protein has the potencial to decrease the viral fitness by interfering with Gag processing. The mechanism is not related to miRNA718. This work was finnacial supported by CAPES and CNPq.

Palavras-chaves: Nef, HIV-1, Genetic, VIRAL PROTEASE, miR-718

Antibody production by B lymphocytes is essential to control neurological disease caused by Oropouche virus in murine model

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Resumo

The emergence of diseases caused by arboviruses have impacted on public health, particularly in the last 30 years. The recent Zika virus epidemic in Brazil and the large outbreaks of the Oropouche virus (OROV) in the Amazon region exemplify the impact that arboviruses can have on a population. Oropouche fever, the disease caused by OROV, is characterized by
the onset of high fever, rashes, polyuria and photophobia. In addition, this infection lead to neurological complications in approximately 5% of symptomatic patients. However, the pathogenetic details and roles of adaptive immunity in the protection from virus infection and the development of neurological complications are not yet fully elucidated. Thus, the aim of this project was to evaluate the role of B and T lymphocytes in the control of OROV neuropathogenesis through the antibody production. We determined morbidity, mortality and viral tropism in C57BL/6 Rag1−/− mice, lacking mature B and T lymphocytes, challenged with OROV after sera transfer from wild-type (WT) mice previously infected with OROV. It was observed that the sera from infected WT animals was able to protect Rag1−/− mice in the challenge with OROV, preventing the virus from reaching to the central nervous system and leading to symptoms of neurological disease, as in Rag1−/− mice only infected with OROV who presented paralysis and impairment of balance. Our results suggest that the production of anti-OROV antibodies by B lymphocytes at the beginning of infection is essential to restrict viral tropism to the central nervous system and lead to neurological disease in murine model, a phenomenon potentially associated with the development of neurological diseases in humans.

Financial Support: São Paulo Research Foundation (FAPESP)

Palavras-chaves: Antibody, Neuropathogenesis, Oropouche virus

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**Antiviral activity of silymarin against Mayaro virus and protective effect in virus-induced oxidative stress**

**Autores**
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**Resumo**

*Mayaro virus* (MAYV) is a neglected arbovirus belonging to the family Togaviridae. Its infection leads to Mayaro fever, with clinical manifestations such as fever, myalgia, headache, rash, arthralgia, vomiting, and diarrhea. The most prominent complaint from infected person is the long-lasting arthritis/arthralgia. The treatment for Mayaro fever is mainly symptom-based and there are no vaccines or antiviral drugs currently available, thus, natural products with anti-MAYV activity may provide a potential alternative. Recent evidences suggest that oxidative stress plays an important role in MAYV infection and compounds capable of modulating oxidative stress could represent a novel therapeutic approach in modulating MAYV-associated oxidative cellular damage. Silymarin is a complex extracted of *Silybum marianum*, or milk thistle, and its major active compound is silybin, which has a remarkable biological effect. Its antioxidant and antiviral effects, including its antiviral activity against the *Chikungunya virus* (CHIKV), prompted us to think whether silymarin could also reduce the replication of the MAYV and restore the pro-oxidant/antioxidant balance in the context of MAYV infection, leading to reduced cellular oxidative stress. We assessed the antiviral activity and protective effect of silymarin against oxidative stress in MAYV-infected HepG2 cells. Cytopathic effect inhibition, viral replication, and plaque reduction assays were used to determine the anti-MAYV activity of silymarin. Additionally, we determined
whether silymarin could reduce MAYV-induced oxidative cell damage. Briefly, silymarin exhibited potent antiviral activity against MAYV and reduced MAYV-induced ROS formation and levels of malondialdehyde (MDA) and carbonyl protein, which are biomarkers of oxidative stress. In conclusion, the ability of silymarin to inhibit MAYV replication and attenuate MAYV-induce oxidative stress warrants further investigation of this compound as a novel therapeutic approach to Mayaro fever disease.

**Palavras-chaves:** Antiviral activity, Mayaro virus, Oxidative stress, Sylimarin

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**POTENCIAL ROLE OF MACROPHAGE MIGRATION INHIBITORY FACTOR IN BRAIN DAMAGE INDUCED BY ZIKA VIRUS INFECTION**

**Autores**

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**Resumo**

The Zika virus (ZIKV) is a flavivirus that promotes a mild febrile condition similar to other arboviruses, such as dengue virus. However, ZIKV infection has been also associated with severe neurological complications such as the Guillain-Barré syndrome in adults and congenital fetal malformation in newborns after maternal infection during pregnancy. The mechanisms of virus pathogenesis are largely unknown, but are associated with an increase in the levels of pro-inflammatory mediators. The macrophage migration inhibitory factor (MIF) is a well described cytokine, produced by different cell types, that is able to enhance the inflammatory response, and is therefore associated with the severity of several infectious diseases, including in the increase of endothelial permeability in dengue. Recently it was demonstrated that ZIKV vaginal infection of Rhesus monkey female, promotes an increase of MIF and others inflammatory mediators. Our hypothesis is that, like in dengue, MIF could contribute to the exacerbation of tissue damage induced by ZIKV. Thus, the aim of this study was to investigate whether ZIKV infection promotes an increase in MIF levels, as well as its contribution to viral dissemination and central nervous system damage induced by infection. For this we use a neonatal murine model characterized by our working group where the active replication of ZIKV in the brain promotes neuroinflammation, decreased brain area and histopathological consequences such as necrosis. Three days old C56/BL6 mice were infected subcutaneously with $10^6$ PFU of ZIKV (50 µL) or with cell culture supernatant (control). We evaluated the weight gain, survival and collected various tissues at 12 days post-infection (dpi) to evaluate viral spread and cytokines expression by qPCR. Infected mice gained less weight compared to control and had a death rate of 60%. The viral load in the brain ($10^6$ Eq. PFU/mg) were higher than in liver ($10^3$ Eq. PFU/mg), spleen ($10^4$ Eq. PFU/mg) and muscle ($10^3$ Eq. PFU/mg). The qPCR analysis of the brain showed that MIF expression is higher in the brains of the infected animals in relation to the controls, together with others pro-inflammatory mediators, such as TNF. This result indicates a potential role of MIF in ZIKV pathogenesis. Experiments using MIF deficient mice (MIF<sup>−/−</sup>) are in progress in our laboratory to assess the role in ZIKV dissemination to central nervous system and in neuroinflammation induced by ZIKV infection.

**Financial support:** FAPERJ

**Palavras-chaves:** zika, mif, brain
FUNCTIONAL ANALYSIS OF A FUSION GLYCOPROTEIN GENE FROM A RECENTLY DISCOVERED THOGOTOVIRUS (ORTHOMYXOVIRIDAE) INSERTED INTO THE GENOME OF A BACULOVIRUS

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Resumo
Thogotovirus is a genus of single stranded segmented RNA viruses that, together with Isavirus, quaranjavirus and Influenza A, B, C and D, compose the Orthomyxoviridae family of viruses. These viruses hosts range from arthropods to humans, causing a variety of illnesses. Although classified together in the same family, the thogotoviruses’ envelope fusion proteins share very little similarities with the ones present in the other members of the Orthomyxovirus family, such as Hemaglutinin and Neuraminidase found on influenza viruses. Phylogenetic and structural analyses of these viruses’ glycoproteins indicate that these are most closely related with the glycoproteins found in the (double stranded DNA) Baculoviridae family such as the GP64 glycoprotein of Autographa californica multiple nucleopolyhedrovirus (AcMNPV), that is essential for cell entry and budding. While the direct relationship between these very different viruses is still unclear, this data suggest a common evolutionary ancestor that gave rise to both of these viruses’ envelope fusion proteins. In order to investigate the functional relationship between the glycoproteins found in these two families we have utilized the baculovirus expression system to insert the gene coding for the fusion glycoprotein of a recently discovered bee infecting thogotovirus into a stably transformed plasmid (Bacmid) containing the genome of AcMNPV with a deletion in its GP64 coding gene. The transfection of the whole baculoviral DNA in susceptible cells is enough to cause the production of viral particles, and so we transfected the recombinant viral DNA into insect cells and showed that the virus was able to infect and replicate in transfected insect cells. We are now analyzing the ability of the thogotovirus fusion protein to fully rescue AcMNPV’s the infectious ability serving as a functional analog to its own GP64.

Financial Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Apoio à Pesquisa do Distrito Federal (FAPDF) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)

Palavras-chaves: Baculoviridae, Fusion glycoprotein, Orthomyxoviridae, Thogotovirus

NEW ANTIVIRAL COMPOUNDS FROM PLANTS AGAINST ZIKA VIRUS

Autores
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Instituição

Resumo

NEW ANTIVIRAL COMPOUNDS FROM PLANTS AGAINST ZIKA VIRUS

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Instituição
Zika virus (ZIKV), an arbovirus belonging to the genus Flavivirus of the Flaviviridae family, is transmitted through Aedes mosquito bites, although sexual and post-transfusion transmissions have been reported. Patients experience fever, skin rash, arthralgia, myalgia and conjunctivitis. Furthermore, ZIKV infection has been linked to congenital malformations, including microcephaly and other severe neurological diseases, such as Guillain-Barré syndrome. Plant extracts are considered potential sources for the development of antiviral drugs for treatment of viral diseases. The aim of this study is to identify natural products useful for the development of antiviral drugs against ZIKV and investigate their mechanisms of action. ZIKV was added to monolayers of Vero cells in 96 wells microplate at m.o.i.= 2 followed immediately by solutions of plant extracts to attain the final concentration of 25 µg/mL. Cytotoxicity (CC50) and the antiviral effect (EC50) were analyzed by MTT. Active plant extracts were fractionated using an UPLC (Nexera-Shimadzu) coupled to a HRMS (MaXis-Bruker) the column effluent (400 µL/min) being collected in 96-wells plates (200 µL/well), which were evaluated in the bioassay. Analysis of the HRMS spectra of the active fractions often allowed the identification of the active compounds, which structures were confirmed by comparison with authentic samples or by isolation and analysis of its NMR spectra. Some plant extracts tested showed antiviral activity against ZIKV with EC50 values ranging from 1.1 to 132.8 µg/mL. However, their selectivity index, that is, the ratio between their antiviral (EC50) and their toxicity to Vero cells (CC50) were in the range 0.8-12. Extracts obtained from the plant Hippeastrum glauscescens (Amaryllidaceae) afforded the antiviral alkaloids lycorine and pretazetine, which showed EC50 of 1.1 and 2.4 µM, and SI of 2.7 and 4.9, respectively. To our knowledge, this is the first report on the antiviral activity of these compounds against ZIKV and other flaviviruses. Further experiments will be conducted to elucidate their mechanism of action. Financial support: IRR - Fiocruz, PROEP/P3D CNPq, CAPES, Fapemig

Palavras-chaves: antivirals, Zika virus, plant extracts, screening, new compounds

New insights into Human Papillomavirus type 16 E7 variants from clinical samples

Autores
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Resumo
Cervical cancer is caused by persistent high-risk Human Papillomavirus (HPV) infection. In particular, polymorphisms in viral oncogenes have been associated with an increased risk of malignant progression. Here we investigate the presence of HPV variants from cervical cells in Brazilian Northeastern patients as well we have performed functional analysis of E7 variants into an NF-κB study model. We have accessed mRNA expression levels from cultured cells and performed E7 variants interaction with the IKK complex in silico. Also, we performed RNA secondary structures prediction and codon usage analysis. Experimental groups of transfected cells included three E7 variants (N29S, I76I and T78T) simultaneously, E7 variant with a single polymorphism, or without any SNP (E7 gene reference). NF-κB activity was measured by luminescence. The E7 mRNA expression levels were calculated. Protein interaction was checked as well RNA secondary structures and codon usage. Only the E7 with all variants have dramatically reduced NF-κB pathway activity, while none of the others variants have disturbed NF-κB pathway. N29S mRNA expression was 3-fold higher than E7 reference, while none of the others variants have reached an E7 mRNA expression 1-fold higher than E7 reference.
could affect the IKK complex recognition. Although functional changes due polymorphisms may not necessarily be related to differential expression levels or codon usage events, E7 transcript with all variants exhibits less complex RNA secondary structures, which may result in improved efficiency scanning process during translation. This study provides one of the first evidence that these polymorphisms may be relevant to NF-κB signaling pathway. HPV-16 E7 gene variants in which the three polymorphisms are present may perform a strong suppressive effect on the NF-κB pathway in HPV-infected patients with implications to infection persistence and progression. However, our mRNA evaluation indicates that E7-mediated NF-κB effects resulting from the polymorphisms may not necessarily be related to the differential expression levels of the variants but to differential mRNA scanning efficiency. Our study collaborates with a research effort on HPV infection profiles in the population and provides important data for a better understanding of how variants can be distinguished under its clinical consequences.

Financial Support: FACEPE, CAPES and CNPq

Palavras-chaves: HPV, variants, NF-kB, E7, oncogene

MOLECULAR INVESTIGATION OF FLAVIVIRUS IN COLLECTIONS OF CULICIDAE FROM MINAS GERAIS

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Resumo

The viruses within the genus *Flavivirus* are a great health issue around the world. In Brazil zoonotic arboviruses have been reported, among them Dengue virus, types 1 to 4 (DENV 1-4), St. Louis encephalitis virus (SLEV), Yellow Fever virus (YFV), Zika virus (ZIKV) and West Nile virus (WNV). These viruses are transmitted by several species of mosquitoes (Order Diptera, Family Culicidae, Subfamily Culicinae). The WNV and SLEV are transmitted by mosquitoes of the genera *Culex* and, in humans, they cause acute febril illness and encephalitis. The aim of this study was to investigate the presence of SLEV and WNV in *Culex quinquefasciatus* collected in Belo Horizonte (Minas Gerais), from 2010 to 2014. A total of 12,477 specimens (female) of *Culex quinquefasciatus* were collected using BG-Sentinel Full® traps, in 3 sanitary districts (East, North and Pampulha) of Belo Horizonte, Minas Gerais. The mosquitoes were collected during 4 days/week, during 128 epidemiological weeks (Dec/2010-May/2011, Oct/2011-May/2012, Oct/2012-May2013, and Oct/2013-May2014. The mosquitoes were separated into pools (up to 30 individuals/pool), according to epidemiological week and region of collect. Next, the mosquitoes were macerated and the RNA was extracted using the RNease® mini kit, Qiagen. Total RNAs obtained from 221 pools were then used for flavivirus investigation using a pan-flavivirus RTqPCR and GoTaq® 1-step RT-qPCR System. A total of


42 pools were positive and presenting an amplicon with Tm raging from 82.75 to 84.25. From 42 positive samples, the amplicons were purified and sequenced in both directions by dideoxynucleotide method. Further phylogenetic analyzes indicated that the viruses belonged to Culex flavivirus species. The vector abundance for the whole period of collection is 110.38 mosquitoes/night, which shows the large quantity of Culex sp. present in the studied. The percentage of positive pools is 19.0% and the infection rate obtained for Culex quinquefasciatus infected with Culex flavivirus is 0.012. The vector index is 0.18 infected mosquitoes/night. Although we did not detected zoonotic flavivirus infecting Culex quinquefasciatus in the analyzed pools in Belo Horizonte, the entomological surveillance is of great importance as it allows the detection of zoonotic arboviruses, even before their transmission to vertebrates. This allows preventive measures to be taken, before the onset of an outbreak.

Financial support: FAPEMIG, CAPES, CNPq, UFMG.

Palavras-chaves: Culex quinquefasciatus, entomological surveillance, Flavivirus

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**Isolation of hantavirus in cell culture**

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**Resumo**

Orthohantavirus is a genus in the Hantaviridae family, which are polymorphic agents. It is enveloped with three negative single stranded RNA genome. Hantaviruses are zoonotic and kept in nature in reservoir hosts of the orders Rodentia, Soricomorpha and Chiroptera. There are 8 hantaviruses genotypes recorded in Brazil, 6 of them cause severe human disease and high lethality, among which Araraquara orthohantavirus (ARQV), a genotype of Andes orthohantavirus, occurs in the Southeastern Cerrado (including Ribeirão Preto) and Central Plateau. ARQV stands out for producing higher lethality among all existing hantaviruses and has as host the wild rodent Necromys lasiurus, which transmits to humans by inhalation of contaminated aerosols from their excreta. In this work, we detected the genome of hantavirus in Necromys lasiurus and Desmodus rotundus (bat tissues), both captured in the Northeast of the State of São Paulo. Of these animals, attempts were made to isolate hantavirus from tissue samples of Necromys lasiurus and Desmodus rotundus. In our biosafety level 3+ we inoculated in VERO E6 cells, and viral infection was observed after 96 hours post-infection. Thus, it was possible to isolate hantavirus from the rodent heart lysate supernatant, which was confirmed by the presence of the viral genome of the isolate by RT-PCR of the cell culture supernatant and the viral antigens in the Vero E6 cells by Indirect Immunofluorescence and Western Blot. The isolation process was reproducible, 3 times, for the same tissue of the rodent. These results indicate that this methodology for hantavirus isolation should be tested more often. The isolated Hantavirus, should provide important information about its complete genome, as well as providing several future studies.

Financial support: Conselho Nacional de Desenvolvimento Científico e Tecnológico; Fundação de Amparo à Pesquisa do Estado de São Paulo

Palavras-chaves: Hantavirus, ARQV, Viral isolation

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**FUNGI AS POTENTIAL SOURCE OF ANTIVIRALS AGAINST ZIKA VIRUS**

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Zika virus (ZIKV) disease are generally mild and include fever, rash, conjunctivitis, muscle and joint pain. Infection during pregnancy can cause infants to be born with microcephaly and other congenital malformations. There is no treatment available for ZIKV infection or its associated diseases. Fungal secondary metabolites are a fertile source for the discovery of antiviral compounds that can be used for developing drugs to treat infections caused by ZIKV. Thus, the aim of this study was to identify antiviral substances produced by fungi isolated from different environments. To bioassay the antiviral activity of fungi extract, ZIKV was added at m.o.i= 2 to monolayers of Vero cells in 96 wells microplate, followed immediately by addition of solutions of fungi extracts to attain the final concentration of 25 µg/mL. Cytotoxicity (CC_{50}) and the antiviral effect (EC_{50}) were analyzed by MTT. Active extracts were fractionated using an UPLC (Nexera-Shimadzu) coupled to a HRMS (MaXis-Bruker) the column effluent (400 µL/min) being collected in 96-wells plates (200 µL/well), which were evaluated by the bioassay. Analysis of the HRMS spectra of the active fractions often allowed the identification of the active compounds, which structures were confirmed by comparison with authentic samples or by isolation and analysis of its NMR spectra. We screened more than two-hundred extracts obtained from fungi against ZIKV using the described bioassay. Five fungal isolates, which are in process of taxonomic identification, were active on ZIKV. One fungal extract, isolated from a marine mollusk occurring in Antarctica, presented interesting effective concentration 50 (EC_{50}) values of 34 µg/mL, with cytotoxic concentration (CC_{50}) > 100 µg/mL and selectivity index (SI) >2.9. This extract was fractionated and a single fraction showed protection against ZIKV. This fraction contains a single compound which [M+H]^+ ion presented m/z 421.0797, for which the molecular formula C_{19}H_{17}ClN_{2}O_{7} was calculated. Since there is no natural product with this formula described in the literature, we cultivated the fungus in larger scale to isolate the compound in sufficient amount for its identification by NMR spectroscopy to further determine the structure of the active compound. Our results indicate that fungi extracts can be a promising source of antiviral agents. Financial support: IRR - Fiocruz, PROEP/P3D CNPq, CAPES, Fapemig

Palavras-chaves: antivirals, fungi, Zika virus
Flaviviridae family, which includes other viruses like Dengue and Yellow Fever, that cause epidemics in Brazil. Usually, the infection is characterized by mild and unspecific symptoms; however, the ZIKV infection has been recently associated with the neurological syndrome in newborns and Guillain-Barré syndrome in adults. Because pregnant women are an important target population for immunization against ZIKV, it is important to develop safe recombinant and inactivated vaccines. The ZIKV RNA is translated into a single polyprotein in association with intracellular membranes in eukaryotic cells. The viral envelope contains two structural proteins, prM and E; both interact with the membrane of the endoplasmic reticulum. The heterologous expression of these proteins alone generates virus-like particles (VLPs) that are liberated from the host cells membranes. These particles are structurally similar to virions, but they are devoid of viral RNA and are not infectious. VLPs can be utilized to study protein function, morphogenesis, and structure. Additionally VLPs can be used as vaccines and applied in viral detection kits. In these work, we developed a ZIKV VLP expression platform based on Baculovirus vector in Sf21 cells. We expressed two constructs, one containing prM-E protein and other just the E protein containing the membrane attachment sequences. After 4 days post-infection, the cells were harvested and the expression confirmed by Western-blot using anti-E ZIKV protein antibody. Both constructs were successfully expressed, and the prM-E construct was entirely processed by cellular proteases. The VLPs were purified by continuous sucrose gradient and characterized by electron microscopy. The next steps will focus on optimizing the purification of the insect-derived VLPs and compare its immunogenic properties with mammalian cells ZIKV VLPs and inactivated virions. Financial Support: CAPES, CNPq e FAPERJ.

Palavras-chaves: ZIKV, Virus-like particles (VLPs), Baculovirus expression system, Flavivirivirus, Vaccine

GENETIC CHARACTERIZATION OF CHIKUNGUNYA VIRUS ISOLATES FROM SERGIPE

Autores

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Resumo

Lately, Chikungunya virus (CHIKV) has been the component of Alphavirus genus with the most importance to the human health, infecting thousands of people around the world. CHIKV has three lineages: Asian, West African and East/Central/South African (ECSA). In Brazil, the Asian and ECSA lineages have been found, but in the Northeast, only the ECSA was identified until 2016. In Sergipe, CHIKV has maintained a high number of cases with 332 cases in 2017 which makes it necessary to know the genetic variability of the circulating viruses to understand if the current cases are caused by the genotype already identified or if a new lineage was introduced in the State. The aim of this study was to perform a genetic characterization of 10 CHIKV isolates obtained from human samples from the state of Sergipe. A fragment of 594 bp of the structural protein E1 was amplified using RT-PCR with primers previously described, followed by Sanger sequencing. Low quality regions in the sequences were excluded using the Standen Package, with phred value 30. Sequences and lineages were identified by the BLAST. A phylogenetic tree was generated using the maximum-likelihood method as implemented in MEGA software, with 1000 bootstrap replicates. The number of conserved and variable sites was analyzed in the nucleotide and amino acid sequences. Phylogenetic analysis based on partial
sequences of E1 showed >99% nt similarity among the sequences and all of them was classified as ECSA lineage. The analysis with other Brazilian CHIKV strains revealed 98.9% nt conserved sites and absence of InDel sites. At the variable sites, it was observed an alteration in the site 135 where all the sequences of this study had an A-C transversion that is not present in most of the Brazilian sequences, except for one isolate from Rio de Janeiro. This transversion is responsible for a non-conservative substitution of the lysine for threonine at position 49 in the amino acid sequence. In this study, the phylogenetic analysis showed that the Sergipe viruses were closely related to other Brazilian viruses, which may be explained by the large flow of travel within the country, which accelerates the spread of closely related strains. The data found here corroborate previous studies that identified the ECSA genotype in Sergipe demonstrating that the circulating viruses responsible for the outbreaks in the State belong to a monophyletic group which may indicate that CHIKV is already stabilized by a wild cycle.

Palavras-chaves: arboviruses, chikungunya, genetics, sergipe

PREVALENCE OF IL-10 - 819 C/T (rs1800871) AND -1082 A/G (rs1800896) POLYMORPHISMS IN DENGUE, CONTROL AND ASYMPTOMATIC POPULATION IN NORTHEAST OF BRAZIL.

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Resumo

Interleukin 10 (IL-10) is an anti-inflammatory cytokine encoded by gene located on chromosome 1q31-32. Single nucleotide polymorphisms (SNPs) -819 C/T and -1082 A/G in have been associated with the susceptibility or protection of dengue with warning signs (DCSA) or without warning signs (DSSA). Considering these findings, the present study aimed to investigate the prevalence of in patients with dengue, control and asymptomatic group in –a population of Northeast Brazil, associating them as a susceptibility factor, protection or risk of progression to the most severe disease. SNPs were investigated by Real-Time PCR, in 119 dengue patients, 129 negative controls, and 85 asymptomatic individuals. The data analysis was performed in BioEstat 5.0, adopting pHaploview 4.2. The A/G genotype of the -1082 A/G SNP was associated with susceptibility to dengue, DSSA and DCSA in comparation to asymptomatic patients. Also, G/G genotype was associated with dengue and DCSA susceptibility when compared with asymptomatic patients. The A/G + G/G model was associated with susceptibility to dengue, DSSA and DCSA versus asymptomatic group. The G allele was associated with susceptibility to dengue, DSSA and DCSA in relation to asymptomatic patients. In the haplotypic analysis the C-G haplotype was associated with susceptibility to dengue, DSSA and DCSA versus asymptomatic groups. In addition, the C-A haplotype was also associated with protection against DCSA in asymptomatic group. In the analysis of SNPs and the clinical manifestations of dengue, the presence of the T allele of the SNP -819 C/T showed susceptibility to headache and edema, and protection against chills.. In this study, we showed a relation of A/G, GG genotype and G allele of -1082 G/A of IL-10 with development of symptomatic dengue. Our data showed that SNP -1082 A/G possibly influences the dengue phenotype. A similar scenario was seen when combined with the SNP -819 C/T, which corroborates the evidence in the literature about the additive effect of polymorphisms.
PREVALENCE OF 174 G / C (rs1800795) POLYMORPHISM IN THE IL-6 GENOME IN A DENGUE POPULATION FROM THE NORTH OF PIAÚI STATE

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Resumo

Dengue is the most frequent arboviral disease which affects humans and it is endemic in tropical and subtropical countries. Studies indicate that single nucleotide polymorphisms (SNPs) in genes related to dengue immunity molecules may be involved with risk and / or protection of this disease. This study investigated the prevalence and influence of 174 G / C polymorphism in the IL-6 gene in dengue patients, from 2016 to 2018. Data were obtained by collecting blood samples from patients attended at public health agencies in Parnaíba-PI. Laboratory confirmation of suspected dengue fever cases was done by rapid immunochromatographic tests and molecular methodologies. Genomic DNA was extracted and genotyped for the SNP by using Real-Time PCR. Statistical program BioEstat 5.0 was used to analyze data, with a significance level of p = 0.043, OR= 0.56, p* = 0.06. Our data suggest that GC genotype and C allele are related to protection for dengue cases in this population. They also contribute to a better understanding of Dengue virus pathogenesis and genetic factors related to host in this disease.

Palavras-chaves: Dengue virus, Polymorphism, IL-6

Resistance mutations of NS3 and NS5b in treatment-naïve patients infected with Hepatitis C virus in the State of Santa Catarina

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Resumo

Hepatitis C virus (HCV) infection is a worldwide health problem, according the global hepatitis report from the World Health Organization (WHO), approximately 71 million people have chronic HCV...
infection, and nearly 399,000 people die each year, mostly due to cirrhosis or hepatocellular carcinoma. Nowadays, direct-acting antiviral agents (DAAs) are the main treatment; however, the high level of variability of HCV lead to development of resistance-associated variants (RAVs). Thus, look into the RAVs among infected patients is an important tool for monitoring the efficacy of the therapy. The aim of our study was to investigate the presence of naturally occurring resistance mutations in HCV NS3 and NS5 regions in treatment-naïve patients. Ninety-six anti-HCV positive serum samples from blood donors at Center of Hematology and Hemotherapy of Santa-Catarina State (HEMOSC) were collected retrospectively, and evaluated in this study. HCV subtypes 1a, 1b and 3a were found. We did not observe a significant difference (p=0.6) between the mean viral loads of the genotypes found in this study. The frequency of patients with RAVs in our study was 6.9%. The HCV NS5b sequencing reveled 1 sample with L320F mutation and 6.35% with the polymorphism C316N. The analysis of the NS3 region revealed mutations D168A/G/T (3.45%), S122G (1.15%) and V55A (2.3%). All samples from genotype 3a presented the non-synonymous mutation V170I/V. In conclusion, we have shown that mutation in NS3 and NS5b domains are present in Brazilian isolates from therapy-naïve patients, in this case, blood donors with unknown HCV infection. The presence of these natural polymorphisms could be important for better understanding viral-variant dynamics and predict the response to antiviral therapy.

Palavras-chaves: HCV, NS3 and NS5b mutations, resistance mutations, Santa Catarina

Functional evaluation of Human papillomavirus 31 E6 and E7 variants

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Resumo

Cervical cancer is caused by persistent high-risk Human Papillomavirus (HPV) infection. HPV oncogenicity is strongly associated with viral types and their variants, whose polymorphisms in viral oncogenes are involved in the risk of malignant progression. The presence of polymorphisms can alter biological function leading to clinical consequences. In addition to impairing the control of the cell cycle mediated by pRB and p53, E6 and E7 activities include perturbation of the NF-κB pathway affecting an important anti-viral cellular pathway. Once we already have identified polymorphisms from HPV-31 E6 and E7 positive clinical samples in Northeast Brazil, our current study performed one of the first functional analyzes based on the NF-κB activity of these polymorphisms. HPV-31 E7 gene polymorphisms at positions H23Y, E46Y, K62E and its respective prototype sequence were cloned into pCI vector. Similarly, HPV-31 E6 polymorphisms at positions T64A, K123R, A138V, and its respective prototype sequence were cloned into pCI vector. The plasmids were transfected into HEK-293 cells together with the luciferase reporter gene under NF-κB pathway control. Twenty-four hours post-transfection, cells were TNFα-incubated during six hours. Cell extracts were read by luminescence. The E6 or E7 effect on NF-κB pathway was compared to that obtained by the prototype and the control experiment. We found a difference between polymorphic E6 and the control, but non-significant effect between polymorphic and prototype, or the control. The H23Y, E46Y, K62E E7 variants did not demonstrated repressive activity on the NF-κB pathway as compared to the control culture. These results add important data for the studies on the E7 variability, which could contribute to a better understanding of the HPV diversity and infection.
EXPRESSION AND PURIFICATION OF HEPATITIS E VIRUS pORF2 FRAGMENT FOR IMMUNOLOGICAL AND STRUCTURAL CHARACTERIZATION

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Resumo

Hepatitis E is one of the major public health concerns in developing countries, being responsible for sporadic or epidemic outbreaks of icteric acute hepatitis, that poses higher risk for pregnant which has a high mortality rate (24-27%). The disease is caused by the enterically transmitted hepatitis E virus (HEV). HEV is a non-enveloped, single-stranded, positive RNA virus. It's genome is composed of three open reading frames (ORFs) that includes the ORF2 that is responsible for the expression of the capsid protein (pORF2). pORF2 is composed of 660 residues that are organized as domains, S, M and P. The P-domain is placed at the c-terminal region of the pORF2 that comprises residues from 452 to 660 that are exposed on capsid surface establishing a dimeric conformation that also displays antibody neutralization epitopes that are good target for vaccine development. The aim of this work is to clone, express, purify and solve the structure by Solution-State NMR and Crystallography of the P-domain heterologously expressed in E coli. Here, we will present the results obtained up to the initial structural characterization of the P-domain. The cloning was confirmed by sequencing using Big-dye technology. The protein expression was optimized and confirmed by Western blotting using specific antibody against the P-domain. The protein was soluble and purified by salting- out followed by IEX chromatography. The secondary structural content was obtained by circular dichroism technique and displayed a mixed of beta and alpha structures. The results obtained are in agreement with structure prediction obtained by in silico analysis of the P-domain. Those results are the initial step and suggests that the protein is structured even after the purification steps. Our group have on going experiments to obtain crystals for Crystallography and the expression of isotopically labeled P-domain that will be analyzed by Solution-State NMR is already achieved.

Financial Support: CNPq

Palavras-chaves: Hepatitis E virus, ORF2, P-Domain, Structure
In spite of its significant health impact, the mechanisms of assembly and budding of human respiratory syncytial virus (HRSV) are only partially clear. HRSV envelope glycoproteins traffic through the secretory pathway, but little is known about how the non-glycosylated M and N proteins reach virus assembly sites. In the present study confocal microscopy revealed that HRSV M and N proteins in virus inclusion bodies co-localize with the viral glycoprotein F and giantin, a marker of cis and medial Golgi, suggesting that M and N proteins are already included in the assembly process before the virus reaches the plasma membrane. This was further confirmed by the in situ interaction assay PLA, which demonstrated that HRSV F and N proteins interacts in Golgi compartment. The trans Golgi network marker TGN46 appeared surrounding HRSV inclusion bodies containing proteins N and M, a pattern that was enhanced over time post infection. HRSV N and M proteins co-localized extensively with the early endosome marker sorting nexin-2 (SNX2), which is important for retromer formation and shaping of tubular structures. Moreover, PLA revealed that SNX2 and RSV M protein interact, reinforcing that the of HRSV non-glycosilated protein happens in endosomes. In addition, the Vacuolar Protein Sorting-Associated 26 (VPS26), which is also part of the retromer is localized in HRSV inclusion bodies, indicating that the retromer can participate in the formation of HRSV inclusion bodies or in the traffic of HRSV proteins. Labeling for GLUT1, a well-known transmembrane protein, appeared concentrated in the vicinity of M and N proteins, showing that HRSV inclusion bodies may contain endosome membranes. TGN46 also co-localized with HRSV M and N proteins in HRSV filamentous structures projecting out of the cell surface, furthermore, mass spectrometry of purified HRSV revealed the presence of TGN46 in virions, what gives support the involvement of the trans Golgi in early stages of HRSV budding. Surprisingly, SNX2 localized to HRSV budding filaments, and was detected by mass spectrometry of HRSV purified particles, suggesting that it may play a retromer-independent role in the HRSV budding. The data indicate that HRSV structural proteins M and N are directed to the secretory pathway at early stages of HRSV inclusion body formation in endosomal vesicles.

Palavras-chaves: HRSV , Secretory Pathway, Non-glycosylated Matrix , Non-Glycosylated Nucleoprotein, Sorting Nexin-2 SNX2

HERPES VIRUSES DETECTION IN BATS FROM BUTANTAN PARK IN SÃO PAULO, BRAZIL.

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Resumo

While emerging infectious diseases may spillover from various wildlife species, bats (Order Chiroptera) have been found to be a primary reservoir for numerous recent zoonoses of global concern. Therefore, understanding the spectrum and characteristics of viruses that bats carry may help prevent and control potential emerging bat-borne diseases. Zhengli (2010) lists an identification over 80 different viruses identified in samples from bats, including RNA and DNA viruses. Herpesviruses (HV) are enveloped double-strand DNA viruses that may infect skin, mucosal membranes, lymphatic system and nervous
system of animals, disseminating in various vertebrate hosts, including humans. The *Herpesviridae* has 3 subfamilies: *Alphaherpesvirinae*, *Betaherpesvirinae* and *Gammaherpesvirinae*, all three already found in bats, as well as many herpesviruses that remain unidentified. Oral and rectal swab samples were collected from 47 bats, captured between 2017 and 2018, with mist nets in the Butantan Park, in São Paulo city, Brazil. Swabs were placed in cryotubes containing 500µL of VTM and kept in ultra-freezer (-80°C). DNA extraction was automatically performed with the “MagMAX™ Express” (Applied Biosystems®) using the “MagMAX™-96 Total Nucleic Acid Isolation Kit” according to the manufacturer’s instructions. For an enhanced sensibility, detection was made using two different Nested-PCR protocols, van DeVender (1996) and Chmielewicz et al (2001) and Human Herpesvirus type 1 was used as positive control. Sequencing was performed with Sanger’s technique using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and ABI PRISM 3130XL DNA Sequencer (Applied Biosystems). A rectal swab from a adult male *Sturnira lilium* (E. Geoffroy, 1810) showed to be positive for a herpesvirus still not classified, closely related to the one found in a *Scotophilus kuhlii* Leach, 1821 in southern China in 2015. Therefore, the results of this work are in agreement with the literature since the Herpesvirus found is related to another bat species and further studies will be needed to define its ecological implications and zoonotic potential.

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Palavras-chaves: Herpes, Herpesviridae, Bats

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**ANALYSIS OF THE INTERACTION BETWEEN DENGUE VIRUS NS1 PROTEIN AND HUMAN PLASMINOGEN**

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**Resumo**

**Introduction:** Dengue virus belongs to the family *Flaviviridae* of genus *Flavivirus*. Its genome is made up of a single positive-stranded RNA and is translated into a poliprotein, which is then processed by both host and viral proteases, originating 3 structural proteins and 7 non-structural proteins. Among those, the NS1 glycoprotein stands out, being the only non-structural protein to be secreted. Previously, our group mapped the interaction between NS1 and several hepatic proteins, such as the plasminogen, which is converted into plasmin through the cleavage by its activators uPA and tPA. The main function of plasminogen is to degrade fibrin clots and other components from the extracellular matrix, acting in angiogenesis, cell migration and also complement inhibition, being essential to the homeostasis. Thus, the goal established for this work is the investigation of this interaction, in order to understand its effects in the context of a DENV infection. **Methods and Results:** Firstly, we confirmed the interaction by performing an ELISA using NS1 with both purified plasminogen and plasminogen present in the serum. Later, in a cleavage assay, we incubated plasmin with NS1, collecting samples...
every hour during 4 hours after incubation, and observed, by a western blotting, that NS1 was being cleaved by plasmin. However, when the same experiment was carried out with NS1 present in the serum, the cleavage did not occur, leading us to hypothesize that NS1 was interacting with regulatory proteins of plasminogen. So, we demonstrated the interaction between NS1 and uPA by ELISA, by adsorbing the latter one and adding different concentrations of NS1 on it. At last, BHK-21 cells were cultured and then treated with NS1 for one hour. After that, pre-activated plasmin was added and, by flow citometry, we could verify that plasmin was able to cleave and remove the NS1 attached to the cell membranes. **Conclusion:** Therefore, through these results we could confirm that NS1 interacts not only with plasminogen (both purified and present in the serum), but also with uPA. Besides, we observed that plasmin is able to cleave NS1 in vitro and remove the NS1 attached to cell membranes. We plan to carry out to elucidate its role during viral infection.

Financial support: FAPERJ, CNPq, CAPES E ICGEB

**Palavras-chaves:** Dengue, NS1, Plasminogen

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**EVALUATION OF LECTIN AFFINITY CHROMATOGRAPHY PROCEDURES FOR PURIFICATION OF RABIES VIRUS GLYCOPROTEIN**

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**Resumo**

Rabies is a zoonotic viral disease that causes more than 59,000 human deaths annually all over the world. Its causative pathogen, *Rabies lyssavirus* (RABLV), is a neurotropic virus, consisting of five genes nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and the RNA-dependent RNA polymerase (L). The G protein is responsible for the recognition of specific cell surface receptors and induces the production of neutralizing antibodies (VNA). Since G is a glycoprotein, the main of the present study was to evaluate the lentil lectin chromatography procedures for G protein purification. For this, BHK-21 cells grown in MEM supplemented with 10% FCS in flasks of 150 cm², and were infected with RABLV (CVS strain /DIC 100). After cell infection, a virus titer of 4.85 x 10¹¹ FFU/ml was obtained. *Rabies lyssavirus* was recovered by (NH₄)₂SO₄ precipitation and subsequent centrifugation at 1000 x g for 30 min, following by G solubilized using CHAPS detergent. Subsequently, the solubilized G protein was submitted to antigen-immunoaffinity chromatography (IAC) using lectin from *Lens culinaris* (lentil/Sigma-Aldrich) as stationary phase (CNBr-activated sepharose 4B; GE), according to the manufacture’s protocol, obtaining coupling efficiencies in the range of 80%. The purity and identification of protein obtained was analyzed by 10% SDS-PAGE under non-reducing conditions, ELISA and lectin-binding assay, this last test using the following lectins: *Sambucu nigra*, *Ulex europeaus*, *Lens culinaris*, *Maackia amurensis*, *Concanavalin A*, *W. floribunda*, *B. purpurea*, *L.polyphemus*, *D. stramonium* and *Erytrina cristagali*. As results, we observed that G protein purified revealed a molecular mass of approximately 65 KDa and the presence carbohydrate (sialic acid, fructose, galactose, mannose and fucose), addition was recognized by rabies virus glycoprotein monoclonal antibody (Abcam). In conclusion, the lentil lectin-sepharose procedure described is rapid,
inexpensive and results in the efficient separation and recovery of RABLV glycoprotein. Future studies are necessary to analyses if the G protein purified conserved most of its native properties and conformation. Financial support: Instituto Pasteur/ São Paulo/ Brazil

**Palavras-chaves:** Rabies virus, glycoprotein G, purification, lectin, chromatography

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**A PLANT-BASED PLATFORM FOR THE EXPRESSION OF AN ANIMAL VIRUS DERIVED VLP WITH GENE AND DRUG DELIVERY PROPERTIES**

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**Resumo**

Viral particles can be seen as highly efficient nanomachines, able to assemble around the genome, escape the immune system, attach to susceptible cells and specifically release their cargoes. All these features can be applied to nanotechnology, inspiring innovative nucleic-acid/drug delivery or antigen presenting systems. We explore the use of a nonenveloped insect virus (NuV) as RNA and drug delivery nanosystem. NuV can efficiently penetrate cell membranes, evading the endocytic pathway that often hinders drug or gene delivery. So far, NuV VLPs, which exhibit the same biophysical functional characteristics of the virion, are generated through heterologous expression of the capsid protein in Spodoptera frugiperda cells using a baculovirus vector. Recently, new expression vectors in plants allow the production of heterologous proteins with a high yield in a cost-effective and simplified way. NuV capsid gene was cloned in the Nicotiana benthamiana expression vector pEAQHT that combines a strong promoter for production of the recombinant gene to the expression p19 protein for suppression of the plant RNAi response. Agrobacterium tumefaciens bacteria were transformed with the recombinant vector and used for agroinfiltration of N. benthamiana leaves. After 4 days, the agroinfiltrated leaves were harvested, weighed and crushed in purification buffer. The filtered leaf extract was ultracentrifuged against continuous sucrose gradient for isolation of the particles. Analysis of the material by SDS-PAGE revealed a pure 70 kDa band, the expected molecular weight of NuV capsid protein. Moreover, the plant-derived NuV VLPs showed maturation and lytic properties similar to insect-derived VLPs. Our work confirmed the efficiency of NuV VLP expression in N. benthamiana using the pEAQHT vector and opens the way for other eukaryotic virus like-particles to be produced using the same technology. Support: FAPERJ, CNPq.

**Palavras-chaves:** Nudaurelia capensis omega virus, Nanoparticle, Virus-like particle, Expression in planta, Nicotiana benthamiana

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**IN SILICO STUDIES RELATED TO PORCINE CIRCOVIRUS TYPE 2b (PCV2b) CAPSID ASSEMBLING**

Autóres: Angelo José Magro, Antoniel Augusto Severo Gomes, João Pessoa de Araújo Jr., David Perahia

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Swine breeding has achieved a high development based on genetic improvement, nutrition, management and sanity. However, due to the intensive breeding methods, swine have become more susceptible to a higher number of infectious diseases. Among the most important pathogens that affect the swine world industry is the porcine circovirus 2 (PCV2), a small, icosahedral, non-enveloped virus, ambisense single-stranded circular DNA, composed by 1,767-1,768 nucleotides. This virus is highly resistant to environmental variations and disinfecting agents, endemic worldwide and has been associated to several distinct clinical manifestations related to important economic losses. One of the factors implicated in PCV2 pathogenicity and immune response is the Cap protein, a structural protein codified by ORF2 from PCV2 genome. In addition to these roles, Cap protein is essential for viral replication, since this molecule is the fundamental unity that composes the PCV2 capsid. Therefore, a better comprehension of the Cap protein key interactions for capsid assembling is very interesting for potential biotechnological applications. Our studies involving in silico molecular simulations indicated that certain interactions seem to drive capsid cohesion and, possibly, PCV2 assembling and replication in host cells. These data are crucial for further experimental investigation and, potentially, they could be important for the development of future vaccines and antiviral drugs. Besides, the techniques and procedures developed for this work can be applied for capsid assembling studies of several virus which cause diseases in humans and other animals.

**Financial support:** FAPESP and CAPES

**Palavras-chaves:** porcine circovirus, PCV2b, capsid assembling, molecular simulation
infected with rabies influences viral internalization and viral growth kinetics in neuroblastoma cells, and if the viral load affects mortality in mice after intradermal infection. We noted that high initial viral loads in brains (group II) were unfavourable for increasing viral titers during serial passages in neuroblastoma cells when compared to low initial viral loads in brains (group I). In addition, group I strains showed higher viral growth and enhanced internalization efficiency in neuroblastoma cells than group II strains. However, we observed that the dominant virus subpopulation in group II promoted efficient viral infection in the central nervous system in the new host, providing a selective advantage to the virus. Our data indicate that rabies infection in animal models depends on not only the virus strain but also the amount of virus. This study may serve as a basis for understanding the biologic proprieties of Rabies lyssavirus strains with respect to the effects on viral replication and the impact on pathogenesis, improving virus yields for use in vaccine development. Financial support: Instituto Pasteur/São Paulo/Brazil

Palavras-chaves: Rabies, Replication, Virus production, Viral internalization, Growth kinetics

INHIBITORY EFFECT OF MICROALGAE AND CYANOBACTERIA EXTRACTS ON INFLUENZA VIRUS REPLICATION AND NEURAMINIDASE ACTIVITY

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Autorens


Resumo

The influenza virus can cause seasonal infections with mild to severe symptoms, circulating worldwide, and it can affect people in any age group. Therefore, this infection is a serious public health problem that causes severe illness and death in high risk populations. Every year, 0.5% of the world's population is infected by this pathogen. This percentage can increase up to ten times during pandemics. Influenza vaccination is the most effective way to prevent disease. In addition, anti-influenza drugs are essential for prophylactic and therapeutic interventions. The oseltamivir (OST, a neuraminidase inhibitor) is the primary antiviral used in clinics during outbreaks. However, OST resistant viruses may emerge naturally or due to antiviral pressure, with a prevalence of 1-2% worldwide. Thus, the search for new anti-influenza drugs is extremely important. Currently, several groups have been developing studies describing the biotechnological potential of microalgae and cyanobacteria, including antiviral activity of their extracts. In Brazil, this potential is poorly known and explored. With the aim of increasing the knowledge on this topic, 38 extracts from microalgae and cyanobacteria isolated from marine and freshwater biomes in Brazil were tested against: cellular toxicity; OST-sensitive and resistant influenza replications; and neuraminidase activity. For this purpose, Madin-Darby Canine Kidney (MDCK)-infected cells were treated with 200 µg/mL of each extract. A total of 17 extracts (45%) inhibited influenza A replication, with 7 of them resulting in more than 80% inhibition. Moreover, functional assays performed with viral neuraminidase revealed 2 extracts (from Leptolyngbya sp. and Chlorellaceae) with IC 50 mean

Palavras-chaves: anti-influenza extracts, cyanobacteria, microalgae, neuraminidase inhibition, OST-sensitive and resistant viruses
Anti-apoptosis activity analysis of US-3 and LR bovine alphaherpesvirus 5 genes

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Resumo

Bovine alphaherpesvirus 5 (BoHV-5) is an important agent of meningoencephalitis in cattle. The current geographical distribution of BoHV-5 infection is mainly restricted to South America, especially Brazil. BoHV-5 is genetically and antigenic related to bovine alphaherpesvirus 1 (BoHV-1) (the prototype of alphaherpesviruses). The BoHV-1 genome have most of its genes homologous to BoHV-5 genome. Previous studies have reported that US-3 and LR BoHV-1 genes are anti-apoptotic genes, however no study have been done so far on the function of these same genes in BoHV-5. Thus, the aim of this study was to analyze whether the LR and US-3 BoHV-5 genes have anti-apoptotic activity. The sequences of the US-3 and LR gene of the BoHV-5 strain EVI88 / 95 were amplified by polymerase chain reaction (PCR) and chemically synthesized, respectively. These fragments were then cloned into the transfer vector pFB-PG-H-PA and then used to construct recombinant baculoviruses. The resulting vectors (pFB-PG-H-PA-US-3 / pFB-PG-H-PA-LR) were used for site-specific transposition into the genome of the baculovirus Autographa californica multiple nucleopolyhedrovirus (AcMNPV) inside an Escherichia coli strain (DH10BAC P35del) as a large plasmid (bacmid). The AcMNPV genome inside the DH10BAC P35del has a deletion in the anti-apoptotic baculovirus gene p35. The selected colonies had their DNA extracted (bacmid-pFB-PG-H-PA-US-3 / bacmid-pFB-PG-H-PA-LR) and confirmed by PCR. Next, both recombinant viruses were obtained after transfection of insect cells derived from Spodoptera frugiperda (Sf21) and Trichoplusia ni (Tn5B). After 96 h post-transfection (p.t.) it was possible to observe polyhedra production and absence of cellular apoptosis which indicates that both genes were able to rescue the AcMNPV P35 anti-apoptotic activity. Studies for the quantitative evaluation of BoHV-5 gene expression and caspase activity will still be performed.

Financial support: Fundação de apoio à pesquisa do Distrito Federal (FAPDF)

Palavras-chaves: apoptosis, BoHV-5, LR gene, US-3 gene, Baculovirus

New Isatin-derived Compounds Exibit Antiretroviral Activity Against The Human Immunodeficiency Virus

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Resumo

Even after nearly 40 years of the first AIDS report, there is still no cure for this pandemic disease. The highly active antiretroviral therapy is currently the most effective AIDS therapy available. However, the emergence of drug-resistant strains in HIV individuals on treatment, and the consequent therapeutic failure, makes necessary the constantly development new therapeutic options, presenting better profiles of activity and toxicity. To this end, our group evaluated the efficacy and toxicity of 24 compounds as new antiretroviral candidates, designed by Professor Núbia Boechat group from Laboratório de Síntese 1 Farmanguinhos/Fiocruz. Using molecular hybridization as a tool of Medicinal Chemistry in the design of new drugs, the compounds were elaborated based on indolin-2,3-dione core of the Isatin, a highly synthetic synthetic molecule that has been successfully used by this group to obtain new molecules with antiviral activity against HIV-1. In order to obtain these new candidates, different groups of acetylenes were inserted into this Isatin derivative with the objective of investigating the contributions of the acetylenes in establishing hydrophobic interactions between the new compounds and HIV-1 reverse transcriptase, as similar as previously observed as the mechanism of action of Efavirenz (EFV). First, we performed cytotoxicity assays to determine the maximum non-toxic concentration (MNTC) of each compound in the HEK293T and MT4 cell lines. To test the antiviral potential of each compound, two different infectivity assays were performed: one to assess the inhibitory effect of viral replication in the late stages of HIV-1 life cycle, and another to assess the antiviral effect in the complete viral life cycle. The preliminary results obtained showed that, compared to the untreated control, compounds PP93/16A, PP23/16, PP84/16 and JN8.094/14PA, showed inhibitory capacity for viral replication (about 50% to 98% inhibition), either acting directly on reverse transcriptase or interfering with the assembly of viral particles, similar to what has already been described for EFV. PMMA026/16F5-25, PMMA027/16F5-24, PMMA031/16F141-169 and PMMA049-FIM also presented inhibitory capacity for viral replication (about 60% to 85% inhibition) in lymphocyte cell lines (MT4), by inhibiting directly TR activity, but without interfering on the assembly of the new particles.

Palavras-chaves: HIV-1, Antiretroviral, Isatin, compound, therapy

Evidence of natural Zika virus infection in neotropical non-human primates in Brazil

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Resumo

Non-human primates (NHPs) participate actively in the cycle maintenance of some arboviruses, including yellow fever virus (YFV). In Africa, Old World Primates are involved in the maintenance of sylvatic circulation of ZIKV. However, in Brazil, the hosts for the sylvatic cycle remain unknown. We hypothesized that wild common marmosets (Callithrix sp.) might play a role in urban ZIKV dynamics; thus, we performed ZIKV investigation using molecular identification, histopathological and
immunohistochemically analysis in 82 free living marmosets from different urban and periurban of São José do Rio Preto (SJRP-SP) and from cities of Minas Gerais States. Together, we collected *Ae. aegypti* and *Ae. albopictus* mosquitoes in the same neighborhood and same time were some NHPs were found in SJRP-SP. Additionally, we inoculated four *C. penicillata* with a ZIKV isolate from a human patient and re-infected them eight months after the first inoculation. We observed a total of 32 ZIKV positive NHP and sequences obtained from 4 NHPs were phylogenetically related to the newly emerged American lineage of ZIKV. We found a cluster between *Ae. aegypti* mosquitoes and NHP positive for ZIKV. Mosquitoes and NHPs were negative to YFV. The experimentally infected NHPs demonstrated that marmosets had a sustained viremia and did not develop viremia after second infection suggesting sterilizing immunity. The natural and experimental infection of NHPs with ZIKV, support the hypothesis that NHPs may be a vertebrate host in the maintenance of ZIKV transmission/circulation in urban tropical settings. The direct detection of the ZIKV genome in free-living NHPs that are in close contact with humans in urban areas is extremely alarming, as it is an indication that these animals are susceptible to virus infection and develop a similar pathogenicity to that demonstrated in humans. The co-occurrence of ZIKV in *Ae. aegypti* and NHPs suggested the possible transmission between these urban vectors and primates, which could contribute to transmission in the area. Further studies are needed to understand the role they may play in maintaining the urban cycle of the ZIKV and how they may be a conduit in establishing an enzootic transmission cycle in tropical Latin America.

Financial Support: FAPESP, CNPq, INCT-Dengue, CAPES, NIH

**Palavras-chaves:** ZIKA, non-human primates, Brazil

THE ROLE OF RAB27A ON OROPOUCHE VIRUS REPLICATION

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**Resumo**

Oropouche virus (OROV) is an arbovirus that belongs to the *Peribunyaviridae* family and *Orthobunyavirus* genus. It is the etiological agent of Oropouche fever, an illness that has a great relevance in public health. Large outbreaks of Oropouche fever have been reported in Central and South America since its first isolation, in 1955, demonstrating its high emerging potential. Despite its relevance, most of the knowledge related to Orthobunyaviruses comes from studies with the Bunyamwera virus, and specific aspects of OROV replication cycle are not well elucidated. Due to the ability of Orthobunyaviruses to subvert cellular endomembrane machinery to efficiently produce viral progeny, we hypothesized that OROV could manipulate cell secretion system and hijack host proteins, such as GTPase Rab27a, to produce new viral particles. Based on this hypothesis, this work aimed to assess if Rab27a protein was involved in OROV replication cycle. To verify the subcellular distribution of Rab27a along OROV infection, monolayers of HeLa cells were infected with OROV at an MOI = 1, fixed at different times post-infection, and subjected to indirect immunofluorescence assay. After 24h p.i., confocal microscopy analyzes revealed a partial colocalization between Rab27a and viral proteins at cell periphery. In order to test if this GTPase had a role on virus replication cycle, the expression of Rab27a was depleted by interference RNA in HeLa cells prior to infection. Indeed, there was a small reduction on viral production upon knock-down of Rab27a when compared to control cells. Therefore, our data suggests that Rab27a has a role on virus replication cycle and the release of viral progeny. Further experiments should be performed in order to confirm this hypothesis and stablish the specific function of Rab27a regarding OROV cycle.
ZIKA VIRUS INHIBITS INTERFERON TYPE I RESPONSE IN INFECTED HUMAN BRAIN MICROVASCULAR ENDOTHELIAL CELLS

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Resumo

Zika virus (ZIKV) infection is associated with neurological manifestations and the virus was detected in the brain of infected patients, indicating that it crosses the blood-brain barrier (BBB). Activation of IFN I response is essential for the ZIKV control, as evidenced in IFNAR knockout mice (IFNAR -/-). On the other hand, it was demonstrated that ZIKV replication inhibits IFN response in certain cell types. Here, we evaluated whether ZIKV induced the production of IFN I and III in human brain microvascular endothelial cells (HBMECs) as an in vitro BBB model and if viral replication would counteract the IFN-mediated antiviral response in this cell type. We observed that HBMEC infected with African or Asian ZIKV strains (ZIKV_MR766 and ZIKV_PE243) presented increased expression of IFN-β, IFN-λ mRNA and of pIκB-3. In order to investigate IFN-mediated response, we used a reporter HBMEC, stably transfected with a plasmid containing a luciferase gene controlled by an ISG promoter (lucHBMEC). Cells were infected with ZIKV_MR766 or ZIKV_PE243, in the presence or absence of IFN-β. Experiments performed with lucHBMEC demonstrated a higher luciferase activity when the cells were infected with ZIKV when compared to uninfected cells, confirming that virus infection induced IFN response in this cell type. However, exogenous IFN mediated response was diminished in ZIKV-infected cells, indicating that virus replication blocked IFN-mediated signaling. Accordingly, IFN mediated pSTAT1 was decreased in ZIKV-infected cells, which may be a mechanism associated to IFN response inhibition. Addition of IFN-β to the cultures, did not affect virus replication, nor cell viability, corroborating that ZIKV escapes from this response. On the other hand, when the cells were treated with IFN-β 24 hours prior to ZIKV infection we observed a decrease in viral replication, indicating that uninfected cells may become resistant to infection upon IFN-β treatment. Those 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, controlling virus dissemination and vascular lesion.

Instituições de fomento: CAPES, CNPq e FAPERJ

Palavras-chaves: Blood brain barrier, Endothelial cells, HBMECs, Interferon, ZIKV
ANTIVIRAL EFFECT OF THE SEAWEED Osmundaria obtusiloba AGAINST THE ZIKA VIRUS

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Resumo

Zika Virus (ZIKV), a mosquito-borne member of the family Flaviviridae, is a human pathogen of global significance. Recently, ZIKV, has become a public health problem with increases in numbers of cases and a strong association between ZIKV outbreaks and the spread of cases of Guillain-Barré Syndrome and microcephaly. In this study, we evaluated extracts of the seaweed Osmundaria obtusiloba (O. obtusiloba) (native to the Brazilian coast) against ZIKV using Vero cells. The seaweed extract tested inhibited ZIKV replication in a dose-dependent manner at low concentrations with EC₅₀ values of 1.82 µg/mL and a selective index (SI) of 288. Other results showed that this extract had significant virucidal effects. In addition, when the extract and Ribavirin were used concomitantly there was a significant synergistic effect. Our promising results suggest that extracts of O. obtusiloba are excellent candidates for further studies, and that marine algae are potentially important sources for the development of novel anti-ZIKV agents.

Financial support: CNPq, CAPES, FAPERJ, UFF (PROPRI)

Palavras-chaves: ANTIVIRAL, ZIKA VIRUS, Osmundaria obtusiloba

A novel method to estimate the quasispecies complexity of RNA viruses based on information theory.

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Resumo

RNA viruses replicate at high error rates generating a cloud of mutant genomic sequences commonly referred to as quasispecies. Accessing and quantifying the intrahost genetic diversity is crucial to the study of viral quasispecies. The normalized Shannon entropy (Sn) is a widely used estimate of intrahost genetic complexity, varying from 0 to 1. In this approach, the entropy contained in the quasispecies distribution is calculated by using the frequency of each haplotype (i.e. genomic variant) as probabilities, where the maximum diversity is reached when all haplotypes are equiprobable. Considering the high error rate of the RNA polymerase, this estimate will, theoretically, tend to 1 when analyzing large fragments or full-length haplotypes since every sequence will be unique by at least one single mutation. Hence, Sn is not optimal to estimate and compare the complexity of distinct genomic regions with different sizes. Herein, we propose a novel estimate of intrahost genetic complexity of RNA viruses. In our approach, designated Hn, each single nucleotide polymorphism (SNP) is analyzed independently. Hn is normalized by the maximum entropy of the sequence, and also varies from 0 to 1. This estimate has the advantage of taking into consideration both the proportion of polymorphic sites and the frequency of each SNP. As a proof of concept, we calculated Sn and Hn using varying fragment lengths for 9 public
datasets of hepatitis virus B (HVB), a DNA virus that replicates via a RNA intermediate. Each dataset is derived from a different patient infected with HVB, and they are composed of ~20 full-length haplotypes sequenced by the Sanger method each. As expected, the correlation between fragment length and quasispecies complexity was higher for Sn (mean $R^2 = 0.51$) than for Hn (mean $R^2 = 0.23$), and the correlation coefficient $R$ for Sn was always positive, whereas it was negative for Hn in 6 datasets. Additionally, Hn varied according to dataset, there is, some patients exhibited more complex HVB quasispecies ($Hn \sim 0.02 - 0.027$ for all ORFs) whereas a few patients showed a less complex HVB intrahost population ($Hn \sim 0.008 - 0.01$ for all ORFs). Sn, on the other hand, was always higher for larger ORFs. Our analyses confirm the robustness of the Hn estimate in face of varying sequence sizes. Also, the ease of implementation of Hn into quasispecies analysis pipelines make it a useful and practical estimation of quasispecies complexity.

Palavras-chaves: bioinformatics, information theory, quasispecies

Molecules of innate immune response against Zika virus

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Resumo

Our objective was to characterize biologically two Brazilian strains of ZIKV, and compare the cellular immune response triggered by them, using an African isolate adapted to mice as control. The kinetics of multiplication of both Brazilian isolates was assessed in Vero and C6/36 cells infected with M.O.I. 0.1 or 0.01. Irrespective of the used M.O.I., PE243 strain attained higher titers in VERO cells when compared to SPH strain. In addition, although SPH displayed a delayed kinetics in C6/36 cells infected with 0.1 M.O.I, peaking at day 7, the viral titers attained in C6/36 cells were higher than those found in cells infected with PE243. In contrast, we found no differences when C6/36 cells were infected with 0.01 M.O.I of either isolate. We also performed transmission electron microscopy (TEM) on Vero and C6 / 36 cells infected with 0.1 M.O.I. of both Brazilian isolates. As already reported for other flaviviruses, the ZIKV forms vesicles called vesicular replication compartments. In TEM images we observed viral particles from both isolates within vesicles, measuring about 50-100 nm in diameter. The cellular immune response was assessed from CHO cells transfected with TLR2 or TLR4 when infected with ZIKV and the activation of these TLRs was observed by flow cytometry. Additionally, intraperitoneal macrophages of C57BL/6 mice and KG-1 cells were infected with both isolates, 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 and low viral multiplication occurred in mouse intraperitoneal macrophages. It has also been shown that the two ZIKV isolates trigger completely different immune responses. The PE243 triggered a stronger immune response, with nitric oxide production, increased cytokine production and activation of TLR2, whereas SPH produced no nitric oxide and lower cytokines levels, without the activation of TLR2. C57BL/6 mice were infected by intracranial route with 400 p.f.u. of PE243, SPH, and MR766. The mice infected with MR766 lost weight, presented conjunctivitis, paralysis and died between 7 and 10 d.p.i. The mice infected with Brazilian isolates don’t die nor presented signs, but the mice infected
with PE243 gain less weight compared to mock.

**Palavras-chaves:** Zika virus, innate immune response, molecules, cytokines

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**POTENTIATED ACTION OF A FLAVONOID DIMER AGAINST THE Mayaro virus**

**Autores**
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**Resumo**

The Mayaro fever, caused by *Mayaro virus* (MAYV), is a sublethal disease with symptoms that are confused to Dengue, Chikungunya, Zika and other arboviruses, exception is made to symptoms of arthritis and persistent polyarthralgias. It has had for decades limited to South America in wild mammals or riverine humans. However, Mayaro fever outbreaks in metropolises have been reported, and its potential to be transmitted by the *Aedes aegypti* put as an emerging virus. Yet, in addition to the lack of an efficient diagnostic system, there is no therapy or vaccine available to this virus. In previous studies by our research group, we found a flavonoid (EPQ) with promising anti-MAYV activity target on the capsid protein (pC) and due to reports in the literature that flavonoid dimers and oligomers have been shown to be more active, so we evaluated *in vitro* the antiviral activity of a heterodimer of flavonoid (EPQ-MEP) isolated from Celastraceae against Mayaro. In a preliminary assay, neither the concentration of 1000 µg/mL of EPQ-MEP was able to affect the viability of Vero cells (ATCC CCL-81). In the global antiviral activity assay, using moi 0.1, the effective concentration of 50% of infected cells (EC₅₀) by the MAYV was 23.15 ± 2.11 µg/mL, being three times more active than the monomer evaluated previously (EPQ). As recommended by the literature, the selectivity index (SI) was greater than 10 and EPQ-MEP can be considered safe, once there is a significant window between the toxic and effective dose. When increasing the multiplicity of infection (moi) to 1, 5 and 10, the EC₅₀ values were 35.34 ± 4.11 µg/mL, 60.35 ± 3.39 µg/mL and 89.59 ± 7.59 µg/mL, respectively. In this way, as was expected, as the number of viral particles increased, the greater the concentration of EPQ-MEP required inhibiting MAYV infection. However, for a 100x higher moi, only a 4x higher concentration of EPQ-MEP was required. In addition, regardless of the moi used, EC₉₀ (effective concentration for 90% of infected cells) was able to reduce viral infection by 7 log units, making it almost null. Thus, this study showed how flavonoid dimers have their action potentiated when compared to their monomer and that EPQ-MEP is very promising in the development of drugs against the MAYV and other arboviruses.

**Palavras-chaves:** Alphavirus, Antiviral, Anti-MAYV, Celastraceae

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**STUDY OF ANTIVIRAL RESISTANCE EMERGENCE IN A MURINE MODEL OF IMMUNOMODULATION DURING INFLUENZA A INFECTION**

**Autores**
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Resumo

Influenza virus infection is a major cause of worldwide morbidity and mortality, affecting thousands of people annually. Severe cases are treated with viral Neuraminidase inhibitors like Tamiflu. However, high genetic variation could lead to emergence of antiviral resistant strains, limiting the treatment effectiveness. Antiviral-resistant viruses may arise spontaneously or by inappropriate antiviral use. Our overall objective was to establish a murine model to study the emergence of influenza A virus resistant to Tamiflu and to investigate the effect of an immunosuppressive treatment with Dexamethasone in combination with a subtherapeutic dose of Tamiflu on immunopathology and resistance emergence. C57/BL6 mice infected with Influenza A/PR/8/34 H1N1 (PR8) were treated with Tamiflu in different doses - 0.1, 1 or 10mg/kg, or vehicle - after two days of infection, and were monitored for weight loss and lethality for 21 days. Alternatively, mice were euthanized after 7 days of infection to perform bronchoalveolar lavage (BAL), viral isolation, titration and oseltamivir resistance test by Neuraminidase activity assay (NA-Star). In addition, we evaluated leukocyte recruitment, lung injury and proinflammatory cytokine production. In a new assay, infected mice were treated with dexamethasone before infection or 2 days after infection, alone or in combination with Tamiflu (1mg/kg). After 7 or 10 days of infection, they were euthanized and the same analyzes were performed. Treatment with the 10mg/kg of Tamiflu, but not the other doses, reduced lethality, weight loss and viral titers in the lungs compared to vehicle. No reduced susceptibility to Oseltamivir were found in different treatments when compared vehicle or PR8. 10mg/kg of Tamiflu reduced total leukocytes, neutrophils in BAL and lung and IFN-γ in BAL. The dose of 1mg/kg partially reduced lethality rates and inflammation. Dexamethasone induced immunosuppression. Only the group post-treated with dexamethasone plus Tamiflu were protected from lethality, weight loss and neutrophil numbers in BAL compared to vehicle group. Viruses isolated from all groups at day 7 did not show reduced susceptibility to oseltamivir. After ten days of infection, no virus were isolated. Suboptimal doses of Tamiflu did not lead to increased antiviral resistance or viral persistence but prevented inflammatory parameters induced by the infection, especially in combination with Dexamethasone.

Palavras-chaves: Antiviral, Influenza A, Inflammation, Resistance

INHIBITION BY MARINE ALGAE OF CHIKUNGUNYA VIRUS ISOLATED FROM PATIENTS IN A RECENT DISEASE OUTBREAK IN RIO DE JANEIRO

Autores

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**Resumo**

Chikungunya virus (CHIKV) infection is one of the most challenging re-emergent diseases caused by a virus and with no specific anti-viral treatment it has now become a major public health concern. In this investigation, 25 blood samples were collected from patients with characteristic symptoms and submitted to a virus isolation protocol, of which 3 isolates were possible to isolate CHIKV. Samples were evaluated by sequencing for the characterization of the strain and its homology to viruses circulating in Brazil during the outbreak. These viruses were used for the development of antiviral assays. After initial analysis, the inhibitory effects of seaweed extracts on CHIKV replication were studied. The marine species of algae tested were, Bryothamnion triquetrum, Caulerpa racemosa, Laurencia dendroidea, Osmundaria obtusiloba, Ulva fasciata and Kappaphycus alvarezii all found in different countries including Brazil. The results revealed high levels of CHIKV inhibition including by extracts of algae with inhibition values of 1.25ug / mL for O. obtusiloba algae with a selectivity index of 420 which shows very promising. Viral inhibition was dependent upon the time of addition of extract of O. obtusiloba to the infected cells with the optimal inhibition occurring up to 16 h after infection. These results indicate that the algal extracts may be promising novel candidates for the development of therapeutic agents against CHIKV infections.

**Financial support:** CNPq, CAPES, FAPERJ, UFF (PROPRI)

**Palavras-chaves:** antivirais, CHIKUNGUNYA, MARINE ALGAE

**Evaluation of Reactive Oxygen Species Production and Oxidative Stress in U-87MG Cells Infected with Zika Virus**

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**Resumo**

Zika virus (ZIKV) is a member of Spondweni serocomplex of the Flavivirus genus and the Flaviviridae family. This virus was first isolated in the Zika forest in 1947, Uganda, from a Rhesus monkey. ZIKV is an arbovirus which is transmitted by mosquitoes from Aedes genus, mainly Aedes aegypti and Aedes albopictus. In 2015, the first cases of ZIKV infection in the American continent, especially in Brazil, were reported. Zika disease has characterized by fever, headache, joint pain or rash and conjunctivitis. The 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 defense system. Since 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, we investigated whether ZIKV infection causes this event in U-87MG human glioma cells. In this work, to better understand some mechanisms that are operational in host cells following exposure to ZIKV, we monitored ROS production and two oxidative stress biomarkers – malondialdehyde (MDA) and Protein carbonil – at different time points after infection. ROS production increased in infected cells in times 15 and 24 hours post infection (hpi). Additionally, ZIKV infection of U-87MG cells resulted a significant increase in the MDA and protein carbonil levels at 15 and 24 hpi. Since MDA is a by-product of lipid peroxidation and protein carbonil is an oxidative modification in proteins marker, we believe that oxidative stress occurred during ZIKV infection. Considering the results
presented in this work, we can infer that ZIKV infections induce oxidative stress and this event may be important for its pathogenesis. Although, we still intend to perform more experiments to better understand the relationship between ZIKV pathogenesis and oxidative stress.

**Palavras-chaves:** Oxidative Stress, Reactive Oxygen Species, U-87MG cells, Zika virus

**IN VITRO STUDIES ON THE INHIBITION OF REPLICATION OF ZIKA AND CHIKUNGUNYA VIRUSES BY DOLASTANE ISOLATED FROM SEAWEED Canistrocarpus cervicornis**

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**Resumo**

An increasing number of reports of Zika virus (ZIKV) and chikungunya virus infections are not associated with specific antiviral therapy or vaccines, showing the importance of searching for effective therapy. Studies with the marine brown alga *Canistrocarpus cervicornis* showed antiviral potential. Hence, the aim of this work was to evaluate the anti-ZIKV and anti-CHIKV activity of a marine dolastane isolated from brown alga *C. cervicornis* and its crude extract. VERO cells were used to antiviral assays, submitted to ZIKV and CHIKV, and treated with different concentrations *C. cervicornis* extract or dolastane. The inhibitory effect was evaluated by the inhibition of the viral plaques. In virucidal evaluation a viral suspension was submitted to the compounds for 2 hours and later added to the Vero cells; the inhibitory effect was determined after 48 hours. In Time of drug addition (TOA) test cells were treated at different times up to 24 hours post infection. The synergistic effect infected cells were submitted to sub doses of the compounds associated with Ribavirin and the synergistic effect was evaluated at 48 to 72h after treatment. In the antiviral activity we observed that the crude extract of *C. cervicornis* showed an inhibitory potential of both ZIKV and CHIKV with EC50 of 3.3μg/mL for ZIKA and 3.1 μg/mL for CHIKV. However, the isolated compound showed a much more significant and promising EC50 inhibitory effect of 0.95μM for ZIKA and 1.3μM for CHIKV; this was more efficient when compared to Ribavirin, which was used as the control. When evaluating the virucidal activity we observed that the dolastane was very efficient on the CHIKV, being able to inhibit around 90% of the viral infectivity in the treatment with 10μM of the compound. For the ZIKV the effects were somewhat lower, although interesting, at around 64% in this same concentration. In TOA we observed that both the extract and the dolastane were able to inhibit the replication of ZIKV and CHIKV at different times of addition post infection, remaining efficient even if added after 16 hours post treatment, but declining soon after. A synergistic effect using sub doses of the extract and isolates was associated with Ribavirin, inhibiting above 80% replication even at the lowest concentrations. These studies suggest that said compounds may be potentially studied for use in the prevention and treatment of ZIKV and CHIKV infections.

**Financial support:** CNPq, CAPES, FAPERJ, UFF (PROPP1)

**Palavras-chaves:** ANTIVIRAL, CHIKUNGUNYA VIRUS, ZIKA VIRUS, MARINE ALGAE
SINGLE-CELL IDENTIFICATION OF OROPOUCHE VIRUS INFECTION BY RNA PRIMEFLOW ASSAY

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Resumo

Oropouche virus (OROV) is an Amazon arbovirus with potential to cause outbreaks in others regions. In addition to the usual arbovirus disease symptoms, this virus is able to cross the blood-brain barrier, leading to infection of the central nervous system. However, the mechanisms associated with neuroinvasion by OROV are not fully understood. Here we present a recent developed assay that can be used to detected productive and persistently infected cells, allowing us to identify possible trojan horse cells. This method, named RNA primeflow, uses in situ hybridization and branched DNA technology, for genome and antigenome identification by flow cytometry. To apply this methodology for OROV, specific complementary probe sets for the virus genome and antigenome, based on the viral L segment sequence of strain BeAn19991 available on GenBank (KP052850.1), were designed. Vero E6 cells were infected with OROV, in a MOI of 1, for 24 hours. The cells were harvested, washed with PBS enriched with 5% Fetal Bovine Serum, fixed with 1X fixation buffer, permeabilized, and submitted to hybridization with the probes. The following steps were performed for target probe detection: preamplification and amplification of the signal, according to the manufacturer's standards, for later acquisition in a flow cytometer. In addition, there were a control group of non-infected cells. Using this technology, we could demonstrate a new and innovative way to track OROV infection in single-cells events through the identification of both viral genome and antigenome. It opens the possibilities of application in more complex experiments, such as the identification of specific cells which are susceptible and permissive to OROV infection, previously targeted with cells surface or intracellular markers. It also helps to amplify our means to study OROV, and other virus, where available tools are still limited.

Palavras-chaves: Oropouche virus, RNA PrimeFlow, flow cytometry

POXVIRUS-HOST INTERACTIONS: THE ACTIVATION OF COMPONENTS OF THE HOST’S UNFOLDED PROTEIN RESPONSES (UPR) DURING INFECTIONS BY THE VACCINIA VIRUS STRAINS GUARANI P1 AND PASSATEMPO

Karine Lima Lourenço 1
Vaccinia virus (VACV) is a member of Poxviridae family and its replicative success is dependent on its ability to evade antiviral responses by the host. Poxviruses multiplication and maturation are closely associated to the endoplasmic reticulum (ER) and their membranes. The RE is able to respond to perturbations through the unfolded protein response (UPR) pathway. This work aims to investigate the effects of infections by zoonotic VACV Brazilian strains, Guarani P1 virus (GP1V) and Passatempo virus (PSTV), on the activation of UPR pathway sensors in comparison to the VACV Western Reserve (WR) prototypical strain. The importance of ATF6 signaling to the GP1V, PSTV and WR replication cycles was determined by plaque phenotype assays and one-step / multi-step growth curves using wild-type (WT) and knockout (KO) mouse-embryo fibroblasts (MEFs) for ATF6. Plaques formed after all viruses replication in ATF6-KO MEFs were smaller when compared to MEF-WT. Nonetheless, virus yield in WT or KO cells were similar for WR and GP1V, whereas PSTV was shown to be less productive in KO cells. Activation of ATF6 during virus infection was confirmed by gene reporter assays. We also evaluated the activation and importance of the XBP1 sensor during virus infections. Treatment of BSC-40 infected cells with the XBP1 inhibitor Kira6 (that disrupts the kinase domain) resulted in smaller plaques for all tested VACVs when compared to non-treated cells. On the other hand, disruption of the XBP-1 RNAse domain by the 4µ8C inhibitor caused no alteration in plaque sizes for any of the viruses. In summary, the ATF6 sensor appears to be important for VACCINIA VIRUS replication and so is the kinase domain of the XBP1 sensor. In contrast, the XBP-1 RNAse domain seemed to be unimportant for virus replication.

Financial Support: CAPES, FAPEMIG, CNPq.

Palavras-chaves: GP1V, PSTV, UPR, VACCINIA VIRUS, WR
β2-adrenergic receptor participates in controlling immune response to Influenza A virus infection in the murine model.

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Resumo

The sympathetic nervous system and the immune system are two integrative systems that work together to detect threats, provide host defense, and to maintain/restore homeostasis. In Influenza A infections (IAV), that is a leading cause of morbidity and mortality worldwide, the immune responses triggered has being explored in many valorous studies. However, its intersection with the nervous system did not receive the same attention. Hence, in the presente work, we investigated the relevance and integration of adrenergic signaling through the β2 adrenergic receptor (B2R) and the immune response to infection by Influenza A Virus. To this goal we infected male B2KO mice intranasally with sublethal IAV dose (250 PFU). 6 days post infection (d.p.i.) we analysed the respiratory parameters of infected mice. They were euthanized, bronchoalveolar lavage fluid (BALF) were collected and leukocytes counted. In another set of experiments, B2KO mice were infected with the same dose (250 PFU) and lethality and weight loss were monitored for 21 days. In all analyses male C57Bl6 mice were used as controls. We could observe that B2KO mice displayed increased susceptibility to IAV infection, accompanied by loss of lung complaciance and larger pulmonary dysfunction when compare to WT mice. Although, no diference in viral load has being observed in the lung of infected animals. The absence of B2R led to a increased recruitment of lymphocytes and reducti on of neutrophils in the airways in sixth d.p.i.. All together, it led to a reduction in lung integrity, translate into more protein in BALF. In summary, our preliminary findings strongly suggest that B2Ri is not only critical for pulmonary mechanics, but also participates in the cellular recruitment process of during the inflammatory response to IAV. Financial Support: CAPES, CNPq, FAPEMIG e INCTV.

Palavras-chaves: adrenergic receptor, Influenza, β2-adrenergic receptor, immune response, murine model
dispersion routes and demographic history of CRF31_BC, this study analyzed all HIV-1 CRF31_BC and Brazilian BC mosaic publicly available sequences. Bayesian phylogeographic and phylodynamic model approaches were used to reconstruct the spatiotemporal and demographic history of 95 sequences identified as CRF31_BC-like by using the Bayesian Skyline, GTR + G4 + I, uncorrelated relaxed molecular clock models and asymmetric transition model with BSSVS. Porto Alegre was estimated to be the origin and center of the dispersion of the CRF31_BC for most of the analyzed locations. However, some viral transitions independent from Porto Alegre were observed in other cities from the Rio Grande do Sul state and also in other Brazilian states. It was also observed that CRF31_BC epidemic grew exponentially in the first 7-8 years, followed by a stabilization. The estimated CRF31_BC epidemic growth rate was similar to subtype C and B in Brazil. Our findings suggest that Porto Alegre, in addition to being the place of origin of the recombinant form CRF31_BC, played an important role in the dissemination of this new HIV-1 variant to other locations in Brazil. However it was also possible to observe that CRF31_BC circulates in some urban centers independently to new introductions from Porto Alegre or Rio Grande do Sul state. Our findings unveil the emergence of CRF31_BC strain from a local variant to a nationally spread lineage, and highlights the importance of studies in viral epidemic surveillance. Financial Support: This study was supported by the FAPERGS, FAPESC, CNPq, VIROGENESIS project and Flagship grant from the Medical Research Council of the Republic of South Africa.

Palavras-chaves: Brazil, HIV-1, Phylogeography, Recombinant CRF31_BC

FROM FOOD TO MEDICINE: THE ANTIVIRAL POTENTIAL OF JAKFRUIT LEAVES

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Resumo

Artocarpus heterophyllus, the jackfruit, is an edible fruit tree largely used in Asia and Brazil as source of nutrients and in the traditional medicine. The tea from leaves is used to calm down and to treat kidney stones. Scientific results point to antitherpesviral action of the lectin extracted from seeds. Nevertheless, no antiviral action from leaves extracts were found in the literature. Thus, the objective of this study was to evaluate the cytotoxicity and antiviral activity of A. heterophyllus leaves extracts against animal herpesvirus. Leaves were collected in Atlantic Forest area in the municipality of Itabuna, Bahia, identified and registered at Universidade Estadual de Santa Cruz plant collection under voucher number HUESC 23.705. First, ethanolic extract was prepared from dried and powered leaves, followed by methanolic and hexane liquid-liquid fractionation. Solvents were evaporated under reduced pressure to obtain dry extracts. Ethanolic (EtOH) extract and methanolic (MeOHf) and hexane (Hexf) fractions were solubilized with dimetylsulfoxide at less than 0,1% and cytotoxic and antiviral tests were performed with doses ranging from 500 to 5,24 ug.mL⁻¹. Vero and MDBK cells were used and cellular morphology was evaluated 72 h after treatment to determine the maximal non-cytotoxic concentration (MNCC). The antiviral test was performed using the MNCC of each extract and it was expressed as percentage of inhibition and antiviral index (AI). Virus tested were bovine alphaherpesvirus type 1 (BoHV-1), equid alphaherpesvirus type 1 (EHV-1) and suid alphaherpesvirus type 1 (SuHV-1). Results showed MNCC for Vero cells of 6,24, 5,24, and 5,74 ug.mL⁻¹ for EtOH, MeOHf and Hexf, respectively. The MNCC for MDBK cells were 7,8 for EtOH, and 15,6 for MeOHf and Hexf. The EtOH inhibited 58,51% (AI = 0,38) and MeOHf inhibited 94,38% (AI = 1,17) of SuHV-1 virus titer. On the other hand, EtOH had no action against EHV-1 in opposition of MeOHf that inhibited 58,31% (AI = 0,38). No effect against SuHV-1 and EHV-1 was observed with Hexf. The inhibition of BoHV-1 by EtOH, MeOHf, and Hexf was 82,2 (AI = 0,75), 45,04 (AI = 0,26), and 61,98% (AI = 0,42), respectively. Altogether, the best antiviral result was the broad viral spectrum and the suitable inhibition of A. heterophyllus leaves MeOHf indicating the antiviral potential of this fruit tree extract.

Palavras-chaves: antitherpesvirus, Artocarpus heterophyllus, cytotoxicity, Moraceae
MOLECULAR CHARACTERIZATION OF Dengue virus IN PIAÚI, 2014-2015

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Resumo
Arboviruses are associated with high morbidity and mortality rates, being a major public health problem in Brazil and in the world. Even though there are other arboviruses with potential to disseminate in Brazil, the three arboviruses Dengue virus (DENV), Zika virus (ZIKV) and Chikungunya virus (CHIKV) are predominant. There are four antigenically distinct DENV (DENV1-4), which produce different clinical manifestations that range from mild symptoms to severe forms, including hemorrhagic and neurological alterations. In the period of 2014 to 2015, 114 serum samples from patients with classical arboviral symptoms were analyzed. The samples were collected in public and private health institution of Piauí - Brazil. The viral RNA was extracted and submitted to transcriptase reverse with random hexamers. Previously described primers were employed for amplification of C/prM and E gene regions of DENV, by the PCR method. Selected positive samples were purified and sequenced. Phylogenetic inference from gene E was performed by maximum likelihood method, using MEGA6. Of the analyzed samples, 22 were positive by RT-PCR, with identification of DENV-1, DENV-2 and 3. Two patients were co-infected with DENV1 and 2. Two DENV-1 samples were sequenced, and the genotype V was identified by phylogenetic analysis. Moreover, two distinct DENV1 lineages were also identified; lineage 1 and lineage 6. Piauí is a hyperendemic region for DENV, which increases the chance of epidemics and development of severe forms of the disease. The genotype V is the same identified in other regions of the country, and the only one described since 1982. Molecular characterization has become a valuable tool for monitoring the circulation of viruses, providing information to aid public health authorities in their decisions.

Financial Support: UFPI, FAPEPI, CNPq and LACEN-PI.

Palavras-chaves: Dengue, Arbovirus, phylogeny, detection

PAN-SIMBU VIRUS DETECTION BY qRT-PCR IN SERUM FROM NON-HUMAN PRIMATES

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Simbu serogroup viruses of the Peribunyaviridae family are arthropod-borne viruses associated with outbreaks in humans of the Amazon region, including countries such as Brazil, Peru, Colombia, Venezuela and Trinidad and Tobago. Moreover, the recent detection of Oropouche in the Northeast and Southeast of Brazil have indicated the need to establish epidemiological surveillance programs to detect viruses from simbu serogroup in all regions of Brazil. Thus, we developed a broad-range qRT-PCR by Taqman assay for detection of all members of this group, using degenerated and overlapping primers for conserved regions of the Small (S) sequence of the viral genome of several viruses, such as Iquitos, Madre de Dios, Jatobal and Oropouche (OROV) viruses. RNA dilutions obtained from OROV infected cells demonstrated that this PAN-Simbu assay is more sensitive for OROV detection than previously described sets of primers/probe. PAN-Simbu assay was able to detect OROV even at concentrations as low as 10 viral copies/mL. In addition, PAN-Simbu assay was also able to identify three positive samples in 52 serum samples from non-human primates collected at a zoo from Sorocaba city. All these samples were negative when tested for OROV with previously described set of primers/probe. Cloning and sequencing DNA reactions are being performed to characterize these detected viruses. Thus, we have established a new PAN-Simbu detection assay with high analytical sensitivity for PCR-based detection of members of Peribunyaviridae family. Financial Support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Palavras-chaves: Detection, Oropouche virus, Peribunyavirus, qRT-PCR, Simbu

INFLUENCE OF SALIVA FROM Aedes aegypti IN INFECTION AND CYTOKINE INDUCTION OF ZIKA VIRUS IN VITRO

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Resumo

The Zika virus (ZIKV) is an arbovirus and its main form of transmission is through the mosquito bite vector Aedes aegypti (Ae). Studies have indicate that the saliva of Ae (SAe) exerts some immunomodulatory effects during the arbovirus infection in the mammalian host. However, there are no enough information about mechanisms of Sae in the ZIKV infection. This knowledge is important to understand the immunopathology of Zika when the virus is transmitted without the vector. The objective of this study is to evaluate the influence of SAe on the infection (entry and replication) of Vero cells with ZIKV in vitro, as well as to quantifying the cytokine production (TNF and IL-1β) in RAW macrophages also infected with Zika presence or not of SAE. Vero cells were grown in 24 well plates to monolayer, and infected with ZIKV obtained from culture supernatant, dilutions of 1/2, 1/3, 1/4 and 1/8 of viral supernatant were used, with title of 4,3x10² PFU/mL. To determine the influence of the saliva on cell infection 0.5, 1, 2 or 4 units of Ae salivary gland extract were added to the cultures at the moment of infection. Plates were incubated by five days the cytopathic effects quantified by TCID. To determine the induction of TNF-α an IL-1β, RAW macrophages was cultured in 24 well plated, infected with Zika 4,3x10³ PFU/mL and treated with 0.5, 1, 2 or 4 Ae salivary glands extracts. Five days after infection the cytokines were quantified by capture ELISA. A progressive increase in the number of cytopathic from treatments from 0.5 to 4 glandules was observed 4,3x10³ PFU/mL up to 1,3X10³ PFU/mL. The levels of TNF-α were also progressive elevated in macrophages infected and treated
with Ae salivary glands from 0.5 to 2 units. There was no difference in IL-1β among the
groups. These preliminary data indicate that the SAe increases the infectivity of Zika
and induces TNF-α production in vitro. The quantifications
of the virus by qPCR are being carried out.

Financial Support CNPq / PIBIC

Palavras-chaves: Aedes aegypti, Arbovirus, Cytokine, Zika

BX-795 INHIBITS POXVIRUS REPLICATION

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Resumo

Poxviruses are complex dsDNA genome viruses capable of modulating different cell signaling
pathways, such as the pathways of type I interferon (IFN-I) which leads to important antiviral actions in
the cell. The TBK1 kinase is an important signal transducer in the IFN-I pathways and thus is targeted
by the action of viral proteins. Among the viral proteins described as capable of inhibiting TBK1 is C6
protein. This protein is functional in vaccinia virus (VACV) strain WR and blocks TBK1 activation during
virus infection. However, C6 is truncated in VACV strain IOC and not expressed by Cotia virus (COTV).
Our hypothesis is that TBK1 may be active during infection with VACV-IOC and COTV. Therefore, the
aim of this work is to investigate the involvement of TBK1 activation during infection of cells with COTV
or VACV (WR or IOC) using the commercial inhibitor of TBK1 activation, BX-795. Western Blot analysis
detected TBK1 activation in cells infected with either COTV or VACV-IOC, which was strongly inhibited
in the presence of BX-795. And, in accordance with the literature, TBK1 activation was not observed
during VACV-WR infection. Total TBK1 and β-actin remained constant at all time points. To evaluate
the effect of BX-795 on the production of virus infectious particles, cells were infected with COTV,
VACV-IOC or VACV-WR in the absence or presence of several concentrations of BX-795 and virus titer
was determined by plaque assay. During COTV infection, 10 µM BX-795 inhibited the production of
infectious progeny by 94.9%. Similarly, the yield of VACV-IOC was inhibited by 89.1% and 97.0% at 5
µM and 10 µM BX-795, respectively. Surprisingly, the yield of VACV-WR was inhibited by 90.7% and
98.8% at 5 µM and 10 µM of the inhibitor, respectively, suggesting that BX-795 has another target in
addition to TBK1. The inhibition of VACV-IOC and VACV-WR progeny production by BX-795 was
confirmed by one-step growth assay. MTT assays have shown low citotoxicity at BX-785
concentrations of 5 µM and 10 µM. To map the steps of the replicative cycle affected by BX-795 and
involved in TBK1 activity, both pre- and post-replicative stages were analyzed by the immunodetection
of virus early and late proteins. No inhibition or delay was observed on the accumulation of different
virus proteins that could justify the strong inhibition of virus yield, suggesting that viral morphogenesis is
the probable target of BX-795.

Financial support: Faperj, Capes, CNPq, and CNPq/PIBIC
INVESTIGATION OF THE VERTICAL NATURAL TRANSMISSION OF CHIKUNGUNYA, DENGUE AND ZIKA VIRUSES IN AEDES AEGYPTI AND AEDES ALBOPICTUS IN DIFFERENT MUNICIPALITIES OF THE AMAZONAS STATE.

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Resumo

Arbovirus is the designation for the viruses transmitted by hematophagous arthropods. In addition to the cycles involving vertebrate and arthropod vectors, vertical transmission is considered as another form of maintenance of these viruses in nature. In the case of invertebrates, this phenomenon occurs when infected females transmit the virus to their eggs. Chikungunya virus (CHIKV) belongs to the Togaviridae family and the genus Alphavirus. Dengue virus (DENV) and ZIKV virus (ZIKV) belongs of the Flaviviridae family, Flavivirus genus. Four serotypes of DENV are recognized and three lineages are recognized for ZIKV. These three viruses are mainly transmitted by the bite of infected female Aedes mosquitoes and outbreaks have occurred in several countries worldwide. The objective of this study was to identify the natural vertical transmission of CHIKV, DENV, and ZIKV, evaluating Aedes eggs collected in the Amazonas State, Brazil. Between, February 2016 and June 2017 Aedes aegypti and Aedes albopictus eggs were collected using ovitraps in nine municipalities of the Amazonas State (Borba, Guajará, Itacotíara, Lábrea, Manaus, Novo Aripuanã, Parintins, Presidente Figueiredo, Tabatinga). These eggs were allowed to hatch, and larvae were then identified and grouped into pools with 1 to 30 larvae. Total RNA was extracted and subsequently subjected to probe-based RT-qPCR reaction targeting CHIKV, DENV, and ZIKV. The positive pools were submitted to nucleotide sequencing to further confirm the detected viral species and genotyping. A total of 698 larvae pools were analyzed, of which, four were positive, three for the ZIKV (mean Cts 31.04; 32.6; 36.45) and one for the CHIKV (mean Ct 35.89) all collected in the municipality of Itacoatiara, from February to April 2016. Nucleotide sequencing and bioinformatics analysis successfully identified that the ZIKV isolates belong to the pandemic Asian genotype. On the other hand, the CHIKV sample could not be sequenced. The presence of CHIKV and ZIKV genome in field-collected Aedes aegypti eggs strongly support that vertical transmission occurs in nature. These results sustain that vector surveillance can be a useful tool in addition to human epidemiological surveillance.

Palavras-chaves: Natural Vertical Transmission, Aedes aegypti, Aedes albopictus, virus zika, vírus chikungunya
The Zika virus (ZIKV) belongs to the family Flaviridae, genus Flavivirus. These viruses are phylogenetically related to other flaviviruses of public health importance, including Dengue, Yellow fever, West Nile, St. Louis and Japanese encephalitis. In 2015, during Brazilian Zika outbreak and the effective relation between infection and microcephaly, Zika was considered a world public health emergency. It is of great importance the development of animal models that make possible to study the pathogenesis, therapeutics, as well as the vaccine development. In the present work, specific pathogen-free (SPF) fertilized White Leghorn chicken eggs were infected, on ninth day of development, with 5x10^3 PFU of ZIKV Rio U1 strain, and collected at 48, 72 and 96 hours post infection. A pool of five animals per point was homogenized and analyzed by quantitative RT-PCR and PFU. The amniotic and chorioallantoic liquid of these animals were also collected and analyzed by quantitative RT-PCR. Chicken embryos trunks were cleaved transversely in 3 mm sequential sections and separated from the head, wings and legs. All fragments were fixed in Carson’s formalin-Millonig for 48 hours at room temperature, and processed according to standard histological techniques for paraffin embedding, and stained with hematoxylin-eosin. Sections of all paraffin blocks from five infected and five control embryo specimens with 96 hours post infection were submitted to immunofluorescence assay and analyzed under an LSM 710 confocal microscope. Viral RNA was detected 96 hours post infection in the embryonic extract (mean 1.6x10^8 copies/ml), in the amniotic fluid (mean 2.8x10^8 copies/ml) and in the chorioallantoic liquid (mean 9.5x10^4 copies/ml). The ZIKV infection was revealed by immunofluorescence in skeletal muscle cells and in fibroblasts. Muscular fibers were strongly positive, but only in isolated areas. In all analyzed embryos (n=5), few isolated foci of these infected cells were observed throughout the embryo, with no apparent preference for any specific muscle groups. The infection was not spread and in none of the analyzed points it was associated with any inflammatory infiltrate. Our work pave the way for the use of these animals as a tool for viral propagation, possible attenuation by serial passages, and for future tests with vaccine candidates and viral constructs.

Financial Support: FAPERJ, CNPq, FIOCRUZ.

Palavras-chaves: Zika, chicken embryos, Pathology, animal model
Resumo

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. Until now, 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 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. Most viruses suppress the stress response pathway of host to promote a cellular environment conducive to viral replication. The objective of this work was to evaluate the effect of ZIKV infection on the activation of the sensor IRE1 of the UPR pathway in T98G Cell Line human. For this, T98G cells were infected with two lineages of ZIKV, Asian and African lineage, 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. During ZIKV infection the IRE1 sensor is activated and splicing of XBP1 is time-dependent, with greater activation of the pathway taking place 3 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: FAPEMIG, CNPq, CAPES.

Palavras-chaves: Zika Virus, Unfolded Protein Response, XBP1, Endoplasmic Reticulum, Stress

IN VITRO AND IN VIVO ANTIVIRAL ACTIVITY OF NUCLEOSIDE ANALOGS PRODRUGS AGAINST ZIKA VIRUS

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Resumo

Zika virus (ZIKV) is a member of the Flavivirus genus within Flaviviridae family. This virus can be transmitted to people through the bite of an infected Aedes species mosquito and vertically by placental passage during pregnancy, being related as the etiological agent of the congenital Zika syndrome. The lack of specific treatment or vaccine against ZIKV makes it necessary to develop effective drugs to control infection. We aimed to evaluate the antiviral activity of the nucleoside analog prodrug 2’-C-β-methylguanosine (1), which inhibits HCV RdRp as previously demonstrated and the pronucleotides generated from it: 2’-C-β-Me-G phosphoramidate monoester (9) and 2’-C-β-Me-G phosphoramidate diester (6). Prodrugs 9 and 6 were designed to enhance intracellular delivery of monophosphorylated nucleoside analogs. Cytotoxicity of the prodrugs was measured by the neutral red uptake assay and the maximum non-toxic concentration (MNTC) determined.

Vero, HBMEC and Neuroblastoma cells were infected with a ZIKV Brazilian isolate ZIKV-PE (MOI 0.05) and treated with prodrugs at the MNTC. Viral replication was assessed by qPCR after 72 hours. All the prodrugs were able to reduce 90 to 99.9% of ZIKV replication in all cells. In Vero cells prodrug 6 presented the lowest IC₅₀ value (0.31 µM), however IC₅₀ value of prodrug 3 (1.25 µM) was higher than IC₅₀ value of 1 (0.44 µM). For Neuroblastoma, prodrug 6 presented the lowest IC₅₀ value (0.17 µM) and inhibited completely the formation of the dsRNA intermediated as evaluated by IF with J2 antibody. IC₅₀
of 1 and 9 was 0.62 µM and 1.02 µM, respectively. Newborn wt Swiss mice were infected at PN3 with 10^5 PFU via i.p. Mice were treated with each prodrug (25 mg/Kg/day) or vehicle (Mock) i.p from PN2 to PN9 and weighed daily. At PN9 mice were perfused with 1X PBS followed by brain and eyes removal. ZIKV replication in the tissues was assessed by qPCR and ZIKV infectious particles were quantify by plaque reduction assay. All mice treated with compounds 9 and 1 died before PN9 probably due toxicity of these compounds. Treatment with compound 6 did not impact on mice weigh and significantly reduced the number of viral particles and ZIKV replication in brain and eyes compared to mock. We characterize prodrugs which efficiently inhibit ZIKV replication in different cellular models. Moreover, prodrug 6 is promising since it protected newborn mice from infection. Financial Support: CNPQ, FAPERJ-Rede Zika, CAPES.

Palavras-chaves: Antiviral, Arbovirus, Nucleoside Analogs, Prodrugs, Zika virus

Skeletal muscle cells of Gallus gallus domesticus (Linnaeus, 1758) embryos produce Yellow Fever virus 17DD after experimental infection

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Resumo

Yellow fever (YF) is a disease associated with a flavivirus infection. Production of the YF vaccine have been done since 1937 by YF17DD virus inoculation into embryonated chicken eggs, which results in a high level of chicken proteins per human dose. In this model, skeletal muscle is the main target in which the viral replication occurs. In the present work, we analyze the skeletal muscle tissue of chicken embryos infected by YF17DD virus to characterize the first infected cells and describe the end of infection. Also, we evaluate the in vitro susceptibility and permissiveness of skeletal muscle cells of chicken embryos to YF17DD infection, determine the best condition to the infection establishment and describe the kinetic of viral production in culture system. To aim this, chicken embryos were infected with YF17DD virus at 9 day of development (dd) and were collected at 48 to 216 hours post infection (hpi), subjected to histopathological analysis and indirect immunofluorescence for detection of muscle specific and YF virus proteins. Also, primary cultures of muscle cells obtained from chicken embryos with 11 dd were infected with YF17DD virus under several protocols. In each condition, the cells were subjected to indirect immunofluorescence between 24 and 72 hpi for the detection of muscle specific and YF virus proteins. The cell extract was submitted to a PCR technique using specific primers to detect the genomic and replicative intermediate RNA and the culture supernatant was used to determine viral triter by PFU. Immunofluorescence assay of skeletal muscle tissue of chicken embryos between 14-18 dd revealed the end of infection after 168 hpi, while brightfield microscopy analysis showed disorganization of bundle muscles of 120 hpi infected embryos which were also solved after 168 hpi. Immunofluorescence assay of skeletal muscle at 48 hpi showed that cells compromised with myogenic lineage (Pax7⁺) were the first to be infected. In vitro, Pax 7⁺ cells and fully differentiated fibers (myosin⁺ and desmin⁺) were susceptible to infection. In addition, the infection was confirmed by molecular analysis and the permissiveness of the culture were observed with peak production in 48 hpi at 0,002 MOI. The data of this work contributes to the understanding of chicken embryos infection and gives support for a development of an alternative method to produce YF live attenuated vaccine with
TERT PROMOTER MUTATIONS IN HEPATOCELLULAR CARCINOMA AND NON-TUMORAL LIVERS OF PATIENTS INFECTED WITH HEPATITIS C VIRUS

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Resumo

Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related death worldwide. Most cases of HCC in Brazil are associated with cirrhosis related to chronic hepatitis C virus (HCV) infection. Since HCC prognosis depends on tumor stage at diagnosis, lower survival rates have been observed among patients at the advanced stage, highlighting the importance of the identification of biomarkers for early liver cancer detection. The telomerase reverse transcriptase (TERT) gene encodes the catalytic subunit of telomerase. This enzyme replaces short bits of DNA known as telomeres, which are otherwise shortened when a cell divides via mitosis. Although the expression of telomerase is repressed in normal somatic cells, its reactivation is a common feature in HCC, mainly due to mutations in the promoter region of TERT. The aim of this study is to determine whether TERT promoter mutations -124C>T and -146C>T are associated with HCC development in Brazilian patients with chronic HCV infection. Human DNA was extracted from formalin-fixed paraffin-embedded liver tissues and mutation analysis was performed by Sanger sequencing of PCR fragments. Until now, 34 (18 HCC, and 16 non-HCC) liver tissue samples were analyzed. None of the samples had the -146C>T mutation. On the other hand, mutation -124C>T was more frequently found in HCC (5/18, 27.8%) than non-HCC (1/16, 6.3%) tissue samples. Our results suggest that mutation -124C>T in the promoter region of TERT contributes to hepatocarcinogenesis, and may be a valuable biomarker for early diagnosis of HCC in Brazilian HCV infected patients.

Financial support: CNPq, FIOCRUZ.

Palavras-chaves: Hepatocellular carcinoma, Hepatitis C virus infection, telomerase overexpression, TERT promoter mutations

ANÁLISE DE USO DE CÓDON EM ESTRANHOS GENÉTICOS DE VARIÂNCIA DE VÍRUS DO PAPILÔMA HUMANO

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Resumo

Cervical cancer is the second most common cancer in women. The major cause of cervical cancer is the infection by a high-
risk human papillomavirus (HPV). One of the mechanisms responsible for the evolution of HPV is the usage of synonymous codons. However, most of the codons used by the virus are not the same as those used by the host. Therefore, the understanding of the mechanisms of evolutionary change in the viral genome becomes important to understand how viruses diversify and thus create effective intervention strategies of treatment against the diseases caused by HPV. Consequently, the objective of this study was to investigate the role of the synonymous codon usage in the evolution of HPV and its host, assessing different viral variants to identify adaptive patterns associated with its pathogenicity. The relative synonymous codon usage, effective number of codons, principal component analysis, correlation analysis and cluster analysis were calculated. It was possible to observe a bias in the use of HPV codons, evidencing that the codon frequency is not the same for the equivalent amino acid, for serine, we observed 87% of UCG and 0.5% for UCU. The Alphapapillomavirus genus showed a different pattern of codon usage than the other genera, 76.3% of UUU and not UUC, for Phe for example, which presented only 7.3%. This genus presents the high-risk HPV types, and this differentiated usage of codons may be related to its adaptation to the host, allowing them to have higher degrees of pathogenicity. Other studies still need to be performed so we can establish the relationship among the HPV codon usage patterns, their diversification, and association with the different levels of pathogenicity.

Financial Support: CNPq, CAPES e FAPITEC/SE

Palavras-chaves: Codon usage, Genetic variants, HPV

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Permissiveness of bats cell lines propose infection models to study DENV-4, ZIKV and HHV-1 infections

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Resumo

Bats are described as asymptomatic reservoirs of several high profile viral pathogens of medical, veterinary and zoonotic importance. Limited availability of tools restricts the understanding of molecular mechanisms of bats-born viral persistence, pathobiology and transmission dynamics to susceptible hosts. In this study, we sought to expand the limited number of permissive cell lines capable of supporting virus replication by assessing two bat cell lines obtained from Rousettus aegytiacus (RoNi/7) and Eidolon helvum (EidNi/41). Initially, growth kinetics of Dengue virus serotype 4 (DENV-4), Zika virus (ZIKV), Human respiratory syncytial virus (hRSV) and Human herpesvirus type 1 (HHV-1) were assessed by the magnitude of cytopathic effects. Thereafter, the permissiveness of bats cell lines was compared with previously characterised mammalian cell lines for individual viruses, including C6/36 (for DENV-4 and ZIKV), HeLa (for hRSV) and Vero (for HHV-1). Collectively, estimation of virus growth kinetics proposes that RoNi/7 and EidNi/41 cell lines were permissive and substantially supported the replication of DENV-4 and HHV-1. However, ZIKV was only able to replicate in EidNi/41 cell line and hRSV was unable to replicate in either of the cell lines. These findings provide foundations to elucidate the cellular determinants that modulate replication, assembly and egress of bat-borne viruses with different genetic background.

Palavras-chaves: Bat cell lines permissiveness, RoNi/7, DENV-4 EidNi/41, ZIKV Arboviruses, Herpesvirus
MODULATION OF THE KALLIKREIN-KININ SYSTEM DURING DENGUE VIRUS INFECTION AND ITS POTENTIAL ROLE IN VIRAL REPLICATION AND INFLAMMATION.

Autores

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Resumo

The kallikrein-kinin system (KKS) presents physiological role in the coagulation extrinsic pathway and inducing inflammation due to the generation of kinins. Altered vascular function and coagulation are hallmarks of Dengue virus (DENV) infection, suggesting that the KKS pathway may be involved in dengue pathogenesis. Bradykinin (BK) is a vasoactive peptide with vasodilatory and hypotensive action and it is an important inflammatory modulator in different infectious diseases. Also, we have demonstrated that BK modulated the replication of Sindbis virus infection in endothelial cells (HBMEC) in vitro and in vivo. BK generation may be initiated by the activation of factor XII (FXII) by anionic polymers, which culminates in the activation of prekallikrein (PKa) in kallikrein (K), leading to the cleavage of kininogen (HK) and BK generation. We established an ex vivo system to investigate contact phase activation, by incubating plasmas from DENV patients or healthy donors with dextran sulfate (DXS), as a surrogate of anionic molecules. Then, the plasmas were incubated with fluorochrome-conjugated PKa substrate and its cleavage was evaluated by spectrophotometry, or the cleavage of FXII was analyzed by western blot. Plasmas obtained from DENV patients showed lower activation of the KKS pathway (about 75% lower than healthy donors) when challenged with DXS, regardless of clinical form. This event correlated with the presence of 90-100% of cleaved FXII in these plasmas, suggesting that the pathway is being activated in very early stages of the infection. In addition, we observed that the infection of HBMECs with DENV increases 50% the expression of BK receptors in these cells. Addition of BK increased 4-fold DENV replication in endothelial cells, by mechanisms involving decreased nitric oxide (NO) production and delayed cell death. Taken together, our data suggest that DENV infection induces activation of the KKS pathway, leading to an increase in BK generation, which may contribute to viral replication and inflammatory response in dengue.

Palavras-chaves: KKS system, Dengue virus, Bradykinin, Inflammation

GENERATION OF A POXVIRUS VECTOR AS A TOOL FOR EXPRESSING A RECOMBINANT GLYCOSYLATED HORMONE IN MAMMALIAN HOST CELL LINES

Autores

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Resumo

Some specific glycosylated hormones are known to increase fertility in cows and other mammals, leading to economic gains especially in the livestock business. Production methods for most of these hormones are based on their purification from animal tissue extracts, what may lead to sanitary and ethical issues. This project offers an alternative tool to double chain glycosylated hormones mass-
production based on a heterologous expression system using a recombinant poxvirus associated with eukaryotic cells. The foreign genes coding for both chains of the glycosylated hormone were inserted into a nonessential region of the poxvirus genome by homologous recombination using a transfer plasmid designed to contain the expression cassette and a reporter gene coding to green fluorescent protein flanked by a homologous region of the poxvirus DNA. In this construction, the expression cassette contains genes coding for each chain located back to back to each other and individually controlled by strong promoters in order to achieve high expression levels of both chains using a single vector. Signal peptides and his-tags were also inserted into this construction to allow secretion and facilitate detection and purification of the recombinant protein, respectively. To generate the recombinant viral vector, the transfer plasmid was transfected with lipofectamine 2000 in mammal cells infected with a wild-type poxvirus. The recombinant viruses were isolated through successive rounds of plaque purification. The isolated recombinant virus was amplified in permissive cells and purified by ultracentrifugation in sucrose cushion, achieving titers as high as \(>10^{10}\) PFU/mL. Using PCR and RT-PCR assays we detected the presence of the exogenous genes in the viral genome and its specific mRNAs transcription in infected cells, respectively. Finally, the recombinant virus driven-expression of the protein of interest in infected cells was detected by a serological method. These recombinant proteins were purified by HPLC and its biological activity will be evaluated by in vivo assays.

**Funding:** CNPq, CAPES, FAPEMIG and INCTV

**Palavras-chaves:** Viral vector, recombinant protein, heterologous protein production

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**In vitro evolution of Zika virus in Aedes albopictus cells**

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**Resumo**

The Zika virus (ZIKV) is an arbovirus of the *Flaviviridae* family, genus *Flavivirus*, with two genotypes recognized, one Asian and one 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 rapidly, central nervous system injuries were reported. This work aimed to evaluate the ZIKV evolution after successive passages in invertebrate cell culture. A human ZIKV sample was inoculated in *Aedes albopictus* C6/36 cells, and the cell supernatant was continued inoculated in new cell cultures over 30 rounds of successive passages. Every five passages, viral supernatants were removed and submitted to real-time PCR to evaluate productive infection. Both passages P1 and P30 were submitted to low-speed centrifugation to remove cell debris, followed by nuclease treatment with DNases and RNase to digest unprotected nucleic acids. The viral RNA was extracted with a commercial kit and submitted to whole transcriptome amplification. A paired-end NGS library was produced with NexteraXT for running in the MiSeq sequencer. A total of four non-silent mutations were observed between P1 and P30. The first mutation was observed at nucleotide 4173 (relative to the RefSeq NC_035889, NS2A coding sequence), in the first position of the codon, changing from adenine to guanine and replacing the amino acid from asparagine to aspartate. A second mutation was observed at position 4317 (NS2B), in the first position of the codon, changing from guanine to adenine and substituting the amino acid from valine to methionine. The third mutation occurred at position 6274 (NS3), at the second position of the codon, changing from cytosine to thymine and also altering the amino acid from threonine to isoleucine. The last mutation was observed at position 9915 (NS5), changing from a guanine to adenine and also replacing the amino acid from a valine to an isoleucine. Further studies are on course to evaluate the impact of these mutations in the Zika virus fitness.

**Palavras-chaves:** Zika virus, evolution, Aedes albopictus cells, Mutations
ANALYSIS OF THE INTERACTION BETWEEN DENGUE VIRUS NS1 PROTEIN AND HUMAN CD14 PROTEIN


Instituição: UFRJ - Universidade Federal do Rio de Janeiro (Cidade Universitária)

Resumo

Introduction: Dengue virus (DENV) belongs to the family Flaviviridae, genus Flavivirus and has four serotypes (DENV1, 2, 3 and 4). After infection, viral RNA is recognized by cellular machinery and translated into a viral polyprotein which is processed into three structural proteins (C, prM and E) and seven non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5). NS1 protein is essential for DENV replication and can be secreted and found in sera from infected patients since the onset of the symptoms. It has been shown that NS1 protein is able to interact with monocytes and macrophages, leading to activation, by a mechanism not yet fully elucidated. Our group identified that NS1 protein can interact with several human proteins, including CD14 protein, a membrane receptor found in monocytes and macrophages. In these cells, CD14 is one of the main proteins responsible for leading to endocytosis and signal transduction of the Toll-Like 4 membrane receptor. In this context, it is interesting to evaluate the role of NS1 protein in modulating signaling and activation by CD14, as it may reveal a new target for the development of drugs or vaccines for dengue. Objective: Our aim was to characterize the interaction between DENV2 NS1 and CD14 to understand the importance of such an interaction during cell activation. Methods and results: First, transduced U937 cell line with the ns1 gene was induced with doxycycline to express the NS1 protein. The cell supernatant was used to confirm the interaction with the commercial CD14 protein by co-immunoprecipitation and enzyme-linked immunosorbent assay (ELISA). It was observed by molecular docking and CD14 proteins, obtained from Protein Data Bank, that NS1 and bacterial lipopolysaccharide (LPS) have the same binding site to CD14. Our findings showed colocalization of NS1 and CD14 proteins in NS1-treated primary monocytes, as well as activation of those monocytes, observed by increased expression of the HLA-DR presenter molecule. Conclusion: We confirm the interaction between NS1 and CD14, as well as the activation of NS1-treated expressing monocytes. However, the NS1-mediated immune response still needs further investigation.

Financial Support: FAPERJ, CNPq, CAPES, ICGEB.

Palavras-chaves: CD14, Dengue, Monocytes, NS1

INHIBITORS OF CAP DEPENDENT TRANSLATION HAVE DIFFERENT IMPACT ON HIV-1 INFECTIVITY

Sara Mesquita Costa, Marcos Romário Matos de Souza, Luciana Jesus da Costa

Autores

INHIBITORS OF CAP DEPENDENT TRANSLATION HAVE DIFFERENT IMPACT ON HIV-1 INFECTIVITY

Financial Support: FAPERJ, CNPq, CAPES, ICGEB.

Palavras-chaves: CD14, Dengue, Monocytes, NS1
Mayaro virus in vitro replication is inhibited by acridone FAC-19

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Resumo

The Mayaro virus (MAYV) is an arbovirus of the genus Alphavirus, family Togaviridae, enzootic in South America, being kept in a wild cycle involving vertebrates and Haemagogus mosquitoes. Cases of MAYV are sporadic and occur in people with history of recent activities within or near forests, however the possibility of adaptation of MAYV to urban mosquitoes such as the Aedes aegypti, associated with the greater use of forest areas and the high mobility of the population causes the MAYV to become a serious risk to public health. In addition, there is still no antiviral therapy specified for the treatment of these infections. In this study, we report the inhibition of virus replication in Vero E6 cells by plaque reduction assay. Cells were infected with 25 PFU of MAYV for 1h at 37°C, following overlay with MEM+1% carboxymethylcellulose (CMC) with or without acridone FAC-19 at a concentration of 2μM. Treated cells showed efficient inhibition of the viral replication at
concentrations that presented minimal toxicity to the cells. The assays showed that the acridone exhibited a >90% inhibition of MAYV replication with no effect on cell viability. Besides, we evaluated the virucidal effect of FAC-19 in Vero E6 cells, and at a concentration of 2µM the acridone FAC-19 inhibit above 90% of virus replication. Our results suggest that this is a promising molecule to be studied with potential antiviral activity against the Mayaro virus.

**Palavras-chaves:** Mayaro virus, INHIBITION, acridones, treatment

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**IMMUNOPROTEASOME IS ACTIVATED DURING CHIKUNGUNYA VIRUS INFECTION AND COULD CONTRIBUTE TO PATHOGENESIS**

**Autores**  
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**Resumo**

The Chikungunya (CHIKV) virus is an alphavirus, usually transmitted by mosquitoes, which induce a febrile condition associated with severe arthralgia and myalgia that could persist for months or even years. CHIKV replication in articular and muscular tissue leads to macrophage and T cell infiltrates, increase of pro-inflammatory cytokines and persistent damage. The immunoproteasome (IP) is a multi-proteolytic complex activated by TNF-α and Interferon-γ. Recently it was demonstrated that in inflammatory myopathies and rheumatoid arthritis, IP is usually over expressed, which increases inflammatory signals, tissue damage and self-antigen presentation. The aim of this work was to investigate the involvement and the role of IP activation during CHIKV infections. To this we infected 12 days-old SV129 mice with 10^5 pfu of CHIKV in the left hind limb footpad. Infected mice exhibited a reduction of weight gain, locomoters difficulties and marked footpad edema comparing to mock-infected mice. Viral quantification at 4 days post infection by plaque assay showed a systemic distribution of CHIKV with muscle and footpad achieving the highest loads, 10^7 and 10^8 pfu/gram of tissue, respectively. Besides that, we observed an increase in the expression of IP subunits β1i, β2i and β5i and its activators TNF-α and Interferon-γ in skeletal muscle of posterior pad of infected mice by qPCR.

Treatment of mice with daily doses of bortezomib (0.1 mg/kg), a general proteasome inhibitor, in the course of infection completed abrogated the CHIKV induced footpad edema and improved the clinical score. Histological analysis and treatment with specific inhibitors of IP are currently in progress in our lab. Next, to accesses mechanism of IP activation during CHIKV infection, we infected C2C12 myoblast differentiated into myofibers with a MOI of 1. After 24 h, 98% of cells were infected by CHIKV, promoting a reduction of cell viability of 63% and viral load of 3x10^7 pfu/ml at culture medium. However, the expression of IP subunits and TNF was not affected by CHIKV infection, indicating that CHIKV did not directly activate IP in muscular cells and is probably dependent of cellular infiltrates. Analyses of the dependence of the cellular infiltrate for activation of the immunoproteasome are ongoing in our laboratory.

**Financial Support:** CAPES, CNPQ, FAPERJ

**Palavras-chaves:** Chikungunya, Muscle, Immunoproteasome, Mouse
COMPARATIVE STUDY OF MUTATIONS PROFILES IN HIV-1 PROTEASE GENE AND ENVELOPE V3 REGION IN INDIVIDUALS UNDER ANTIRETROVIRAL THERAPY FOR MORE THAN 10 YEARS IN BELÉM, PARÁ

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Resumo

In Brazil, it is estimated that around 455 thousand people living with HIV/AIDS (PLHA) have adhered to treatment and use antiretroviral therapy (ART). However, as a consequence of prolonged use of ARVs, secondary resistance episodes, also called Acquired Drug Resistance (ADR), may occur due to the effect of selective drug pressure. The aim of the present study was to evaluate the variability of the V3 region in the HIV-1 env gene and also the protease region of the pol gene in order to describe the mutations of resistance to protease inhibitors in subjects under ART for more than 10 years. Epidemiological, laboratory and sequencing data were collected from 17 PLHA during two periods, in the first one in the years 2001-2002 and in the second one in 2017, and all the individuals underwent clinical and laboratory follow-up at the Specialized Reference Unit on Infectious and Parasitic Diseases. A new collection of blood was performed, followed by DNA extraction, amplification and nucleotide sequencing of the envelope (env) and protease (pro) regions, and the results were confirmed by phylogenetic analysis. According to laboratory results, there was an increase in the mean CD4+ T cells count and a drastic reduction in viral load when compared to individuals in the years 2001-2002 for the year 2017. There was also a decrease or disappearance of resistance mutations to protease inhibitors when comparing the two periods studied. Subtype B was the most frequent for both gene regions, as well as its GPGR variant in relation to the env gene was the most frequent among the study subjects, in both periods. However, due to our small sample size, it is evidenced that more studies are needed to better understand the genetic variability of circulating viruses in the state of Pará today.

Palavras-chaves: ART, HIV, molecular epidemiology

DENGUE INFECTION OF ENDOTHELIAL CELLS PROMOTES ALTERATIONS IN OXYGEN CONSUMPTION AND ROS PRODUCTION, WHICH AFFECTS CELL VIABILITY AND VIRAL REPLICATION

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Resumo

Dengue infection of endothelial cells promotes alterations in oxygen consumption and ROS production, which affects cell viability and viral replication.
Dengue virus infection is associated with vascular alterations, such as vasodilation and increased vascular permeability, indicating that endothelial cells are a critical element in the development of inflammatory response. Our group has previously demonstrated that endothelial cells are permissive to dengue virus and that the infection activates cytoplasmic RNA sensors, inducing the production of interferon and proinflammatory cytokines and cell death. Viral sensing may be associated with response to cellular stress, including mitochondrial dysfunction and the production of reactive oxygen species (ROS). In addition, ROS production may regulate cell activation and survival. Here, we investigated whether DENV infection affects mitochondrial physiology and ROS production and the relation of these events of virus-induced cell death and activation. Human brain microvascular endothelial cells (HBMECs) were used as an endothelial cell model. The cells were infected with DENV2 (16681 strain) and oxygen consumption was measured by high resolution respirometry and ROS production by 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 qRT-PCR and ELISA. We observed that dengue virus infection in HBMECs induced a decrease in basal oxygen consumption and maximal respiratory capacity. Virus replication also resulted in increased ROS production, including mitochondrial ROS and NADPH oxidase-dependent ROS. Inhibition of mitochondrial ROS and ROS produced by NADPH oxidase resulted in diminished cell death and cytokine secretion, indicating that ROS-associated signaling pathways are involved in cellular activation and death. Interestingly, pharmacological inhibition of ROS production also resulted in decreased virus replication. These data suggest that DENV-induced mitochondrial stress may contribute to the production of ROS in HBMECs, and this may be an important signal for viral replication and cellular activation, contributing to the development of vascular lesions and endothelial death.

Financial Support: CNPq, CAPES, FAPERJ

Palavras-chaves: Dengue virus, Endothelial cells, ROS, Cell viability, Viral replication

### Blocking Chikungunya Virus-induced Autophagy upon Efavirenz treatment inhibits viral replication.

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**Resumo**

Chikungunya virus (CHIKV) is a mosquito-transmitted virus, member of Alphavirus genera, Togaviridae family and causes an acute febrile and exanthematous illness characterized by high fever, intense polyarthralgia, myalgia, headache rash and also joint swelling, which could persist for several months as a clinical outcome known as post-chikungunya chronic polyarthralgia. CHIKV urges for alternative therapeutic strategies able to block viral replication, alleviate symptoms, and avoid unfavorable outcomes of infection. Therefore, this proposal is based on the characterization of drug repurposing of the clinically approved antiretroviral Efavirenz (EFV) to treat CHIKV infection. CHIKV infection in human cells induces autophagy, which has proviral effects. On the other hand, the anti-HIV drug EFV has
several effects on mammalian cells including induction of oxidative stress, ER stress and increasing levels of the autophagic receptor p62 in an autophagy-independent way. To characterize the role of Efavirenz on CHIKV replication, initially, EFV citotoxicity was determined for human cell lines Hela and HBMEC. The Brazilian CHIKV isolate, Ibex-1, was used to infect these cells with a multiplicity of infection of 0.02. Infected cells were treated with 1, 5 and 10 µM of EFV. Infectious virus particles were quantified by plaque assay in Vero cells 24 hours after treatment. Levels of protein shutoff and p62 protein contents were analyzed by Western blotting of cell lysates. Presence of the dsRNA replicative intermediate in HBMEC cells was evaluated by IF using the J2 antibody. Levels of p62 and LC3 and distribution were also evaluated in CHIKV-infected HBMEC cells. Our results show that at 1µM EFV inhibited 86% of CHIKV infectious particles in both cells. This data correlates with western blotting for analyses of protein shut-off: while CHIKV infection induces a profound global protein synthesis inhibition in the lowest concentration of EFV was observed the highest recovery of expression of cellular proteins. Moreover, we observed that levels of p62 are reduced by CHIKV infection, and with EFV treatment, these levels tend to achieve mock levels. Moreover, we observed punctas of p62 and LC3 in CHIKV infected HBMECS, and reduction of punctas upon treatment. Taken together, our results point that autophagy inhibition upon EFV treatment could prevent CHIKV replication and thus EFV could be a potential alternative of drug repurposing to treatment of CHIKV fever.

Palavras-chaves: Chikungunya virus, arboviruses, antiviral therapy, Efavirenz, Autophagy

OSTEOPONTIN AND ITS SPLICING ISOFORMS EXPRESSION IS ASSOCIATED WITH SEVERITY AND MORTALITY IN INFLUENZA A(H1N1) PDM09 CASES.

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Resumo

Introduction: Influenza virus causes a highly contagious respiratory infection with high morbidity and mortality. The role of host factors associated with severe disease is still poorly understood. Osteopontin (OPN) is a glycoprotein with biomarker potential in infections and tumors. OPN levels were increased during influenza infection, correlating with lung injury. OPN generates isoforms (A, B, C, 4 e 5), through alternative splicing. Our objective is to evaluate total OPN and its isoforms expression during influenza infection, which is not yet described. Methodology: We studied 176 influenza A(H1N1)Pdm09 positive and 65 negative samples (nasopharyngeal swabs and post mortem specimens). Additionally, murine OPN expression was assessed in lungs of C57/BL/6J mice infected with A/PR/8/34 virus (5x103 PFU), which were sacrificed 3 and 5 days post-infection (pi) (N = 5 per group). For detecting OPNs in clinical samples, reverse transcription PCR with specific primers to the isoforms was performed. For assessing their expression levels, real time PCR was used. Quantitation of relative gene expression was performed using the ΔΔCT method. GAPDH gene was used as constitutive control. Results: Total OPN and OPN4 expression was more frequent in influenza positive than negative samples. OPN4 and OPNC expression was more frequent in influenza severe cases, when compared to mild cases. Total OPN expression was more frequent in influenza fatal cases, when compared to non-fatal ones. In influenza positive cases, O2 saturation

Palavras-chaves: Biomarkers, Influenza, Isoforms, Osteopontin
THERAPEUTIC EFFICACY OF EXTRACT FROM MARINE ALGA Osmundaria obtusiloba AGAINST CHIKUNGUNYA VIRUS

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Resumo

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus that can cause fever and chronic arthritis in humans. Currently, there is no antiviral drug or vaccine commercially available for the treatment of chikungunya fever. Thus, there is a need for effective therapy against CHIKV. Our group’s studies with the ethanol extract of red seaweed Osmundaria obtusiloba (native to the Brazilian coast) showed the antiviral potential of this alga and its low acute toxicity in BALB/c mice. Hence, this study examined the therapeutic efficacy of O. obtusiloba ethanol extract against CHIKV. Two months old female C57BL/6 mice were infected subcutaneously via footpad inoculations with 1×10^5 PFUs of CHIKV. The infected animals were divided into three groups and treated orally with O. obtusiloba extract (200mg/kg, n=5), Ribavirin (50mg/kg, n=5) or saline (n=5) twice a day. Their behaviors, weight and footpad swelling were monitored for a 14 day period. Animals from all groups had difficulty supporting the hind and front paws; however, the negative control group animals (saline) had a higher severity of symptoms. CHIKV infection did not cause a significant change in weight, but caused swelling of the paw, reaching a peak of swelling on the sixth day and a progressive reduction in the days afterwards. The group treated with O. obtusiloba extract showed a more pronounced reduction of swelling at the end of the experiment when compared with control groups. These results suggest that the O. obtusiloba extract may be useful in reducing the severity of CHIKV symptoms and has a good potential in the development of a new anti-CHIKV drug.

Financial support: CNPq, CAPES, FAPERJ, UFF (PROPRI)

Palavras-chaves: ANTIVIRAL, CHIKUNGUNYA VIRUS, MARINE ALGAE
Congenital Zika Syndrome

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Resumo

Zika virus (ZIKV) is an arthropod-borne virus, member of the Flaviviridae family, which recently brought 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. The unveiling of the cellular miRNAs modulated by ZIKV may aid the identification of cellular pathways modulated by ZIKV and other arboviral infections. Here we studied human miRNAs induced by the ZIKV infection in neuroblastoma cell-line (SH-SY5Y). 754 cellular miRNAs were screened using the QuantStudio™ 12K Flex (Life Technologies) platform. Further, we searched for target-genes of all the differentially expressed miRNAs modulated by ZIKV infection and performed the Gene Set Enrichment Analysis on the putative targets of the differentially expressed miRNAs. We validate the target genes through TaqMan Gene Expression Array. The selected miRNAs were confirmed by specific qPCR in brain tissues of stillborn children with Zika-induced microcephaly (n = 8) and controls (n=3) children of the same age. We found seven miRNAs (miR-99a*, miR-126*, miR-190b, miR-361-3p, miR-522-3p, miR-299-5p and miR-1267) downregulated during ZIKV infection, and one upregulated (miR-145). We also found miRNAs exclusively expressed in ZIKV-infected cells (miR-148a, miR-342-5p, miR-598 and miR-708-3p) or in non-infected mock cells (miR-208, miR-329, miR-432-5p, miR-488, miR-518b, miR-520g and miR-767-5p). The GSEA analysis reveal that targets of miR-145 were enriched in biological processes related to neurogenesis and neuronal migration. Targets for the downregulated miRNAs, include pathways involved with cytoskeleton and cell adhesion. The upregulation of miR-145 was also confirmed in three ZIKV positive brain post-mortem samples (Fold induction = 4,35). The miR-148a was also found to be upregulated in the same set (Fold induction = 3,2). Histopathological and target genes expression analysis at the brains confirmed the regulation of microcephaly, neurogenesis and neuronal migrations pathways disturbed by Zika infection during pregnancy. Overall, our data reveals that these miRNAs could have an important role in the ZIKV congenital syndrome, promoting microcephaly and others CNS lesions.

Palavras-chaves: Zika, miRNAs, neuroblastoma, microcephaly

MAPPING OF ANTI-VIRAL RESISTANCE MUTATIONS USED IN THE TREATMENT OF CHRONIC HEPATITIS B

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Resumo

Regarding the treatment, two therapeutic approaches have been used for the treatment of HBV infection: immunomodulators and nucleo(t)ides analogues. One of the most important factors in therapeutic failure is the onset of mutations, which can confer resistance to
antivirals. In addition, genetic variability of the virus may influence the therapeutic response. Thus, it is essential to identify resistant strains to guide clinical decisions to increase treatment efficiency and to prevent multiresistant viruses. This research aims to identify resistance mutations to antivirals used in the treatment of chronic hepatitis B as well as circulating genotypes. In the Viral Hepatitis Outpatient clinic linked to CEP, 100 patients will be selected to participate in the study. A nested-PCR was applied to amplify a 1300 bp fragment corresponding to the partial region of the HBV S/P gene. To date, 57 samples were selected, where 52 were positive and sequenced. The HBVseq online tool (HIV Drug Resistance Database, Stanford University, USA) was used for analysis of the P gene in relation to antiviral resistance mutations. Based on the partial results, the following genotypic distribution was observed: A1 16 (30%), D1 1 (2%), D2 7 (14%), D3 14 (27%), D4 7 (14%), F2a 7 (14%). The prevalence of genotype D and subgenotype A1 in the Amazon region was verified and corroborate with studies already performed. Regarding analysis in the P gene, the mutations rtL80V, rtL180M and rtM204V were identified in only one sample, which is strongly related to resistance to lamivudine, telbivudine and partial resistance to entecavir. Therefore, it is important to consider that the single health care system (SUS) for more than 10 years provides antiviral drugs for the treatment of chronic hepatitis B, but a system for the monitoring and evaluation of resistance to these drugs is not available.

Financial support: CHAMADA MS/CNPq/FAPER/SESU N. 003/2016 - PPSUS

Palavras-chaves: Hepatitis B, Mutation, Resistance, Antiviral drugs

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**DIAGNOSTIC IMAGING OF ENCEPHALITE / MENINGOENCEFALITE CAUSED BY THE DENGUE VIRUS: AN INTEGRATIVE REVIEW**

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**Resumo**

**Introduction:** Dengue is the most important and most common systemic arboviruses in humans. In addition to the commonly manifested symptoms, when the infection is aggravated by certain clinical factors, it can lead to cases of encephalitis or meningoencephalitis. Such a condition develops some serious conditions for the central nervous system and the human body, which may result in sequelae or death to the patient. Therefore, diagnostic imaging tools are important to observe changes effectively.

**Objective:** To determine the main/possible clinical findings in cases of dengue encephalitis/meningoencephalitis in diagnoses by computed tomography and magnetic resonance imaging, as well as to identify the best imaging method to identify such condition.

**Methodology:** This is an integrative review of the literature, with article search in the LILACS, SCIELO and PUBMED databases. The descriptors "Dengue encephalitis magnetic resonance", "Dengue encephalitis tomography", "Dengue meningoencephalitis magnetic resonance imaging" and "Dengue tomography meningoencephalitis" were used. Inclusion criteria: scientific articles that addressed the theme, published in the period 2004-2018; in Portuguese, English and Spanish. **Results and Discussion:** 195 articles were identified, however, only ten were included in the inclusion criteria. The studies observed that MRI is more sensitive and accurate than computed tomography when the evaluation is related to patients with dengue and early neurological manifestations. All studies have
demonstrated abnormal intensities of the MRI signals. Lesions appeared hyperintense in T2-weighted sequences. Commonly affected areas are the basal ganglia, thalamus, cerebellum, cerebral cortex, and white matter. The signs/symptoms present described in the studies were: fever, headaches, joint pains, malaise, lack of appetite, drowsiness, dizziness, vomiting, sensitivity to light, confusion, disorientation, abnormal reflexes, muscle weakness, speech problems and stiff neck. **Conclusion:** magnetic resonance imaging is the best option for the identification of DENV encephalitis, but secondary laboratory tests to confirm the virus infection are always necessary. The observation of the described findings may indicate to the clinician an initial suspicion of DENV infection, providing a better management of the patient. **Financial support:** Programa de Pós Graduação em Ciências Aplicadas à Saúde – Universidade Federal de Jataí.

**Palavras-chaves:** magnetic resonance imaging, meningoencephalitis, dengue, encephalitis

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**INTRINSIC STUDY BASED IN THE ACTIVATION OF BAX ASSOCIATED WITH THE APOPTOSIS PHENOMENON IN FATAL CASES OF MICROCEPHALY INDUCED BY THE ZIKA VIRUS**

**Autores**

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**Resumo**

Zika virus (ZIKV) is a member of the single-stranded positive-sense RNA virus *Flaviviridae* family that was first isolated from the blood of a rhesus macaque (*Macaca mulatta*) in 1947 in the Zika Forest, Uganda during a sentinel study. In recent years, ZIKV has become a global concern because of increased incidences of Guillain-Barré syndrome, microcephaly, and other congenital malformations. With regards the development of microcephaly, apoptosis appears to contribute to pathology via the elimination of infected cells with little tissue damage. The BAX is a pro-apoptotic BCL2-family protein that resides in the cytosol and translocates to mitochondria upon induction of apoptosis. It has not been found any study about ZIKV demonstrating the *in situ* activation of BAX and its implications over the intrinsic cascade development in fatal cases microcephaly caused by the ZIKV. To address this, brain tissue samples were collected from 10 individuals, five of whom were diagnosed as ZIKV-positive with microcephaly and a further five were flavivirus-negative controls that died because of other causes. The expression of the BAX *in situ* was based biotin-streptavidin-peroxidase method. Statistical analysis was performed in GraphPad Prism 5.0 using student t test. The quantitative analysis of the BAX *in situ* revealed that immunolabelling was predominant in neurons, glia cells, and astrocytes in ZIKV microcephaly cases compared to control. Notably, BAX has been shown to induce mitochondrial damage and the formation of transmembrane pores, leading to the release of cytochrome C that binds to APAF-1 to form apoptosomes in ZIKV microcephaly cases and induce apoptosis.

**Financial support:** Ministry of Science, Technology and Innovation/National Council for Scientific and Technological Development.

**Palavras-chaves:** Apoptosis, BAX, Microcephaly, Pathology, ZIKV
ANTIVIRAL ACTIVITY OF IMIDAZOLE SALTS AGAINST MAYARO VIRUS

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Resumo

Viral infections are a major worldwide problem, including those caused by drug-resistant strains already in clinical use. The characterization of new compounds with properties to act against several viruses has become a necessity, especially in viral infections caused by (re) emergent viruses with a high degree of infectivity, such as Zika (ZIKV) and Mayaro (MAYV). Imidazole salts (SI) are substances consisting of anions associated with the imidazole ring of the cationic nucleus, and a long alkyl chain. Such properties facilitate the electrostatic interaction with biological systems, evidencing the importance of these constituents for the pharmaceutical industry. The cytotoxicity and antiviral activity of three SI (SI16MIC1, SI16PyrCl and C4MImCl) were evaluated from mitochondrial and lysosomal functionality by reducing the MTT methyl tetrazolium salt and Neutral Red dye in VERO cells. Cell cultures were exposed to serial dilutions at concentrations of 0.156 to 40µM and for the control group, cells without SI exposure were considered as 100% viable cells. After the incubation time, the MTT and VN test were performed. Furthermore, the potential antiviral activity of the imidazole salts was evaluated by the virus plaque-reduction assay in Vero cell lines infected with MAYV. Results from the evaluation of cytotoxicity showed a reduction of cytotoxic effect in the concentrations of 3.9 µM to 2.6 µM, therefore we selected the concentrations of 10 µM to 0.312 µM to perform the antiviral assay. It was observed that from the three molecules analyzed, the SI16PyrCl exhibits a cytotoxic effect in 99.32% in its highest concentration, while in its lower concentration it appears in about 30% of the cells. In addition, the concentration of 20 µM presented 2.90% viable cells. The SI16MImCl demonstrates at its lowest concentration 100% cell viability and the C4MlmCl has a cytotoxic effect lower than 10% even at its highest concentration. In the antiviral assay, the C4MlmCl compound showed a greater reduction in the number of plaques reaching 41% of virus inhibition. The compound SI16PyrCl presented cytotoxic effect in the highest concentration and showed 13% of viral inhibition at the concentration of 1.25 µM. It is concluded that the compounds have a concentration-dependent toxicity curve and two of them presented antiviral activity. Further studies are necessary to understand the mechanism of action of these compounds against MAYV.

Financial support: CAPES, CNPq, DCIT-MS

Palavras-chaves: Antiviral, Cytotoxicity, Mayaro

Development and Validation of Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) for Rapid Detection of ZIKV in Mosquito Samples from Brazil

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Resumo

The rapid spread of Zika virus (ZIKV) represents a global public health problem, especially in areas that harbor several mosquito species responsible for virus transmission, such as Brazil. In these areas, improvement in mosquito control needs to be a top priority, but mosquito viral surveillance occurs inefficiently in ZIKV-endemic countries. Quantitative reverse transcription PCR (qRT-PCR) is the gold standard for molecular diagnostic of ZIKV in both human and mosquito samples. However, the technique presents high cost and limitations for Point-of-care (POC) diagnostics, which hampers its application for a large number of samples in entomological surveillance programs. Here, we developed and validated an one-step reverse transcription LAMP (RT-LAMP) platform for detection of ZIKV in mosquito samples. The RT-LAMP assay was highly specific for ZIKV and up to 1.000,000 times more sensitive than qRT-PCR. Assay validation was performed using 60 samples from Aedes aegypti and Culex quinquefasciatus mosquitoes collected in Pernambuco State, Brazil, which is at the epicenter of the Zika epidemic. The RT-LAMP had a sensitivity of 100%, specificity of 91.18 %, and overall accuracy of 95.24%. Thus, our POC diagnostics is a powerful and inexpensive tool to monitor ZIKV in mosquito populations and will allow developing countries to establish better control strategies for this devastating pathogen. Financial Support: FACEPE – APQ-0154-2.12/16

Palavras-chaves: diagnostic, Culex quinquefasciatus, Aedes aegypti, point-of-care, arbovirus

EPIDEMIOLOGIC ANALYSIS OF HUMAN RABIES CASES IN THE STATE OF PARÁ ON THE FIRST SEMESTER OF 2018

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Resumo

INTRODUCTION: Rabies is a zoonotic infectious disease, its aetiology is viral and belongs to the Lyssavirus genus. The disease causes acute progressive encephalitis with an almost 100% of lethality. In developing countries, the rabies is considered a public health issue. In Brazil, the largest reports of transmission are by dogs and bats of the genus Desmodus rotundus. MATERIAL AND METHODS: It was realised an epidemiologic research of human rabies in the state of Pará on the first semester of 2018. The cases were notified by the SESPA (Department of Health of the state of Pará). RESULTS: It was notified 14 human rabies cases in the semester, all of them were in the city of Melgaço In the State of Pará. Among the cases, 3 (21.4%) were discarded, 7 (50%) were confirmed and 4 (28.8%) are suspicious cases which are still under investigations due to no laboratory results. The suspicious and confirmed cases occurred between May and April of 2018. Among the 11 affected people, the majority
(63.64%) were male, with an average age of 8 years and age range of 2 to 39 years. Furthermore, all
the patients had history of aggression by bats and no one received rabies post-exposure prophylaxis;
thus, all of them evolved to death. The diagnose was based on blood serum samples, cerebrospinal
fluid, lingual swab, hair follicle of the patients’ neck and encephalon fragments of the patients who died.
In addition, the samples were sent to Central Laboratory of State of Pará (Lacen/PA), where were
prepared and sent to reference laboratories such as Institute Pasteur/São Paulo (hair follicle and
lingual swab) and Evandro Chagas Institute/Pará (encephalon fragments). Among the 7 confirmed
cases, 6 were positive in direct immunofluorescence of encephalon and/or RT-PCR of lingual swab
and/or hair follicle. One of the cases was confirmed by Rt-PCR of lingual swab and hair follicle once
that it was not possible to collect the encephalon fragment. From the 11 cases, it was realized the
 genetic sequencing in four samples resulting in the rabies virus from the Desmodus rotundus lineage.
**CONCLUSION:** Since in Brazil there is a high prevalence of rabies virus in bats, population awareness
is required so that prophylaxis against the infection and control measures of the virus can be effective.
Financial support: Rural Federal University of the Amazon.

**Palavras-chaves:** disease, epidemiology, lethality, occurrence, zoonosis

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**RETROSPECTIVE STUDY OF SEXUALLY TRANSMITTED DISEASES IN A BRAZILIAN NORTHEAST MATERNITY HOSPITAL IN 2017**

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**Resumo**
Sexually Transmitted Diseases (STD) encompass a series of infectious illnesses caused by micro-organisms
whose preferential way of transmission is the sexual one. It is estimated in the world about 1 million new
cases of STD every day, representing the second bigger cause of loss of healthful life among women. This
work aims to investigate the prevalence of the main causing agents of STD that can be detected in cervical
smears with sexual behavior characteristics and distinct socioeconomic aspects. For that, a retrospective
study was accomplished in 5326 women sexually active from 11 to 61 years old, among 3385 no-pregnant
and 1441 pregnant attend in the MESM (Maternidade Escola Santa Mônica) in 2017. Among those who were
not pregnant, 35,53% presented with Staphylococcus aureus. Among pregnant woman, 57,45% presented
with Condyloma acuminata. The prevalence of these and other STDs in those grups were very high, which
means that health care policy needs to be renewed for this groups.

**Palavras-chaves:** Epidemiology, Sexually Transmitted Diseases, Pregnancy
CHARACTERIZATION OF ZIKI VIRUS INFECTION IN PREGNANT WOMEN DURING 2016 OUTBREAK IN CAMPINAS/SP

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Resumo

The main purpose of this research work was to characterize the presence of Zika virus (ZIKV) by qRT-PCR in different placental regions collected from pregnant women who had symptoms of arboviruses infection during pregnancy. For this, we established a systematic scheme for placental sampling, which included obtaining biopsies of five different regions of the placenta, such as umbilical cord (CU), amniotic membrane (MA), basal membrane (MB), chorionic plate (PC) and chorionic villus (CV), all collected after the delivery. Of 77 pregnant women initially included in this study, 21 (27.3%) women were excluded to have other clinical conditions that could explain the initial symptoms, such as urinary infection. Of the remaining 56 pregnant women, 17 (30%) presented symptoms and/or serological or qRT-PCR results compatible with Zika infection during gestation. Of these 17 pregnant women, 13 pregnant women presented positivity in different placental regions by qRT-PCR assay, 04 in CU, 06 in MA, 06 in MB, 07 in PC and 12 in VC, totaling 35 positives in 84 placental samples. Thus, these results indicate that ZIKV can infect different regions of the placenta of naturally infected pregnant women. In addition, the ZIKV detection in placenta after several months of initial symptoms, same in mothers without ZIKV in serum or urine by qRT-PCR, suggest that this tissue can be a site for viral persistence during pregnancy.

Financial Support: Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP, Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq.

Palavras-chaves: Arbovirus, Placenta, Pregnancy, qRT-PCR, Zika Virus

CYTOTOXICITY EVALUATION AND ANTIVIRAL ACTIVITY AGAINST ZIKA VIRUS OF PHTHALIMIDE-TRIAZOLE-NAPHTHOQUINONE HYBRIDS

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Resumo

The development of antivirals that inhibit some steps in the virus life cycle without cause significance cellular damages is a challenge. Zika virus (ZIKV) has emerged in the Western hemisphere as an important cause of developmental defects and neurological disease, such as the Guillain-Barré syndrome. The virus belongs to Flaviviridae family and possesses positive-sense single-stranded RNA and is a arboviruses transmitted by mosquitoes of Aedes genus. In this study, we aimed to develop novel antivirals against this important pathogen by synthesizing new molecules derived from triazole, naphthoquinone and phthalimide groups. Fourteen compounds were
ENHANCEMENT OF ZIKA VIRUS INFECTION MEDIATED BY DENV-IMMUNE SERA IN VERO E6 CELLS DOES NOT OCCUR WHEN BOTH ANTI-ZIKA AND ANTI-DENGUE IgG ANTIBODIES ARE PRESENT IN A PLAQUE REDUCTION NEUTRALIZATION TEST (PRNT)

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Resumo

Zika virus (ZIKV) is a mosquito-borne Flavivirus which caused a huge outbreak in the years 2015/2016 in the Brazilian northeast and since then became a serious problem of public health worldwide. The diagnosis of ZIKV infection is a complex process since up to 80% of cases are asymptomatic and when symptomatic, acute illness symptoms presents high similarity to those caused by other arboviruses. In addition, a high degree of cross-reactivity with other flavivirus as Dengue virus (DENV) can be present in commercial serological tests thus hindering the precise in vitro diagnosis of this viral infection. Generally, plaque reduction neutralization test (PRNT) is a very sensitive and specific serological method used for viral diagnosis. Initially, our aim was to standardize a 90% plaque reduction neutralization test (PRNT90) to use as complementary diagnostic test to detect neutralizing antibodies against ZIKV in convalescent serum samples collected from patients with past ZIKV infection from Maceio city, Alagoas state. However, DENV is highly endemic in Alagoas, being able to reach a rate of DENV-IgG positivity upper than 70% in some populations. Therefore, we investigated if antibodies against DENV present in human sera samples could promote an enhancement phenomenon similar to antibody-dependent-enhancement (ADE) in ZIKV-infected cell culture system in vitro. For this, Vero E6 cell monolayers at 70% confluency were infected with a mix of ZIKV plus 3-fold dilutions of the heat-inactivated test sera, incubated at 37°C for 1 hour and covered with carboxymethylcellulose overlay followed by incubation at 37°C for 6 days. In this study, we tested 10 serum samples by PRNT90 collected from volunteers with clinical diagnosis for ZIKV fever up to 1 year post-infection, 8 of them tested DENV-IgG positive by ELISA. Moreover, 6 samples were ZIKV-IgG positive and 4 were negative in the rapid test assay. Interestingly, an enhancement of ZIKV infection at low serum dilutions (1:4 and 1:8) was detected in three ZIKV-IgG negative/DENV-IgG positive sera. In contrast, 4 others samples ZIKV-IgG positive/DENV-IgG positive led to neutralization of infection in both low and high serum
dilutions. In conclusion, it seems that enhancement of ZIKV infection mediated by DENV-immune serum in Vero E6 cells does not occurs when both IgG antibodies are present (anti-DENV and anti-ZIKV) but occurs only in the presence of anti-DENV IgG in the PRNT system.

Financial Support: FAPEAL

Palavras-chaves: Antibody-dependent-enhancement, anti-Dengue IgG, PRNT90, Zika virus

INVESTIGATION OF HUMAN HERPESVIRUSES 6 AND 7 INFECTIONS IN RENAL TRANSPLANTATION

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Resumo

The survival of patients after renal transplantation has been evaluated frequently in the last decades. The frequency of acute rejection has decreased, while infectious disease concerns have increased and remain responsible for approximately 15% to 20% of death cases. In pediatric or adult renal transplant recipients, Human Herpesvirus 6 (HHV-6A / B) and Human Herpesvirus 7 (HHV-7), also called Roseolovirus, often react after transplantation. Until now, there is few information about prevalence and excretion of these viruses. However, it has been reported that the latency and persistence of Roseolovirus can occur in the salivary glands. The reactivation of these viruses in solid organ receptors has been associated with serious diseases and deaths. Therefore, the aim of this study was to evaluate the prevalence of HHV-6 and HHV-7 in saliva samples from transplant recipients; 155 saliva samples from renal transplant patients were analyses. The detection and quantification was performed by duplex qPCR with a synthetic standard curve; 119 (77%) and 127 (82%) were positive for HHV-6 and HHV-7 respectively; in 108 (69.5%) samples HHV-6 and HHV-7 were detected simultaneously. Viral load remained high to both viruses, suggesting an active infection. Besides that, coinfection of Roseolovirus can be considered a factor of evolution of serious diseases. Therefore, the results found highlight the importance of the investigation of these viruses in renal transplant patients and suggest that the high positivity and prevalence of the samples indicate the persistence of HHV-6 and HHV-7 in saliva mainly in immunosuppressed patients. To confirm the active infection, it is necessary to perform mRNA detection to evaluate viral replication in saliva samples.

Palavras-chaves: Herpesviruses, qPCR, Roseolovirus, Saliva, Renal Transplantation

ZIKA VIRUS AND INCREASE OF GLUCOSE LEVEL: A “FATAL” COMBINATION?

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Resumo

Zika virus (ZIKV) is an arbovirus, from Flaviviridae family and Flavivirus genus. ZIKV is transmitted to humans by Aedes aegypti mosquito such as Dengue virus and Yellow Fever virus. The first confirmed case of ZIKV infection in the Americas was reported in Northeast Brazil in May 2015. ZIKV rapidly spread across Brazil and to more than 50 other countries and territories on the American continent. The ZIKV infections in Brazil were associated with an increase of microcephaly incidence, as well the increasing number of others central nervous system (CNS) anomalies such as Guillain Barré syndrome and encephalitis. Despite serious clinical manifestations, most infections are asymptomatic or self-limiting. The interferon (IFN) type-I as well as interferon-stimulated genes (ISGs) are important players in ZIKV infection control. For same acute viral infections individuals who have a pre-existing chronic illness, such as diabetes mellitus, can develop a severe disease but there are no studies of this relationship with ZIKV. The aim of this work was investigated the interference of glucose in ZIKV infection and for ISGs expression. For this, ZIKV replication in Vero Cells was analyzed in different concentrations of glucose [high-0.4% (HG) and normal 0.1% (NG)]. The viral load and the intracellular RNA were analyzed by qPCR (targeting NS5 region) in 6, 12 and 24 hours post infection. To verify the influence of glucose and ZIKV replication in IFN system was analyzed the OAS and PKR gene expression in Vero Cells (knockout IFN type I). The high glucose levels increase seven times the viral load at 24 hours post infection and 7.5 times in the intracellular viral RNA level. Thus, the high concentration of glucose maximizes viral replication. Just as well the ISGs OAS and PKR were more expressed in the infected HG group than compared to infected NG group. These results suggest that the glucose level is important for virus replication and for stimulates the ISGs by IFN independent pathway. In this way these results suggest that individual alteration of glucose metabolism (e.g. diabetes mellitus) can interfere in the virus replication and became these individuals more susceptible to the damages caused by this virus. Financial Support: Universidade Federal dos Vales do Jequitinhonha e Mucuri.

Palavras-chaves: glucose, interferons, ISGs, Zika virus

Macrophage necroptosis as a novel pathogenesis mechanism during Respiratory Syncytial Virus infection

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Resumo

Respiratory Syncytial Virus (RSV) is a negative-strand RNA virus classified in the family Pneumoviridae and it is an important cause of bronchiolitis in infants. Alveolar macrophages have an antiviral sentinel role during respiratory infections, but they can also enhance the pathogenesis. Necrosis is a pro-inflammatory mode of cell death, since it leads to the extravasation of damage-associated molecular patterns, increasing the severity of the inflammatory process. Necroptosis is a programmed form of necrosis and
involves RIPK1, RIPK3 and MLKL proteins, also called the necrosome. Therefore, we hypothesize that macrophages undergo necroptosis during RSV infection, worsening lung inflammation. Thioglycollate-elicited macrophages and alveolar macrophages from BALB/c mice, wild type and RIPK3 knockout (KO) macrophages derived from mice and human monocytes were stimulated with RSV for 6h at 37°C, stained with a viability dye to label necrotic cells and analyzed by flow cytometry, or assessed for cytotoxicity. To evaluate the role of the necroptotic machinery on RSV-induced macrophage death, levels of gene expression of Ripk3 and Mlkl were measured. Alternatively, cells were incubated with selective inhibitors of RIPK1, RIPK3 and MLKL. To analyze the role of alveolar macrophages during RSV infection in vivo, BALB/c mice were treated with clodronate intranasally (for macrophage depletion) 24h before RSV infection with $10^7$ PFU/animal. After 5 days, animals were euthanized and bronchoalveolar lavage (BAL) and lungs were collected. RSV induced peritoneal and alveolar macrophage necrosis in vitro, in a concentration- and time-dependent fashion. Infected cells showed higher levels of Ripk3 and Mlkl mRNA expression. RSV-triggered macrophage necrosis was dependent on RIPK1 and RIPK3, since the treatment of cells with NEC-1 and GW blocked cell death. Additionally, NSA treatment of human monocytes abrogated RSV-induced cell death. Furthermore, RIPK3 KO macrophages were protected from RSV-induced cytotoxicity. During in vivo infection, mice pretreated with clodronate did not show the loss of weight and the expressive viral load observed in the group without treatment. We conclude that RSV induces necroptosis of macrophages and this process may be relevant to disease pathogenesis. More experiments must be performed to better understand this phenomenon.

Palavras-chaves: respiratory infection, human monocytes, RIPK1, RIPK3, MLKL

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**EPIDEMIOLOGICAL ANALYSIS OF DENGUE IN MACEIO ON 2017**

**Autores**

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**Resumo**

This study is an observational, epidemiological study of dengue monitoring in Maceió, Alagoas in 2017. The disease is a major public health issue in the world, reaching mainly tropical countries due to the hot and humid climate, which provides the ideal environment for its vector proliferation. Several socioeconomic and environmental factors are related to incidence levels of the disease. This research aims to describe the epidemiological situation of the dengue virus in a capital of northeast state of Alagoas, Maceió, presenting incidence of confirmed cases by age and gender. Secondary data obtained from the information and health surveillance aggravations system of Health Department, which were analyzed in a systematized manner. There were 5335 cases of Dengue in 2017, the incidence coefficient was 571.6 / 100,000 inhabitants. There was a higher incidence in women. The results show that 58.8% of cases are among people between 15 and 39 years old. Among the notifications were 3985 cures, 1349 were unaccompanied and 1 died. Therefore, it is important to highlight that the study emphasizes the importance of knowledge production on this subject for many health fields, but mainly in basic care, with the clear aim of providing material that promotes discussion and orient strategies to control this disease.
Prevalence of Chronic Viral Hepatites in the Indigenous Population of the Western Amazon

Fabianne Araújo Gomes dos Santos Alves, Alcione de Oliveira dos Santos, Adriana Maria de Andrade Andrade, Suyane da Costa Oliveira, Lourdes Maria Pinheiro Borzacov, Juan Miguel Villalobos Salcedo, Deusilene Souza Vieira.


Resumo:
Viral hepatitis is one of the most important health problems in the world, reaching several segments of the population and causing great impact of morbidity and mortality. The main etiological agents of viral hepatitis are classified in alphabetical order of A-E. Viral hepatitis chronic B, C and D 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, Brazil. The study was subsidized in a retrospective documentary survey of information regarding rapid immunochromatographic tests for HBV and HCV and immunoassays for Anti-HDV performed in the indigenous area covered by the DSEI Porto Velho / RO, from 2009 to 2017. The study present has approbation Ethics in Research with Human Beings. Based on the results the totals of 12,537 indigenous people, 10,203 are distributed in 175 villages and 2,334 in urban areas. The overall prevalence of hepatotropic virus was 1.83% (229 / 12,537), being 1.49% (187/ 12,537) HBV, 0.20% (25/12,537) HBV/HDV, 12,537) HCV, and 0.02% (3/12,537). Viral prevalence was concentrated in only three of the five base poles of the Porto-Velho DSEI, consisting of 1% (21/2095) Ji-Paraná, 2.8% (168/5908) Guajarara-mirim / RO and 3.8 % (40/1043) High Forest of the West, 0% in Humaitá and Porto-Velho, respectively. Of the patients with hepatotropic virus, 61.1% (140/229) were male and 38.8% (89/229) were female. Age groups showed a fairly even distribution between groups, demonstrating that there is no correlation between age and viral hepatitis, except for HBV / HDV co-infected individuals who were more common in individuals older than 48 years, expressed as P> 0,05 (48-58 p =0.0116, greater than 58 p)

Financial Support: CAPES / FAPERJ

Palavras-chaves: prevalence, viral hepatitis, indigenous peoples.

Arboviruses Analysis in Serum and Urine of Symptomatic Patients

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Palavras-chaves: Arboviruses, Dengue, Epidemiology.
RESUMO

Recent increases in cases and outbreaks confirm arboviruses as a worldwide public health problem, especially in tropical countries. Studies focused in monitoring the circulation of these viruses in the population should be encouraged. Arboviral infections are constantly reported in Mirassol/SP, placing the city at risk due to the high *Aedes aegypti* index verified in the last years. Therefore, we are conducting a study to investigate arbovirus infection in serum and urine of symptomatic patients. Blood and urine samples from 119 symptomatic patients for arboviruses were collected at the Emergency Care Unit (UPA) in Mirassol. After collection, samples were processed and stored. RNA extraction was performed and RT-qPCR assay was performed for DENV, ZIKV, CHIKV and YFV. Overall, 82 cases of arboviral infection were reported: 71 of DENV (86.6%), 10 of ZIKV (12.2%) and 1 of CHIKV (1.2%). Serum and urine samples of 106 patients were tested for DENV. Regarding DENV positive samples, 98.6% (70) were DENV2 and 1.4% (1) DENV1. We tested the urine samples of DENV2 positive patients in serum (67) and the virus was found in 40.3% (27) of the samples. We also found 3 patients positive for DENV2 only in urine samples. For ZIKV, 119 patients’ serum and urine samples were tested. From the 10 patients positive for ZIKV, 7 cases were detected only in serum samples and 3 only in urine samples. So far, 79 samples of serum were tested for CHIKV and YFV. CHIKV was reported in only one case (imported from Rio de Janeiro, RJ) and no positive sample for YFV were found. Co-infection of DENV-ZIKV was found in 7 cases, 3 patients were positive for DENV (serum) - ZIKV (serum), 1 patient DENV (serum and urine) - ZIKV (serum) and 3 patients were positive for DENV (serum and urine) - ZIKV (urine). Most of the patients presented with fever and muscle pain. Our results confirm the presence of DENV, ZIKV and CHIKV in the city. Epidemiological studies concerned with concurrently using serum and urine samples for arbovirus diagnoses are important to reveal positive cases of underreported arboviruses in patients. Our data is also important to assist in vector control campaigns. Financial Support: FAPESP, CNPq, CAPES.

Palavras-chaves: Arboviruses, Symptomatic patients, Urine, Serum

THE EMERGENT NOROVIRUS VARIANT GII.P17/GII.17 KAWASAKI DETECTED IN BRAZIL: DETECTION AND MOLECULAR CHARACTERIZATION

Autores

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Resumo

Norovirus (NoV) is the major cause of viral acute gastroenteritis (AGE) and is responsible for almost 50% of AGE outbreaks worldwide. In Brazil, after the rotavirus vaccine introduction in 2006, NoV became the leading cause of AGE outbreaks and hospitalizations of children under 5 years old. Here, we report the detection and molecular characterization of a emergent variant of NoV (GII.P17/GII.17 Kawasaki_2014) in Brazil. During the period of May and September 2015, we analysed stool samples of AGE cases by RT-qPCR and detected NoV GII 28% (51/178) of samples received in the laboratory for diagnosis. NoV-positive samples were amplified using primers targeting ORF1/2 junction region. Sequencing was performed using Sanger method and genotype was assigned using Norovirus Automated Genotyping Tool. Phylogenetic dendrogram was constructed based on partial 5′-end of ORF2 region by neighbor-joining method using a matrix of genetic distances established under Kimura-two-parameter model using MEGA v.6. In order to characterize the origin of NoV GII.17 Kawasaki_2014 strains detected in Brazil, a Bayesian coalescent-based analysis was performed in BEAST v1.8.3 package.
We detected six NoV strains classified as GII.P17/GII.17 from children under two years of age and adults, from three Brazilian states. The full sequence of viral capsid gene was characterized and the Brazilian strains showed high similarity to the most recent emergent GII.P17/GII.17 strains, that caused outbreaks in Japan, China, Hong Kong and Taiwan and strains of Italy and USA. Comparative analysis of amino acid changes compared to a GII.17 representative strain, revealed three changes on a P1 domain (V256A, S281T and N512D). Phylogenetic and molecular clock analyses of the complete sequence of viral capsid showed four independent introductions of the emergent NoV GII.17 in Brazil, all of them originated from Hong Kong and the estimated date between July and December 2014. The estimated date of origin, almost one year later of detection in Brazil, coincides with a peak of international tourism, boosted by the FIFA World Cup. NoV is able to rapidly spread around the globe, so the monitoring of GII.17 spread and evolution is essential to public health.

Financial support: CAPES/ CNPq/ FIOCRUZ-IOC/ Plataforma Genômica de Sequenciamento PDTIS-FIOCRUZ

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Presentation format: Poster

Palavras-chaves: Norovirus, emergent, variant, GII.17, Brazil

MOLECULAR IDENTIFICATION OF HEPATITIS B VIRUS IN INDIGENOUS WARI POPULATION IN WESTERN AMAZON, BRAZIL

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Resumo

The hepatitis B virus (HBV) is enveloped, composed of an icosahedral capsid, with double-stranded circular DNA genome. HBV is classified into 10 genotypes (A-J) and divided into several subgenotypes. The Brazilian Amazon region has a high prevalence of cases of HBV infection and previous studies have confirmed the endemicity of the infection among indigenous groups. In addition to epidemiological factors, virus genotypes, host-related factors, such as interleukin (IL) 28B gene-related polymorphisms, influence the response to infection. In view of the above, the study aims to identify HBV by qualitative and quantitative real-time PCR (qPCR), as well as the genotypic characterization of the rs8099917 SNP of the IL28B gene. The study was carried out with the Wari indigenous population of the municipality of Guajará-Mirim, due to the absence of researches involving indigenous population, mainly in this region of the country, due to the great difficulty of interaction, such as cultural issues and geographical location, since part of them still live in rural areas. Seventy (70) samples of the indigenous population Warí do Pólo de Guajará-mirim-RO, attended at the Viral Hepatitis Clinic of the
Research Center for Tropical Medicine of Rondônia (CEPEM / RO) were analyzed, male and female patients aged 18 to 70 years, with or without symptomatology. The study was approved by the Ethics Committee (Number 1,718,840). Samples were analyzed using Real Time PCR using the SYBR Green and TaqMan systems, the genotypic characterization of SNP rs8099917 by conventional PCR and RFLP method. Of the analyzed, 32.9% (23/70) were female and 67.1% (47/70) were male, 52.9% (31/70) were older than 31 years. Thus 11.4% (8/70) were detectable by SYBR Green and 53.8% (7/13) so far, by TaqMan. The viral load profile ranged from 1.0 to 3.9 log10 (mean 2.5 log10) among the analyzed samples. The SNP rs8099917 is classified in TT, TG and GG, with the frequency found in the indigenous population of 83.9% (26/31) for TT, 12.9% (4/31) TG and 3.2% (1/31) GG in 31 samples tested. Therefore, it was possible to identify a higher frequency of the virus by means of the qPCR method using the TaqMan system, observing a frequency of 66.7%, and for the SNP rs8099917 the TT genotype was the most frequent in the study population.

Financial Support: CAPES / FAPERO

Palavras-chaves: Hepatitis B, Real-time PCR, Indigenous

CONSTRUCTION OF HUMAN Fab ANTI-ZIKA VIRUS: A METHODOLOGICAL APPROACH

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Resumo

The construction and selection of conformational libraries of antibodies are an alternative to the use of the murine monoclonal antibodies. However, the technology used in the construction of these libraries has been divergent and different protocols were described depending on the experimental material tested and specificities of each case. The aim of this work was to test and evaluate several methodologies used in the different stages of the process to obtain a Fab fragment in the adequate concentration from clinical samples of patients with active ZIKA virus infection. RNA extracted from blood was used as the source for RT-PCR reaction for amplification of the genes from light (κV) and heavy variable (HV) chain of the immunoglobulins. Cloned human Fab template was used as the source for amplification of the constant fragments of the immunoglobulins (κC and HC). PCR overlap was used to join κV+κC and HV+HC. A second overlap PCR reaction was used to join both fragments obtained. The products were purified from agarose gel. The results showed that the protocols described in the literature are difficult to reproduce from clinical samples and all steps require technical changes, depending on the sample. Even with the use of high performance extraction kits it was necessary the treatment with DNAses after RNA extraction. In cDNA synthesis, the use of primers specific for IgM and IgG may improve the efficiency of subsequent PCR reactions. The use of fluorimeters in the quantification of both RNA and amplified products showed better performance when compared to the spectrometric reading. Purification of cDNA before of the PCR reactions was also essential for efficiency of PCR reactions. The use of high specificity enzyme with hot-start activity was
important to obtain the amplified products. In the purification step of the PCR products from agarose gel, the results showed that the high concentration of these products was not efficient for the separation of the amplified fragments, suggesting that the DNA could be forming secondary structures in the gel that could hamper or delay their displacement. Thus, we observed the need for an initial input of PCR product of the 100 ng into the gel for the purification step. After all these methodological changes it was possible to propose a new protocol to obtain a suitable Fab in quality and concentration for the construction of conformational library of anti-ZIKV antibodies. Financial Support: Fapesp and CNPq

**Palavras-chaves:** antibody library, PCR overlap, Zika virus

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**INCREASED DIAGNOSTIC YIELD IN RESPIRATORY SAMPLES WHEN USING THE BIOFIRE® FILMARRAY® RESPIRATORY PANEL**

**Autores**

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**Resumo**

Acute Respiratory Infections (IRAs) are among the most common diseases in all age groups, and are a major cause of large numbers of medical visits and hospitalizations. Thus, a rapid and accurate diagnosis of the infectious agent is necessary to provide the appropriate treatment to the patient. Until May 2017, the laboratory offered a test for respiratory virus research based on the molecular biology methodology of the CLART® PneumoVir platform (GENOMICA), with three processing stages and a time of about 9 hours to release the result. After this period, the BioFire FilmArray RP was implemented, which requires only 2 minutes of hands-on time to load the sample and approximately 1 hour to perform the test. The objective of this work was to evaluate the increase in the diagnostic yield using the FilmArray considering the sample were not evaluated by both tests. We analyzed 2050 respiratory samples from January 2015 to May 2017, processed on the CLART® PneumoVir platform (GENOMICA), and 1322 respiratory samples from June 2017 to July 2018, processed on the BioFire FilmArray RP platform. The percentage of positivity was increased in the BioFire platform (52.3%) when compared to GENOMICA (43.5%). With the implementation of the BioFire platform, 53 samples belonging to three types of Coronavirus were identified: 19 to type HKU1, 16 to type NL63 and 21 to type OC43. Three bacterial species were identified in 14 samples: 5 *Bordetella pertussis*, 1 *Chlamydia pneumoniae*, 8 *Mycoplasma pneumoniae*. Influenza A H3 virus was identified in 63 samples (7.8%). An increase in positivity samples was observed in Adenovirus (5.8%), Coronavirus 229E (0.2%), Metapneumovirus (77.2%), Parainfluenzae 1 (136.6%) and Rhinovirus/Enterovirus (16.9%). Influenza A outbreak, from April to July/2015, Influenzae A H1N1/2009 outbreak, from February to May/2016 and Human Respiratory Syncytial Virus outbreak, from February to April/2017 interfered in the analyzes of these pathogens. Bocavirus is not detected by the BioFire FilmArray platform. In this study, we describe an 8.8% increase in the positivity of respiratory samples when using the BioFire FilmArray RP Panel platform, as well as the identification of not previously searched...
Herpesvirus infections are ubiquitous. About 60-95% of the human population is infected with at least one of such viruses. *Herpes simplex virus type 1* (HSV-1) is associated particularly with oral infections, characteristic of herpesviruses, HSV-1 can establish latent infections that can occasionally be reactivated, a process that may be triggered by a number of factors, including stress, immunosuppression and fever. Acyclovir and related drugs have for long been used as treatment of HSV induced signs of infection. However, indiscriminate use and prolonged administration can lead to resistance, what makes the search for new anti-herpetic drugs a relevant issue. In the present study, the anti-herpetic activity from bufotenine was evaluated. Bufotenine, a tryptamine alkaloid resulting from the methylation of serotonin, is a common metabolite spread throughout different living organisms, that can be found, for instance, in the skin secretion of many Brazilian toads of *Rhinella* genus as well as in plants of Leguminosae family. The maximum tolerated concentration (MTC) of the drug was determined on African green monkey kidney cells (Vero). Titration of HSV-1 strain KOS and an acyclovir resistant HSV-1 strain was performed in Vero cells in the presence and absence of bufotenine. Infectious virus titers were significantly reduced in presence of bufotenine, particularly against the KOS strain. These preliminary results suggest that natural alkaloid bufotenine may have a potential antiviral action herpesviruses, which will be further investigated.

Financial support: Instituto Pasteur

**Palavras-chaves:** Alkaloid, antiviral, herpesviruses, natural compound, resistance

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**NEW APPROACHES FOR DENGUE VIRUS DIAGNOSIS USING GOLD nanoparticle**

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Resumo

DENV, ZIKV, YFV and other Flavivirus, and CHKV and MAYV, both of the Alphavirus genus, are transmitted by Aedes aegypti and have similar symptomatology. Direct virus detection in mosquitoes enables more accurate virus surveillance in vector populations over time, influencing arbovirus prevention, control programs and epidemiological studies, which are even more necessary due to the co-circulation of these viruses. However, the currently available tests, despite having high sensitivity and specificity, are expensive methodologies and may not provide rapid diagnosis. Therefore, a new biosensor technology should offer benefits compared to traditional methods regarding time of analysis, sensitivity, and simplicity of manipulation. Thus, the aim of this work is to develop and analyze the effectiveness of gold nanoparticle (AuNPs) functionalization methodologies with specific anti-flavivirus antibodies and their binding to antigens. For this, gold nanorods (GNR) were synthesized and then functionalized to 0.3% PEI, conjugated to 0.1; 0.2; 0.4; 0.8 and 1.6 µg/mL of antibodies and incubated with \(10^3\) PFU/mL virus. The results were analyzed through the reading of their plasmon resonance in a UV-Vis scanning spectrometer. The synthesis of GNRs resulted in absorption peaks at different wavelengths, suggesting the synthesis of nanorods with different aspect ratios. A peak absorption shift was observed when biosensors were incubated with DENV1 and DENV2 but not after incubation with MAYV and the best results were seen at concentrations of 0.2 and 0.4 µg/mL of antibodies. In the presence of mosquito maceration containing or not virus solution, DENV1 was detected at the 1: 4 dilution of the solution. Therefore, it is suggested that gold nanoparticles can be used for an even faster, more precise and more practical diagnosis than the existing techniques and also contribute to virology surveillance studies in mosquitoes.

Financial support: CAPES, CNPq, FAPEMIG, UFOP

Palavras-chaves: Dengue virus, Gold nanorod, Plasmon resonance

ETHIOLOGICAL CHARACTERIZATION OF THE ARBOVIROSES IN PORTO VELHO, RONDÔNIA.

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ETHIOLOGICAL CHARACTERIZATION OF THE ARBOVIROSES IN PORTO VELHO, RONDÔNIA.
Resumo

Arboviruses are infections caused by viruses transmitted through hematophagous arthropod vectors. They currently represent a major public health problem due to the impacts of morbidity and mortality. The main arboviruses of clinical importance are transmitted by mosquitoes of the genus Culex and Aedes. According to data from the Ministry of Health, 22% (1153) of Brazilian municipalities have a high rate of Aedes aegypti circulation and another 2,069 municipalities are on alert, thus being at risk of dengue fever, Zika, and chikungunya. Thus, the objective of this study is to analyze the presence of RNA from Zika, dengue, and chikungunya in symptomatic patients. The research is being carried out at the malaria outpatient clinic of the Center for Research in Tropical Medicine (CEPEM) in Porto Velho, Rondônia. Individuals with fever up to the fifth day and malaria-negative were considered suspected to be arboviruses and invited to participate in the study. From January 2018 to the present, samples of serum, saliva, and urine were collected from 92 individuals. Of these, viral RNA was extracted from 66 specimens and tested by molecular biology using the real-time PCR kit one-step ZDC (Institute of Molecular Biology of Paraná, Brazil), following the manufacturer’s instructions. Reactions were performed on the real-time PCR platform of Fiocruz Rondônia (Applied Biosystems 7500). The number of positive individuals totaled 04 (6.0%) for the Dengue serotype 1 virus. The individuals in question are all males, aged between 24 and 59 years, living in the south and east of the city of Porto Velho - RO. The main symptoms presented are: fever, arthralgia, headache, retroocular pain, nausea, myalgia and chills, with onset of symptoms between 1 and 4 days before the date of care. The data presented here are preliminary, it is important to consider that the region of study is endemic for Aedes’ circulation and that the population presents symptomatology characteristic of arboviruses. Thus, the low number of positivity reinforces the importance in the investigation of the circulating etiologic agent, thus it is intended to broaden the identification of other viruses and to apply different techniques for greater robustness of the study.

Financial support: MCTI/CNPQ/CAPES/FAPS Nº 16/2014 - PROGRAMA INCT and IPEPATRO

Palavras-chaves: Arboviruses, Chikungunya, Dengue, Zika, Aedes

HUMAN BOCAVIRUS VIREMIA AND SHEDDING PATTERNS IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT PATIENTS

Autores
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Resumo

Human Bocavirus (HBoVs) are classified in the Paroviridae family and are associated with respiratory and gastrointestinal symptoms. Viral infections are an important cause of morbimortality in immunocompromised patients such as allogeneic hematopoietic stem cell transplantation (allo-HSCT) recipients. The aim of the present study was to evaluate the positivity rate and loads of HBoVs in clinical samples (feces and sera) of patients who were subjected to allo-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 consecutive 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, targeting the NP1 gene. The results showed that 53.4% (11/21) of the patients were male, aged between four 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 42.9% (9/21) of the
patients and 77.7% (7/9) were positive in both fecal and serum samples. Viral loads in fecal samples were higher than in sera and prolonged fecal shedding was observed, with two patterns: one intermittent and another continuous. Of all HBoV positive patients, six (66.6%) had the first positive sample before the transplantation, and a rise of the viral loads after the allo-HSCT occurred when comparing to the loads before the allo-HSCT. Furthermore, on most cases the highest viral loads were detected during the first 100 days after the allo-HSCT. Considering the symptoms presented by the patients, 66.6% (6/9) had diarrhea at the same period of the viral genome detection in feces, but no association was observed. Three fecal samples were characterized as being HBoV-1, with more than 99% of nucleotide identity among them. The present data shows a high occurrence and loads of HBoVs, as well as higher and prolonged fecal shedding, in allo-HSCT recipients. These results highlight the importance of including HBoV specific tests during allo-HSCT patients’ follow-up.

Financial Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Universidade Federal de Goiás (UFG); Fundação de Amparo à Pesquisa do Estado de Goiás (FAPEG).

Palavras-chaves: bocavirus, immunocompromised patient, bone marrow transplant

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SEROLOGICAL AND MOLECULAR DETECTION OF HEPATITIS A VIRUS IN PATIENTS AT A REFERENCE LABORATORY IN THE EASTERN BRAZILIAN AMAZON (1982-1983)

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Resumo

Introduction: The hepatitis A virus (Hepatovirus A, HAV) is a relevant agent causing acute hepatitis around the world. Although it has been discovered since 1973, the virus infection remained with limited diagnosis until the end of the 1980's, due to limitations of serological and/or molecular methods applicable to routine laboratory diagnosis. The study aimed to evaluate the serological and molecular frequency of HAV infection in cases of acute hepatitis attended in a reference laboratory in the eastern Brazilian Amazon during the years 1982 and 1983. The study evaluated 421 cryopreserved (-20º C) serum samples of patients with clinical suspicion of acute hepatitis (jaundice, abdominal pain, fever, vomiting and / or elevated serum transaminases AST and ALT), attended during the period from February 1982 to December 1983, in the Hepatology Section of the Evandro Chagas Institute, located in the city of Belém, Pará state, Brazil. The 421 serum samples were screened for anti-HAV IgM antibodies by enzyme immunoassay and/or electrochemiluminescence. When positive for anti-HAV IgM, the samples were also submitted to total RNA purification, later used in nested RT-PCR assays for amplification of the VP1-2A region (522 bp) of HAV-RNA. The study was approved by CEP/IEC/SVS/MS (approval n. 1.728.095). Anti-HAV IgM antibodies were detected in 68.5% (277/421) of the tested samples. Anti-HAV IgM antibodies were observed predominantly in males (58.1%, 161/277). Anti-HAV IgM antibodies were detected in individuals aged 1 to 47 years old, and most frequently observed among children up to 10 years of age (55.6%, 154/277). The HAV-RNA was detected in 74.7% (207/277) of the tested samples. We observed a high frequency of anti-HAV IgM antibodies, which indicates the significant participation of HAV in the etiology of acute hepatitis among the evaluated cases. The epidemiological profile of the patients with hepatitis A was similar to that observed in other regions of Brazil in the same period. The detection of HAV-RNA in a significant frequency of cases will allow the following genotyping of the isolates, thus contributing to a better understanding of the genetic variability of HAV circulating in the eastern Brazilian Amazon in the early 1980's.

Palavras-chaves: hepatitis A, Amazon, human
ANALYSIS OF TLR4 POLYMORPHISMS IN ZIKA VIRUS INFECTION IN THE STATE OF PARANÁ

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Resumo

Zika virus (ZIKV), a mosquito-borne infection, has recently been drawing public and scientific attention due to its rapidly spread worldwide and its association to neurological and autoimmune disorders, cooperating to the increase on the morbidity rates. The pathogenesis of ZIKV infection varies among individuals, suggesting that genetic factors may influence the development and severity of the disease. Toll-like receptors (TLRs) are important mediators of the innate immunity, responsible for triggering the immune response against a range of viruses through proinflammatory cytokines and type I interferons synthesis. However, single mutations in TLR genes have been associated with impaired cytokines production and impaired virus clearance. The aim of this study was to investigate the implication of genetic variations of TLR4 gene on Zika-host interaction. Two single nucleotide polymorphisms (SNPs), rs4986790 (Asp299Gly) A>G and rs4986791 (Thr399Ile) C>T, in the TLR4 gene were genotyped through PCR-RFLP in ZIKV patients (RT-PCR positive) and healthy controls. So far, the study enrolled 16 ZIKV patients with a mean age of 39 years (SD ± 15.4) and 30 healthy controls with a mean age of 38 years (SD ± 14.3). All subjects recruited were residents of the northwest region of Paraná. Statistical analysis was performed using the software SNPstats. The sample evaluated is in HWE. The majority of the studied participants were females, 81.3% (cases) and 83.3% (control). The allelic and genotypic analyses showed no statistical difference among the groups in both analyzed SNPs. Regarding the genotypic distribution, for TLR4rs4986790, the A/A wild-type genotype was the most frequent among ZIKV patients (100%) and controls (80%). The heterozygous A/G genotype was observed only in control subjects (20%). Similarly, for the TLR4rs4986791, the most observed genotype was the wild-type C/C among patients (100%) and controls (87%). The C/T genotype was present only in the control group (13%). No mutated G/G and T/T genotypes were observed. Although no association was yet observed, studies with Dengue virus shows that TLR4SNPs plays a minor role on susceptibility and severity of the infection. In these preliminary analyses, no influence of TLR4 polymorphisms was encountered. However, a large case-control group is being
studied for better understand the role of these SNPs in ZIKV infection and host interaction.

**Financial Support:** FA; CAPES; CNPq; LIG-UEM.

**Palavras-chaves:** Toll-like receptor 4, genetic polymorphism, Zika, infection

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**GOLD NANORODS AS A TOOL FOR DIAGNOSTIC DEVELOPMENT FOR ZIKA VIRUS**

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**Resumo**

Among the arboviruses that have the largest circulation in Brazil, Zika receives greater attention due to its association with fetal abnormalities and Guillain-Barré syndrome. The symptomatology of this arbovirus is similar to other febrile diseases, hindering the clinical diagnosis and making essential a laboratory diagnosis. Existing diagnostic methods are limited due to the short viraemic period and the cross-reactivity of antibodies among members of the genus Flavivirus. Thus, there is a need for a diagnostic method for the Zika virus (ZIKV) which aggregate specificity, sensitivity and is also readily available. New technologies have emerged to meet these needs and the use of nanotechnology has been promising because of high sensitivity, specificity and lower cost for application in the diagnosis of diseases. The use of gold nanoparticles, due to their unique physico-chemical characteristics, can help as a diagnostic tool. Thus, the aim of this work is to seek a new approach for the diagnosis of ZIKV using gold nanorods. For this, they were synthesized by the seed method and functionalized with polyethyleneimine (0.3%). After functionalization, the nanorods were incubated with different concentrations (1.6, 0.8, 0.4, 0.2, 0.1 µg / mL) of the anti-Flavivirus monoclonal antibody to test the detection ability when incubated with the virus. The analyzes were performed with the viral titer 10³ PFU / mL for the ZIKV and also for the Mayaro virus (MAYV) which was used as a negative control. The UV-Vis spectra of the solutions were obtained for shift analysis at the peak of the gold nanorods extinction band. In each process, the observed displacement indicates their effective bonding to nanorods. After incubation with the viruses, only lower concentrations (0.1 and 0.2 µg / ml) showed significant shift demonstrating that at higher concentrations the displacement generated by antibody binding may be masking the change in target binding. In addition, the concentration of 0.1 µg / mL showed the best shift relation between the positive control (ZIKV) and the negative control (MAYV). Thus, the concentration of 0.1 µg / ml of anti-Flavivirus antibody is ideal for use in subsequent experiments with gold nanorods for the detection of ZIKV.

**Financial Support:** UFOP, CAPES, CNPq, FAPEMIG

**Palavras-chaves:** Gold nanorods, Surface plasmon resonance, Zika virus

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**Technical development of real-time RT-PCR (RT-qPCR) for detection of rabies virus (RABLV) genetic lineage compatible with canids (Agv2)**

**Autores**
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Despite the decline in the number of cases of human rabies transmitted by dogs in Brazil, there are still records of occurrence, mainly in the north and northeast Regions. Between 2011 and 2016, six cases of human rabies were diagnosed with the genetic lineage of canids. The present study had as purpose the development of a real time RT-PCR (RT-qPCR) in samples of rabies virus (RABLV) genetic lineage compatible with antigenic variant characteristic of canids (AgV2). Seventeen samples were selected from central nervous system of mice which were inoculated with AgV2 RABLV. The extraction of total RNA was initially performed using TRizol, and taken transverse transcription followed by polymerase chain reaction (RT-PCR) using specific primers for the protein coding gene N. The amplicons were then purified and sequenced using the same primers used in RT-PCR. For the development of the RT-qPCR to AgV2 samples, genetic sequences were aligned and a preserved and common area was chosen. Using the software Primer Express was defined the most appropriate place for the synthesis of primers and probe. From the product of reverse transcription was performed RT-qPCR technique, using the primers and specific probe designed for AgV2. A sensitivity test was performed on the techniques used and Fisher's exact test (p > 0.05) was used for the proportion of positives. All samples were tested positive in RT-PCR, while in RT-qPCR, 15 (88.2%) were positive and two negative. When evaluating the results obtained in both techniques, the diagnostic sensitivity of RT-qPCR was 89.5%. The comparison of the techniques of RT-PCR and RT-qPCR made by Fisher exact test as to the proportion of positives detected was not statistically significant (p = 0.48). The RT-qPCR technique developed in this study proved to be efficient in detecting the rabies virus in AgV2 samples with the advantage of being a faster technique that makes possible the early identification of positives samples without need of DNA sequencing, providing a fast decision-making thereby leading a effective epidemiological control of this important zoonosis.


Palavras-chaves: Antigenic variant 2 (AgV2), Canids, Rabies Virus, Real Time PCR (RT-qPCR), RT-PCR

OROPOUCHE VIRUS INFECTION IN PATIENTS FROM MANAUS, AMAZONAS, BRAZIL

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Resumo: Arthropod-borne viruses (arboviruses) are important etiological agents of human illness, affecting mainly those living in the tropical and subtropical regions. Natural cycles comprising arthropod vectors and vertebrate hosts maintain these viruses in nature. In Brazil, several arboviruses of the Flaviviridae family, including Dengue virus (DENV), Zika virus (ZIKV), Yellow fever virus (YFV), and Rocio virus (ROCV) have already caused human outbreaks. Besides these viruses, Chikungunya virus (CHIKV) and Mayaro virus (MAYV) of the Togaviridae family and Oropouche virus (OROV), which belongs to the...
Human pegivirus (HPgV), formerly known as GB virus C, is a member of the Flaviviridae family of single-stranded, positive-sense RNA viruses and has genomic similarity to hepatitis C virus (HCV). However, unlike HCV, HPgV is lymphotropic (non-hepatotropic), establishes a subclinical infection and is not related to hepatitis or any other disease. Epidemiological data indicate that HPgV is highly prevalent in populations worldwide. The viremia in general populations varies, being lower (1–5%) in developed countries and higher (up to 20%) in developing ones. Due to the shared transmission route, co-infection in individuals with underlying conditions as HIV, HCV, patients receiving haemodialysis and people who inject drugs is common and HPgV viremia up to 45% has been reported. Several studies reported that HPgV infection is associated with delayed HIV disease progression as indicated by higher CD4 cell counts, lower HIV RNA levels and longer disease-free survival. Conversely, in HCV-infected individuals, studies have indicated that HPgV infection is likely to be associated with slower HCV clearance, leading to a higher likelihood of persistent infection. To better understand the impact of HPgV in co-infections, it is needed to know epidemiological characteristics of this virus. In Brazil, most HPgV studies were performed in São Paulo and in HIV co-infection. Data about HPgV on triple co-infection (HPgV-HCV-HIV) and its influence on the natural history of HCV-HIV is rare. The aim of this study was to determine the prevalence and genotypic distribution of HPgV in patients attended at a hospital in Rio de Janeiro. Serum samples were collected from 56 HCV/HIV co-infected patients and were analyzed by RT-PCR for specific amplification of 5’UTR region of HPgV genome. Triple infection was identified in 8 samples (14.3%) and 6 of them were successfully sequenced. Phylogenetic analysis revealed the presence of genotypes 2a (16.7%), 2b (50%) and 3 (33.3%). Our findings demonstrate the high frequency of HPgV among HCV/HIV co-infected patients in Rio de Janeiro and the circulation of genotypes that have already been described in past Brazilian studies. Additional HPgV screening in groups of patients mono-HIV and mono-HCV infection and evaluation of clinical information, are needed to assess the impact of HPgV in the course of HCV and/or HIV disease and to contribute with data about epidemiological characteristics of this virus in Rio de Janeiro.
POLYMORPHISMS ANALYSIS OF CHRONIC CARRIERS OF THE HEPATITIS DELTA VIRUS

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Resumo

Interleukin (IL) 28-B polymorphisms has been related to interferon response in the treatment of infection hepatitis C, but its role in chronic hepatitis B (CHB) and Delta is still poorly understood. Report studies single-base substitution polymorphisms (SNPs) next to the IL28-B gene on chromosome 19 as predictors of treatment response. The hepatitis Delta virus (HDV) has a ribonucleoprotein with a circular, negative, single-stranded RNA (ssRNA) of approximately 1,700bp that encodes only a viral antigen HDAg. It is considered a satellite virus because it requires the surface antigen of hepatitis B virus (L-HBsAg; M-HBsAg; S-HBsAg) to form its viral envelope and become an infecting particle in the host. Due to the need to identify the genotype present in the SNP named rs8099917 existing in the proximities of the Interleukin-28B gene in individuals with HDV infection, this study aimed to determine the genotypic frequency of the IL28B polymorphism and relate the profile of the biological variables in indigenous in the Amazon region. For the selection of samples, a total of 22 participants were included in the Guajará-Mirim/RO base station belonging to the Indigenous Health District - DSEI/Porto-Velho-RO, male and female, aged 18 years and younger than 70, who had anti-HDV Reagente serological markers, and who also consented to participate in the study through the Free and Informed Consent Form-TCLE. Nested PCR and RFLP techniques were performed, and finally, electrophoresis was performed to determine the genotype in the SNP rs8099917 and the viral genotypes of HDV and HBV. We analyzed and determined genotypically SNP rs8099917 up to the time of 15 samples, where the highest frequency was 80% (12/15) of the TT genotype, 13.3% (2/15) GG genotype and 6.7% (1 / 15) TG genotype for the analyzed region rs8099917. From the total of 15, 9 samples were analyzed to determine viral genotype so far. Being 7 of these F genotypes and 2 HBV genotype A, the frequency of HDV genotype 3 was already 100% (9/9). The present study verified the highest genotypic frequency of the IL-28B SNP rs8099917 gene in the indigenous individuals with the HDV virus of the TT genotype in the analyzed group, the presence of different genotypes for HBV, and predominance of genotype 3 for the virus HDV.

Financial Support: CAPES / FAPERO

Palavras-chaves: Delta Hepatitis, IL28B, Polymorphisms, SNP

INFECTION OF TCD4, TCD8 AND B LYMPHOCYTES IN HUMAN LYMPHOID TISSUES BY...
Human metapneumovirus (HMPV) belongs to the family Pneumoviridae, and has been recognized as an important agent of acute respiratory infections (ARI), a leading cause of morbidity and mortality worldwide. Primary HMPV infection occurs early in childhood but re-infections are common throughout adulthood. Very little is known regarding the pathophysiology or immune response associated with HMPV infection. Although HMPV tropism for the respiratory epithelium is recognized, HMPV RNA has been detected by RT-PCR in tonsillar tissues taken from children without symptomatic ARI. The present study investigates whether HMPV replicates in lymphoid tissues from asymptomatic children, thus contributing to the elucidate whether the virus pathogenesis includes persistence in such tissues, which could be source of virus shedding and spread in the community. Adenoids and palatine tonsils from children 3 to 13 years old with chronic tonsillar disease and without of acute respiratory infections (ARI) were obtained post adenotonsillectomy. RT-PCR assay was performed to detect HMPV in lymphoid tissues using Taqman® Universal PCR Master Mix Kit (Applied Biosystem). β-actin was used as endogenous control for gene expression analysis. Paraffin embedded tissue slices were analyzed by serial immunohistochemistry. Briefly, each round of staining was scanned on ScanScope at 40× magnification (Aperio Technologies; Vista, CA) and erased by treatment with alcoholic dehydration followed by incubation in H₂SO₄/KMnO₄ solution. Full-slide scans of stained tissue were pseudocolored and overlaid on the hematoxylin background using Photoshop Cs5 and ImageJ Softwares. 17 samples were positive for HMPV in human lymphoid tissues from 155 children with chronic tonsillar hypertrophy by real-time PCR. The rates of detection in palatine tonsils and adenoids were 15,5% and 10,85%, respectively. Immunohistochemistry of HMPV distribution in palatine tonsils showed positive signal for viral antigen in epithelial cells. Positive IHC staining for HMPV was also observed in the follicular and interfollicular regions. Immunophenotypic analysis indicated that the infected cells with HMPV were positive for CD3, CD4, CD8 and CD19 expression. HMPV infects TCD4+, TCD8+ and B lymphocytes in tonsils from children without ARI symptoms.

Financial support. CNPq.CAPES.FAPESP.

Palavras-chaves: Metapneumovirus, Human, Palatine tonsils, Adenoids, Lymphocytes
Resumo

**Background:** *Aedes (Stegomyia) aegypti* (*Ae. aegypti*) transmits arboviral diseases of high public health importance, including those caused by *Zika virus* (*ZIKV*), *Dengue virus* (*DENV*), *Chikungunya virus* (*CHIKV*) and *Yellow fever virus* (*YFV*). Barreiras is a city with 157,638 inhabitants in the West of the State of Bahia, in the Northeast of Brazil. The climate is dry, with well-determined and concentrated seasons of rains. The city is crossed by a Federal Highway and by the Rio Grande river.

**Methods:** In this study, we aimed to understand the dynamics of mosquito vectors and arboviral diseases in Barreiras. Specific aims were: i) to investigate the relationship among rains, mosquito infestation and arboviral diseases in the city and ii) to understand the influence of both, a highway and a river on the distribution and variability of *Ae. aegypti* in the urban area of Barreiras. We used correlation statistics to investigate a possible relationship among rains, mosquito abundance and transmission of diseases. In addition, we used geometric morphometrics to compare mosquitoes from areas limited by the highway and the river.

**Results:** We found that i) arboviral diseases are transmitted in rain-dependent cycles and that ii) both, the river and the highway segregate populations of *Ae. aegypti* in different areas of the studied city.

**Conclusions:** Our results indicate that it is necessary to treat anthropic containers with mosquito breeding capacity during both, the dry and rain seasons. In addition, it is necessary to better understand vector dynamics in the city: segregated and distinct populations can respond differently to an only control strategy.

Financial support: International Society for Infectious Diseases and Fundação de Amparo à Pesquisa do Estado da Bahia-PIBIC

**Palavras-chaves:** *Ae. aegypti*, rainfall, transmission, barriers, segregation

HIGH PREVALENCE OF HUMAN PARVOVIRUS B19 INFECTION IN CHRONIC KIDNEY DISEASE PATIENTS UNDER HEMODIALYSIS

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Resumo

Human Parvovirus B19 (B19V) infection is usually acute and self-limited, and its common related to a respiratory transmission, through personal close contact. After acute infection, B19V DNA may remain in bone marrow, kidney, liver, myocardium and other organs. Chronic kidney disease (CKD) patients under dialysis treatment have disruptions in their immune system due to the immunosuppressive effects of uremia, deficient erythropoietin production, and significantly decreased erythrocyte survival, which increase the susceptibility to acute and chronic anemia after B19V infection. The aim of this study was to evaluate the frequency of B19V infection among Brazilian CKD patients under dialysis. A study was conducted among 120 CKD patients under dialysis recruited at three Brazilian dialysis units: 32 patients were from dialysis unit of a Central region of Rio de Janeiro, collected at 2013 (unit 1), 38 were from dialysis unit of North region of Rio de Janeiro, collected at 2014 (unit 2), and 50 were referred from Ceará State unit, collected at 2015 (unit 3). B19V DNA was investigated in serum samples by real time PCR (qPCR). Demographic, clinical and hematological data were obtained from these patients and p-value ≤0.05 in all of the dialysis units. It was not observed a significant relation between the presence of B19V DNA and anemia, mean duration of dialysis, blood transfusion, transplantation, age or gender (p>0.05). The persistent B19V infection was commonly found in the Brazilian dialysis units evaluated. Although, in this study, it was not observed a clinical significance of B19V infection in patients under dialysis, the persistent B19V viremia (10^4 IU/mL) should be considered as a potential risk through the contamination of dialysis equipment and subsequent threat to dialysis patients during transplantation procedures.

Palavras-chaves: Chronic kidney disease, Hemodialysis, Human Parvovirus B19, Prevalence, Real time PCR

Factors associated with the dengue outbreak after the worst environmental disaster in Mariana-Brazil (2016)

Autores

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Resumo

In November 2015, the iron mine dam spilled 60 million m^3 of mud in the Rio Doce Basin (RDB), contaminating its water with iron residues, as well as transporting human and animal sewage to this river. This was the worst environmental disaster in Brazil. Currently, dengue has been the most important epidemic disease in Brazil since 2014 and in this area the dengue cases was increased in 200%. In this context, the present study examined the relationship between the low sanitation of Barra Longa after the disaster and the emergence of new cases of dengue. With this event it was possible to prove how low sanitation is an important element for Aedes aegypti proliferation. Municipal database and Geoprocessing dengue cases points and contamination of water supply were correlated during one year (2015-2016). Until 2015 Barra Longa was one of the cities of Minas Gerais with a lower incidence of dengue fever. However in 2016, after the disaster, the dengue cases was three times higher than in previous years. The work of the epidemiological surveillance in Barra Longa in the last two years have shown that the incidence of dengue has increased as well as the vector Aedes aegypti.
detection and this event was correlated to increased contamination of water supply observed too. Other indicators of low sanitation were also determined in Barra Longa as an increase in the volume of waste in the streets. Municipal database and Geoprocessing dengue cases points and contamination of water supply has been used as the actions of the Contingency Plan in the Barra Longa as an innovative model of action in Epidemiological Vigilance for containment and prevention of dengue. Financial Support: CAPES, CAPES-PNPD, UFOP, UFMG.

Palavras-chaves: Database, Epidemiology, Dengue, Mine dume, Low sanitation

MOLECULAR CHARACTERIZATION OF HEPATITIS B VIRUS IN PATIENTS WITH CHRONIC INFECTION IN GOIÂNIA-GOIÁS

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Resumo

Hepatitis B virus (HBV) infection remains a worldwide concern, despite the availability of an effective vaccine for many years and specific antiviral therapy. HBV genotypes may interfere with treatment response, progression from disease to chronic form, cirrhosis and hepatocellular carcinoma. The aim of the present study was to investigate the socio-demographic, clinical and molecular characteristics of patients with chronic hepatitis B and hepatocarcinoma in Goiânia-Goiás. Blood samples (n = 52) were collected from patients diagnosed with chronic HBV infection who were treated at a referral hospital in Goiânia-Goiás. An interview was conducted with a structured questionnaire on sociodemographic data, possible characteristics associated with the risk of HBV acquisition and previous vaccination against hepatitis B. Other information such as the use of antiviral therapy and the results of complementary tests were collected in the medical record. All samples were submitted to HBV-DNA extraction and a semi-nested PCR was performed using primers complementary to the Pre-S/S (PS1, S2 / S22 and SR) and Pre-C/C regions (X1, C2, X4 and C3) of HBV. After this step, the nucleotide sequencing was performed (S and Pre C/C regions of HBV). The analysis of the results showed that the majority of the patients evaluated reported alcoholic beverage use (n=38/52), irregular condom use (n=38/52), had clinical features such as cirrhosis (n=27/52), chronic HBeAg negative infection (n=47/52) and the use of antiviral therapy (n=27/52). Sequencing of nucleotides was performed successfully in 22 samples, and the genotypes / subgenotypes A1 (n =10/22), A2 (n=01/22), D3 (n =07/22), D2 (n =01/22) and F2 (n=03/22) were found. Furthermore, the analysis of the sequences revealed that among the samples studied, 22 had at least one mutation as T1762, A1764, V207L, Y221F, Q130P, W153R, Y126R, N118T, I91L, K122R, T131N, Y100C, S114T and I68T. The presence of these mutations also has been reported in other studies involving patients with chronic infection. Moreover, the data of the present study ratify the predominant circulation of genotypes A, D and F in Brazil and in our Region.

Palavras-chaves: Hepatitis B, Chronic hepatitis B, Mutations, Genotypes

AVIDITY AND NEUTRALIZING TITLES FOR ANTI-DENV IgG ANTIBODIES FROM SOROLOGICAL SAMPLES OF HYPERENDEMIC REGION.
Dengue fever is a painful, debilitating mosquito-borne disease caused by dengue viruses (DENVs), consisting of four serotypes (DENV 1 to 4). Diagnosis in public health can be done based only on clinical symptoms in endemic areas, and it is a compulsory notification disease. Severe cases have been associated with the presence of non-neutralizing antibodies that can occur in successive heterologous infections. The neutralization capacity in vivo is multifactorial and depends of the antibodies (Abs) avidity, titer and accessibility of the epitopes targeted by Abs against DENV. The avidity of anti-flavivirus monoclonal Abs was shown to positively correlate with neutralization activity in vitro, however, the relation between neutralizing activity in polyclonal samples avidity is still unclear. The goal of this study is to evaluate the viral response and avidity profile of serum samples from Caratinga-MG, a hyperendemic DENV region. DENV positive samples were analyzed in correlation with the avidity, time post infection and the titers of neutralizing antibodies. To verify the DENV virus response, 26 sera (2010 to 2011) collected within three months after the symptoms period, were screened by ELISA IgM/IgG. PRNT was performed to analyze specific response per serotype and neutralizing Abs titer. Avidity was evaluated by a modified Elisa, which includes a chaotropic agent (treated well) or PBS (untreated well) before the reaction with secondary Ab-peroxidase. The avidity rate was calculated by: (% = (chaotropic well OD/PBS well OD)*100). Statistic analyses were performed using GP Prism 6 and Excel. Through IgM/IgG ELISA and PRNT, it was possible to verify the population response for the 4DENV serotypes. These data were corroborated by avidity Elisa analysis, showing that patients with less than 10 days of symptoms already had IgG and high avidity. Through avidity versus time analysis, it was possible to observe that only 3 of the patients which were positive for only one serotype in PRNT, had low avidity within the 3-month period. Here, we also observed a high correlation between avidity and neutralizing titles (p-value =0.0063). The correlation between serum avidity and neutralization capacity, showed greater avidity to a previously infecting DENV serotype as compared to the current infecting DENV serotype in the early phases of infection.

Financial support: CNPq, FAPEMIG, FAPESP, CAPES, DECIT-MS

Palavras-chaves: DENV, Avidity, IgG antibodies, Sorological, Hyperendemic

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**Evaluation of the Hepatitis C Virus Load in platelet-rich plasma and platelet-free plasma**

Resumo
Hepatitis C Virus (HCV) is an RNA virus from the Flaviviridae family associated with Chronic Hepatitis C. The disease treatment has been significant advances with the introduction of the direct acting antivirals (DAA). The use of the DAA in the treatment of the Chronic Hepatitis C has led to sustained virologic response (SVR) in infected patients. However, the use of DAAs has been dependent on the HCV load in plasma from infected patients. The Brazilian Ministry of Health has indicated the Viral Load test using plasma from HCV infected patients. This plasma is obtained from venous blood sample by centrifugation at 1312xg, then, this plasma used to perform the HCV viral load is with platelets (platelet-rich plasma). Recently, studies have been demonstrated that HCV is able to bind human platelets in HCV infected patients and these viruses would be adsorbed to the platelet and not free in plasma. Then, the goal of this study was to compare the HCV load in platelet-rich plasma and platelet-free plasma. Blood total samples were obtained from HCV infected patients and it was centrifuged at 1,312xg for 3 min to obtain platelet-rich plasma. One aliquot from this plasma was centrifuged 1,600xg for 5 min to obtain platelet-free plasma. The HCV load was performed using qRT-PCR. The experiment was performed in quadruplicate. The results suggest that HCV viral load in platelet-rich plasma and platelet-free plasma is no different, despite of the HCV-platelet interaction. The amount of the HCV bound in platelets seems does not interfere with plasma HCV viral load. Other studies have been performed with more samples to infer about the significance of these findings. Financial Support: This study was funded by Ministry of Health, Federal Government of Brazil.

Palavras-chaves: HCV, Plasma, Platelets

ANALYSIS OF THE ZOONOTIC POTENTIAL OF ROTAVIRUS STRAINS OF SPECIES C

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Resumo

Rotaviruses (RVs) are etiological agents of acute diarrhea in several species including birds and mammals. RVs are classified into 10 species, A-J, being species A (RVA) the most studied while the other species are neglected. Its genome consists of 11 segments of double stranded RNA. The segmented genome allows the virus to undergo reassortment, which consists of the exchange of genomic segment(s) between different strains. Reassortment of viruses from different species could lead to the emergence of chimeric strains bearing RNA segments of both parental viruses. It is believed that several RVA strains isolated from humans have emerged from reassortment events between human and animal strains. It is believed that when RVs cross the interspecies barrier they need to adapt to the new host in order to effectively spread among the new host species. However, once an animal strain acquires genome segments from pathogenic human strain, the resultant chimeric virus might have better chance of infecting and spreading efficiently through the human population. That is to say, this mechanism of genetic evolution allows combining the advantages of the replication and infection of the RV strains of human and animal origin for the emergence of strains with the potential to cause zoonotic infections. This study aims to analyze, through genomic sequencing, the occurrence of genomic reassortment in RVC strains. Thus far, 15 RVC strains detected in fecal swine samples from Rio de Janeiro and Minas Gerais were analyzed by sequencing the genes coding for NSP4 and NSP5. Phylogenetic analyzes of NSP4 revealed that 13 out of 15 strains had identity of 99.8-100% between themselves and 85-91.6% with strains of swine. Strains from Rio de Janeiro and Minas Gerais formed a single clade (99.6-100% identity) suggesting a spreading of these viruses to different regions of the country in a period of 5 years. Two strains from Rio de Janeiro had a higher nucleotide and amino acid identity with human reference strains (94.6-98.9% nt, 93.3-98% aa) than with swine strains (67.8-72.4 % nt; 60.7-64.7% aa). Analysis of the NSP5 sequences revealed that all strains showed high identity with swine strains. These results suggest the possibility of occurrence of genomic reassortment of the NSP4 gene, the virus enterotoxin, between RVC strains from humans and swine. The analysis of the other genomic segments will confirm this hypothesis.
**HEPATITIS B VIRUS INFECTION IN INDIGENOUS POPULATION OF THE WESTERN AMAZON, BRAZIL: EPIDEMIOLOGICAL ASPECTS**

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**Resumo**

Viral hepatitis are one of the most important public health problems in the world. They are caused by hepatotropic viruses, the main ones being classified as A, B, C, D and E. The Western Amazon has characteristics of a region of high endemicity, mainly for the virus B. However, there are still few studies involving indigenous populations and due to this lack the epidemiological profile is still not well defined. The present study aims to describe the epidemiological profile of B virus infection in the indigenous population of western Amazonia, through the clinical and serological analysis of each individual. Clinical data were collected from 70 individuals from an indigenous population, attended at the Viral Hepatitis Clinic of the Rondônia Research Center in Tropical Medicine (CEPEM / RO). The present study was approved by the Ethics Committee (Number 1,718,840), where male and female patients aged 18 to 70 years, with or without symptomatology, were included in the study. The 70 indigenous people are distributed among 17 villages, among which are the villages of Rio Negro Ocaia and Aldeia Ricardo Franco and Sagaran, with the largest number of individuals participating in the study, with a total of 17.1% (12/70) and 11.4% (8/70) respectively. Among the 14 ethnic groups represented, the most frequent were Oro Nao with 25.7% (18/70) followed by the Oro Mon ethnic group 15.7% (11/70). The study population is comprised of 32.9% (23/70) female and 67.1% (47/70) males, the majority (52.9%) being over 31 years of age and 37.1% (26/70) present a family history of viral hepatitis. The serum samples collected were subjected to a serological screening using rapid tests (VIKIA®) for detection of the HBsAg antigen, where 62.9% (44/70) were reagents. Subsequently, ELISA assays were performed as a confirmatory test for serological markers of HBV Virus. The distribution of the markers were 71.4% (50/70) subjects HBsAg reagent, 80% (56/70) total anti-HBc reagent, all non-reactive HBeAg, 65.7% (46/70), anti-HBe reagent. As to the symptomatology, only 27.1% (19/70) reported symptoms. Therefore it was possible to confirm the presence of Hepatitis B in the indigenous population, being all patients with chronic hepatitis B, being the majority asymptomatic. They do not present anti-HBC IgM reagent nor evidence of viral replication (HBeAg negative).

**Financial Support:** CAPES / FAPERO

**Palavras-chaves:** Hepatitis B, Epidemiology, Indigenous
Histopathological characterization of Swiss-Webster neonate mice brains infected with different Zika virus strains

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Zika virus (ZIKV) is an arbovirus member of the Flaviviridae family. Although it was first characterized in 1947 in Uganda, ZIKV infection emerged as an international public health concern during the epidemics in Brazil and its spread through the Americas in 2015 and 2016. In this outbreak, viral infection was linked to fetal abnormalities, given the increase in reports of microcephaly and other fetal malformations in areas where pregnant women were exposed to ZIKV. Even though it is now well established that ZIKV can cross the placental barrier and reach the fetus, causing a broad spectrum of neurodevelopmental defects, the mechanisms underlying lesion generation by ZIKV and how they progress in the developing brain are not fully understood. To conceive these questions, many researchers around the world worked on establishing animal models susceptible to ZIKV infection. Different mouse strains and routes of inoculation have been tested, exhibiting variations in reproducibility and applicability. To further characterize the histopathological findings in the developing brain exposed to ZIKV, we analyzed brain tissue from Swiss-Webster neonatal mice infected via intraperitoneally with three ZIKV strains: the mice-adapted neuroviral African strain (MR766) and two clinical isolates from the epidemics in Brazil - one from the state of Pernambuco (ZIKV-PE) and other from Espírito Santo (ZIKV-AB). As expected, MR766 was highly virulent, as all infected pups died between 5 and 6 days post-infection, showing serious body growth impairment and intense brain inflammation, as seen in tissue slices. When compared to MR766, clinical onset and time-to-death was delayed in animals infected with the Brazilian ZIKV strains, however, both ZIKV-AB and PE generated severe clinical outcomes, such as seizures, hind limb paralysis and imbalance. Survival curves were also similar, as all subjects died from 14 to 25 days post-infection. Their brains also displayed critical lesions, including areas of calcification, edema, and neuronal death. Moreover, neurological damage in neonates infected with ZIKV-AB or PE was observed even when viral titer was no longer detectable by plaque assay. To better establish this neonatal murine model of ZIKV infection, we will compare these murine brain samples with human tissues obtained from cases of congenital ZIKV syndrome after the detailed histopathological characterization is completed.

Palavras-chaves: Zika virus, mouse, histopathology, neuropathology, animal model

Genomic surveillance of Zika virus transmission in the Amazonas State, Brazil.

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Resumo

Zika virus (ZIKV) has caused an unprecedented epidemic linked to severe congenital syndromes in the Americas. ZIKV was first confirmed in May 2015 in northeast Brazil although it was likely introduced at least 12 months before its detection. Manaus, the capital city of the Amazonas State, the largest territory of any state in Brazil and the main economic center in the northern region, reported between 2016-2017 more than 2,327 suspected cases of ZIKV infection. To gain insights into the timing, source, and the routes of ZIKV introduction in the Brazilian Amazon, we sequenced and analyzed novel ZIKV whole genome sequences from Manaus. Using portable nanopore sequencing we generated 56 Brazilian ZIKV complete genomes from Manaus. On the basis of available sequences of isolates from the Americas, most Manaus isolates fell within a single strongly supported monophyletic clade (bootstrap support = 99%, posterior support = 1.00) that comprised isolates from 2015 to 2017. Molecular dating analysis indicates that the outbreak was caused by a single founder strain introduced in Manaus in or around February 2015. Spatial analysis of genetic data further indicates that the northern neighbourhoods of the Manaus regions acted as a source location for virus spread within the municipality. Our work illustrates that genomic surveillance in the field can augment traditional approaches to infectious disease surveillance and control. Our data indicates the persistence of the virus in Manaus across a period of 2 years, consistent with year-round transmission of arboviruses in the region. Our spatiotemporal analyses recover the hotspots of ZIKV transmission within a single city and provide a better understanding of ZIKV transmission ZIKV in new geographic regions.

Palavras-chaves: ZIKV, Genomic Surveillance, MinION

Molecular Detection of Arbovirus in Human Samples Collected in Central Brazil

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Arboviruses (arthropod-borne viruses) are an ecological group of viruses transmitted mainly by hematophagous mosquitoes, involving a complex cycle. Consisting of viruses of medical importance, its main representatives belong to the genus Flavivirus. It is noteworthy that in several tropical and subtropical countries, including Brazil, arboviruses have emerged and represent one of the main public health problems because they cause constant epidemics and outbreaks. In addition, the constant epidemics increase the number of people who seek medical care and cause overload in the public health system, resulting in high economic costs for the affected countries. Identification of genetic material of important non-dengue South American arboviruses from samples of individuals suspected of dengue, but later discarded by laboratory methods. After approval of the ethics committee on research involving human subjects (Nº seim 1.101.056), the samples from Goiânia were screened in the Virology Laboratory of the Federal University of Goiás. For this purpose we used multiplex-nested-RT-PCR. Thus, primers for Mayaro, equine Encephalitis east/west and venezuelan, Chikungunya, Yellow fever, West Nile, Encephalitis Saint Louis, Aura and Ilhéus viruses were used during the reactions. After molecular assay carried out on 52 samples, two Yellow Fever virus infections were identified in individuals aged 13 and 47 years. Confirmation was performed through nucleotide sequencing. The main clinical findings of the patients were fever, myalgia, prostration, arthralgia and abdominal pain, common to both patients. However, other signs and symptoms were present as headache, retro-orbital pain, nausea, vomiting, diarrhea, dizziness, pruritus and eyelid edema (50%). These cases were reported as dengue by the health system, because at the sampling period an epidemic of dengue occurred in the city. By these facts, it is necessary to a differential diagnosis between dengue and yellow fever, mainly due to the higher mortality rate caused by the YFV. This study points cases of retrospective yellow fever infection that are underreported. Further surveillance studies are needed, since other non-dengue flaviviruses may cocircular in overlapping geographic areas. 

Financial support: Fundação de Amparo a Pesquisa do Estado de Goiás, Universal 005/2012.

Palavras-chaves: arboviruses, yellow fever virus, dengue virus, RT-PCR

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**HIV INFECTION SUSCEPTIBILITY: POSSIBLE INVOLVEMENT OF FOXP3 -3279 A/C**

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**Resumo**

The FOXP3 gene, located on the X chromosome, encodes a transcription factor that determines the identity, developmental rule, maintenance and function of regulatory T cells. The protein of this gene is able to activate a transcription of other proteins that characterize the processes that, similarly, exert their function of modulation of the immune system. The polymorphisms in the FOXP3 gene can change, quantitatively or functionally, the FOXP3 factor, causing a dysfunction of Tregs cells and consequently cause the development of autoimmune diseases. Polymorphisms, such as rs3761548 on the FOXP3 show characteristics of susceptibility to autoimmune diseases, allergies, and infertility. In HIV infection, the genetic variants have not yet been evaluated. A total of 356 HIV + and 325 HIV- (control groups) were selected, of which 472 are of Euro-descendant origin and 154 Afro-descendants, 360 are female and 269 are male. Mean age HIV- 36.9 ± 12.2 and HIV + 40.8 ± 10.2. These
polymorphisms were genotyped using TaqMan probes by qPCR StepOne® - Applied Biosystems®. Frequencies allelic and genotypical were estimated between groups by 2x2 contingency table for Fisher’s exact test. For significance analyze we used chi-square, odds ratio using Woolf’s approximation. These frequencies showed a significant difference between HIV+ and controls ($p = 0.0739$). These results can be indicating a possible association of susceptibility to HIV infection. Moreover, other analyze more robust is necessary to confirm this hypothesis.

Financial Support: FAPERGS funding agencies, Capes, Feevale.

Palavras-chaves: FOXP3, HIV, SNP, Susceptibility, Tregs

INVESTIGATION OF NATURAL POLYMORPHISMS OF RESISTANCE IN THE REGION NS3 IN PATIENTS WITH CHRONIC HEPATITIS C IN THE STATE OF PARÁ, BRAZIL.

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Resumo

Introduction: The development of new drugs, called collectively antiviral agents of direct action or direct acting antivirals (DAAs), created new perspectives for the treatment of chronic hepatitis C, with an increase in the rates of sustained virologic response (SVR) in patients. Several studies with DAAs also revealed the presence of variants of amino acids associated with viral resistance or resistance associated amino acid variants (RAVs) that are naturally produced during the life cycle of the hepatitis C virus (HCV). Objective: To Determine the prevalence of polymorphisms of the natural resistance to inhibitors of protease NS3 in patients with chronic hepatitis C in the state of Pará, in the period February 2015 to December 2016. Material and methods: Molecular analysis was performed in a total of 32 samples of serum/plasma pre-treatment, selected patients diagnosed with chronic infection by HCV (genotype 1). Total ARN was extracted from the samples. After extraction of ARN was carried out the step of RT-PCR and the first and second amplification of the region NS3, with the use of primers specific for each subtype viral (1a and 1b). Subsequently, reactions of sequencing nucleotidic were developed by the method of Sanger, in both directions of the region NS3, aiming to identify RAVs. Results: 13 positions that have RAVs clinically important were analyzed. In positions 122, 132, and 170 has been identified substitutions S122T, V132I and V170I, with the prevalence of 3.1% (1/32), 15.6% (5/32) and 6.2% (2/32), respectively. These mutations were identified in subtype 1b. The replacements V132I and V170I, are not associated with resistance to inhibitors of protease NS3. The replacement S122T consists of a variant associated with resistance to protease inhibitors Simeprevir. The prevalence of natural this RAV was 3.1% (1/32), representing the prevalence of natural global RAVs of the present study. Conclusion: The molecular analysis identified substitutions of amino acids associated with and not associated with the resistance of HCV to protease inhibitors NS3. The identification of the mutation of resistance to Simeprevir (S122T), showed the natural presence of RAVs (3,1%) in our study.

Palavras-chaves: hepatitis C, viral Resistance, polymorphisms
ANALYSIS OF CCR5Δ32 (RS333) IN INFLUENZA A(H1N1)PDM09 CASES FROM THE BRAZILIAN NORTHEAST, SOUTHEAST AND SOUTH.

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Resumo
Introduction: About 500,000 people die annually worldwide due to influenza infection, caused by a virus that is highly contagious. It mainly affects children, the elderly and immunocompromised individuals. In the 2009 pandemic, healthy young people were mostly affected by severe influenza. Therefore, there is a need to investigate host factors that may be associated with disease severity. A 32 bp deletion in the CCR5 chemokine receptor (CCR5Δ32) causes loss of receptor function. The correlation of this mutation and severe influenza is still controversial. Our objective is to investigate CCR5Δ32 in influenza positive cases from Brazilian population and its correlation with severity of disease. Methodology: We investigated 432 influenza A(H1N1)Pdm09 positive clinical samples (nasopharyngeal swabs or aspirates and postmortem specimens), collected from 2012 to 2018 in different Brazilian regions: northeast, southeast and south. Patients were classified according to their clinical status, into the following groups: influenza like illness (ILI) (n=153); severe acute respiratory infection (SARI) (n=173) and fatal (n=106) cases. Genomic DNA was extracted from the clinical samples, followed by PCR and gel electrophoresis. In gel analysis, wild-type (WT) homozygosity (WT/WT) was detected as one band (178 bp); heterozygosity (WT/Δ32) was detected as two bands (178 and 146 bp), and homozygosity for the deletion (Δ32/Δ32) would present a unique band (146 bp). This last genotype was not detected. A subset of samples was Sanger sequenced (n = 273) to confirm the electrophoresis profile. Results: There was no difference in the distribution of genotypes (WT/WT and WT/Δ32) and CCR5Δ32 allele frequencies among ILI, SARI and fatal cases. Additionally, there was no association between distribution of CCR5Δ32 different genotypes and clinical factors related to clinical severity, such as dyspnea, O₂ saturation

Palavras-chaves: Biomarkers, CCR5, Influenza, SNP

THE RESPONSE OF NLRP3 AND AIM2 IN INFLAMMASOME ACTIVATION AND THIS IMPLICATIONS IN FATAL ZIKV MICROCEPHALY CASES

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Resumo
Zika virus (ZIKV) is an arbovirus belonging to the family Flaviviridae and genus Flavivirus. It is mainly transmitted through the bite of an infected Aedes aegypti mosquito, but also through blood transfusion, vertical transmission, and sexual contact. ZIKV was initially isolated in 1947 from the serum of a febrile sentinel rhesus monkey (Macaca mulatta) in Zika Forest, Uganda. Inflammasomes, are multi-protein complexes that induce inflammation and pyroptosis, are predicted to contribute to the immune response to this flavivirus. In situ immune responses are not yet understood, but inflammasome activation in the neural parenchyma is hypothesized to play a role in the development
of microcephaly in response to ZIKV infection. Brain tissue samples were collected from eight babies, including four ZIKV-positive microcephalic neonates who died after birth and four flavivirus-negative neonatal controls who died of other causes and whose central nervous system (CNS) architecture was preserved. The expression of the receptors (AIM2 and NLRP3) in situ was based biotin-streptavidin-peroxidase method. Statistical analysis was performed in GraphPad Prism 5.0 using student t test. The quantitative analysis of AIM2 and NLRP3 in situ revealed that immunolabeling was predominant in neurons, glia cells, and astrocytes in ZIKV microcephaly cases compared to control. Recognition ZIKV pathogen-associated molecular patterns can induce the activation AIM2 and NLRP3 and consequently the production of caspase 1, thereby promoting the cleavage of pro-IL-1β, pro-IL-18, and pro-IL-33 and the conversion of these cytokines to their bioactive forms. At last this relation can aggravate the neuroinflammatory process and the pyroptosis in ZIKV microcephaly cases.

Financial support: Ministry of Science, Technology and Innovation/National Council for Scientific and Technological Development.

Palavras-chaves: AIM2, Inflammasome, Microcephaly, NLRP3, ZIKV

Molecular detection of Chikungunya virus in urine samples of patients with acute infection in Brazil

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Resumo

The Chikungunya virus (CHIKV) is a re-emerging arbovirus 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. The symptoms associated with CHIKV include fever, chills, nausea and vomiting, swollen glands and above all, it is characterized by severe joint pain (arthralgia). The laboratory diagnosis of CHIKV infection, established by the Ministry of Health, can be performed directly, through viral isolation and identification of viral RNA, or indirectly through the analysis of specific antibodies. Recently, studies showed that viral RNA can be identified in the urine, but no attempt has yet been made to isolate this virus from this clinical specimen. Flavivirus such as Zika virus (ZIKV) and Yellow Fever virus (YFV) can be detected in the urine for more than 10 days after the onset of acute infection. Nevertheless, diagnosis is not regularly conducted using urine samples. In this context, our objective was to detect CHIKV genomic RNA in urine samples by means of real-time PCR (qRT-PCR). In addition, we also attempted to isolate CHIKV from urine samples. We used qRT-PCR and ELISA to analyse a total of 50 serum and 50 urine samples from patients, with an acute clinical presentation suggestive of CHIKV infection. Forty-two (84%) of the total serum samples were ELISA and qRT-PCR positive. Of the patients that were CHIKV seropositive, only four (8%) were positive in the urine by qRT-PCR, with cycle threshold values (Ct) from 30.9 to 36.8. All urine samples were used for viral isolation attempts but only one urine sample looks promising. In conclusion, our findings confirm the importance of serum for the direct diagnosis of CHIKV and that the virus can be identified in the urine, but the low viruria appears to make CHIKV isolation hard in cell culture hard.
**Resumo**

Oropouche virus (OROV) is the most prevalent Orthobunyavirus in Brazil. OROV was isolated for the first time in 1955 in Vega de Oropouche, Trinidad and Tobago. Since 1960s, virus was responsible for outbreaks in Trinidad and Tobago, Panama and South America. In Brazil, the first outbreak caused by OROV was in Belém, Pará. Outside the North region, the virus caused only sporadic cases of infection.

The present study reports the circulation of OROV or OROV-like infection in Ji-Paraná, Rondônia state. Serum samples of patients presenting dengue symptoms were collected in 2013 and screened for the presence of most common arboviruses in Brazil. Viral RNA was extracted with QiAmp Viral RNA Mini Kit and tested for the presence Flavivirus and Alphavirus by RT-PCR that were likely to be causing disease in the area. Additionally, samples were screened for OROV virus. Positive samples were sequenced in ABI377 automated sequencer and aligned using BLAST. We were able to perform phylogenetic analysis with an amplicon of 500 bp of S protein in 13 samples that were positive for OROV. The cladogram containing our samples resembles previous phylogenetic studies with this virus. The likely migration route for ours strains is from Acre, since the closest nucleotide sequence related to ours belongs to an OROV isolated in the state. Although the S protein is not the best choice for the phylogenetic analysis or detection of OROV, due to reassortment with other orthobunyaviruses such as Madre de Dios and Iquitos viruses, we were able to demonstrate that an Orthobunyavirus was causing febrile illness in a city from Rondônia State. However, due to previous circulation of OROV in the area, we believe that febrile illness in Ji-Paraná was caused the virus.

Financial Support: Brazilian Ministry of Health, PADC/FCF – UNESP and CAPES.

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Palavras-chaves: Orthobunyavirus, Phylogenetic analysis, Rondônia
INVESTIGATION OF ZIKA AND CHIKUNGUNYA VIRUS IN SERUM AND URINE SAMPLES FROM PATIENTS ATTENDED AT A UNIVERSITY HOSPITAL FROM 2015 TO 2017

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Resumo

In the last years, infections with the Zika (ZIKV) and Chikungunya (CHIKV) viruses have left the country on alert. In 2016, the WHO declared the infection by ZIKV a public health emergency of international importance, given the various severe clinical manifestations reported, such as congenital malformations and neuropathies. However, the signs and symptoms of these arboviruses are similar, which makes clinical diagnosis difficult. Therefore, the laboratory diagnosis has great importance in the identification of the etiological agent. The aim of this study was to evaluate the occurrence of ZIKV and CHIKV infections in patients with symptoms suggestive of arbovirus infection assisted in the University Hospital (HUCFF/UFRJ) from 2015 to 2017. We evaluated serum and urine samples from 235 patients. The samples were collected in different follow-up periods, totaling 824 clinical samples (412 serum and 412 urine). All samples were submitted to RNA extraction, real-time Reverse Transcription-PCR (real-time RT-PCR) for ZIKV and CHIKV RNA detection. We considered positive sample for ZIKV and/or CHIKV when at least one of the serum and/or urine samples had been amplified for ZIKV and/or CHIKV. Of the 235 patients assisted, 38 (16.2%) were positive ZIKV, 39 (16.6%) were positive CHIKV and 30 (12.8%) amplified for ZIKV and CHIKV, characterizing co-infection. Considering the serum and urine samples analyzed, we observed that amplification of the CHIKV cDNA occurred in only in the serum samples of 25 patients, in urine samples occurred in 7 patients and in 7 others had amplification in both samples. In the evaluation of amplification for ZIKV, 14 patients were positive in serum samples, 9 in urine samples and 15 were positive in both samples. Of the cases of co-infection found, 8 patients were positive only in the serum samples, 2 in the urine samples and 20 were positive in serum and/or urine samples. In this study, we identified a greater number of cases of co-infection between ZIKV and CHIKV when we analyzed both samples. In addition, we observed that the number of urine samples with amplification for ZIKV was higher than in the cases of CHIKV infection, indicating a great importance of collecting both samples, thus increasing the detection sensitivity of ZIKV in suspect cases. However, in the cases identified for CHIKV, we observed that the serum sample can be determined as the one of choice for the early diagnosis by real-time RT-PCR.

Palavras-chaves: Zika virus, Chikungunya virus, molecular diagnosis, early diagnosis
TLR9 GENE POLYMORPHISM AND SUSCEPTIBILITY TO HERPES SIMPLEX VIRUS TYPE 1 AND 2 INFECTION AMONG PREGNANT WOMEN IN A SOUTHERNMOST POPULATION OF BRAZIL

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Resumo
Toll-like receptors (TLRs) are important innate immunity regulators that can be activated upon recognition of bacterial and viral ligands known as pathogen-associated molecular patterns (PAMPs). These receptors are involved in several immune functions, such as the protection of the female reproductive tract against invading pathogens, especially those that cause sexually transmitted infections. TLR9 is localized intracellularly, mostly in the endocytic compartments and recognizes unmethylated CpG motifs present in bacteria and virus. Genetic polymorphisms in TLRs are involved in susceptibility or resistance to infection. Single nucleotide polymorphisms (SNP) −1237T/C (rs5743836), located in the promoter region of TLR9, have been correlated with infection. The aim of research was evaluate the role of SNP rs5743836 variant of TLR9 in the development of Herpes simplex Virus (HSV) 1 and/or 2 infection among a Brazilian pregnant women group. Placental tissue samples from 225 women (maternal-side) collected at the obstetric ward of a University Hospital were studied. Human and viral Genomic DNA was extracted from placental tissue and HSV-1 and HSV-2 were detected by means of nested PCR. Genotypes in TLR9 SNPs were estimated by PCR amplification of bi-directional specific alleles (Bi-PASA), and were confirmed by direct DNA sequencing. From all placental samples, 100 of them were positive for HSV 1 and/or 2, and 125 negative for such viral infections, which were considered as pregnancy control samples. Similar distribution of alleles and genotypes in TLR9 SNP were observed between HSV infected placental tissues and uninfected ones. The allele C of the rs5743836 variant was correlated with an increased risk of the viral infection, however, the difference were no statistically significant (OR 1.41, 95% CI 0.8–2.4; p=0.26). The role of −1237T/C polymorphism in Toll-like receptors 9 and the interactions between them in HSV infection needs further evaluation.

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Palavras-chaves: Toll, PAMP, Pregnancy, sexually transmitted infections

Serodiagnosis of symptomatic patients for Zika Virus in the city of São Paulo

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Resumo

Zika virus (ZIKV) is an emergent arbovirus that belongs to the Flaviviridae family and it is transmitted by Aedes spp mosquito, as well as Dengue, Chikungunya and Yellow Fever viruses. ZIKV was isolated in Rhesus monkeys in the Zika Forest, Uganda, in 1947. The first ZIKV outbreak took place in 2007 on Yap Island in Micronesia. However, it was only in May 2015 that Brazil encountered the emergence of the ZIKV in the northeast of the country. ZIKV has spread to the Americas since then, with Brazil being the most affected country at the time. The Brazilian Ministry of Health reported a greater number of cases of microcephaly, mainly in the northeast region of the country. The clinical picture of ZIKV infection is similar to that of Dengue and Chikungunya, manifesting fever, headache, myalgia, arthralgia and rashes. Due to the symptoms, specific diagnosis for each of the arboviruses may be impaired. The diagnosis is made mainly through molecular methods, mainly by real-time PCR, for its sensitivity and specificity. However, antibody detection is an extremely important tool for understanding the history of the disease in the country, as well as a Zika virus infection that has a short viral period and several asymptomatic patients without notification. Our laboratory received from January 2016 to April 2018, 239 samples of blood, cerebrospinal fluid or oral swab from patients suspected of Zika virus in the city of São Paulo. Of these total, 228 were tested by real-time PCR (qPCR) for 4 arboviruses: Zika virus, Dengue virus (DENV), Chikungunya virus (CHIKV) and Yellow Fever virus (YFV). In addition, of the 239 samples, 67 were submitted to qualitative serology for the presence of anti-ZIKV IgG antibodies. Of the 228 samples tested by qPCR, 211 were negative for all the viruses (92.5%), 3 were positive for DENV (1.3%), 6 for CHIKV (2.6%), 2 for YFV (1.0%) and 6 for ZIKV (2.6%). In serology, of the 67 samples tested, 52 were negative (78%) and 15 positive (22%). Out of the 15 positive samples for ZIKV, 9 were negative for the 4 arboviruses tested by qPCR, 3 were positive for ZIKV and 3 were not tested by qPCR. These data trace an epidemiological profile and help mapping these diseases in the largest city in Brazil, revealing an anti-ZIKV IgG seroprevalence of 22% of the suspected ZIKV samples.

Financial Support: FAPESP, Cnpq, CAPES

Palavras-chaves: Diagnosis, qPCR, Serology, Zika

Molecular characterization of circulating dengue virus in Amazonas (2011-2016)

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Resumo

Dengue is considered the most important arbovirus that affects humans. During 2017 in Brazil, 252,054 probable cases of dengue were reported, of which, 141 resulted in death. In the same year, almost 4,000 cases were registered in Amazonas, leading this state to the third position in the number of reported cases in the northern Brazilian region. Despite the incidence data, there is little information about the serotypes/genotypes circulating in the North region. Thus, this study aimed to
characterize DENV isolates that circulated in the Amazonas state, between 2011 and 2016. We analyzed 608 DENV suspected samples from 26 municipalities of Amazonas. The Viral RNA extraction was performed using a commercial kit according to the manufacturer's instructions. Firstly, samples were submitted to RT-qPCR to confirm the presence of DENV, followed by a semi-nested PCR for serotyping. Among the analyzed samples, 80 were positive for DENV-4: Boa Vista do Ramos (2015); Borba (2013 and 2014); Itacoatiara (2014); Manacapuru (2013); Manaus (2011-2016); Maúes (2013 and 2015); Novo Airão (2013); Parintins (2015); São Gabriel da Cachoeira (2014); Tefé (2013, 2014 and 2015); nine for DENV-2: Manaus (2011 and 2016), Novo Airão (2013); Tefé (2014); and seven for DENV-1: Manaus (2011, 2015 and 2016) and Parintins (2015). Genotyping was conducted by phylogenetic analyzes using the maximum likelihood (ML) method implemented in the PhyML (3.0) software. Until now, 29 samples were sequenced and analyzed, one DENV-1 genotype V, collected in Manaus (2016), one DENV-2, Asian/American genotype also from Manaus (2011) and twenty-seven DENV-4, genotype II, collected in six municipalities (Itacoatiara, Manaus, Maúes, Novo Airão, São Gabriel da Cachoeira and Tefé). An overall similarity of 99-100%, with 140 variable sites for nucleotides, and eight for amino acids was observed for the DENV-4 sequences. We also conducted a phylogeographic analysis with BEAST (v1.10.1) software that supported at least two DENV-4 independent introductions in the Amazonas state. Furthermore, our data also showed the DENV-4 spread beginning from Manaus to the other municipalities inside the Amazonas state. This study contributes to strengthening the arboviral surveillance in the Amazonas state, reporting not only the DENV serotypes and genotypes from different municipalities but how the spread of DENV-4 GII occurred in this Brazilian state. Financial support: CNPq, CAPES, FAPEAM, ILMD.

Palavras-chaves: Amazonas, Dengue, Genotypes

IMIDAZOLIUM AND PHOSPHONIUN-BASED SALTS AS ANTIVIRAL AGENTS: IN VITRO CYTOTOXICITY, ANTIVIRAL ACTIVITY AND IN SILICO STUDIES AGAINST MAYARO VIRUS

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Resumo

The Mayaro virus (MAYV) is an arbovirus and presents structural similarity with the Chikungunya virus, co-infection with Dengue and, thus, the possibility of triggering an epidemic. Currently, there is no approved drug for the treatment of viral infections caused by this etiological agent. Based on literature, imidazolium (IS) and phosphonium salts (PS) are of great interest due to theirs satisfactory chemical properties and express constituents that can be used to improve physicochemical properties and interact with enzymes and receptors. This study aimed to evaluate four imidazolium salts (IS01-IS04) and two phosphonium salts (PS01-PS02) against MAYV using in silico tools, in vitro cytotoxicity and antiviral activity in primary screening. Initially, to perform cell viability, cell cultures were exposed to serial dilutions at concentrations 0.312 to 40 µM and a control group with cells without IS and PS exposure were considered as 100% viable cells. After the incubation period, the cytotoxicity of compounds was measured through the neutral red uptake assay in Vero cells. Then, the potential antiviral effect of the compounds were performed by the virus plaque-reduction assay in Vero cell lines infected with MAYV. In addition, ADMET prediction were investigated in ADMETlab and DataWarrior. The molecular docking between the compounds and C Protein of MAYV were performed in AutoDock Vina. In view of the preliminary results, compounds express low cytotoxicity, considering toxicity indices below 50% of cell viability. In the antiviral assay, the PS01 showed significant reduction - about 47.8% - in the number of plaques in its lower concentration when compared to the viral control. The other compounds showed moderate antiviral activity when compared to viral control inhibiting about 20% of viral replication. Docking analyzes revealed the most favorable bind free energy at -5.4 kcal/mol, -5.1 kcal/mol and -4.6 kcal/mol for IS04, IS01 and PS01, respectively. ADMET prediction characterized the compound IS01 with the most suitable pharmacokinetic and toxicological profile followed by PS01 and PS02. Based on results of cytotoxic, antiviral activity and in silico analysis against MAYV, the present findings associated with further in vitro studies indicate the compounds tested are subject to structural optimization to become potential antiviral drug
Evaluation of IgG anti-Tax in HTLV-1 carriers in a longitudinal study from GIPH cohort

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Resumo

The Tax protein plays a major role in the pathogenesis of HTLV-1, interacting with many host proteins that regulates viral and cellular expression of genes interfering in apoptosis, cellular proliferation, immune response and inflammation. Tax is the major protein recognized by CTL from HTLV-1 carriers and along with its antibodies, is involved in HAM/TSP pathogenesis, by promoting cellular proliferation and neuronal damage. Higher HTLV-1 proviral load (PVL) levels are suggestive of increasing the risk for HTLV-1 associated diseases. However, several asymptomatic carriers (AC) have remained with high PVL levels for long period of follow-up by GIPH cohort (Minas Gerais State, Brazil) without developing HAM/TSP. Thus, it is necessary to identify new markers for risk of developing neurological symptoms. Once AC individuals are a heterogeneous and complex group and in order to advance in the characterization of a useful HAM/TSP risk marker, the present work aimed to prospectively analysis anti-Tax IgG levels in sera/plasma from AC and HAM patients using an Indirect ELISA performed with recombinant C-Terminal Tax. We enrolled 18 AC (9 with high PVL [>1%]; 9 with low PVL)

Palavras-chaves: HTLV-1, Tax, Prognostic, ELISA
Resumo

Bats are recognized as reservoirs of a variety of viruses, including well-characterized zoonotic pathogens like Rabies lyssavirus (RABV). Their biological and ecological behaviors support the environmental maintenance and dispersion of viruses among them and to other animals. The increasing human-bat contact, following the anthropogenic change of wild habitats, alert to viral surveillance to identify pathogens with zoonotic potential. Although viral diversity in these animals remains largely unknown, more recent studies using high throughput sequencing (HTS) helps improving the identification of viruses in biological samples. The aim of this study was the identification of circular DNA viruses through HTS analyses. Six lungs of Glossophaga soricina and twenty lungs of Molossus molossus were obtained from animals sent to rabies diagnostic. The samples were processed to isolate encapsidated DNA in two pools, one for each species. The viral enrichment were performed with phi29 DNA polymerase and amplicons were pair-end sequenced using Illumina MiSeq. The reads were DE NOVO assembled with SPAdes assembler and overlapping regions were identified with PRICE. Sequences generated were identified by NCBI Blastn and Blastx tools. In Molossus molossus lungs two complete genomes of anellovirus (2387 and 2305 nt long) related to Torque teno Tadarida brasiliensis virus, were identified. Still in these samples, a complete genome of a circovirus (1878 nt long) was identified, belonging to the recently suggested new genus Krikovirus. In Glossophaga soricina a 1233 nt long sequence related to ORF1 from Torque teno desmodus rotundus virus was identified. In addition, various sequences related to Bovine papillomavirus type 8 were identified, which are still under analysis. These results help to understand the viral diversity in these bats and reinforce the importance of viral monitoring to detected potential zoonotic viruses present in the environment.

Financial support: FAPESP (Processo 2015/25367-0) e CNPq

Palavras-chaves: Bats, Circular DNA viruses, High throughput sequencing

CARDIOPULMONARY SYNDROME BY HANTAVIRUS (CSBH): Case report

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Resumo

The Hantavirus is a zoonosis transmitted by a RNA virus from the Bunyaviridae family; it is a rare disease withonly a few reported cases in Brazil until now. It is transmitted by the inhalation of aerosols released by the excrement of contaminated wild rodents. The risk population is composed mainly by men, previously healthy, involved in farming activities in rural area. The lesion of the disease occurs through the immunological response of the host, which results in the classical conditions of early leukocytosis, pulmonary infiltration by TDCD8 lymphocytes, thrombocytopenia and hematocrit reduction. Due to the unspecific symptoms and the lack of knowledge by professionals, the diagnosis is late most of the time, which contributes with the fast evolution of the disease into the cardiopulmonary phase. Goal: to report a case of Cardiopulmonary Syndrome by Hantavirus in a previously healthy patient. Case Description: Male patient, 35 years old, salesman of farming products, from Tapejara/RS, presented weakness in the lower limbs, fever and dry cough; the symptoms initiated about five days prior to the search for medical assistance. The diagnosis hypothesis was of bacterial pneumonia, and the treatment began with azithromycin and ceftriaxone. After 5 days of treatment he was admitted into the HSPV Hospital in Passo Fundo since the symptoms persisted. During the hospital stay, he presented regular general state. Vital signs PA 96/67 mmHg, FC 123 bpm, FR 23 mm, temperature 37.3 °C, SaO2 87%. Laboratory exams: HT 54,1%, Leukocytes:25.520 cells/µL and IgM for hantavirus: reagent (result obtained only after the patient's death). Chest radiography: pulmonary infiltration bilateral diffuse, opacities in mat glass and smooth interlobular thickening. After 5 days of treatment he was admitted into the HSPV Hospital in Passo Fundo since the symptoms persisted. During the hospital stay, he presented regular general state. Vital signs PA 96/67 mmHg, FC 123 bpm, FR 23 mm, temperature 37.3 °C, SaO2 87%. Laboratory exams: HT 54,1%, Leukocytes:25.520 cells/µL and IgM for hantavirus: reagent (result obtained only after the patient's death). Chest radiography: pulmonary infiltration bilateral diffuse, opacities in mat glass and smooth interlobular thickening. The patient evolved into a progressive apnea with significant decrease in O2 saturation, being taken to the ICU, where he was sedated with analgesia, antimicrobial drug with the use of norepinephrine and antiarrhythmics, evolving on the second day to a condition of SARA with cyanotic extremities. PAM: 61
mmHg, body temperature: 40 °C, FR:163 bpm, consistent with the condition of shock. It evolved into asystole, resuscitation maneuvers were performed according to ACLS for 20 minutes, without return of the circulation and the death was verified.

**Conclusion:** The early diagnosis of the infection by hantavirus is fundamental for the prognosis of the disease.

Financial Support by the authors.

**Palavras-chaves:** Hantavirus, zoonosis, rodents

**Serum levels of HspBp1 and anti-HspBP1 as a predictor for HIV progression**

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**Resumo**
The heat shock protein 70 (Hsp70) is a molecular chaperone with several biological functions. The Hsp70 binding protein-1 (HspBP1) is a co-chaperone that inhibits the Hsp70 activity. Previous studies have shown that HIV-infected individuals present high levels of circulation anti-HspBP1 IgG compared to uninfected-HIV subjects. This observation is corroborated by the fact that 53 % of HIV negative individuals lack anti-HspBP1, however only 24.3% of HIV-infected lack these antibodies. Thus, the aim of this research was to quantify the levels of circulating HspBP1 and IgG anti-HspBP1 in HIV-infected individuals and to correlate them with T CD4 counts and viral load, as well as to determine the kinetics of those proteins during acute HIV infection. For this, sixty serum samples from HIV+ outpatients, thirty with high viral load and thirty with low viral load, were analyzed. The HspBP1 was quantified by capture ELISA and anti-HspBP1 by direct ELISA. To investigate the kinetic of HspBP1 and anti-HspBP1 during the acute phase, these proteins were quantified in a commercial seroconverting HIV curve. All dosages were also compared according to the CD4 and CD8 T cell counts. The results showed that the mean of blood circulating HspBP1 found in HIV-infected outpatients with high viral load (5.44 ng/mL ± 0.60) was significantly higher compared to HIV negative individuals (2.54 ng/mL ± 0.66). Moreover, the levels of anti-HspBP1 IgG also increased in HIV-infected outpatients with high viral load (optical density = 0.257 ± 0.007), compared to HIV+ with low viral load (D.O = 0.178 ± 0.016) or HIV negative individuals (D. O. = 0.160 ± 0.019). When the systemic concentrations of HspBP1 and anti-HspBP1 IgG were analyzed according to the number of CD4+ T cells, it was observed that the levels of HspBP1 were higher in individuals with

Financial Support: CNPq, UFCSPA

**Palavras-chaves:** HIV, HspBp1, Prognostic

**EVALUATION OF ADHERENCE TO ANTIRETROVIRAL THERAPY IN HIV-POSITIVE PATIENTS UNDER DIFFERENT THERAPEUTIC DIAGRAMS**

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Resumo

Currently in Brazil, HIV-positive patients, regardless of their viral load and CD4 + T cell counts, are able to adhere to antiretroviral treatment immediately upon diagnosis. The Ministry of Health recommends that the initial treatment should preferably be composed of two nucleoside reverse transcriptase inhibitors together with an integrase inhibitor, Dolutegravir being the most used, except for women in the fertile period. This amendment aims to increase adherence levels, yet numerous therapeutic regimens have been administered, which have been related to different acute and chronic adverse effects. The objective of this work is to establish a possible relationship between therapeutic regimen and levels of adherence to treatment. A total of 59 records of patients from Porto Alegre were analyzed, with the first withdrawal of drugs registered in the SICLOM system between January 2015 and December 2017. Adherence was classified as: sufficient adhesion for the drug dispensation with a delay of up to six days last withdrawal of the antiretrovirals, insufficient for more than six days and abandonment to more than 100 days of delay. Four therapeutic combinations were observed among patients, of whom 11 used protease inhibitor schemes, 41 used non-nucleoside and 7 integrase inhibitor. Of the 59 patients, 16 made unjustified therapeutic exchanges, without genotyping examination or reason described in the clinical file. There was no significant association between the type of therapy and the level of adherence. However, there was a higher percentage of non-nucleoside users among patients with insufficient adherence or abandonment (73%), which corroborates previous findings that associate this class of drug with different adverse effects, and consequently lower adherence levels. It should be emphasized that this study is in the initial phase, and it is intended to increase the sample number for 500 patients, which may demonstrate more clearly the existence or not of correlation between the type of drug and the improvement of the adherence.

Finacial support: Feevale, Fapergs, Capes

Palavras-chaves: adherence, antiretroviral therapy, hiv, non-nucleoside, therapeutic diagrams

SEMEN OF ZIKA VIRUS INFECTED INDIVIDUALS: SHEDDING PATTERN AND CYTOKINE AND CHEMOKINE PROFILES.

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Resumo

Zika virus (ZIKV) is an emerging mosquito borne viral disease with recent outbreaks associated with congenital neurologic birth defects and microcephaly. In addition, sexual transmission and prolonged viral shedding in semen was described, highlighting it importance in the diagnostic for males with ZIKV disease and the necessity of understand the viremia, virus shedding in biological fluids, the potential for persistence and the immune response involving in the infections. Here we describe ZIKV presence over time in the genital tract and the immune response of symptomatic patients from Sao Paulo city. The weekly collection of serum and semen specimens from 2 males started around 2 weeks after the onset of symptoms and it has prolonged for 298 days and 155 days for ZIKV17 and ZIKV19,
respectively. The presence of ZIKV RNA and infectious virus was done by quantitative reverse transcription (qRT-PCR) and by isolation in C636 cells, respectively, and the cytokine and chemokine profiles were determined by Cytometric Bead Arrays kits*. Nether virus nor cytokines and chemokines were detected in serum during the period. On the other hand virus in semen was shedding for several months for both patients (ZIKV RNA for 158 days, and infectious virus for 117 days for ZIKV 17 and ZIKV RNA for 95 days for ZIKV19). The level of IL-6, IL-8, IL-10, INF γ, IP-10, RANTES, MCP-1 and MIG were increased compared to normal control allied with statistical correlation between the ZIKA RNA load and the increase of IL-8, IFN γ, MCP-1 (p<0.0001). IgM specific antibodies in serum were detected by ELISA for 54 and 95 days for patient ZIKV 19 and 17, respectively, and IgG ∆NS1 specific antibodies were detected by ELISA during all the period. The presence of antibody response and the rapid clearance of ZIKV from blood appear to indicate that the immune system can handle systemic infection. However, the lasting presence of infectious virus in sperm cells could indicate that male sexual reproductive organs turns into a longer time virus replicative sanctuary, in which although there is an effort of the local immune system to combat virus replication, this is not enough for quick virus cleaning. Besides, the alteration in the genital cytokine milieu could play an important role in replication and transmission of the virus as has been seen for other viruses, as HIV-1 that could considerably increase the risk of ZIKV sexual spread and could potentially have a synergistic role in virus spread.

Palavras-chaves: Zika virus, semen, cytokine, chemokine, Brazil

RESPIRATORY VIRUSES IN PRIMARY CHOLESTEATOMAS

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Resumo

Cholesteatoma is an abnormal chronic tissue growth of keratinized squamous epithelium, with collections of keratin, debris, cysts and inflammatory reactions in the middle ear. The formation of cholesteatoma is attributed to inbalances of pro-inflammatory cytokines, growth factors and the presence of pathogens. The treatment of cholesteatomas is based on surgery to remove the abnormal tissue, but recurrences are frequent. Respiratory viruses have been detected in middle ear effusion from patients with chronic otitis media, a process that frequently precedes cholesteatoma, but its etiology still remains unknown. The aim of the present study was search for a panel of respiratory viruses in primary cholesteatoma tissues by real-timePCR and immunohistochemistry (IHQ). For that, 28 samples of paraffin-embedded primary cholesteatoma tissues removed from patients undergoing surgery at the University of Sao Paulo Hospital, in Ribeirão Preto, Brazil were tested for a panel of 13 respiratory viruses. At least one virus was detected by PCR in 7 of 28 (25%) tissues: human rhinovirus (HRV) in 14%, human enteroviruses (HEV) in 11%, and human metapneumovirus B (HMPV B) in 4%. Coinfection of HEV and HMPV B was observed in one patient. IHQ was positive for the tested viruses.
To the best of our knowledge, this is the first study of respiratory viruses in cholesteatomas, and the results raise concern that they may participate in the triggering and maintenance of inflammation. In addition, cholesteatomas may be a niche for persistence of respiratory viruses in the middle ear.

Financial support: FAPESP, CAPES and CNPq

Palavras-chaves: cholesteatoma, virus, tissue

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EVALUATION OF GENE EXPRESSION OF CYTOSOLIC PROTEINS TREX-1 AND SAMHD-1 IN ART-FREE INDIVIDUALS AND AIDS PATIENTS

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Resumo

In HIV-1 immunopathogenesis, cytosolic recognition sensors are important components of innate immunity against infection. SAMHD-1 (sterile alpha motif domain containing 1) and TREX-1 (three prime repair exonuclease 1), act in the degradation of free nucleic acids in the cytoplasm, inhibiting viral replication. Changes in gene expression of these sensors can result in proinflammatory cytokine production, favoring disease progression. Therefore, the aim of this study was to evaluate the expression levels of TREX1 and SAMHD1 in different HIV-1 infection profiles. Forty-two HIV-1 positive individuals were evaluated, they were organized in the following groups: Group I: 28 individuals with recent diagnosis of infection, without antiretroviral therapy (ART), assisted by the reference center CASA DIA; Group II: 14 individuals with AIDS, hospitalized at the João de Barros Barreto University Hospital (HUJBB). The individuals were then divided into two groups according to viral load (log): Group III: Subjects with log 4. Expression of TREX1 and SAMHD1 and plasma viral load tests were performed by real-time PCR. The CD4+ T Lymphocytes (CD4+ TL) count was performed by flow cytometry. Group II presented lower levels of expression of SAMHD1 and TREX1 when compared to group I (p=0.0423 and p=0.0018, respectively). It was also observed that individuals in group IV had lower levels of expression of SAMHD1 and TREX1 when compared to group III (p=0.0043 and p=0.0453, respectively). The results showed that there was a positive correlation between the expression of SAMHD1 and TREX1 and the CD4+ TL count, for both group I (pSAMHD1 and TREX1 are downregulated mainly in AIDS patients with high viral load. Therefore, cytosolic proteins play a key role in the regulation of HIV-1 infection and are suggested to relate to the decrease of proinflammatory cytokine production, which in an exacerbated response may induce chronic inflammation and accelerated progression to AIDS. Work funded with LabVir own resources - UFPA virology laboratory.

Palavras-chaves: HIV-1, SAMHD1, TREX1, Expression, AIDS
ANALYSIS OF HEPATITIS C VIRUS NON STRUCTURAL PROTEINS (NS5A AND NS5B) USING DRIED BLOOD SPOT SAMPLES

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Instituição

Resumo
Hepatitis C virus (HCV) infection affects 70 million people worldwide and is one of the leading causes of chronic liver disease. Currently, the treatment of HCV is made by using direct antiviral agents (DAA) where drugs could inhibit HCV NS5A or NS5B proteins. Some studies have shown 10 to 15% of antiviral resistance in chronic HCV cases probably due to genetic heterogeneity of HCV. Dried blood spot (DBS) is a minimally invasive sampling method suitable for collection, storage and transportation of samples in resource-limited areas. This study aims to detect and evaluate HCV non-structural proteins (NS5A and NS5B) using DBS samples among coagulopathy patients. A total of 10 HCV chronic cases presenting hereditary coagulopathy were recruited from August 2012 to March 2015 in Northeast region of Brazil and gave paired serum and DBS samples. HCV RNA was extracted from serum and DBS samples using commercial assay and qualitative RT-PCR along to primers for NS5A and NS5B regions were used. For DBS, RNA sample volume was 1.5 fold increased in RT-PCR. Positive samples were submitted to Sanger nucleotide sequencing and sequences were analyzed using MEGA® 7.0 software and Geno2pheno (HCV) v0.92 (Saarbrücken, Germany) to identify baseline resistance mutations. HCV positivity for NS5B was observed in seven paired serum and DBS samples where mean HCV serum viral load was 5.92 ± 0.22 log IU/mL, 5 samples were classified as subtype 1a and 2 samples were classified as subtype 1b. In addition, it was possible to amplify NS5A region of 4 out of those 7 serum samples (mean HCV serum viral load was 5.92 ± 0.16 log IU/mL) and paired DBS were negative. One DBS sample was positive for NS5A (HCV serum viral load of 5.71log IU/ml and genotype 1b) and other was positive for NS5B region (HCV serum viral load below 1.48 log IU/ml) without positive results in paired serum samples. Concordant results between serum and DBS for NS5A and NS5B were 50% and 90%, respectively. Two serum samples showed resistance mutations to HCV treatment for NS5A region where one demonstrated L31M mutation and confers reduced sensitivity to daclatasvir and resistance to ledipavir and another sample had a P32A mutation and confer resistance to ledipasvir. In conclusion, it was possible to detect HCV NS5B and NS5A in DBS samples, but the highest sensitivity was found for NS5B region.

Palavras-chaves: hepatitis C virus, molecular virology, dried blood spot

MAYARO VIRUS INDUCES INFLAMMASOME ACTIVATION IN MURINE MACROPHAGES

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Resumo
Mayaro virus (MAYV) infections have been mainly restricted to Amazon basin region, however this virus is considered to be silently emerging. Clinical symptoms are similar to Chikungunya virus (CHIKV) infection, and there have been several links between chronic disease and a stronger inflammatory response to acute infection. Macrophages play an important role during the arthralgia phase of CHIKV infection. However, little information is available about the inflammatory process
caused by MAYV and whether inflammasome activation is an important event during viral infection. Inflammasome activation is an essential component of the innate immune response and is critical for the pathogenesis of infectious diseases. Therefore, we investigated how macrophages respond to MAYV infection in terms of inflammation and cell death. Our results demonstrated that MAYV could effectively infect C57BL/6 mice BMDMs and induced IL-1β release 24 and 48 hours after infection, in a MOI-dependent manner. Additionally, we observed that MAYV can trigger caspase-1 activation by FLICA assay. We also observed cell death by LDH release and pore formation of BMDMs upon infection with MAYV, and these were independent of inflammasome activation. We have seen the inflammasome activation by MAYV is dependent upon NLRP3, ASC and Caspase 1/11. We are currently evaluating inflammasome role during virus infection clearance in mouse models. Taken together, these results will contribute to a better understanding of the inflammatory process during MAYV infection pathogenesis.

**Palavras-chaves:** MAYV, inflammasome, Caspase-1, IL-1β production

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**Recurrent Herpetic Keratitis: Case Report.**

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**Resumo**

Introduction: Herpetic Keratitis, which principal etiological agent is Simplex Herpetic Virus type I, is the principal cause of corneal opacities, being the most blinding infectious disease. The infectious findings are in the superficial layer of the cornea, typically with dendritic lesions, but can involve more deep layers, like corneal stroma. Late diagnosis can lead to damage vision and sometimes lose de eye. The primary infection is usually asymptomatic and limited. In the other hand, recurrent infection can cause conjunctivitis, blepharitis and keratitis. Objective: case report of recurrent herpetic keratitis in a healthy patient. Case description: male patient, white, from Passo Fundo, seller, consulted with complaint of burning, mild conjunctival hyperemia and reduction of visual acuity of the left eye (OE). At ocular examination: visual acuity (AV) 20/20 = 1.0 and OE= 20/40, 0.5, with superficial corneal opacities associated with mild irregularity of the corneal epithelium evidenced with topical dye (fluorescein) in left eye. There was an apparent reduction in corneal sensitivity. We opted for topical treatment with antibiotic and non-hormonal anti-inflammatory. In the reevaluation after seven days, a dendritic-like corneal herpetic lesion was observed and was started with topical acyclovir ointment in the left eye and oral Valaciclovir. In 12 years the patient presented 3 recurrent episodes of herpetic keratitis, with good response to specific treatment, without visual impairment. Conclusion: Early diagnosis of corneal herpes simplex associated with specific topical and systemic treatment often presents a good response, contributing to a lower number of recurrences and ocular complications.

Financial Support by the autors.

**Palavras-chaves:** Herpetic Keratitis, corneal, opacities

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**Genomic Surveillance of Chikungunya virus from 2014 to 2016 in Bahia, Brazil**

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Resumo

Background: The Chikungunya East Central South African virus lineage (CHIKV-ECSA) was first detected in the municipality of Feira de Santana (FSA), Bahia state, by mid 2014. Since then, 5,782 CHIKV cases have been notified in FSA. Here we examine the genomic diversity of CHIKV strains circulating in FSA until 2016 to inspect whether the virus has acquired mutations associated with increased transmission in Aedes mosquito populations.

Findings: Viral RNA was isolated from clinical samples and submitted to probe-based RT–qPCR. Using next generation sequencing approach (Illumina) we generated 5 novel Brazilian CHIKV complete genome sequences from the Serraria Brasil neighbourhood in FSA. Phylogenetic analysis revealed that the new FSA genomes belongs to the ECSA genotype isolates and falls within a single strongly supported monophyletic clade that includes older FSA sequences (bootstrap support = 99%), suggesting persistence of the virus during distinct epidemic seasons. After single nucleotide polymorphism (SNPs) and minority variants analysis, known mutations that increase CHIKV transmission by other mosquito vectors were not found. However, several non-synonymous mutations were found in non-structural proteins that need to be further evaluated.

Conclusion: Phylogenetic analysis indicates that new generated sequences belongs to CHIKV-ECSA genotype which is circulating in the northeast region of Brazil, particularly in the Bahia state. These findings reinforce the importance of continuous genomic surveillance to track viral adaptations and to identify main sources of transmission for improved public health control.

Palavras-chaves: Arboviruses, CHIKV , ECSA , Genomic surveillance

A TALE OF TWO CITIES: SAINT LOUIS ENCEPHALITIS VIRUS ISOLATED FROM HUMAN AND MOSQUITOS

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Resumo

Saint Louis Encephalitis (SLEV) is a *Flavivirus* that was identified in 1933, during an encephalitis outbreak in Saint Louis, Missouri. Over the past few years, the virus has been responsible for infection cases in different countries of South America. In Brazil, SLEV was isolated for the first time in 1964; the first outbreak occurred in 2006, in São José do Rio Preto, São Paulo. We were able to detect SLEV in four serum samples of patients presenting dengue symptoms and in one mosquito pool. The samples were collected in Araraquara, São Paulo and Sinop, Mato Grosso, between 2011 and 2016. Viral RNA was extracted from mosquito pools and human serum respectively with an in-house and a commercial method. We screened our samples with RT-PCR to detect members of *Flavivirus* and *Alphavirus* genera. Positive SLEV samples were sequenced in ABI377 automated sequencer. Amplicons were aligned using BLAST. We detected SLEV in a *Culex quinquefasciatus* female. *Culex* sp mosquitoes are considered major vectors of SLEV in United States and Argentina. We detected the virus in serum samples of three female and one male patient. One sequence from Pará is intimately related to the viruses that circulated in Araraquara and Sinop. Our sequences clustered in genotype V. Our result indicates that SLEV is spreading throughout the country over the years. The samples were collected in settings where major outbreaks caused by dengue, zika and chikungunya viruses were occurring. In such epidemiological scenarios, other viruses such as SLEV can have unreported circulation or be reported as other arbovirus due to similarity of initial symptoms.

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Palavras-chaves: Saint Louis Encephalitis virus, Araraquara, Sinop

EVALUATION OF IMMUNOLOGICAL ADHESION AND RECOVERY OF HIV / AIDS PATIENTS FROM GREAT PORTO ALEGRE.

Autores

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Resumo

Adherence is defined as the behavior of the individual according to the plan of the health professional (use of medications among others). However, few studies evaluate adherence to drug treatment and immune recovery. The present study aims to evaluate adherence to antiretroviral treatment and immunological recovery of patients with HIV / AIDS, based on their viral load data. Sixty patients who started antiretroviral treatment in January 2015 were randomly selected from two municipalities in the Metropolitan Region of Porto Alegre, previously included among those defined as priorities by the Ministry of Health (MS) and with a higher rate of HIV / AIDS infections. The collection of data was done through the consultation of medical records and public databases. The SISCEL database provided data on viral load and CD4 cell count. The SICLOM database provided information on the dispensing of antiretroviral drugs. All the data collected corresponded to the reports between 2015 and 2017. The evaluation of adherence obeyed the criteria defined by the MS, which classifies adherence as sufficient (dispensing of medicine with a maximum delay of 20% referring to the last dispensation), insufficient (dispensing more than 20% delay) and abandonment of drug treatment (more than 100 days without dispensing drugs). Of the selected patients, 38 are male and 22 female. The age of men ranged from 22 to 67 years
(mean of 39.5 years) and women between 7 and 75 years (mean of 36.5 years). Of the total, only 38 patients adopt the 3 in 1 regimen (Lamivudine, Tenofovir and Efavirenz). The therapeutic regimen of other patients varied in the period. Values of CD4 T cells ranged from 0 to 1,229 cells / µL (median of 605.5 cells / µL). The observed viral load ranged from 0 (undetectable) to 505,000 copies / µL (median of 41.5 copies / µL). The data allowed to categorize adherence as sufficient 24 patients, insufficient 33 patients and as treatment of abandonment only 3 patients. In addition, considering the frequencies of the exams, therapeutic adherence was observed in the period and the immunological recovery seems to be related. The complexity related to adherence to therapy involves numerous factors (side effects, behavior of the individual). This preliminary study used indicators to assess the quality standards of HIV / AIDS treatment, but new indicators will be selected for a broader understanding of the factors influencing adherence to medication for individuals with HIV / AIDS.

Palavras-chaves: Adherence, Treatment, Antiretroviral, Indicators

Identification of Current Rotavirus Genotypes in Children in Macapá, Amapá, Brazil in Support Surveillance Epidemiologic


Resumo

It is estimated that in developed and developing countries there are 23 million outpatient visits and 2.3 million hospital admissions per year and that the group A rotavirus (RVA) causes death in more than 215,000 children each year, affecting mainly children 5 years of age, particularly in developing countries. RV are classified in the genus Rotavirus, Reoviridae family being composed of 11 segments of double-stranded ribonucleic acid (dsRNA) which are inserted into the viral core. RV are classified into nine groups or species designated A through I, the species J, found in bats, has recently been proposed. The objective of this study was to detect the RVA in diarrheal and non-diarrheal children 0-5 years in the city of Macapá, Amapá. The study involved 161 fecal samples from children up to five years from January 2014 to August 2015 with or without diarrhea treated at outpatient and inpatient levels in the city of Macapá, Amapá. The RVA was identified by enzyme immunoassay (EIA) followed by reaction Polymerase Chain preceded by reverse transcription (RT-PCR) and Nested PCR to amplify the VP7 genes (types G) and VP4 (P type). The positivity found for RVA was 18.6% (30/161), only being found throughout the electrophoretic profile in the studied samples (60%). There was a predominance of emerging genotype G12P [8] circulating in children Macapá, State of Amapá. Of diarrheal children, 43.3% (13/30) were associated with fever and vomiting to viral clinical picture. Regarding the vaccine frame, there was a significant association between infection RVA and the number of incoming VORH of doses (p

Palavras-chaves: diarrhea, Rotavirus, children, G12P [8], Amapa
HIV controllers (HIC) are individuals that naturally suppress HIV-1 viremia to low levels for several years. Whether these individuals are able to control viremia and maintain the stability of immunologic parameters after HIV-1 superinfection (SI) is unclear. Here, we conducted a deep longitudinal analysis of two HIC dually infected with subtypes B and F1 (EEC09 and VC32) to estimate the timing and impact of inter-subtype HIV-1 SI on viremia, reservoir reseeding, viral evolution and disease progression. Analysis of env sequences in proviral DNA and plasma RNA compartments revealed a triple and dual HIV-1 infection in subjects EEC09 and VC32, respectively. EEC09 was initially infected with a CCR5-tropic subtype B strain and sequentially superinfected with a CXCR4-tropic subtype B strain (between 2006-2007) and with a subtype F1 variant (between 2012-2013). VC32 was initially infected with a subtype B strain and superinfected with a subtype F1 variant (around 2014-2015). In both subjects, SI with subtype F1 variant leads to a fairly increase in viral load levels and an extensive turnover of viral population in plasma. The impact of SI on viral DNA was different in these individuals; whereas a transient increase in the proviral load and a near complete turnover of the viral DNA quasispecies was observed in EEC09, no significant impact in the size and composition of the DNA proviral reservoir was detected in VC32. Despite the slight increase in plasma viremia detected after inter-subtype HIV-1 SI, both subjects maintained virologic control.

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Palavras-chaves: Disease progression, HIV controllers, Reservoir, Superinfection, Viral load
Resumo

Recent epidemics of Zika, Chikungunya and Yellow Fever viruses have shown the need for intense and frequent surveillance of arboviruses. In a context in which febrile diseases are generally diagnosed as dengue, it is important to evaluate the etiology of febrile diseases in medium sized cities, which can be influenced by the circulation of mosquito-borne viruses. *Aedes aegypti*, which is present in almost all Brazilian cities, is the main vector of several arboviruses. Matão is located at the central portion of the state of São Paulo and presents a flow of people to/from neighboring cities due to economic and educational purposes. Matão has reported dengue epidemics in recent years as well as cases of zika, chikungunya and yellow fever. The aim of this study is to assess arbovirus causing febrile disease in patients from Matão. Samples of febrile patients with clinical diagnosis of dengue were collected for arbovirus screening. Viral RNA was extracted with commercial method and analyzed by RT-PCR. During the collection of the clinical samples, participants answered a socio-epidemiological survey. We evaluated 14 samples and six were positive for DENV-4. Among the positive patients, 83.3% were female and mean age was 39 years. The most frequent symptoms were myalgia, arthralgia, exanthema, rash, fever, nausea, prostration, and back and stomach pain. Half of the patients had history of travel approximately 15 days before the onset of the symptoms. This is the first report of arbovirus surveillance in Matão. We were able to detect DENV-4 causing febrile illness in the city, which is a pattern that is similar to other cities in the state of São Paulo. However, DENV-1 has been the main serotype circulating throughout the country in recent years. The intense flow of people among cities of metropolitan areas has an important role in the spread of arboviruses. We emphasize the importance of permanent arbovirus surveillance to identify introduction of different arbovirus and to help health policy makers initiating control measures to restrain the spread of the virus with public health importance.

Financial Support: CAPES and Ministry of Health of Brazil.

Palavras-chaves: Aedes aegypti, Arboviruses, Dengue, Public Health, Surveillance

Liver damage assessment in HIV-positive patients in antiretroviral therapy in the municipality of Carazinho/RS

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Resumo

According to the National Program on Sexually Transmitted Diseases (STD) and AIDS, it is estimated that there are 600,000 HIV virus carriers in Brazil. Currently, without knowledge of HIV infection and a major evolution in the drug treatment for HIV / AIDS have provided an increase in the survival of patients after the contagion. The introduction of Highly Active Antiretroviral Therapy (HAART) enables. An increase in quality of life, as well as a reduction in hospitalizations originated by individuals and, consequently, by a reduction of AIDS deaths. The study is based on the toxicity of antiretroviral drugs and possible liver damage in patients within the municipality of Carazinho - RS. Samples were
collected from patients under 18 years of age and treated for more than half a year. Exclusion criteria were non-agreement on participation in the study, use of antituberculosis agents and anti-HCV antibody positivity, still being submitted to a questionnaire and laboratory tests of AST and ALT dosage. As results, the prevalence of the female sex was verified, with 66.6%; mean age of 38.7 years; 44.5% reported heterosexual infection mode of infection. Evaluating the therapy, 44.4% used 5 to 8 years; 55.5% used triple therapy, and 66.6% had side effects. At the end of the research, normal values were found in the reference standards, with no significant changes to the liver, which shows the relevance and quality of the medications in the current treatments.

**Palavras-chaves:** Antiretroviral theraphy, Liver damage, AST, ALT

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**Design of a novel, aptamer-based rapid POC test for the detection of the Zika virus**

**Autores**

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**Instituição**


**Resumo**

More than 50% of the viruses in the genus Flavivirus, family Flaviviridae, causes disease in humans, including dengue fever, yellow fever, and zika. The Zika virus was described in 1947 and presents the lineages of East and West Africa. The infection caused by this virus is a global concern since, in pregnant women, it can lead the baby to present neurological sequelae such as microcephaly, as well as cases of Guillain-Barré syndrome and others. The objective of this study was to produce antigenic proteins, such as NS1 and NS5 for *E. coli* and use them, together with active virus, in aptamer selections that can be utilized in the development of diagnostic assays for antigen / virus detection during the initial stages of Zika infection. NS1 and a region of NS5 identified by modeling as non-homologous between zika and dengue were cloned and expressed in *E. coli*. Proteins were present in inclusion bodies, and were purified and solubilized in buffer with the presence of urea or l-laroyl sarcosyl. Purified proteins and active virus, grown in Vero cells and purified in two stages of purification, were used for the selection of aptamer, nucleic acids capable of binding in their target with high affinity and specificity, using the SELEX methodology. Several aptamer sequences were selected from the selections against the target proteins, whilst only two sequences were selected against the active zika virus, following negative selections using viruses from the four serotypes of dengue and the yellow fever vaccine virus. The selected aptamers were analyzed for homology and propensity to form secondary structures. The aptamers against the active virus, and those that appeared with the highest frequency and the lowest free energy of the secondary structure, were selected for analysis. Fluorescence spectroscopy and ELISA were used to evaluate the affinity and specificity of the selected aptamers, and biotinylated aptamers were used for sandwich ELISA and rapid test/POC, with the antigen-specific aptamer as a capture agent and the flavivirus 4G2 monoclonal antibody to reveal the interaction. Promising results indicate that aptamers may offer a solution for the Zika diagnosis in the early stages of flavivirus infection, where similarity of symptoms do not allow elucidation of a clear clinical diagnosis.

**Palavras-chaves:** aptamers, diagnostic, zika virus, POC

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**YELLOW FEVER VIRUS DOES NOT INFECT HUMAN FETUS – CASE REPORT ABOUT FATAL**
YELLOW FEVER VIRUS INFECTION IN A PREGNANT WOMAN

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Resumo

Despite its extreme medical importance, current health science lacks many important information about Yellow fever virus (YFV). From December 2017 to April 2018, 81 cases of death were attended by the Service of Verification of Deaths of the Capital - University of São Paulo (SVOC – USP). One of them was a young pregnant woman, who died despite intensive medical care. Post-mortem examination of her and fetal tissues, which we describe here, allowed us to study effect and dissemination of YFV in fetus directly for the first time. For mother and fetus, we collected samples from heart, lung, brain, kidney, spleen, pancreas, liver, blood, uterus, ascitic fluid, amniotic fluid, amniotic sac, placenta, umbilical cord and insertion umbilical-cord tissues. The total RNA from the tissue samples were extracted using the TRIzol® reagent, following the manufacturer's instructions. The molecular diagnosis of the virus was performed using qRT-PCR with virus-specific primers and probe. Sera samples were evaluated with an in-house ELISA which detects anti-YFV IgM antibodies. RNA positive sample was submitted to viral genomic sequencing using the Illumina platform. Consensus genomes were assembled with paired-end reads using Bowtie2 version 2.0.6. Viral phylogeny based on coding sequences were estimated using Maximum Likelihood implemented in IQ-TREE. Briefly, the RT-qPCR showed that YFV occurred in high quantities in all tested maternal tissues. Virus concentration was low in placenta, umbilical cord and in insertion umbilical-cord tissues when compared with other maternal tissues. No virus was detectable in amniotic fluid and any fetal tissue. Similar results were obtained with Immunohistochemistry analysis. ELISA showed that maternal serum was positive for antibodies against YFV, and no IgM antibodies was detected in fetal serum. We observed that the complete YFV sequence was phylogenetically related to previously isolated viruses from Minas Gerais and Espírito Santo states in 2017, and belong to the South American I genotype. In conclusion, to our knowledge this is the first case of detection of YFV in a pregnant woman, associated with the sylvatic virus, and without detection of viral RNA in the fetus.

Financial support: CNPq and FAPESP
EVALUATION OF ELISA δNS1 ZIKV- IgG ASSAY IN THE DIAGNOSIS OF MOTHERS IN SALVADOR CITY.

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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.

Considering its clinical implications in developing unborn baby, the serological tests for ZIKV are extremely important for the clinical diagnosis in pregnant women and for population serological studies. In this context, the serological diagnosis against ZIKV is expected to be sensitive and highly specific. However, the high cross-reactivity observed in vitro in currently available serological tests hinders the wide use of the tests. Seventy-two sera sample from mothers of Salvador city collected between 2016 and 2017 were tested against the ZIKV. Antibodies were detected by the ELISA method using as an antigen a region of the NS1 protein of the Zika virus. The samples were simultaneously submitted to the gold standard test PRNT90 as described by the CDC (Center of Control Disease). Compared to the standard methodology the deltaNS1 ZIKV IgG test showed sensitivity of 84% (95% CI 67% - 95%), specificity of 87% (95% CI: 73% - 96%) PPV 84% (95% CI: 67% - 95%) and VPN 87% (95% CI 73% - 96%).

The test showed high sensitivity, high specificity, and reproducibility in the detection of IgG class antibodies against Zika virus. Currently, the development of the test is in the stage of transformation into a commercial kit.

Palavras-chaves: ELISA, COMPARATION, PRNT, SENSIBILITY, SPECIFICITY

A (new?) Torque Teno virus identified in the plasma virome of acute febrile patients in Amazonas, Brazil.

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Resumo

The detection of an infectious agent may be challenging, especially for those that cannot be detected by classical PCR methods. The extensive use of next-generation sequencing (NGS) opening a new area in the study of medical microbiology.
with unknown pathogens. We report the detection of a (new?) Torque Teno virus in the plasma virome of patients with acute febrile fever of unknown etiology, attended in a sentinel hospital during the emergence of Zika virus (ZIKV) in Manaus, Amazonas, Brazil, between February - June 2016, including but not limited to exanthema, edema, pruritus, and conjunctival hyperemia with or without fever. Different biological samples were collected and tested for ZIKV, dengue virus (DENV), chikungunya virus (CHIKV), mayaro virus (MAYV), and oropouche virus (OROV). Among the negatively tested samples, 18 were randomly chosen to be submitted to a metagenomics protocol, focusing in the virome analysis. Then treated for host nucleic acid removal with different nucleases, followed by the production of double-stranded cDNA and unbiased whole transcriptome amplification with WTA2-Sigma. NexteraXT libraries were produced and sequenced with MiSeq (2 x 300bp) paired-end run, according to the manufacturer's instructions. Initially, a library with 1,574,942 reads was evaluated with BLASTn against all GenBank sequences. Several reads were assembled to Torque Teno virus (TTV). The TTV RefSeq was used for map to reference tool embedded in Geneious (10.2.2) software to generate a draft genome. The cleaned final sequences have 2,544bp, 13.4 mean coverage and a Q30 score of 92.4%. Subsequently, all complete TTV genomes deposited in GenBank were aligned using the MAFFT algorithm. Then used for phylogenetic analysis with MrBayes 3.2, using the General Time Reversible model with a proportion of invariant sites and a gamma-shaped distribution of rates across sites. The alignment reveals a considerable number of amino acid substitutions throughout the CDS. The Amazonas isolate sequenced in this study belongs to the TTMDV subtype. Although TTVs are not considered human pathogens, and can be found in several human viromes, the protocol applied in this study was able to identify a possible new TTV, supporting the importance of viral metagenomics for virus discovery. Further studies are being conducted to fully characterize this isolate, as well as to identify other viral reads in the virome of acute febrile patients of the Amazonas State.

Palavras-chaves: Metagenomic, NGS, TTMDV, TTV

CHIKUNGUNYA ANTIBODIES SEROPREVALENCE IN A POPULATION PROSPECTIVE COHORT STUDY

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Resumo

The Chikungunya virus (CHIKV) is the pathogenic agent responsible for the Chikungunya fever, which varies largely from asymptomatic infection to rash and severe arthralgia. In recent years, the circulation of CHIKV in Brazil' geographic area increased until their transmission was detected in every state, mainly due to the high prevalence of the vector mosquitoes Aedes aegypti (and, in less range, A. albopictus) in an urban and peri-urban area. The aim of this work was to verify the circulation of CHIKV in Sao Jose do Rio Preto, an area highly monitored for other flavivirus infections with a prospective cohort. For this, sera samples were collected in two different moments: one at the first entrance of the subject at the cohort in 2016, where we also did a sociodemographic survey; and other blood collection one year after to analyze seroconversion of the subject and data update. Those samples were analyzed with EUROIMMUN® ELISA IgG anti-CHIKV. For the first cohort year, we tested 775 samples, of which 772 were negatives (from those 298 were men, and 474 were women). Only 3 samples were IgG positives for CHIKV, two with the age range between 21 to 40 years and one subject from the age range of 41 to 60. For the second year, we tested 775 samples, of which 758 were negatives (from those 297 were men, and 461 were women). In the second year, 17 samples were positives, which those 4 were men and 13 were women - age range between 21 to 60 years. The IgG anti-CHIKV prevalence was 0.4 in the first year, and 2.2 in the second year (incidence of 18.5 cases/1000 habitants). Although we analyzed this cohort for only two years (between 2016 and 2017), we can attest to the asymptomatic circulation of CHIKV in an endemic area for Dengue and Zika virus. The real seroprevalence of CHIKV in this population will be consolidated after analyzing the results for this cohort in following years.
SEROPREVALENCE OF THE ARARAQUARA VIRUS (ARAV) ANTIBODIES IN A POPULATION FROM A PROSPECTIVE COHORT IN SÃO JOSÉ DO RIO PRETO, SP, BRAZIL.

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Resumo

Hantaviruses (family Hantaviridae, genus Orthohantavirus) are spherical and enveloped presenting glycoproteins in the surface with approximately 120 nm in size. The genome consists of a negative and tri-segmented sRNA. The three RNA segments are defined as small (S), medium (M) and large (L) which encode N proteins, enveloped G1 and G2 glycoprotein, and viral RNA polymerase, respectively. In the Americas, the disease caused by hantavirus is presented in different forms, from acute non-specific febrile illness to more severe and characteristic pulmonary conditions, leading to Hantavirus Pulmonary Syndrome (HPS). In South America, a significant cardiac involvement was observed, being renamed to Hantavirus Cardio-Pulmonary Syndrome (HCPS). Hantaviruses have as natural reservoirs wild rodents that can eliminate the virus through urine, saliva and feces. Rodents can carry the virus for life without becoming ill. In Brazil, epidemiological surveys detected the genotype Araraquara virus (ARAV) in Necromys lasiurus rodents, being predominantly found in the cerrado, a predominant biome in the São Paulo State. The aim of this study is to perform a serological survey to detect the presence of anti-ARAV IgM and/or IgG antibodies in patients from a prospective cohort study in a neighborhood of the municipality of São José do Rio Preto, SP, Brazil. A total of 831 patients were randomly selected, and submitted to an interview for investigation of patient's health history, living conditions and workplace. So far 253 sera samples were collected and tested by an IgG/IgM indirect-ELISA using the N recombinant protein of ARAV as antigen, as described previously. The cut-off was established as the mean value + 2 standard deviations control samples and showed a cut-off Optical Density (OD) equal to or greater than 0.300. Our results showed 0.8% (2/253) of positivity to ARAV-IgG, 0.4% (1/253) of undetermined and 99.6% were negative. São José do Rio Preto is endemic to Dengue and other arboviruses; however, several cases of acute febrile disease are negative for these viruses. In this way, we will be able to estimate the prevalence of the hantavirus antibodies, contributing to surveillance in the municipality and with a better diagnosis and patient care.

Palavras-chaves: Hantavirus, cohort, SEROPREVALENCE, ELISA

VIROLOGICAL AND EPIDEMIOLOGICAL CHARACTERIZATION OF YELLOW FEVER OUTBREAK IN JUIZ DE FORA, MG, 2018

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Resumo

Hantaviruses (family Hantaviridae, genus Orthohantavirus) are spherical and enveloped presenting glycoproteins in the surface with approximately 120 nm in size. The genome consists of a negative and tri-segmented sRNA. The three RNA segments are defined as small (S), medium (M) and large (L) which encode N proteins, enveloped G1 and G2 glycoprotein, and viral RNA polymerase, respectively. In the Americas, the disease caused by hantavirus is presented in different forms, from acute non-specific febrile illness to more severe and characteristic pulmonary conditions, leading to Hantavirus Pulmonary Syndrome (HPS). In South America, a significant cardiac involvement was observed, being renamed to Hantavirus Cardio-Pulmonary Syndrome (HCPS). Hantaviruses have as natural reservoirs wild rodents that can eliminate the virus through urine, saliva and feces. Rodents can carry the virus for life without becoming ill. In Brazil, epidemiological surveys detected the genotype Araraquara virus (ARAV) in Necromys lasiurus rodents, being predominantly found in the cerrado, a predominant biome in the São Paulo State. The aim of this study is to perform a serological survey to detect the presence of anti-ARAV IgM and/or IgG antibodies in patients from a prospective cohort study in a neighborhood of the municipality of São José do Rio Preto, SP, Brazil. A total of 831 patients were randomly selected, and submitted to an interview for investigation of patient's health history, living conditions and workplace. So far 253 sera samples were collected and tested by an IgG/IgM indirect-ELISA using the N recombinant protein of ARAV as antigen, as described previously. The cut-off was established as the mean value + 2 standard deviations control samples and showed a cut-off Optical Density (OD) equal to or greater than 0.300. Our results showed 0.8% (2/253) of positivity to ARAV-IgG, 0.4% (1/253) of undetermined and 99.6% were negative. São José do Rio Preto is endemic to Dengue and other arboviruses; however, several cases of acute febrile disease are negative for these viruses. In this way, we will be able to estimate the prevalence of the hantavirus antibodies, contributing to surveillance in the municipality and with a better diagnosis and patient care.

Palavras-chaves: Hantavirus, cohort, SEROPREVALENCE, ELISA
Resumo

Yellow fever virus (YFV) is an enveloped virus with a negative sense single-stranded RNA genome and member of the genus Flavivirus, family Flaviviridae. From July 2017 to 28th February 2018, Brazilian Ministry of Health has reported 732 confirmed cases of YFV in Brazil, including 237 deaths. It is important to notice that the majority of cases were notified in Minas Gerais, with 314 confirmed cases and 103 deaths. Forty patients of Juiz de Fora, Minas Gerais, Brazil were investigated for YFV, Zika virus (ZIKV), Dengue virus (1-4 serotypes) (DENV) and Chikungunya virus (CHIKV) with qRT-PCR and serological assay for IgM using an in-house ELISA test. Plasma, whole blood, serum, saliva, liquor and urine were extracted based on TRIzol® isolation of RNA. RT-qPCR assays were performed using the AgPath-ID one-step RT-PCR kit and molecular assays were done for sylvatic and vaccine strains of YFV and also for DENV, ZIKV, and CHIKV. Molecular tests detected 32.5% (13/40) cases of YFV and one case of coinfection (2.5% 1/40) with CHIKV. Serological results for YFV showed that 26 patients has IgM antibody detected and 28 cases were classified as acute and/or convalectic phase of infection (viral RNA and/or IgM positive). All patients were evaluated with presence of molecular and serological markers in those. Furthermore, in 15 that reported previous vaccination, we found six RNA positives cases, nine IgM(+) all of RNA positive were IgM positive. In conclusion, our results suggested co-circulation of CHIKV and YFV in Juiz de Fora, MG in 2018. Surprisingly, even a small number of patients has already reported vaccination we observed viral RNA and IgM positive in this group. Finally, based on our observation we suggested that the vaccination need to be under investigation.

Palavras-chaves: Yellow Fever virus, RT-qPCR, Serology, Vaccination coverage
YELLOW FEVER VIRUS IN SÃO PAULO, BRAZIL, 2018: EARLY BIOLOGICAL FINDINGS

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Resumo
In its largest outbreak of the 21st century in the Americas, the Yellow Fever virus (YFV) started an intense circulation from 2016 in the southeastern states of Brazil, in sylvatic environments near densely populated areas, such as the metropolitan region of the city of São Paulo. In this preliminary work, we worked with tissue samples from patients who died under clinical suspicion of death associated with YFV. The Service of Verification of Deaths of the Capital-USP investigated 81 deaths from December/2017 to April/2018. Necropsy samples from heart, lung, brain, kidney, spleen, pancreas and liver tissues were collected. The RNA from these samples were extracted using the TRIzol® reagent. The molecular diagnosis, with the identification of sylvatic and adverse vaccine response cases to the virus were performed using qRT-PCR. Some positive cases were submitted to viral genomic sequencing. RNA libraries were constructed and validated using the TruSeq HT, and the sequencing was done using the Illumina MiSeq platform. Consensus genomes were assembled with paired-end reads using Bowtie2 version 2.0.6. Viral phylogeny based on coding sequences were estimated using Maximum Likelihood implemented in PhyML 3. Our preliminary results indicate that 90.1% (73/81) of the total death cases were associated with the presence of the YFV in tissues. In these cases, six patients were positive for the vaccine strain and were therefore associated with an adverse vaccine response. The other cases were caused by sylvatic virus, with cases of infection associated with areas close to epizootics. We sequenced the complete genomes of the YFV from nine patients. We observed that the sequences from São Paulo were phylogenetically related to previously isolated viruses from Minas Gerais and Espírito Santo states in 2017, and belong to the South American I genotype. The isolated sequences of the current Brazilian outbreak 2016-2018 had specific synapomorphic changes [V108I (Capsid), E1572D (NS3), R1605K (NS3), K2607R (NS5), V2644I (NS5), G2679S (NS5), V3149A (NS5) and N3215S (NS5)]. The sequences from São Paulo - 2018, had a single synapomorphy (N1646T) located in the NS3 protein. As many of the mutations can alter the structure of proteins and other biologically significant targets, they should be monitored for impact on clinical features, diagnosis, vaccines, and possible emergence of the virus in an urban environment.

Financial support: CNPq and FAPESP.

Palavras-chaves: Yellow Fever virus, São Paulo, Sylvatic virus, Adverse vaccine response, South American I genotype

HIV-1 RESERVOIR SIZE AND DIVERSITY AMONG ACUTE-INFECTED INDIVIDUALS

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Resumo

Early combined antiretroviral treatment (cART) of HIV infection aims to limit the seeding of the viral reservoir in the initial phase of infection, and, consequently, decrease the intrahost viral diversity. Here, we aimed to measure the effect of the cART in the size and complexity of the proviral reservoir. Peripheral blood mononuclear cell (PBMC) and plasma samples were obtained from ten HIV-infected individuals, diagnosed at the acute phase (Fiebig II-V) of infection, before (PreART) and 12 months (M12ART) after suppressive cART. HIV proviral reservoir size was determined by quantitative real-time PCR while the intrahost viral diversity of the env C2-V3 region was assessed by single genome amplification or next-generation sequencing in PBMC and plasma, respectively. The mean nucleotide diversity (π) and the normalized Shannon entropy (H_{SN}) were used to infer the complexity of the viral population. Overall, we identified the immunological recovery of patients, with CD4+ T cell gain of 31% (P=0.008) and a normalization of the CD4/CD8 ratios (~1.0, P=0.016) after 12 months under cART. We also observed significant decreases in the HIV-1 RNA (~4 log, P=0.004) and DNA (~1 log, P=0.002) levels. The median time to achieve viral suppression was three months. The high intermixing between the sequences from both visits suggests that the HIV-1 DNA reservoir remained remarkably stable. There was a slight reduction in proviral π (PreART=0.20 vs M12ART=0.10; P=0.156) and a significant decrease in H_{SN} (PreART=0.41 vs M12ART=0.25; P=0.019) after one year of cART. We found no correlation between π or H_{SN} at PreART with the rate of HIV DNA decay, T CD4+ cell change or CD4/CD8 ratios presented at M12ART. One year of cART initiated in acute phase was sufficient to reduce the size and complexity of proviral reservoir, and to achieve immunological restoration, independently of the HIV-1 plasmatic viral load, CD4+ T cells count or HIV-1 subtype. The early initiation of cART may favor strategies to achieve post-treatment control of HIV and, ultimately, a functional cure, through the restriction of the pool of variants, allowing a more focused targeting by therapeutic vaccines or other immune approaches.

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Palavras-chaves: Acute infection, diversity, HIV-1, reservoir

INVESTIGATION OF POTENTIAL ENZOOTIC CYCLES OF ZIKA VIRUS IN WEST-CENTRAL BRAZIL

Autores

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Resumo

In 2014, epidemic activity of Zika virus (ZIKV) spread to Brazil and continues to spread throughout the tropical and subtropical regions of the Americas. Despite ZIKV being zoonotic in origin, information
about potential vertebrate hosts and invertebrate vectors for ZIKV in the Americas is scarce. We conducted active surveillance of domestic and sylvatic free-ranging vertebrates for ZIKV in western Brazil, at established field sites up to 4 times per year, where there was evidence of recent or active ZIKV transmission in humans. Trapping efforts focused on abundant wildlife and domestic animals, as these would be candidate-amplifying hosts. We screened whole blood for ZIKV nucleic acid by a universal flavivirus real time RT-PCR and confirmed by ZIKV specific real time RT-PCR and nucleotide sequencing. We also tested plasma samples for presence of anti-ZIKV antibodies by plaque reduction neutralization testing (PRNT). From February 2017 to March 2018, we collected 1,090 animal samples from Cuiaba, Mato Grosso state, and 1,049 animal samples from Campo Grande, Mato Grosso do Sul state. Preliminary test results for a fraction of the samples collected in Brazil reveal 3.1% (33/1,077) of animals are positive for flavivirus by RT-PCR. However, all samples tested negative by ZIKV specific RT-PCR and analysis of nucleotide sequencing of positive samples revealed non-specific results. Of 760 plasma samples tested by PRNT, 68 (8.9%) showed evidence of anti-ZIKV antibodies by PRNT, and are currently being tested for other flaviviruses to discard heterologous reactions. The results of this study will facilitate a better understanding of ZIKV’s ability to establish a sylvatic cycle outside of human transmission.

Palavras-chaves: Zika virus, Brazil, Mato Grosso, Mato Grosso do Sul, enzootic

THE ROLE OF EASTER ISLAND IN THE MIGRATION OF ZIKA VIRUS FROM SOUTH PACIFIC TO THE AMERICAS

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Resumo
Zika virus (ZIKV) is a mosquito-borne flavivirus associated with several recent human outbreaks in the Southern Pacific islands and the Americas. The ZIKV strains circulating in those regions comprise a single lineage (ZIKV-SP-AM) that arose from sequential viral dissemination from Southeastern Asia into French Polynesia (FP) and from Southern Pacific into the Americas. The Easter Island (EI) was recently suggested as a potential staging post in the spread of ZIKV to continental America, but this hypothesis was not properly tested. Here, we evaluated the putative role of EI in the ZIKV spreading to the Americas using a comprehensive dataset of ZIKV sequences. Concatenated E and NS5 fragments of seven new ZIKV strains from EI were aligned with ZIKV Asian genotype complete coding sequences and E sequences from Southeastern Asia, Pacific islands, and the Americas. The spatiotemporal viral diffusion pattern was reconstructed by Bayesian phylogeographic methods. The origin of the ZIKV-SP-AM lineage was traced to FP in early 2013. This lineage exhibited a strong geographical subdivision and two highly supported sub-clades: clade I, comprising strains from FP, the Americas, New Caledonia and Vanuatu; and clade II enclosing strains from the EI and Cook Islands. FP was the main hub of dissemination of the ZIKV-SP-AM lineage and was the most probable source location of the ZIKV clade I strain introduced into the Americas, Vanuatu, and New Caledonia as well as of the clade II strain introduced into EI. The ZIKV introductions in several South Pacific islands and the Americas occurred at around the same time (2013.6-2014.8). These results clearly reject the hypothesis that ZIKV was introduced into the Americas via the EI and further support a longer period of cryptic circulation of ZIKV in the Americas than in Southern Pacific islands.

Palavras-chaves: ZIKV, AMERICAS, SOUTH PACIFIC, PHYLOGEOGRAPHY, SPREAD
HOMOLOGY MODELING AND STRUCTURAL VALIDATION OF C, E2 AND E1 PROTEINS OF MAYARO VIRUS

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Resumo

The Mayaro virus (MAYV; family Togaviridae) is an emerging arthropod-borne virus and is the causative agent of Mayaro Fever, an acute viral disease endemic in Central and South America. Structurally, the MAYV presents a positive-sense single-stranded RNA genome with approximately 11,429 nucleotides and expresses an organization similar to that described for species of the genus Alphavirus. Presently, there are no specific drug therapies or vaccine for MAYV infection. Based on literature, the interaction between a conserved hydrophobic pocket of capsid protein (CP) with citoplasmatic domain of glycoprotein E2 (cdE2) of Alphavirus is characterized as a promising molecular target for block the viral budding stage. The aim of this study was to elaborate the structural C, E2 and E1 proteins of MAYV using homology modeling. Initially, protein sequences of capsid protein, E2 and E1 envelope glycoproteins of MAYV (strain Brazil) were obtained from UniProt (id: Q8QZ72). The search for homologous proteins was performed using BLAST method in Protein Data Bank (PDB). After, the template with best e-value was downloaded and sequence alignment was made in Clustal Omega. The model three-dimensional for MAYV proteins was built in MODELLER 9v15 software and stereo chemical properties was evaluated by PROCHECK in SAVES v5.0 webserver and Z-score in ProSA. From entity-based query in PDB, the best template for CP of MAYV was capsid protein of Chikungunya virus (CHIKV) (PDB id: 5H23) with 87.42% of identity between sequences and residue coverage 108-258. For E2 and E1 protein of MAYV the template was crystal structure of the mature envelope glycoprotein complex of CHIKV (PDB id: 3N41) showed 56.68% and 61.38% sequence identity with residue coverage 5-341 and 1-378, respectively. Structure validation of CP, E2 and E1 models showed good stereo chemical property with 96.1%, 87.8% and 90.0% most favored regions, respectively. The ProSA Z-score for CP (-5.9), E2 (-6.4) and E1 (-7.3) represents the high accuracy of model prediction. In conclusion, the results provide structural characteristics to construct the model of CP-cdE2 complex and, thus, to prospect new insights in the structure and ligand-based rational design strategies against MAYV infection. Financial Support: CAPES/CNPq/DCIT - Ministry of Health – Brazil. Feevale University.

Palavras-chaves: Antiviral targets, Drug discovery, Homology modeling, Mayaro virus

The importance of Influenza monitoring program in Minas Gerais State

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Resumo

Flu is an acute infectious contagious disease of the respiratory tract, globally distributed and it has as the etiological agent, the influenza virus. There are three subtypes of influenza virus (InfA, InfB, InfC). InfC is more associated to less important infections in public health. InfA and InfB viruses are responsible for annual epidemics and outbreaks, being InfA viruses the cause of large pandemics. In this context, the accurate diagnosis of these viruses prove to be an important activity for the elucidation of epidemiological issues that impacts on collective health. The Fundação Ezequiel Dias (Funed-MG), is responsible for the diagnosis of Influenza in Minas Gerais state. The National Influenza Epidemiological Monitoring Program is composed of sentinel surveillance of Gripal Syndrome (SG), Severe Acute Respiratory Syndrome (SARS) and by the Universal Surveillance. Respiratory tract samples are collected at sentinel units and at several hospitals in the capital or interior of the state and sent to the lab, where they are aliquoted for nucleic acid extraction and processed by RT PCR following
the recommended protocol by the (CDC Realtime RTPCR (rRT PCR) Protocol for Detection and Characterization Influenza). Only in 2018 (January to July), 2,016 samples were tested and 18% (n = 365) were positive for Influenza - 22% (n = 82) were InfA H1N1, 44% (n = 162) InfA H3N2, 30% (n = 111) could not be subtyped, 3% (n = 10) were InfB and 4%(n=16) were inconclusive. The results obtained in Minas Gerais go against the latest national surveillance report, where 44.3% of the samples tested positive for H1N1, 37.8% for H3N2, 5.0% were not subtyped and 12.9% were InfB. The amount of non-subtyped influenza A virus is highlighted as a situation that deserves attention by public health authorities, since it creates the hypothesis that there might be a new Inf A virus subtype circulating in the environment. Another hypothesis, would be a viral mutation exactly in the annealing region of the primers used in the diagnosis. Anyway, this is an important issue that involves the subject and it deserves the attention of researchers and health authorities. Influenza virus monitoring actions associated to constant improvement in laboratory diagnosis may help to prevent infectious diseases, and constitutes an important tool to subsidize public policy actions aimed at the protection, promotion and recovery of collective health.

Palavras-chaves: influenza virus, monitoring program, RT PCR, collective health, infectious diseases

DETECTION OF ANTI-RABIES ANTIBODIES IN WILD MAMMALS FROM SOUTH OF BRAZIL

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Resumo

Previous studies and surveillance actions reported the role of wild canids, marmosets, hematophagous and non-hematophagous bats as reservoirs of rabies, thus they are important in the rabies sylvatic cycle, and potential vectors to humans and domestic animals in Brazil. Although different control actions have reduced or even eliminated the circulation of the Rabies Lyssavirus (RABV) in the urban environment, the frequent interactions between men and wild animals, and the possibility of host shift events are a matter of concern for public health. The aim of this study is to identify anti-rabies neutralizing antibodies among wild mammals from the Mata Atlantica Biome, in Rio Grande do Sul - South of Brazil, and to emphasize the importance of surveillance with collaborative projects. The samples were collected at three municipalities where wild animals display a close proximity to humans. Blood from 16 hoary fox and from 10 capuchin monkeys were collected, sera were obtained and
submitted to the rapid fluorescent focus inhibition test (RFFIT). From all samples (total of 26), five were positive (20%) when a cutoff of > 0.1 IU/mL antibody titer was delimited, which is suggestive of a pre-exposition/infection with RABV. The data obtained show the circulation of RABV in wild animals and highlights the implication of different species in the rabies sylvatic cycle in south of Brazil.

Financial support: FAPESP and Capes.

Palavras-chaves: Non-human primates, Canids, RIFFT, Capuchin monkeys, Hoary fox

HUMAN RESPIRATORY SYNCTIAL VIRUS LOAD IN HOSPITALIZED PATIENTS SUSPECTED OF INFLUENZA A(H1N1)2009 INFECTION IN A UNIVERSITY HOSPITAL IN SAO PAULO, BRAZIL.

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Resumo

Human respiratory syncytial virus (HRSV) is recognized as an important cause of respiratory tract infection, mainly in children and elderly, requiring hospitalization of severely affected patients. Generally, these patients are reported to present high viral load with positive correlation to clinical severity. This study was conducted to investigate the viral load of HRSV in positive hospitalized patients at São Paulo University Hospital, previously suspected of pandemic influenza A(H1N1)2009 infection. Nasal aspirate and swab samples were collected from children and adults, respectively, during 2009 to 2014, and tested by real-time RT-PCR aimed to the conserved region of HRSV Matrix gene. RNase P gene was used as internal control and for normalization of threshold cycle (Ct) values. Viral load concentrations were calculated in Log10 RNA copies/mL (LRc/mL). A total of 660 samples were tested, of which 268 were from children and 392 from adults. HRSV was detected in 15.29 % (41/268) of children (ages: 0.08-7 years; mean±SD: 1.62±1.84; median: 0.9), and 3.82 % (15/392) of adults (ages: 24-85 years; mean±SD: 44.86±20.06; median: 36) (Pp=0.28). However, in adults, median age was 36, with 4 adults > 60 years old. 40% of children and 21.95% of adults presented coinfection with other respiratory viruses, with influenza A (FluA), human adenovirus and coronavirus NL63 (hCoV-NL63) more frequent in children, and FluA and hCoV-NL63 in adults. HRSV viral loads were not significant different between children and adults but infection seems to be more frequent in children. Further investigation shall be done to assess other variables such as time of hospitalization, comorbidities, deaths and HRSV genotyping.

Palavras-chaves: Human respiratory syncytial virus, viral load, hospitalized patients

WORLDWIDE SEQUENCE MUTATION IN ZIKA VIRUS prM PROTEIN

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Zika virus (ZIKV) is a mosquito-born Flavivirus (Flaviviridae family). A recent study suggested that the mutation founded in prM gene of ZIKV contributes to fetal microcephaly. Despite this, there are few studies focusing in genetic variations founded in prM gene of ZIKV. Thus, this study analyzed prM gene mutations in ZIKV sequences in worldwide samples. A total of 422 viral genome sequences of ZIKV prM gene, reported since 1947 from 35 countries, were obtained from GeneBank. Comparisons between consensus sequence and isolated samples were performed by using multiple alignments, using CLUSTALW (Mega 7.0) program. Predictions of B-cell and T-cell epitopes were performed by using BcePRED and ProPRED/ProPRED I servers. Phylogenetic trees were constructed by using FastME and PhyML algorithms. 173 single nucleotide changes were founded in prM gene of ZIKV, in which 43/173 were non synonymous mutation. The four most frequent non-synonymous mutations were A521G/S139N (121/422), A691G/T196A (17/422), C473T/A123V (7/422) and A922C/S273R (4/422, founded only in Brazil and Colombia isolates). These above mentioned variations were embedded in T-cell epitopes (MHC-Class I and MHC-Class II). Phylogenetic tree showed that A521G/S139N mutation was founded in Africa and Asian lineages, while A691G/T196A, C473T/A123V and A922C/S273R were founded in Asian lineages. Genetic variations in prM gene of ZIKV may be involved with the ZIKV disease. Further studies are necessary to clarify the involvement of mutations in prM gene of ZIKV and the pathogenicity caused by this virus.

Palavras-chaves: Zika virus, prM gene, Genetic mutations

SEROPREVALENCE OF ANTI-ZIKA AND ANTI-DENGUE ANTIBODIES IN A PROSPECTIVE COHORT IN BRAZIL

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Resumo

The emergence of Zika virus (ZIKV) in Brazil presented new challenges to both clinicians and public health authorities. Brazilian authorities largely rely on clinical and epidemiological data for the diagnosis of most of ZIKV cases, but the clinical presentation of this arboviral infection is diverse, from asymptomatic or mild infections to severe cases with neurological complications. In this study, we performed a serological survey to evaluating seroprevalence of Immunoglobulin G (IgG) anti-Zika and anti-Dengue antibodies in a population cohort in São José do Rio Preto, São Paulo, Brazil, by ELISA.
The rotavirus of the species A (RVA) is one of the main responsible for the gastrointestinal infections in humans, being also able to infect animals. The RVs have a genome with 11 double stranded RNA segments encoding 12 proteins. Studies suggest that RVs of animal origin may serve as reservoirs, their transmission infecting human hosts may be an important pathway responsible for the evolutionary diversity of circulating Rotavirus strains. This high circulating diversity of rotavirus and the characteristic of its segmented genome allow a facility in the reassortment of co-infected cells with strains of different evolutionary origins, characterized as zoonotic. The main objective of the present study is to use phylodynamics to understand the zoonotic evolution between the Rotavirus strains. We sequenced the 11 genes from 09 samples collected in 3 surveillance studies in the state of Pará. The philodynamic approach was performed using the Bayesian methodology. Were tested which are the best evolutionary models, molecular clock and coalescence for the data set and gene analyzed, being included in the analyzes the host of origin and the date of collection for the inferences. The results indicated five different evolutionary origins (Human, Feline, Canine, Swine and Bovine) with samples belonging to the Wa-like, Ds-1 Like and Au-1 constellations (genogroup I, II and III respectively). We found the genotypes G4P[6], G12P[9], G3P[3], G12P[8], G4P[x]. The genotypes G4P[6] and G4P[x], Wa and Ds-1, have evolutionary origins with samples from human and swine strains, with reassortment in the NSP3 gene (T7 genotype) of sample COD424 and in the NSP4 gene (E1) of the sample HSE005 Ds-1 Like. The sample of genotype G3P[3] Wa had evolutionary origin related to felines except for the NSP5 gene, with canine origin (H6), besides in the genes VP2 and NSP2 with feline and human origin respectively. The samples of the genotype G12P[9] and G12P[8] Au-1 showed the highest evolutionary diversity with human, bovine, feline and canine origin. Two samples presented reassortment in the gene NSP5 (H6) and one sample with bovine origin H3. The present study verified the intense event of transmission and reassortment between the strains that have zoonotic potential and indicated that the use of phylodynamics is an adequate tool to identify the evolutionary pattern of zoonotic Rotavirus.
strains, and can assure the zoonotic profile.

Financial support: Evandro Chagas Institute.

**Palavras-chaves:** Phylodynamics, Rotavirus, Zoonotic

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**EPIDEMIC OF HIV/AIDS IN THE METROPOLITAN REGION OF PORTO ALEGRE AFTER 2015 GUIDELINES**

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**Resumo**

**Introduction:** Rio Grande do Sul has the second highest rate of HIV/AIDS (human immunodeficiency virus/ acquired immune deficiency syndrome) detection in Brazil, with 31.8 cases per 100,000 inhabitants. Porto Alegre leads among the capitals with the highest mortality rate, with 22.4 deaths /100.000 inhabitants. Since 2015, WHO (World Health Organization) recommends the start of treatment for all people diagnosed with the HIV virus, regardless immunological tests. The early initiation of HAART (Highly Active Antiretroviral Therapy) was associated with lower rates of mortality and morbidity. However, there are several problems, such as acute and chronic adverse effects and other factors, related to the use of drugs. **Material and Methods:** We cataloged sixty-three patients from municipal HIV/aids treatment centers of Novo Hamburgo and Viamão, who started antiretroviral therapy between the years 2015 and 2017. Personal, social, clinical, and laboratory data from medical records and database were recorded. **Results:** In total, 22 were female and 39 male, and the mean age was 37.6 years. Most of them were Caucasian residents in the cities of Novo Hamburgo and Viamão, and had incomplete elementary school. We identified 7 alcoholics, 11 active smokers and 5 users of illicit drugs with a predominance of cocaine and marijuana. The main way of HIV virus transmission in these individuals was sexual and the following coinfections were recorded: syphilis, herpes, neurotoxoplasmosis, HPV papillomatosis, neurocryptococcosis. The preferred regimens for initiation of therapy and their prevalence were: Tenofovir + Lamivudine + Efavirenz in 77% of the cases; Tenofovir + Lamivudine + Dolutegravir in 8.1%; Tenofovir + Lamivudine + Atazanavir/Ritonavir in 4.9%; Tenofovir + Lamivudine + Darunavir/Ritonavir in 1.6%; Zidovudine + Lamivudine + Lopinavir/Ritonavir in 8.1% of cases. The median of the first CD4 was 405, of first viral load was 6.604,5. The mean time to reach undetected viral load after therapy initiation was 14.2 months. **Conclusion:** This is a pilot of a larger project aiming to draw a profile of the characteristics of patients infected by the HIV virus in the metropolitan region of Porto Alegre, in order to facilitate the understanding of this prevalent epidemic and to evaluate the impact of the early initiation of therapy in these patients. Financial Support: from the author; CAPES scholarship.

**Palavras-chaves:** epidemic, guidelines, HIV, impact, therapy

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**LOW PREVALENCE OF HEPATITIS C VIRUS INFECTION IN CARRIERS DIABETES MELLITUS TYPE 2 IN GOIÂNIA-GOIÁS.**

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The viral infection caused by the Hepatitis C virus (HCV) is one of the leading causes of chronic liver disease worldwide. Some studies have shown that the prevalence of HCV infection in diabetic patients is higher compared to the general population. This study’s objective was to analyze the epidemiological profile of HCV infection in patients with type 2 diabetes mellitus (DM 2). The population consisted of 605 individuals with type 2 diabetes mellitus, followed/attended at the Hospital das Clínicas of the Federal University of Goiás (HC-UFG) and at the Primary Care Units of Family Health (UABSF) to these patients (Hiperdia Program - Ministry of Health). In Goiânia-Goiás the serum were screened for anti-HCV by ELISA. The anti-HCV positive samples were submitted to the detection of viral RNA by Reverse Transcription Polymerase Chain Replication (RT-PCR) and genotyped by Line Probe Assay (LiPA) method. The mean’s average age was 62.1 years old (SD = 11.3), predominantly female, and 78.4% had up to nine years of schooling. Nine samples were positive for anti-HCV, resulting in a prevalence of 1.49% (95% CI: 0.73-2.90) for HCV in patients with DM 2. Viral RNA was detected in four anti-HCV positive specimens and genotypes 1 (2/4) and 3 (2/4) were identified. In the multivariate analysis, blood transfusion before 1994 (p = 0.005) and the use of illicit drugs (p = 0.003) were associated with HCV infection. Male remained marginally associated (p = 0.06). Despite the global prevalence for HCV in patients with DM 2 in Goiania-GO have been similar to the one found in the population in general, more studies are needed to clarify the epidemiology of HCV infection in individuals with DM 2 and provide information which can support prevention measures and control of infection in this group of the population.

Financial Support: CNPq

Palavras-chaves: hepatitis C, prevalence, Diabetes Mellitus Type 2
The collection of socio-demographic data was performed using the data available in the patients' records. In the study period, 815 medical records of the Gastroenterology / Oncology sector of the HC were selected and reviewed, of which 260 individuals had cirrhosis and / or CHC that were included in the study. The mean age was 55 years, 66.5% were male and 33.5% female. Regarding the etiology, of the 218 cases with only cirrhosis, 11.5% of the patients were HBV, 0.9% HBV / ethyl alcohol, 5.5% HBV / HCV coinfection, 22.0% HCV, 4% for HCV / ethyl alcohol, 28.5% for ethyl alcohol, 12.8% for NASH and 17.4% for other causes. For the 42 cases of cirrhosis and HCC, 14.3% of the patients were HBV, 2.4% HBV / ethyl alcohol, 31.0% HCV, 9.5% HCV / ethyl alcohol, 26.2% 9.1% for NASH and 7.1% for other causes. Thus, early detection and prevention of HCC development is, in principle, the most impactful strategy to improve patient prognosis.

Financial Support: CNPQ and FAPEG.

Palavras-chave: HEPATOCELLULAR CARCINOMA, PREVALENCE, RISK FACTORS

PERIPHERAL POLYNEUROPATHY ASSOCIATED WITH INFECTION BY CHIKUNGUNYA VIRUS

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Resumo

Chikungunya virus (CHIKV) is an arbovirus belonging to the genus Alphavirus, first identified during an epidemic of febrile polyarthralgia in Tanzania in 1953. Since then, several large-scale outbreaks have been reported in Africa, India, Southeast Asia, the Western Pacific and the Americas, imposing heavy economic burdens and lost productivity. According to data from the Brazilian Ministry of Health in the period 2015 to 2017, there has been a considerable increase in the number of CHIKV cases in the country, most of them in the northeast region. During an outbreak in the State of Piauí in 2017, a 66-year-old female patient in Parnaíba-PI was admitted to an emergency of a public hospital with a complaint of fever for 4 days, severe arthralgia, myalgia, nausea, diarrhea, rash without pruritus, abdominal pain, ocular sensitization, asthenia, inappetence and reduced range of motion. Clinical and laboratory information were obtained through the application of a semi-structured form. CHIKV infection was confirmed by molecular methods employing Reverse Transcription followed by Polymerase Chain Reaction (RT-PCR) using primers which amplify the E1 and E2 gene regions of the virus. After examination of the hemogram, mild thrombocytopenia and leukopenia were observed. Ultrasonography of the hands, wrists, feet, ankles, femoral and shoulders showed synovitis with bilateral joint effusion, evidencing the presence of bilateral tendinopathy and periarticular edema.
After Electroneuromyography, sensory-motor axonal pattern peripheral polyneuropathy was observed, affecting upper and lower limbs in distal topography. The patient continued to report complaints of severe arthralgia, asthenia, distal edema and body weight reduction, turning to specialized medical care and then after approximately 180 days a gradual remission of symptoms was observed. Considering the presented case, the need to monitor the manifestation of neurological diseases associated with CHIKV infection and the importance of the use of complementary neuroimaging exams to allow the selection of appropriate diagnostic and therapeutic tests is evident, as CHIKV can be a potential cause of disability in chronic diseases. Financial Support: CNPq, CAPES, FAPEPI, and Parnaíba Municipal Council.

Palavras-chaves: Arthralgia, Chikungunya, Detection, Polyneuropathy

YELLOW FEVER OUTBREAK IN THE JEQUITINHONHA VALLEY: THE BEGINNING OF BRAZILIAN EXPERIENCE

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Resumo
The Yellow Fever virus (YFV) is a member of the Flavivirus genus of the Flaviviridae family. The YFV have two types of cycles, the urban cycle and the silvan cycle. In the silvan cycle, the transmission occurs by the bite of the female mosquito of the genus Haemagogus or Sabethes tonon-human primates, which causes the spread of the disease. The human can become infected when entering in the epizootic areas. In urban cycle the human infection occurs through the bite of infected female Aedes Aegypti mosquito. This human infected can propagating the cycle. The first outbreak of Yellow Fever (YF) in Brazil occurred in 1685 in Recife. Because of the high number of people infected with YF in Brazil, in the 1937 the vaccine against YF was available for the brazilian population. In 2016 and 2017 the Brazil has a new YFV outbreak. The virus spread in the regions of the Atlantic Forest biome and the most affected region was the Minas Gerais State (MG). In this state had 475 cases of this disease and 162 confirmed deaths. In Minas Gerais one of the most affected areas was the Diamantina city that is a reference county for tertiary health care in this region. In Diamantina happened 10 cases of YF and 3 deaths. In this way, the goal of this study was the clinical, immune and molecular characterization of YF cases from Jequitinhonha Valley in 2017. The research was developed from 8 patients hospitalized at the Santa Casa de Caridadeof Diamantina who were diagnosed with YFV. The medical records were consulted, processed and tabulated. Clinical samples that was collected from patients during hospitalization were tested to YFV. The RNA was detected by qPCR and IgM by ELISA. Six patients were IgM positive and two were qPCR positive to YF. The viral genome from the positive patient in qPCR was partially sequenced. The virus belongs a South America Genotype I. The cases in Jequitinhonha Valley happened in regions with low vaccine coverage (}
INFLUENZA A/H3N2 IN BRAZIL: GENETIC DIVERSITY, COCIRCULATION AND PERSISTENCE OF GENETIC GROUPS BETWEEN 2009-2017

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Resumo
Influenza viruses presented a rapid evolution due to accumulation of mutations in their genome, generating a high genetic variability that affects the selection of the vaccine strain. The influenza A/H3N2 virus was introduced into the human population in 1968 pandemic and since then it has been causing seasonal epidemics. The aim of this study was to investigate the genetic variability and the events associated to influenza A/H3N2 circulation, as well as to compare the recommended vaccine strains with the genetic groups that circulated in Brazil from 2009 to 2017. A maximum likelihood phylogenetic reconstruction was performed with 457 Brazilian sequences and global references of hemagglutinin gene from influenza A/H3N2 viruses. The sequences were translated into amino acids and compared with A/Brisbane/10/2007 vaccine strain for genetic group classification. The genetic groups found were further classified according to the season of circulation: autumn-winter (March-October) or spring-summer (November-April). Sequences detected in Brazil during the study period were classified into 14 distinct genetic groups and most of the identified amino acid substitutions were in antigenic sites, indicating the selective pressure exerted by the immune response of the host. Although these groups had circulated in different periods, there was a considerable genetic diversity since five genetic groups co-circulated in the same season. In addition, six genetic groups persisted at least for two autumn-winter seasons. Interestingly, a total of five (64.3%) genetic groups were detected for the first time in the spring-summer seasons, the period with lower virus circulation. Furthermore, the vaccine strains recommended have presented genetic divergence from the virus sequences that circulated in most of the seasons. Regarding the vaccines used during the period of this study, only in the autumn-winter seasons 2009, 2010, 2015 and 2016 we observed genetic match between vaccine strains and the A/H3N2 sequences identified. The recommended vaccine strain A/Perth/16/2009 showed larger genetic divergence for the period 2010-2012, showing correspondence only with 2010 Brazilian sequences. Therefore, the high genetic variability observed in this study reinforces the importance of the genetic surveillance for the optimization in selecting a vaccine strain, including the period of low virus circulation.

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Palavras-chaves: Genetic diversity, Influenza A/H3N2, Phylogenetic analysis

ANTIGENIC AND GENETIC CHARACTERIZATION OF STRAINS OF THE RABIES VIRUS, CIRCULANTS IN THE STATE OF PARÁ, FOR THE PERIOD 2010 TO 2017
The antigenic and genetic characterization of rabies virus makes it possible to obtain advances in the epidemiology of disease, because, the association of viral variant and host can elucidate on the maintenance cycle of Rabies lyssavirus. The objective of the study was to characterize antigenically and genetically strains of the rabies virus isolated at the Evandro Chagas Institute (IEC) rabies laboratory, from samples from the State of Pará, received in the period 2010 to 2017. According to the IEC database, between the years 2010 and 2017, 73 samples from the State of Pará were diagnosed as positive for rabies. These isolates were characterized antigenically by the panel of monoclonal antibodies produced and distributed by the CDC/OPAS, and the samples that were not compatible with any panel monoclonal antibody reading pattern were genetically characterized according to the methodology of Barbosa (2007). The 73 positive samples in the period were submitted to antigenic characterization, of which 72.60% (53/73) were compatible with some monoclonal antibody panel reading standard. Of the viral strains isolated from bovine brains, 64.58% (31/48) were compatible with VAg3 reading standard and 35.42% (17/48) did not correspond to any reading pattern. Of the strains of equinates, 84.62% (11/13) were compatible with VAg3 and 15.38% (2/13) did not correspond to any reading pattern, of the canine strains, 100% (6/6) were compatible with VAg2 and the only positive feline sample also corresponded to VAg2. Of the viral strains isolated from bats, 80% (4/5) corresponded to VAg3 and 20% (1/5) did not correspond to any reading standard. Of the 73 samples underwent antigenic characterization, 20 presented a reading profile not compatible with the panel of monoclonal antibodies and were submitted to the partial sequencing of the N gene. According to the phylogenetic analysis, all the sequenced samples were grouped in of VAg3 group, related to the hematophagous bat Desmodus rotundus, where most of the samples are related to samples characterized in previous studies carried out in the State of Pará. The antigenic and genetic characterization allowed to document the change and/or maintenance in the epidemiological cycle of rabies in the State of Pará. The results found in the State of Pará have been similar to those found in other states of Brazil and other Latin American countries, where the variant associated with Desmodus rotundus it has been widely identified.

Palavras-chaves: Rabies lyssavirus, Genetic characterization, Antigenic characterization, State of Pará
wild animals. Especially in the Amazon, where most riverside communities are located far from large urban centers, and exhibit a large shortage of basic infrastructure, in addition to the characteristics of dwellings, which are mostly stilts, with straw cover, facilitating the entry of an aggressor. The Pará has climatic conditions and shelters to keep populations of bats, in relation to cattle density, the State of Pará is the second largest cattle producer in the North Region of the country, these factors, added to disorderly urbanization contribute to the occurrence of rabies in the State. The objective of this study was to describe the prevalence of rabies in the State of Pará between 2010 and 2017. A survey was carried out on the database of materials received at the Laboratory of Diagnosis of Rabies of the Evandro Chagas Institute, from 2010 to 2017, originating from the six mesoregions of the State of Pará. Of 5,051 samples received and analyzed in the laboratory, 1.44% (73/5,051) were positive. Of the 73 positive samples, 65.75% (48/73) were bovine, 17.81% (13/73) equine, 8.22% (6/73) canines, 6.85% (5/73) feline. In the analysis of the distribution of positive cases by mesoregion, it was verified that 52.05% (38/73) belonged to the mesoregion of Southeast Paraense, 38.36% (28/73) to the Northeast of Paraense, 6.85% (5/73) the Metropolitan of Belém and 2.74% (2/73) to the Southwest of Paraense. The southeast of Paraense was the mesoregion that had the highest positivity index and the species with the highest percentage of positivity were cattle and horses. The results presented highlight the importance of the annual immunization of production animals as well as emphasize the relevance of the vaccination campaigns of companion animals for the control of urban rabies in order to reduce the incidence and lethality of the disease.

Palavras-chaves: Rabies lyssavirus, Epidemiology of rabies, State of Pará

MOLECULAR AND EVOLUTIONARY CHARACTERIZATION OF NOROVIRUS GII.17 IN THE NORTHERN REGION OF BRAZIL.

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Resumo

Noroviruses (NoV) are associated with a fifth of all cases of acute viral gastroenteritis occurring in the world. Currently a new variant called GII.17 2014 have been associated to outbreaks of gastroenteritis in several Asian countries and in some cases is replacing the previously dominant variant, GII.4. There are already records of the circulation of this strain in Latin America, but data on the circulation of this new strain are still limited mainly OR principally in Brazil. Therefore the aim of this study was to perform molecular characterization of strains evolving GII.17.2014 NoV strains detected in the northern region of Brazil. Initially, an epidemiological survey was carried out on the number of NoV cases detected during the years 2015 and 2016. Fecal suspensions were prepared at 10% of the obtained samples and viral RNA was extracted using commercial kit. The primers used (LNOV 5KF / 5KR and LNOV 6KF / 6KR) for amplification of VP1 were constructed using GII.17.2014 strains obtained from Genbank. The samples were submitted to one-step RT-PCR reaction. The products with satisfactory quality were purified and submitted to direct sequencing with the kit Big Dye Terminator® v.3.1 Cycle Sequencing Kit. The variations of the nucleotides within and the sequences were verified by the application of the Neighbor-Joining method and the evolutionary analyzes was done using Bayesian inference. Between
the years of 2015 and 2016, 12 samples (12.6%- 12/95) were characterized as GII.17-2014, 3.3% (1/30) in the year 2015 and 16.9% (11/65) in 2016. The Brazilian strains detected in our study were grouped in Clade D. Among clade C and D, the amino acid variation was 4.7%. It was verified that the time to most recent common ancestor (TMRCA) for the C and D clades was 2008 and 2012, respectively. According to the temporal analysis the strains detected in this study had two entrance events in the country. With regard to mutation rates among samples from the Amazon region, values of 4.82x10^{-3} and 4.32x10^{-3} substitutions/site/year were obtained. Comparing the VP1 sequence of Clade C strains with Clade D strains it was observed that 36 changes occurred in several positions and that 61.1% (28/36) happened in the P2 region. These data demonstrate that the NoV GII.17 has undergone great evolution for years and the number of mutations observed on its surface may explain the increase in its prevalence.

Palavras-chaves: norovirus, gastroenteritis, Amazon region

### SERUM-EPIEMIOLOGICAL SURVEY OF MEASLES AND RUBELLA IN THE CITIES OF BELÉM AND ANANINDEUA, PARÁ, BRAZIL

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**Resumo**

**Introduction**: Measles and rubella are infectious diseases that spread through the respiratory route. For measles, the risks of serious complications and death are high in children younger than five years and in immune-suppressed adults. Rubella is transmitted directly or transplacentally, entering fetal circulation and infecting the fetus. After the “Program for Measles Eradication and Rubella Elimination and Congenital Rubella Syndrome Control,” in which there were several strategies and investments in vaccination programs to achieve the goal, the eradications of measles and rubella were certified in 2016 and 2015, respectively. The resurgence of cases of these viruses is a threat to a decades-long work of epidemiological surveillance, which continues to be monitored to maintain eradication. Outbreaks and epidemics of these diseases have been observed around the world, even in continents that are economically developed, because of low vaccine coverage, circulation of the viruses in different parts of the world, case importation, and consequent viral propagation, in addition to primary and secondary vaccine failures. **Objective**: The purpose of this study was to evaluate the presence of antibodies specific to measles and rubella in a population ranging in age from 15 to 39 years as an indication of susceptible individuals over two years (2016 and 2017) in the cities of Belém and Ananindeua. **Material and Methods**: This is a cross-sectional, observational study of students in schools, faculties, universities, and the Evandro Chagas Institute that will include epidemiological information from the participants. A minimum of 2,210 individuals, of which 1,107 will come from Belém and 1,103 from Ananindeua, will be enrolled. From April 2016 to June 2017, a total of 1,059 samples were collected, of which 681 were from Belém and 378 from Ananindeua. **Results**: For the measles virus (VS) 83% and 81% presented immunity in the municipality of Belém and Ananindeua respectively. For the rubella virus (VR)
they accused immunity in Belém 91% and Ananindeua, 86%. The susceptibility to measles and rubella in Belém was higher in the age group of 15 to 19 years old with 22% and 11% respectively, the same occurred with the municipality of Ananindeua with 21% for SV and 17% for RV. Conclusion: Considering the total sampling, the municipalities of Belém and Ananindeua presented a statistically significant percentage of susceptible for the measles virus, but not for Rubella.

**Palavras-chaves:** Measles, Rubella, Epidemiology, Vaccine

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**AN OLD VIRUS PLAYING NEW TRICKS – COULD BOVINE LEUKEMIA VIRUS CAUSE CANCER IN HUMANS?**

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**Resumo**

Bovine Leukemia Virus (BLV) is a retrovirus that causes a neglected, silent lifelong infection commonly found in dairy cattle, named enzootic bovine leukosis (EBL), characterized by persistent lymphocytosis that, in some cases, depending on the animal’s Bola genotype, progresses to B cell lymphoma. BLV infection is widely spread in dairy cows from North and most South American countries including Brazil. Early studies in the 1970’s indicated that BLV was non-infectious to humans; nonetheless, by using more sensitive serological and molecular approaches that theory has now been questioned and recent data linked BLV with breast cancer in women. However, there is no consensus on this subject and, in at least two studies, BLV DNA or anti-BLV antibodies could not be found in women. On this scenario, our study aimed to contribute to this discussion in that breast cancer is a major worldwide issue of public health. By using the polymerase chain reaction (PCR) we investigated the presence of BLV genome in healthy (n=72) and cancerous (n=72) paraffin-embedded samples of breast tissues from women in south Brazil. BLV DNA was found most frequently (30.5%) in breast cancer tissue than in healthy breast tissue (13.8%) (Odds ratio = 2.73; confidence interval = 1.18 – 6.29; p = 0.027). Simultaneously, we used a competitive enzyme-linked immunosorbent assay (cELISA) to detect antibodies to the BLV major envelope glycoprotein (gp51) in serum from healthy blood donors (n = 1.500) and, in contrast, found only 2 (0.13%) positive samples. There was no association between BLV DNA and other tumor prognostic biological markers such as hormonal receptors, HER2 oncoprotein, proliferation index, metastasis in sentinel lymph nodes, and tumor grade and size. The mechanisms by which BLV reaches human beings have not been investigated yet but within cattle, and similar to other retroviruses, transmission requires transferring of cells from infected to non-infected individuals and, as such, meat and milk from infected animals could be potential sources of virus. Our findings suggest that BLV should be considered a potential predisposing factor to breast cancer in women.


**Palavras-chaves:** Cancer, Animal Virus, Zoonosis, PCR, BLV
ANTIVIRAL ACTIVITY OF TRANS-CINAMIC ACID AGAINST HUMAN MASTADENOVIRUS - SEROTYPE 5

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Resumo

Introduction: Human Mastadenovirus - Serotype 5 (HAdV-5) cause about 5% of respiratory infections in the world, leaving the body through the gastrointestinal system and can re-infect if there is no proper sewage treatment. Precisely for this reason, the need to create appropriate treatment for it becomes extremely important, in order to avoid the appearance of more cases of this disease around the world. Trans-cinnamic acid (t-CA) is an aromatic compound found in several plants and has anti-inflammatory, antimicrobial, antitumor, antioxidant and antiviral activities already known. Objective: The present study aims to evaluate the possible antiviral activity of t-CA against HAdV-5. Methodology: Once t-CA was poorly soluble in water, it was first tested for absolute ethanol cytotoxicity in order to dissolve the t-CA and a stock solution of t-CA in absolute ethanol (0.1 g/mL) was prepared for further testing of the cytotoxicity of the extract itself on A549 cells (human lung adenocarcinoma cells). Three independent experiments were performed in 96-well plates, testing acute (24h) and chronic (6 days) toxicity, considering the virus's replication that was previously patterned on lab. The CC₅₀ (50% cytotoxic concentration) was set at 1699.69 µg/mL. For antiviral activity tests, six-well plates were prepared 24h before, inoculated with HAdV-5 in the test wells and culture medium in the control wells for 1h in 5% CO₂ atmosphere at 37 ° C. At the end, the inoculum and the culture medium were withdrawn, the t-CA was added in the required wells (concentrations of 120 µg/mL, 60 µg/mL and 30 µg/mL) and culture medium was added for viral control and cell, returning to incubation. After the 6-day period, the entire contents of the wells were removed and the plate was stained with violet crystal. Results: Preliminary assays demonstrated a reduction in the number of lysis plates formed by HAdV-5 depending on the amount of extract used. New tests are being done to define the percentage reduction of lysis plaques and confirm the anti-HAdV-5 potential. Conclusion: Even though it still needs more tests to confirm the antiviral activity, t-CA demonstrates a great potential for anti-HAdV-5 and once this activity is confirmed, tests are necessary to establish the place of action of the extract in the viral replication cycle. Financial Support: Feevale University

Palavras-chaves: Antiviral, A549, HAdV-5

Muscular replication of Zika virus as a potential injury site and route to access central nervous system

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Resumo

The ZIKA virus (ZIKV) is an enveloped virus that belongs to the Flaviviridae family, transmitted to humans by Aedes mosquitos. During ZIKV infection, severe conditions are associated with neurological complications such as Guillain-Barré syndrome and fetal malformation after intra-uterato 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 and its consequences has not yet been demonstrated. Therefore, the aim of this study was to characterize the impact of ZIKV replication in muscular tissue and the possible use as a route to ZIKV spread to central nervous system (CNS). Thus we used primary culture of human myoblasts isolated from skeletal muscle (HSMM cells) to in vitro studies and an in vivo neonatal mice model of ZIKV infection to access the role for viral spread and muscular damage. HSMM cells were infected with ZIKV at multiplicity of infection of 5. We observed a time-dependent increase of release of infectious ZIKV at culture medium of myoblast (determined by plaque assay) and a reduction of cell viability of about 48% (determined by MTT assay). Analysis of ZIKV replication in differentiated HSMM cells into myofibers demonstrated lower amplitude of ZIKV release and lower percentage of positive cells when labeled with antibody against viral protein E (37% of myoblast and 8% of myofibers, 48 hpi), indicating that myogenic precursors were more susceptible to ZIKV. Infection of wild type SV129 newborn mice (3 days-old) with 10^5 PFU of ZIKV, using subcutaneous route, results in clinical signs such as weight loss, lethargy and difficult of locomotion. We also observed a temporal increase in detection in ZIKV RNA at muscle (determined by ZIKV by qPCR), that was consistent with viral replication. We also observed higher levels of IL-1B and TNF expression when compared to mock-infected animals. Curiously the increase of ZIKV RNA detection in mice brain occurs only after the peak of muscular replication (4 dpi), together with ZIKV RNA detection in peripheral neural structure as dorsal root ganglion and spinal cord. These results indicate that ZIKV induces muscle inflammation and probably use this route to reaches CNS. Immunological detection of ZIKV and histological analysis are ongoing to sustain these observations.

**Palavras-chaves:** Mice model, Muscle infection, Viral spread, Zika virus

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**Antiviral effect of Nitazoxanide drug against ZIKV on Human Placenta and Cervix cells**

**Autores**
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**Instituição**

**Resumo**

Infection by Zika virus (ZIKV) is associated with the congenital syndrome; however, as the virus reaches the fetus is still unclear. It is possible that the transmission of ZIKV to the fetus occurs with the participation of the uterus in addition to hematogenous routes since has been demonstrated sexual transmission. Despite the emerging severity caused by the ZIKV infection, there is still no specific or prophylactic treatment for this disease. The aim of this study was to evaluate the antiviral effect of the drug Nitazoxanide, in the context of drug repurposing, and to investigate its possible immunomodulation in cultures of chorionic primary cells of human placenta and cervix human cells, two important cells for congenital transmission. The antiviral effect was assessed by immunofluorescence, Plaque assay and RT-qPCR in cultures infected with ZIKV and treated with non-toxic concentrations of the Nitazoxanide for 48 hours. The activity of the drug evaluated by screening in Vero cells before and after infection or together with the viral adsorption only showed antiviral effect when the treatment was performed after the adsorption step. Additional trials in this treatment scheme revealed a dose-dependent reduction of infection in chorionic cells that at the concentration of 50 µg/mL was 79%. Interestingly, in the
cervix cells, at the concentration of 12 µg/mL already had a 95% reduction in infection. The EC$_{50}$ values were 23±5 for chorionic cells and 6±0.4 for cervix cells. Analyzing the reduction of infectious particles, the concentration of 25 µg/mL in the chorionic cells and 12 µg/mL in the cervix cells inhibited 100% of the progeny. In addition, the quantification of viral RNA copies/mL in the supernatant of the treated cultures with 50 µg/mL showed a reduction of 93% in chorionic cells and 87% in cervix cell cultures. However, in cells of the ZIKV-infected C6/36 mosquito line, the treatment did not reduce the number of infectious particles in the cultures supernatant, suggesting that the activity of this drug is relate to the response of the host cells. On the other hand, the treatment was also effective against the dengue virus in Vero cells, suggesting that this drug can act against others Flavivirus. Research that applies drug repurposing should be encouraged and may accelerate the discovery of drug to treat ZIKV infection, especially for pregnant women infected or living in endemic areas.

Palavras-chaves: Antiviral effect, Cervix cells, Human Placenta, Nitazoxanide, ZIKV

LEVELS OF PLASMA CYTOKINES IN INDIVIDUALS WITH ACUTE AND CHRONIC HEPATITIS B

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Resumo

The hepatitis B virus (HBV) is one of the leading causes of acute and chronic hepatitis representing a serious public health threat. Cytokines are known to be important chemical mediators that regulate the differentiation and proliferation of immune cells, thus coordinating the responses and progression of inflammatory diseases. The aim of this study was to investigate the presence of pro and anti-inflammatory cytokines (IL-35, IL-6, IL-17A, IFN-$\gamma$) in the plasma of patients with acute and chronic hepatitis B and to verify if there is any association between the production of these cytokines in the plasma with the viral load and the different genotypes of the virus. A total of 57 patient samples were evaluated, 23 of them with acute infection and 34 with chronic infection, confirmed by serological tests, liver function (ALT and AST) and viral load by real-time PCR. All samples included in the study were tested for the presence of HBV DNA by nested-PCR, positive samples were purified, sequenced and genotyped for phylogenetic tree construction. The cytokines were detected by ELISA. HBV DNA was detected by real-time PCR in 93% (53/57) of the samples, the mean viral load quantitative log for acute patients was 4.48 and, for chronic patients, it was 2.81. Regarding the distribution of genotypes, it was observed that 83% of the individuals with acute HBV infection belonged to genotype A and 17% to genotype F. The chronic profile was the one with the highest genotype diversity, 82% of the samples belong to genotype A, 6% to genotype D, 6% to genotype E and 6% to genotype F. No correlation was found between genotype and cytokine production. It was observed that plasma levels of IFN-$\gamma$ and IL-17A were higher in chronic patients compared to the acute ones, and a positive correlation between viral load and IL-17A ($p = 0.045$) and IFN-$\gamma$ ($p = 0.023$) was observed through the Pearson correlation coefficient in both groups. These cytokines could be modulating pro-inflammatory effects and inducing hepatocellular damage. IL-6 levels ($p = 0.052$) were higher in acute patients when compared to the
chronic group, this cytokine would be involved in viral elimination and protection against chronicity. In contrast to the literature for HBV, IL-35 levels were higher in acute than in chronic subjects. Further studies are necessary for a better understanding of the complex regulatory mechanisms of the host antiviral response related to IL-35 during HBV infection.

**Palavras-chaves:** Acute, Chronic, Cytokines, Hepatitis B

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**ISOLATION AND CHARACTERIZATION OF STRICTLY LYTIC BACTERIOPHAGE PARTICLES FOR ERADICATING PSEUDOMONAS AERUGINOSA INFECTIONS**

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**Resumo**

Introduction: Burst of the host bacterial cell induced "from within" is the last step in the bacteriophage lytic replication cycle, being mediated by two special proteins produced in the last stage of the virion progeny assembly, viz. holins (which perforates the inner side of the cytoplasmic membrane) and endolysins (which passes through the newly formed pores and accesses the peptidoglycan layer, degrading it and resulting in destabilization of the murein sacculus), resulting in the lysis of the host bacterial cell with liberation of the virion progeny particles and concomitant release of intracellular components. In the research effort entertained herein, the potential of isolation (from environmental sources) and characterization of novel bacteriophage particles with broad lytic spectrum capable of infecting Pseudomonas aeruginosa, was investigated. Methods: Physicochemical characterization of the isolated bacteriophages included verification of bacterial lysis, amplification of the bacteriophages, titration of the concentrated bacteriophage suspensions, SDS-PAGE electrophoresis, XRD and UV-Vis scannings, whereas biological characterization encompassed evaluation of their lytic spectra, efficiency of plating (EOP) assays, determination of the one-step growth curves (OSGC) for latent period and burst size determinations, and determination of the adsorption curves for calculation of the bacteriophage adsorption rate onto its bacterial host cell. Results and Discussion: The bacteriophages isolated and characterized produced clear (and different) plaques of bacterial lysis and exhibited a broad lytic spectrum against several Pseudomonas aeruginosa strains isolated from (human) clinical samples. Conclusions: The isolated bacteriophages proved to be suitable candidates for eradicating antibiotic-resistant bacterial strains of Pseudomonas aeruginosa.

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**Palavras-chaves:** BACTERIOPHAGE, PSEUDOMONAS AERUGINOSA, CHARACTERIZATION

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**RETROSPECTIVE STUDY OF HUMAN RABIES CASES CONFIRMED BY LABORATORY**
Resumo

Rabies is a viral zoonosis, usually lethal and of significant public health importance. In Brazil, it is an endemic disease that in recent years presented a change in the epidemiological pattern. Over the years, a decrease in the number of cases of human rabies transmitted by dogs and a considerable increase in transmission by wild animals was observed. The knowledge of the spatial and temporal distribution of rabies cases in humans and animals, can contribute to the development of measures for prevention, surveillance and control of the disease. The aim of this study was to evaluate available data of human rabies cases diagnosed at the National Reference Laboratory for rabies diagnosis, Instituto Pasteur (IP) from São Paulo, in the period from 2003 to 2009. We assessed the diagnosis request files and the data registered in the computerized IP system, Inforaiva. Human rabies cases were considered positive when confirmed in at least one laboratory technique. The data were analyzed descriptively considering the epidemiological variables as transmissions as characteristics of the person attacked, the aggression, the aggressor animal, the geographic location and the seasonality. In the evaluated period, 31 cases were detected positive for human rabies in IP, of these 61% (19/31) are men, 25.9% (8/31) are children from 0 to 10 years, 83.9% (26/31) suffered aggression in the Northeast region, and the Maranhão state presented the highest occurrence, with 71% (22/31). In the rural area more human rabies cases (38.8%) occurred than in the urban area (12.9%). The most frequent form of aggression was the bite with 48% (15/31) and the most commonly reached place were the feet, presenting in most cases single and deep wounds (16.2%). In this period, the bat had greater importance in the transmission of human rabies, responsible for 33.2% of the cases. Spring was the period with the highest number of cases (38.7%). It is important to note that among the analyzed datasets, a high frequency of uninformed data was observed, ranging from 29 to 45.2%. The number of human rabies cases diagnosed in the IP declined in the considered period. On the other hand, a greater number of human rabies transmitted by bats was observed, suggesting a tendency of change in the epidemiological profile in relation to the animal species involved in the transmission.

Financial Support: FAPESP (2017 / 15064-5)

Palavras-chaves: Human rabies, Laboratory Diagnosis, Instituto Pasteur
countries, and are associated with time and economic constraints, overtaxing the import process. Here we report a polyclonal IgG antibody designed to react specifically with RABV ribonucleoprotein (RNP), and the evaluation of its applicability for dFA as a diagnostic reagent for rabies. Horse hyperimmune serum against RNP was used for purification of the polyclonal IgG by HiTrap protein-A column, 5mL (GE Healthcare) followed by antigen-immunoaffinity chromatography (IAC) using RNP protein as stationary phase (CNBr-activated sepharose 4B; GE), according to the manufacture’s protocol, obtaining coupling efficiencies in the range of 75%. The purity and affinity of IgG obtained were analyzed by 10% SDS-PAGE (under reducing and non-reducing conditions) and indirect ELISA, respectively. The purified IgG concentration was estimated by methods absorbance at 280 nm. Following the manufactures’ recommendations, anti-RNP IgG (1mg/ml) was conjugated to FITC (Sigma), obtained fluorochrome/IgG protein molar ratio approximately 2.8. The specificity and sensibility of anti-RNP IgG- FITC were tested by dFA in positive (n=20) and negative (n=22) CNS samples for rabies of different species animal (bovine, cat, dog, equine and bat) using a commercial reagent as reaction control. As results, the purified IgG contained one band of molecular weights ranged from 250 to 150 kDa under non-reducing conditions, and two bands, one at approximately of 52-58 kDa (H-chain) and another band at 22-29 kDa (L-chain) under reducing conditions, showing electrophoretic pattern compatible with horse IgG. In addition, it had high-affinity RNP recognition by indirect ELISA. The analyses of samples by dFA revealed that the conjugated produced obtained 100% of diagnostic specificity and sensibility for RABLV detection, demonstrating concordant results with those obtained with a commercial reagent. In conclusion, our results demonstrate that IAC may be a good technique for the purification of polyclonal anti-RNP IgG, which may be used as a diagnostic reagent for rabies. Financial support: Instituto Pasteur

Palavras-chaves: Anticorpo, cromatografia de afinidade, Diagnóstico, Imunofluorescência direta, Raiva
infection in human cells (A549 cells) in a concentration-dependent and ZIKV-lineage independent manner. NAR antiviral activity was also observed when human neuronal cell line (A172 cells) were infected by ZIKV. NAR displayed its antiviral activity when the cells were treated after infection, suggesting that NAR acts on the viral replication or assembly of viral particles. Moreover, a molecular docking analysis suggests a potential interaction between NAR and the protease domain of the NS2-NS3 protein in ZIKV which could explain the anti-ZIKV activity of NAR. Finally, the results support the potential anti-ZIKV effect of NAR, which could be a suitable candidate molecule for developing anti-ZIKV treatments.

Palavras-chaves: antiviral, naringenin, zika, arbovirus

ANTIVIRAL ACTIVITY OF GALLIC ACID AGAINST MAYARO VIRUS

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Resumo

Introduction: The search for new forms of disease prevention is extremely important. The most viruses do not have adequate treatment, even though it has been known about them for years. It makes it difficult to contain them and they end up spreading worldwide. This is the case of the Mayaro virus (MAYV), an arbovirus of Togaviridae family, Alphavirus genus, which may cause a disease called Mayaro Fever: severe fever, headaches, joint pain and chronic potential. Gallic Acid (GA) is a flavonoid that already demonstrated its antitumor, antioxidant, antimutagenic and, in some other studies, antiviral activities.

Objective: This work pretends to evaluate the viral activity of GA on the VERO cell line (African green monkey kidney) against the MAYV. Methodology: First, the cytotoxicity of GA in Vero was assessed by cell viability assays with MTT. Three independent experiments were performed, evaluating viability in 24h (acute toxicity) and 48h (chronic toxicity) exposure to acid, considering the virus’s replication that was previously patterned on lab, resulting in CC_{50}/(50% cytotoxic concentration) of 20µg/mL for 48h exposure. For the antiviral activity assays, two 12-well plates, prepared 24h prior to GA exposure, were inoculated with MAYV on the wells that were tested and culture medium on the cell/cytotoxicity control, remaining for 1h in an incubator. After the period, the inoculum and culture medium were removed, the extract added in six different concentrations of GA (18 µg/mL, 17 µg/mL, 16 µg/mL, 15 µg/mL, 14 µg/mL and 13 µg/mL), being kept for 48h under an atmosphere of 5% CO_{2} at 37°C. At the end, the contents were removed from the wells, the cells were stained with violet crystal and the lysis plates counted. Results: Two independent experiments were performed and they showed reduction of lysis plate formation as the GA concentration in the well increased. A third experiment is being conducted in order to confirm the anti-MAYV effect and the percentages of reduction of the viral effect on the cells. Conclusion: Although a third confirmatory experiment is necessary, it has been found that, the GA can interfere in the cycle of viral replication and tests are necessary to identify the mechanism of action of the GA against the MAYV. Financial Support: Feevale University

Palavras-chaves: Antiviral, Human virus, Mayaro, Vero

PREVALENCE AND RECURRENCE OF RESISTANCE ASSOCIATED MUTATIONS TO DIRECT ACTING ANTIVIRALS IN HEPATITIS C VIRUS INFECTED PATIENTS
Prevalence and recurrence of resistance associated mutations to direct acting antivirals in Hepatitis C Virus infected patients Treatment of Hepatitis C Virus (HCV), in Brazil, is based in direct acting antivirals (DAAs) such as Simeprevir (SMV), a NS3/4A protease inhibitor, Daclatasvir (DCV), a NS5A inhibitor, and Sofosbuvir (SOF), the NS5B polymerase inhibitor. To determine the best combination of drugs during treatment, many variants are considered, and the viral genotype is the most important since neither all the antivirals have pangenotypic activity. This therapy results in sustained virological response (SVR) that varies according to the viral genotype. Genotypes 1 and 3 are the most prevalent in Brazil and, despite the high SVR rates provided by the DAAs, treatment escape is still a problem. This is associated with the occurrence of resistance substitutions in the viral genome. The aim of this study was to evaluate the presence of resistance substitutions within viral proteins NS3, NS5A and NS5B in samples collected from patients infected with HCV genotypes 1 and 3, before treatment with DAA. A total of 117 viral variants from the serum of the corresponding patients (73 from genotype 3 and 44 from genotype 1b) has been analyzed. Serum samples were submitted to RNA extraction, cDNA synthesis, amplification of DAAs target region by Nested-PCR and direct sequencing by Sanger method. Many substitutions were observed for genotype 1b, such as Q80L for NS3 (18.75% of mutations in this region), L28M, R30Q and Y93H (14.3%, 23.8% and 4.8% of recurrence, respectively). For NS5B, substitutions Y448H (8.33%), A421V (16.66%), L159F, C316N and S556G (25% of recurrence for each one) were detected. One curious situation is that mutations L159F and C316N occurred simultaneously, in three viral variants of the same three patients, an indication of a possible compensatory relation between them that corroborates with the literature. For genotype 3 NS5A, mutations A30K, A62S and A62T were observed in 3.6%, 73.2% and 19.6% of all substitutions, respectively, while for NS5B, only L159F, in one single viral genome, was detected. The study and description of the prevalence of these mutations may contribute to the therapeutic targeting in order to increase SVR and reduce treatment escape.

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Palavras-chaves: Direct-acting antivirals, HCV, Resistance mutations, Treatment evasion
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Resumo

Dengue virus (DENV) is an emerging virus involved in numerous outbreaks in Brazil. The association between the virus and vertical transmission with disorders in the placenta has raised a worldwide concern. We described a case of DENV-4 infected pregnant women and her fetus during 2013 dengue outbreak. During the 29th gestational week, pregnant women presented severe complications of dengue infection which leads to abortion. Postmortem analysis of fetal organs demonstrated the presence of DENV by RT-PCR in the fetal brain and in several peripheral fetal tissues such as brain, liver, lungs, spleen as well as in placenta (mainly in Hofbauer cells) using anti-NS3 staining. Histological analysis of the placenta and fetal organs revealed different types of tissue abnormalities, which included inflammation, hemorrhage, edema, necrosis in placenta, as well as tissue disorganization in the fetus, such as spongiform parenchyma, microglial inflammation, steatosis, hyalinose arteriolar, inflammatory cells in the alveolar septa and disorganization of the lymphoid follicle. Increased cellularity (macrophage, Hofbauer cells and TCD8+ lymphocytes), as well as, up-regulation of inflammatory mediators such as IFN-γ, TNF-RANTES/CCL5, MCP1/CCL2 and VEGF/R2 were detected in liver, lung, spleen, brain and placenta supporting placental and fetus peripheral tissues inflammation.

Palavras-chaves: Vertical infection, DENV-4, Stillborn

MOLECULAR SURVEILLANCE AND PHYLOGENETIC ANALYSIS OF DENGUE, ZIKA AND CHIKUNGUNYA IN SYMPTOMATIC PATIENTS FROM SÃO JOSE DO RIO PRETO, SP, BRAZIL

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Resumo

Aiming to detect patterns and mechanisms of viral circulation, various surveillance actions have been intensified, as well as serological investigations and molecular surveillance in different regions of the country. The objective of this study was to analyze the presence of Dengue virus (DENV), Chikungunya virus (CHIKV) and Zika virus (ZIKV), their subtypes and associated genotypes in clinical samples of patients with fever who sought the Health Services of the Municipality of São José do Rio Preto in an active epidemiological surveillance system. For the investigation of these arboviruses specific primers for the envelope gene (ZIKV), NsP1 (CHIKV) and NS5 (DENV) were used by real-time RT-PCR. Between February/2016 and June/2018, 1891 serum samples were analyzed. Two hundred and nine samples (11.1%) were confirmed as positive for ZIKV, 99 (5.24%) for DENV and three (0.16%) for CHIKV. It is important to note that seven cases of DENV-2 / ZIKV co-infection and three cases of DENV-1 / ZIKV co-infection were observed. Samples of the predominant serotype DENV-2 were submitted to the envelope gene sequencing and were used for phylogenetic reconstruction, showing...
the circulation of the American/Asian genotype, but with the presence of genetic polymorphism in relation to the samples detected in the past in our region. DENV-1 samples were genotyped for SNP present at position 2021 of envelope and 8587 of NS5 protein, which differentiate L1 from L6 within genotype V, showing the predominant circulation of L6 when compared to L1, subsequently submitted to sequencing of E gene and phylogenetic reconstruction. Our data allow us to conclude that although Zika is the predominant arbovirus in our region, dengue continues to circulate, with emphasis on serotype 2. The circulation of DENV-1 and DENV-4 is residual from the last outbreaks (with genotypes that previously circulated, genotype V and genotype II, respectively). In addition, CHIKV is circulating in low amounts, which would allow the occurrence of a large-scale epidemic in the future. This work demonstrates the importance of university-health system integration through molecular studies to understand the origin and evolution of arboviruses, emphasizing the importance of using them as a tool to predict epidemics in epidemiological surveillance programs.

Financial support: FAPESP

Palavras-chaves: Arbovirus, Viral Circulation, Genotyping, Phylogenetic Reconstruction, Surveillance

HUMAN CYTOMEGALOVIRUS INDUCES EXPRESSION OF KALLIKREIN RELATED PEPTIDASE 6 IN HUMAN PRIMARY FIBROBLASTS AND U138 GLIOBLASTOMA CELL LINE

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Resumo

The human cytomegalovirus (HCMV) is a widespread viral agent, highly prevalent in the worldwide population. After primary infection, the virus establishes lifelong latency in the host and can be reactivated in immunocompromised individuals, causing serious diseases which can lead to death. In addition, the virus is the main cause of congenital infection diseases in newborns such as deafness, mental retardation and microcephaly. HCMV has tropism for CNS cells and many groups reported its presence in brain tumors. It is suggested that the virus can increase tumor malignity in a process called oncomodulation. Gliomas are the most common SNC tumors. Grande IV astrocytoma, also known as Glioblastoma Multiform (GBM) is a highly malignant and infiltrative tumor, with an extremely low survival rate. Our group has reported a high prevalence of HCMV in GBM tumors of Brazilian patients. Kallikreins (KLKs) are a family of serine-proteases with trypsin and/or chymotrypsin-like specificities. There are 15 tissue kallikreins, named from KLK1 to 15 which are involved in many physiological functions, such as skin dequamation, teeth enamel function, semen liquefaction and neural synaptic plasticity. In the Central Nervous System (CNS), the KLK6 is found to be highly expressed and its deregulation appears to be involved with Alzheimer’s disease and multiple sclerosis. Changes in KLK6 expression are also related to reduced sensibility of glioblastoma (GBM) to chemo e radiotherapy. It is known that HCMV is capable of modulate the expression of many host proteases, however there are no reports regarding the possible effect of the virus in KLKs expression. main goal of this work is to evaluate the expression of KLK6 in U138 GBM cell line and MRC5 primary fibroblasts infected with the HCMV TB40 clinical strain. The data obtained by RT-quantitative PCR indicates that the virus induces the expression of the KLK6 transcript in a time-dependent manner in both MRC5 and U138 cell line.

Financial support: FAPESP, CAPES, UFABC.

Palavras-chaves: Glioblastoma, HCMV, Kallikrein
Next generation sequencing (NGS) is a potential and robust tool for the identification of new/emerging viruses from different samples and helps to understand the circulation and spread of viruses in wild animals, eventually allowing the prevention of zoonotic transmissions. The aim of this study is to analyze the virome of oral swabs samples from New World primates (*Sapajus nigritus*, n=5) collected in a municipality of Southern Brazil, in the Mata Atlantica Biome. The samples were collected using individual swabs and next, submerged in 2 ml of Minimal Essential Medium supplemented with antibiotics. Samples were pooled and the pool was filtered (0.45 µM) and ultracentrifuged at 33 000 x g for 3 h at 4º C. The viral pellet was resuspended in saline buffer, treated with 100 U of DNAse and RNAse and submitted to nucleic acids extraction. DNA and cDNA were randomly amplified with Phi 29 enzyme and products were used for pair-ended sequencing using Illumina sequencing technology. In total, 533,526 reads were assembled by DE NOVO tool with MIRA assembler and overlapping regions were identified with PRICE. The sequences generated were checked first in Blast2Go software with a viral data bank and reviewed manually using a cutoff e-value of 0.001. The preliminary analyses of sequences have identified the partial genomes of *Human papillomavirus* type 4, *Human gammaherpesvirus*, *Bat polyomavirus*, *Callitrichine herpesvirus* and *Densovirus*. Moreover, two complete genomes of *Circovirus* (2329 nt) and *Torque teno virus* (3318 nt) were found. Additional analyses will be performed to further identify the viruses found here and to speculate on their importance for the host’s health or even for public health. These data emphasize the importance of surveillance strategies to detect emerging diseases with potential to spread from wildlife to humans.

Financial support: FAPESP, FAPERGS and Capes.

**Palavras-chaves:** Monkeys, Oral Virome, Circovirus, Torque teno virus, Poliomavirus
PUTATIVE NOVEL DICISTROVIRUS DETECTED IN MOSQUITOS COLLECTED IN TOCANTINS STATE, NORTH OF BRAZIL

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Resumo

Dicistroviridae is a family of multiple non-enveloped viruses with positive sense RNA genomes, having as host aphids, leafhoppers, flies, bees, ants and silkworms. These viruses have been associated with diseases such as the Black Queen Cell Virus, the cricket paralysis virus, the Israeli acute paralytic virus. The objective of the present work was to evaluate the presence of virus in pools of about 1,000 mosquitoes collected in four municipalities of the state of Tocantins. After the collection, the samples were homogenized in water, ultracentrifuged, submitted to viral DNA and RNA extraction and sequenced by next generation sequencing. The sequences obtained were assembled and the contigs submitted the BLASTx tool for viral detection. After identification, the contigs belonging to the same family were extended using the software "PRICE". The contigs showed similarity with the Flaviviridae, Metaviridae, Baculoviridae and Dicistroviridae families. The genes sequences that matched to members of the Dicistroviridae family showed about 50% amino acid identity with proteins from the Bundaberg bee virus. Phylogenetic analysis points to a possible new genus of Dicistrovirus that infect mosquitoes. The role of this putative new virus will need to be further explored to confirm its infection in mosquito hosts. This new putative dicistrovirus is the first mosquito-borne virus identified in Tocantins, Brazil.

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Palavras-chaves: Arboviruses, Cerrado, Culicidae, Potencial, Virome

Infection by Zika Virus of explant culture and human placenta primary cells

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Resumo

In 2015 there was an outbreak of infants with microcephaly and other neurological disorders born from mothers who were affected by Zika fever in Brazil. It is unclear how the virus reaches the fetus, but the placenta represents an important route of transmission. Thus, the objective was to implement the primary culture of human placenta explants and cells to investigate the profile and susceptibility of infection by ZIKV. Fetal membrane and chorionic villi samples from the Placentas were collected to obtain explants or cells by enzymatic dissociation, both morphologically and phenotypically analysed by anti-cytokeratin-7 (CK-7) and anti-vimentin antibody. The explants in culture presented morphological and structural integrity and characteristics CK-7 and vimentine labeling. The amniotic cells showed higher yield (9x10^6/ml) and viability (91%) compared to the chorionic, 3x10^6/ml and 58%, respectively, after dissociation. They maintained their morphological and phenotypic CK-7+ homogeneity in about 100% of the culture, however, the chorionic cells varied their morphology accompanied by a phenotype variation between 72%, 28% and 7% of CK-7+ cells, up to reach 100% vimentin+ cells. Preliminary results of the isolation of trophoblastics cells from spheroid of chorionic villus demonstrated that the protocol used led to the migration of only vimentin+ cells, although the spheroids presented 31±8% of CK-7+ cells and 69±8% of vimentin+ cells. Both the spheroids and the cells that migrate are infected by ZIKV, but it is necessary to confirm which cell type is most susceptible. Regarding chorionic villus explants the data show that the infection occurred only in vimentin+ cells. Although amniotic and chorionic cells have been susceptible to infection, it appears that CK-7+ cells are more permissive to ZIKV than vimentin+ cells, which require high viral loads (MOI 10 and 20) to reach 21%, 56% and 80% of infected cells during 24h, 48h and 72h, respectively, with MOI 20. Even when chorionic cells were infected with MOI 20 for 48 h, a 56% infection difference was observed in vimentin+ cells and 77% in CK+ cells. The results obtained by Electron Microscopy of Transmission of infected chorionic cells revealed important ultrastructural changes and the presence of numerous viral particles in reticulum vesicles. These data show that this experimental model represents a tool to explore biological aspects of congenital transmission by ZIKV.

Palavras-chaves: Human placenta, primary cells, ZIKV
Maternal infections may increase perinatal morbidity and mortality when undiagnosed and treated. Numerous maternal infections caused by microorganisms that can be transmitted to the fetus may occur during pregnancy, with severe sequels for the newborn. In order to ensure the birth of a healthy child as well as to ensure the well-being of mother and child, laboratory tests that detect the main infectious diseases called TORCHS are included in prenatal care, these are: Toxoplasmosis, Hepatitis B and C, HIV, Parvovirus B19, Rubella, Cytomegalovirus (CMV), Herpes simplex (HSV) and Syphilis. The objective of the study was to analyze the soroprevalence of CMV, Toxoplasmosis, Syphilis, Hepatitis C, HIV and Rubella from the examinations requested during the prenatal period, from a population of pregnant women attending a Clinical Analysis laboratory located in the city of Passo Fundo - RS. A retrospective study was carried out, based on the results of serological screening tests in the prenatal period, from January 2014 to July 2018, with ages ranging from 10 to 49 years, where the results of serological tests for detection simultaneous IgG and IgM class antibodies to CMV, Rubella and Toxoplasmosis of these pregnant women. The data will be presented initially in descriptive form. Preliminary results suggest that of the 60 pregnant women who underwent prenatal examinations, 5% presented reactivity to antibodies of the IgM class of CMV, suggesting acute infection, and 86% had regent results for the IgG antibody class. While the reactivity of IgM class anti-Toxoplasma Gondii and anti-Rubella virus was 1.66%, and for IgG class reactivity of 66% and 56% respectively. In addition, screening for syphilis, hepatitis C and HIV tests showed 100% negativity. From these preliminary data we observed the high IgG positive rate against the infections analyzed so far, demonstrating a good immunization of the pregnant women, but later than data will demonstrate other information pertinent to the study objectives, such as the most prevalent infection and the age where the positivity for such infections are more related. However, planning strategies in prophylaxis and treatment of these infections should be encouraged, avoiding their vertical transmission and possible data to the newborns.

Palavras-chaves: Cytomegalovirus, HIV, Rubella, Syphilis, Vertical Transmission Infectious Disease

Phylogenetic and genomic analysis of HBoV-1 in patients with acute respiratory infections

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Resumo

Human bocavirus (HBoV), of the family Paroviridae, subfamily Parovirinae, genus Bocaparvovirus is a small, non-enveloped, ssDNA virus with a ~5.2 kb genome encoding 3 open reading frames. Since its discovery in 2005 HBoV has been detected in association with respiratory and gastrointestinal symptoms.
Phylogenetic analysis identifies 4 HBoV species: HBoV-1, more frequently detected in respiratory secretions from children with acute respiratory infections (ARI), and HBoV-2, HBoV-3 and HBoV-4, more frequent in stools from patients with gastroenteritis. The objective of the present study was to do whole genome sequence analyses of HBoV-1 detected in nasopharyngeal secretions (NPS) from ARI patients. Nineteen HBoV-1 strains from patients seen at the Hospital of the University of São Paulo School of Medicine, in Ribeirão Preto, Brazil from 2005 to 2007 were sequenced. DNA extracted from 250 μL of NPS with Wizard DNA Purification Kit (Promega, USA) was sequenced with BigDye Terminator v3.1 on an ABI Prism 377–(Applied Biosystems, USA). HBoV genomes were assembled using SeqmanTM II software (DNASTAR) and complete genome sequences of 280 HBoV-1 strains were obtained from GenBank. Nucleotide sequences were aligned using Seaview 4.6.2 and temporal phylogeny was carried out by a Bayesian Markov-chain Monte Carlo (MCMC) approach, as implemented in the BEAST 2.4.8 (Bouckaert, et al, 2014). The consensus tree with the maximum product of posterior probabilities (maximum clade credibility tree) for analyzing MCMC data was annotated by TreeAnotator version 2.4.8 with a burnin of 10% and visualized using FigTree, version 1.4.3. In the phylogenetic tree, the Ribeirão Preto HBoV-1 sequences were clustered with other Brazilian sequences, with low levels of nucleotide variation and no evidence for recombinant sequences. Additional whole genome sequence analyses are needed for a better understanding of HBoV evolution and epidemiology in Brazil.

Financial Support: FAPESP, CNPq

Palavras-chaves: HBoV, Phylogenetic analysis, Acute respiratory infections, Genomic analysis

EVALUATION OF ANTIVIRAL AND BIOCIDAL ACTIVITY OF YAM (Colocasia esculenta) EXTRACTS AGAINST ZIKA VIRUS AND Aedes sp. LARVAE.

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Resumo
The morbidity and mortality caused by arboviruses have a great impact on public health worldwide. In the last four years, thousands of cases caused Dengue (DENV), Zika (ZIKV) and Chikungunya (CHIKV) viruses were reported in Brazil. The control of their main vector, Aedes aegypti, is one of the ways to counter their spread. Due to the lack of specific antiviral therapy and the resistance and environmental damage generated by vector control, natural anti-viral and vector control compounds are an important and attractive research aspect. The aim of this study was to evaluate the antiviral activity of yam extracts (Colocasia esculenta) against selected arboviruses and to assess the larvicidal action of the extracts against Aedes aegypti larvae. Yam extracts were produced from leaves and stem of the plant, using water and methanol as solvents. Aqueous extract was produced by maceration and methanolic by soxhlet. Cytotoxicity assays for antiviral experiments were carried out with different
concentrations of the extracts, revealing that aqueous extract presented higher toxicity when compared to methanolic extract. We analyzed antiviral activity by plaque reduction assays in treated C6/36 and Vero E6 cells against ZIKV. The methanolic extract inhibited viral replication at concentrations of 0.014 ppm and 0.007 ppm by approximately 38%. Larvicidal assays were performed in fourth stage Aedes aegypti larvae, using methanolic and aqueous extracts at concentrations of 1 ppm and 2 ppm. Apparently, no larvicidal activity was detected with these concentrations. Methanolic extract of yam seems to have a promising antiviral activity against ZIKV since small concentrations were capable of inhibiting ZIKV. Quantitative real time PCR will be performed to confirm the results of plaque reduction assays.

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Palavras-chaves: Antiviral, Biocidal, Yam, Zika Virus, Extracts
compared to NS1-positive samples (7.0 log10 copies/mL of serum, SD 0.8; p < 0.001). There was also a trend toward lower NS1 detection in DENV-4 cases (p=0.08), but this did not reach significance. This study optimized and applied a multiplex rRT-PCR for the simultaneous detection of DENV, CHIKV and ZIKV with a view toward improving the detection of these arboviruses. Our results confirmed the co-circulation of these viruses in Paraguay in 2016 and identified subsets of dengue cases that may test falsely negative if rapid NS1 assays are relied upon in the acute setting. Financial support: Research was supported by a grant from the Dirección General de Investigación Científica y Tecnológica, Rectorado. Universidad Nacional Asunción (DGICT-UNA).

Palavras-chaves: arbovirus, rRT-PCR, dengue, chikungunya, Zika

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**EPIDEMIOLOGIC INVESTIGATION OF UNEFREPORTED ZIKA VIRUS INFECTION IN PREGNANT WOMEN IN RIBEIRÃO PRETO, BRAZIL IN 2016**

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**Resumo**

The disease linked to Zika Virus (ZIKV) infection was first included to the National Compulsory Notification List (SINAN) of Brazilian Health Ministry in February of 2016 after the increase in microcephaly cases associated with ZIKV infections in Brazil, in 2015. The reporting of a suspected case is based on the presentation of skin rash in the presence of two other symptoms. However, approximately 80% of the population infected with ZIKV do not present any symptoms, making difficult to estimate the percentage of infected individuals by ZIKV, especially in pregnant women, a group of great concern. In this study, we investigated the epidemiological characteristics of pregnant women infected with ZIKV that were not reported to the Ribeirão Preto Health Service during the year of 2016. Based on samples from 1034 pregnant women collected during their prenatal visits to the public health of Ribeirão Preto, a search for these patients was carried out in the CEVES information system and only 391 pregnant women were reported. Investigation on the HYGIA WE System revealed that out of the underreported 643 patients, 34 had spontaneous abortion, 17 in the first trimester, 10 in the second trimester and 7 did not have information about the gestational age. Yet, another 10 patients had newborns with malformations and 2 patients had stillborn babies. Partial results of Neutralization Test (using a cutoff of 1/500 serum dilution) showed that 35 of 67 patients were negative to ZIKV and gave birth to healthy newborns and the other 32 were positive to ZIKV. Out of these, 24 patients had healthy newborns, 3 had newborns with malformations, 4 had a spontaneous abortion and 1 had a stillborn. Considering that these samples were collected during a ZIKV outbreak and that Neutralization Test specifically detects the presence of neutralizing antibodies, it is clear that these patients were infected during their pregnancy. This data suggest that either these patients did not present any symptoms or the reporting was not efficient, showing that we need a better reporting system to guarantee that all cases in pregnant women are included in the system. On the other hand, it is clear that asymptomatic infections also lead to severe fetal infection.

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**Palavras-chaves:** Zika, Epidemiology, Pregnant women

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**MOLECULAR INVESTIGATION OF ENTEROVIRUS AND GASTROENTERITIS VIRUSES IN THE**

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Resumo
Enteroviruses (EV) are responsible for clinical manifestations varying from febrile disease to central nervous system (CNS) syndromes. Acute gastroenteritis is one of the major public health problems in the world, with viruses been considering the main associated pathogens. In addition to the gastrointestinal involvement, gastroenteritis viruses have also been associated with CNS neurological disorders. The present study aims to (i) identify the EV and viruses that cause gastroenteritis (Rotavirus-RVA, Norovirus-NoV, Adenovirus-AdV and Astrovirus-AstV) in cerebrospinal fluid (CSF) samples from patients attended at the reference Hospital de Base of São José do Rio Preto in 2017; (ii) report the prevalence of these viruses associated with CNS disorders, and (iii) describe the patient's anamnesis. We selected 300 CSF samples from the CSF collection of the Hospital. Until now, 143 samples were analyzed (47.7%) and the specimens were screened by ELISA (RVA), RT-PCR (AstV) and qRT-PCR (NoV and EV). EV infection was detected in 4.9% (7/143) of the cases. RVA, NoV and AstV have not been detected so far. The EV-positive patients were numerically identified from 1 to 6. Patient 1 was diagnosed with meningitis; Patient 2 with congenital toxoplasmosis, meningitis by S. aureus and trauma; Patient 3 with trauma and meningitis; Patient 4 with cerebral aneurysm; Patient 5 with trauma and Patient 6 with congenital toxoplasmosis. EVs present global distribution and epidemiological studies confirm its importance as an etiological agent associated with CNS disorders. The global frequency of EV varies from 18 to 44.3%, being higher than that obtained in our study; however, these are still preliminary data. EVs are commonly associated with aseptic meningitis, corroborating the results found in Patients 1 and 5. The present investigation also identified EV infection associated with cases of trauma, cerebral aneurysm and congenital toxoplasmosis. This study will bring important advances for the differential diagnosis and the establishment of the etiological agents associated with CNS disorders in Brazil.

Financial Support: FAPESP.

Palavras-chaves: Enteroviruses, Gastroenteritis, Neurological Disorders
The upsurge of Zika virus (ZIKV) infections in the last decade has become a major global concern, particularly due to the occurrence of microcephaly in newborns from infected mothers. Unlike other arboviruses ZIKV can be spread not only by mosquito vectors, but also by sexual contact. Previous studies have shown that intravaginal ZIKV inoculation induces high titers of ZIKV-specific and neutralizing antibody in serum and the vaginal lumen. Despite those findings, to date, there is not in vitro model to elucidate the sexual transmission of ZIKV. Then, the present study was carried out to evaluate the susceptibility of murine mesenchymal stromal cells (mMSCs) isolated from vaginal tissue as a potential model for ZIKV infections. The mMSCs were isolated from vaginal tissue from FVB/N mice as previously described. Murine adipose tissue-derived mesenchymal stem cells (mASCs) were isolated and maintained as control cells. Cultures were kept in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum and antibiotics. Characterization of mMSCs was performed by morphological analysis, growth kinetics, evaluation of the surface marker profile (CD11b, CD31, CD44, CD45, CD90.2 and Sca-1) and differentiation in the osteogenic and adipogenic pathways. At the fourth passage, mMSCs were infected with a Brazilian Zika virus strain (ZIKV 17SM) at multiplicities of infection (MOI) 1, 0.1 and 0.01 for 2 h. After infection, the inoculum was removed and cells covered with fresh DMEM supplemented with 2% (v/v) heat-inactivated fetal bovine serum. The infected cells were observed daily in search for evidences of cytopathic effect. At 24 h, 48 h, 72 h and 96 h post infection (p.i.), infected cultures were frozen, thawed and aliquoted. The viral load was determined by a quantitative ZIKV real time PCR. An increase in viral loads was observed in mMSCs 48 hours p.i., when compared to those in mASCs. These findings suggest that mMSCs isolated from vagina are permissible to ZIKV infection. Such cells may potentially be used as in vitro models for studies on sexual transmission of ZIKV. Additional studies including mMSCs isolated from ovary and uterus tissues are being performed to evaluate ZIKV replication in such models.

Financial support: CNPQ

Palavras-chaves: zika virus, sexual transmission, susceptibility

Detection and clearance of a mosquito densovirus contaminant from laboratory stocks of Zika virus

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The Zika virus (ZIKV) epidemics that affected South America in 2016 raised several research questions and prompted an increase in studies in the field. The transient and low viremia observed in the course of ZIKV infection is a challenge for viral isolation from patient serum, which leads to many laboratories around the world sharing viral strains for their studies. C6/36 cells derived from Aedes albopictus larvae are commonly used for arbovirus isolation from clinical samples and for the preparation of viral stocks. Here, we report the contamination of two widely used ZIKV strains by Dipteron brevidensovirus 1 viruses, also known as the mosquito densovirus (MDV). MDV contamination was confirmed by molecular and immunological techniques and likely originated from C6/36 cultures commonly used to grow viral stocks. We applied two protocols that successfully eliminated MDV contamination from ZIKV stocks, and these protocols can be widely applied in the field. As MDV does not infect vertebrate cells, we performed serial passages of contaminated stocks using a mammalian cell line and infecting susceptible mice prior to re-isolating ZIKV from the animals’ blood serum. MDV elimination was confirmed with immunostaining, PCR, and analysis of the mosquitoes that were allowed to feed on the infected mice. Since the putative impact of viral contaminants in ZIKV strains generally used for research purposes is unknown, researchers working in the field must be aware of potential contaminants and test viral stocks to certify sample purity.

**Palavras-chaves:** densovirus, zika, contamination

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**Association between Hepatitis C virus epitope genetic diversity and geographical origin of the sequence**

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**Resumo**

Despite all the advances in the Hepatitis C (HCV) infection treatment several questions remains unanswered. The past research approach focusing treatment development do not answered questions about the evolution of the virus across time and epidemiology regarding host-parasite interaction. Host genetic characteristics and its association with viral evolution still remains unclear. The aim of this work was to detect patterns of evolution in three well know NS5A HCV recognition CD8 epitopes and its association with phylogenetic and geographical characteristics. To determine this, 861 sequences of NS5A HCV protein from subtype 1a were retrieved from public databases. Maximum likelihood phylogenetic reconstruction analysis were performed to determine phylogenetic characteristics. Genetic diversity (PhyML 3.0) and genetic complexity (BioEdit, Informational Entropy) were obtained from specific epitope aminoacids positions. The HCV 1a phylogenetic reconstruction demonstrated a separation in two major clades with different geographical distribution (US and non-US sequences). All three epitopes analyzed showed higher genetic conservation when compared with the mean genetic diversity of the protein. Epitope functional structure on HCV appears to be linked with genetic conservation at that regions. Sequences from the same geographical origin (US) presented lower values of entropy at epitope regions (informational entropy) than non-US sequences. These results indicates a possible correlation between the genetic complexity of epitopes recognition regions and geographical origin. Further analyses are needed to determine if the different profile of evolution at this sites are linked to host-parasite interactions and evolutive pressure from immune system.

**Palavras-chaves:** Hepatitis C, Immunology, Genetic Diversity, Epitope

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Resumo

Acute respiratory infections (ARIs) are an important cause of hospitalizations and morbidity in early childhood worldwide. To identify the pattern of circulating viruses, we performed an intensive epidemiological study at the Central Laboratory of Grupo Hospitalar Conceição, one of the largest public health hospitals in the State. Nasopharyngeal samples from 2656 and 2280 from patients of years of 2016 and 2017, respectively, with 70%) of individuals infected with the Influenza A had presented clinical symptoms like a cough, fever, and dyspnea; this last symptom was associated with A(H1N1)pdm09 strain (p=0.00) as well as the presence of pneumopathy (p=0.15) as a comorbidity. An A(H1N1)pdm09 cases had a higher lethality rate in relation to others agents. Although 68% of patients had used oseltamivir most had received no vaccine. While a large number of ARI infection can be identified as one of the common respiratory viruses in children, approximately 60% of children that present ARI is negative for this diagnostic panel. Thus it is highly likely that other respiratory viruses co-circulate in Southern Brazil during the season, and therefore this warrants further investigation. The number of cases is continually growing, and it would be needed big campaigns to raise awareness of the importance of vaccination, especially in children that are included in the group at risk.

Ministério Da Saúde, Fundação Estadual De Produção E Pesquisa Em Saúde And Centro Estadual De Vigilância Em Saúde

Palavras-chaves: Epidemiological Surveillance, Laboratory Diagnosis, Influenza Viruses, Respiratory Viruses

DEVELOPMENT OF MULTIPLEX REAL TIME REVERSE TRANSCRIPTASE PCR ASSAY FOR SIMULTANEOUS DETECTION OF ZIKA VIRUS, DENGUE VIRUS AND YELLOW FEVER VIRUS USING HIGH RESOLUTION MELTING ANALYSIS.

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Resumo

Arboviruses are considered important emerging viruses worldwide. The signs and symptoms of Dengue virus (DENV) may be similar at the early stage of infection to those presented by infections with Zika virus (ZIKV) and Yellow Fever virus (YFV), which are transmitted by the same mosquitoes in...
urban areas. Laboratory diagnosis methods for confirming those viral infections may involve detection of the virus, viral nucleic acid, antigens or antibodies, or a combination of these techniques. Many real-time RT-PCR assays have been developed employing TaqMan or SYBR Green technologies. The aim of this study was to develop multiplex real-time RT-PCR using high resolution melting (HRM) technology-based assay for DENV, YFV and ZIKV detection. We developed a multiplex real-time RT-PCR-HRM assay for differentiation of these viruses in a single tube. These results have important implications for epidemiology and surveillance of emergent flaviviruses in Brazil. This is the first report of application of multiplex real-time RT-PCR-HRM technology for detection of DENV, YFV and ZIKV in clinical samples. The findings demonstrate the potential use of these assay as a sensitive diagnostic test for rapid and real-time virus detection in regions of simultaneous transmission of different arboviruses with similar clinical presentations.

**Financial support:** FAPEMIG/FUNED

**Palavras-chaves:** HRM, Zika virus, Dengue virus, Yellow Fever virus, detection

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**INVESTIGATION OF OCCULT HEPATITIS B INFECTION IN INDIVIDUALS WITH CHRONIC HEPATITIS C TREATED WITH ORAL DIRECT ACTING ANTIVIRALS (DAAS).**

**Autores**  
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**Resumo**

Hepatitis B virus (HBV) infection is a worldwide public health problem, even with the existence of an effective vaccine. The occult B infection (OBI) is determined by the absence of surface antigen (HBsAg) and by the presence of HBV-DNA in the liver or serum from infected patients. The frequency of OBI in Brazil is 3.5% and it has great relevance in the clinical context, since it can develop the development of severe hepatic disease, such as cirrhosis and hepatocellular carcinoma and until death. Viral factors of OBI induction may be associated with mutations, especially in S protein and co-infection with other viruses. Reactivation of hepatitis B virus (HBV) has been reported in patients with chronic HCV in treatment with direct action antiretroviral (DAA). The reactivation can result in fulminant hepatitis, hepatic insufficiency and death. According to the Clinical Protocol and Therapeutic Guidelines for Hepatitis C and Coinfections, individuals with presence of HBsAg detected prior to the start of use of DAAs need to use HBV treatment to prevent their reactivation due to treatment of hepatitis C, however in cases where HBsAg is not detected, treatment with DAAs is released without investigation of the presence of HBV-DNA. The OBI / HCV coinfection in patients treated with DAA has not been investigated yet in Brazil. The aim of this study was investigate the presence of OBI in patients with HCV chronic treatment with DAAS. For this, 80 patients samples prior to the start of treatment with DAAs were selected from a public hospital in Rio de Janeiro. The serum samples were evaluated by immunoenzymatic assay (EIA) of total-HBc and HBsAg. The samples positive to anti-HBc without HBsAg were tested by real-time PCR (qPCR) and PCR. In serological test 31.25% (25/80) samples had anti-HBc positive and HBsAg negative and in 3.75% (3/80) of patients, the HBV-DNA was detected with absence of HBsAg. One samples was positive in S-region amplification by nested-polymerase chain reaction (nested-PCR), this difference in molecular tests is expected due to higher sensitivity of the qPCR technique to detect low viral load. The results of this study are of great relevance to subsidize the data that contribute to the therapeutic guidelines for hepatitis C and coinfections, and to evaluate the importance of OBI detection before and during DAAs treatments.
Viral etiology causing acute respiratory disease among adults attended in a primary health care unit.

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Resumo

Acute respiratory tract infections are a persistent public health problem in developed countries and developing too. They cause a big part of the diseases in the world. The most frequently reported viruses include respiratory syncytial virus (RSV), influenza viruses A and B (IAV, IVB), parainfluenzaviruses (PIVs), human rhinovirus (HRV) and human bocavirus (HBoV). Thus, the aim of this work was to evaluate the aetiology of patient with acute respiratory infections on a primary health care unit in Guarapuava, Paraná, Brazil. Patients that are selected complained about viral acute respiratory infections in this scenery, all adults, that agree with the study. A nasal swab was collected from each symptomatic patients, being collected from 209 samples, during the period of 2013 to 2015. Samples were searching for HRV, IAV, IBV, RSV, and HBoV nucleic acid. 25 samples tested presented a positive result for the HRV, 3 samples were positive for IVB, and no cases of respiratory infection were associated with IVA, RSV and HBoV. The highest detection rate were between May and August. As for the Influenza virus, which in this group presents the greatest variability and infectious potential, are the less frequent pathogen and associated with sporadic cases of infection - fact that can had justified the absence of epidemic Influenza A in the period studied. And still, for RSV and HBoV were not detected cases of respiratory infection associated with the same in all samples, which may represent absence of the virus in the population in question or an insufficient number of samples. Thus, it is concluded that the more relevant period to study the acute respiratory infections is between may and august, and the virus that shows more positives samples in the period its HRV. However, considering that this is the first epidemiological study to investigate the presence of such viruses in patients with respiratory infection in the Guarapuava, more studies are necessary in order to establish strategies for diagnosis and prevention of the disease.

Palavras-chaves: Acute respiratory, viruses, disease

TROPHOBLAST CELLS INFECTED BY ZIKA VIRUS

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Resumo

Trophoblast cells infected by Zika virus.
**TROPHOBLAST CELLS INFECTED BY ZIKA VIRUS**


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In 2015, Brazil started to report high cases of newborns presenting microcephaly and grownups affected by Guillain-Barré syndrome. These cases were then confirmed as related to Zika Virus (ZIKV) infection. ZIKV is an arbovirus that belongs to the Flavivirus genus. It is an enveloped ssRNA(+) virus and it has a 11kb genome. It can be transmitted by 19 female species of Aedes spp mosquito. It is estimated that 2 billion people are in risk of infection around the globe. This project aims to characterize the basic ZIKV infection cycle in vitro using cell lines permissive to African and Brazilian ZIKV strains. Our other goal is to demonstrate if those cells are susceptible to different viral strains through kinetics experiments. ZIKV strains used were the Brazilian strain - gently provided by Instituto Evandro Chagas (IEC) - and the African strain MR-766. These strains were cultivated in Vero cell lines in a series of three subcultures. Viral kinetics was tested by time-point (TP) inoculation to determine viral growth curve in tree cell lines: BeWo, BeWo with forskolin HTR-8, and Huh-7. Viral titre (PFU/mL) was performed, as the detection by RT-qPCR. The tree cell lines were infected by both viral strains, which showed cytopathic effects and formation of syncytia. A series of three passages (TP) in triplicate for both strains were confirmed the infections by RT-qPCR. Our work shows that IEC is less infectious than MR766 extra- and intracellular. The results show in PFU/mL the amount of viral particle by cell line: IEC/BeWo 24 hours post infection (hpi) intra celular=1,7E7; MR766/BeWo 48hpi intra=5,78E6; IEC/HTR-8 72hpi intra=8,14E6; MR766/HTR-8 48hpi intra=3,17E8; IEC/HTR-8 72hpi intra=8,14E6; MR766/HTR-8 48hpi intra=3,6E8. These results show that these cell lines are permissive to ZIKV infection and that there are differences in infectivity between viral strains and even in the same viral strain.

Financial Support: FAPESP and CNPq.

**Palavras-chaves:** Zika virus, Trophoblasts cells, Kinetics

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**CYTOKINES IL-6, IL-10 AND IFNγ EXPRESSION IN PEOPLE LIVING WITH HIV/AIDS WITHOUT ANTIRETROVIRAL THERAPY**

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**Resumo**

**Introduction:** Cytokines are proteins produced by the immune response and may have proinflammatory or anti-inflammatory action. The proinflammatory cytokines, such as IL-6 and IFN-γ, are synthesized and secreted mainly by NK and cytotoxic T cells, macrophages and mast cells, respectively. The IL-10 cytokine is synthesized and secreted by T helper and macrophages cells, and act reducing the inflammation via proinflammatory cytokines inhibition. In people living with HIV/AIDS (PLWHA) occur chronic activation of the immune system, with CD4+ T lymphocytes depletion, as well as increased levels of proinflammatory cytokines, favoring the inflammatory response. **Objective:** Quantify the plasma levels of cytokines IL-6, IL-10 and IFN-γ in people living with HIV/AIDS without antiretroviral therapy and compare with HIV negative individuals. **Material and methods:** Plasma samples were used in patients of both genders attended on Healthcare Service Unified (SOAMU-IEC) in 2015. The total Ig anti-HIV detection was performed by enzyme immunosorbent assay type ELISA (Kit-MUREX AG/AB COMBINATION DIASORIN, UK) and confirmed by rapid immunoblot (Kit- Biomaguinhos-FIOCRUZ). The cytokines quantification was perform by flow cytometry BD™ Cytometric Bead Array (CBA). For the statistical analysis was applied the non-parametric Mann-Whitney test using the Prism program GraphPad software ($\alpha = 95\%$; $p < 0.05$). **Results:** The samples were constituted of 5 positive and 5 negative HIV individuals. The analyze of
Cytokine concentration in plasma samples showed that no statistical differences were observed between HIV positive and negative individuals for IL-6 and IFN-γ concentrations. However, IL-10 concentration showed a significant increase in HIV positive individuals (p = 0.0079). The development of this anti-inflammatory response (increase of IL-10 concentration) in HIV positive individuals without treatment characterizes the transition from the acute to the chronic phase of the infection, and contribute to establish viral latency in long-lived memory CD4+ T cells. **Conclusion:** In this study we observed a high concentration of IL-10 in HIV positive individuals without antiretroviral therapy. The IL-10 can be a chronic infection indicator, in addition, confers to HIV infection advantages due its characteristic of inflammatory response attenuation. However, further studies are needed to elucidate the HIV and IL-10 interaction.

**Financial Support:** Evandro Chagas Institute

**Palavras-chaves:** cytokines, flow cytometry, HIV

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**COMPARATIVE EVALUATION OF INDIRECT RAPID IMMUNOHISTOCHEMISTRY AND DIRECT FLUORESCENT ANTIBODY TESTS FOR RABIES VIRUS ANTIGEN DETECTION**

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**Resumo**

As a member of the genus Lyssavirus in the family of Rhabdoviridae, RABLV is a neurotropic virus that causes fatal encephalitis in warm-blooded animals and humans. Currently, the direct fluorescent antibody (DFA) technique is considered the gold standard for rabies diagnosis. However, complementary diagnostic, such as the rapid immunohistochemistry (RIT) test, have been developed. Based on that, the aim of this study was to compare the DFA and the iRIT techniques for rabies diagnosis. So, we tested 103 Central Nervous System (CNS) samples: cattle (44), horses (19), cats (7), dogs (11), bats (18), marmosets (2), skunks (1) and maned wolves (*Chrysocyon brachyurus*) (1). For each species (excluding bat), 5 anatomic locations in the brains (cortex, cerebellum, hippocampus, brainstem, and spinal cord fragments) were examined by both techniques on glass microscope slides. As primary polyclonal antibody was used: the anti-rabies virus antibody conjugated to FITC (Pasteur Institute) and serum of hyperimmune mice against rabies virus (Instituto Evandro Chagas, Belém, Brazil) for the DFA and iRIT assay, respectively. Exclusively for iRIT assay, the Envision detection system peroxidase (DakoCorporate) and DAB chromogen were used for reaction development, and the blades were observed under an optical microscope. Positive and negative samples were used as controls reaction in both techniques. The samples that obtained divergent results were submitted to RT-PCR, followed by genetic sequencing. As a result, of the 103 samples analyzed, 51 were negative and 52 positive by DFA, and 101 (98.06%) yielded concordant results by iRIT. However, two samples (one feline and one equine species) presented divergent results (negative by DFA and positive by iRIT). These samples were analyzed by molecular biology techniques, confirming the results obtained by iRIT. According to these data, iRIT presented greater diagnostic sensitivity in relation to DFA for the RABLV antigen detection. Our results indicate that it is possible to use iRIT as an effective and rapid auxiliary tool for the diagnosis of rabies, and it can be applied to samples of different animal species. 

**Financial support:** Instituto Pasteur- IP01 / 2014
ADENOVIRUS PREVALENCE IN PEDIATRIC PATIENTS ADMITTED TO AN EMERGENCY SERVICE OF A PRIVATE HOSPITAL IN SANTA MARIA/RS

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Resumo

Introduction: Human adenoviruses (HAdV) play an important role in the etiology of acute respiratory tract infection (ARTI). Regarding pediatric population, these pathogens are known as the second most detected viral agents who is related with up to 15% of all reported ARTI. Despite this agent could be diagnosed by direct immunofluorescence, molecular tools, such polymerase chain reaction (PCR), could be useful to distinguish such viruses from other pathogens and to prevent the inappropriate use of antibiotic therapy. This study aimed to determine the HAdV prevalence in children with severe acute respiratory syndrome (SARS) admitted to a private hospital emergency service of Santa Maria/RS. Material and Methods: Cross-sectional prospective study between September 2017 and July 2018. Were included children aging 0-12 years who presented signs and symptoms of SARS. Combined oropharyngeal and nasopharyngeal swabs were obtained from the oral and respiratory mucosa and maintained in viral transport medium at 4ºC until acid nucleic extraction, what occurred within up to twelve hours after collection. The presence of HAdV was determined by PCR. A human gene (RPL0) was employed as an endogenous control for PCR. Data were analyzed using Chi-square test and significance was determined at α< 0.05. Results: Fifty-five patients were included (34 male and 21 female) with median age of 2.15 years. Most frequent respiratory symptoms were cough (90.91%) followed by rhinorrhea (81.82%) and oropharyngeal hyperemia (45.45%). Fever at admission was detected in 30.91% of children. Among valid tested samples, positivity for HAdV was found in 22 patients, which corresponds to 40% of viral prevalence in this study population. A great number of positive cases was observed during autumn season (10/22) and in patients aged 1-2 years (68.17%). Considering clinical findings, viral presence was associated with cough (p=0.0229) and rhinorrhea (p=0.0176). Other signs and symptoms were not related specifically with HAdV positivity. Conclusion: Clinically, HAdV infection can be related to nonspecific symptoms that can be caused by other pathogens. Our data showed a high prevalence of HAdV in children admitted to an emergency service. The high number of positive cases indicates that is important to monitor HAdV by high sensible methods for correct medical prescriptions and for an effective infection control, even in emergency units. Financial Support: Not applicable.

Palavras-chaves: Adenovirus, Infection Control, Pediatrics, Severe Acute Respiratory Syndrome

ANALYSIS OF RESISTANCE PROFILE TO INTEGRASE INHIBITORS IN NAIVE PEOPLE LIVING WITH HIV/AIDS ANTIRETROVIRAL THERAPY IN BELÉM, PARÁ.

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The class of integrase inhibitor drugs (INI) has recently been introduced into antiretroviral therapy (ART). The presence of mutations associated with resistance to INI may have a direct impact on the success of treatment of people living with HIV / AIDS (PLHA). Thus, present study aims to describe viral subtypes of HIV-1 and the resistance profile to INI of HIV-infected PLHA in the city of Belém, Pará. A cross-sectional study was carried out between June and September of 2016 at the Center for Attention to Health in Infectious Diseases Acquired (CASADIA), and 29 individuals were included in the investigation. After responding to an epidemiological questionnaire and signing the informed consent, venous blood was collected from these individuals, followed by the extraction of deoxyribonucleic acid (DNA), Nested-PCR and nucleotide sequencing of the HIV-1 integrase region. Identification of resistance mutations was performed using the Stanford HIV Drug Resistance database and genetic variability was assessed using the REGA tool. The results were later confirmed by phylogenetic analysis. Most of the evaluated individuals were from the state of Pará, either heterosexual or homosexual, had a higher education level and low family income and single or fixed sexual partners, in addition to partners from other states (Amapá, Maranhão, Tocantins, Bahia, Pernambuco, Rio de Janeiro and São Paulo). All 29 samples analyzed were identified as HIV-1 subtype B and no primary mutation associated with INI drug resistance (raltegravir, elvitegravir or dolutegravir) was found. However, 8 sequences showed at least one resistance related accessory mutation (L74I, V151I, S119R). This was the first study to evaluate the INI class in northern Brazil. All the evaluated individuals were susceptible to INI, evidencing that the use of this class drugs in the northern region of Brazil is a viable alternative, since the secondary mutations found are not able to cause resistance in the absence of major mutations. Financial support: Coordination for the Improvement of Higher Education Personnel (CAPES) and Postgraduate Program in Biology of Infectious and Parasitic Agents (PPG-BAIP).

Palavras-chaves: ANTIRETROVIRAL , HIV, INTEGRASE, RESISTENCE, THERAPY
health system in Cambé. For each patient, the demographic, clinical, NS1 viral protein detection and serology (specific anti-dengue IgM and IgG) data were recorded and analyzed. Samples positive to NS1 and/or IgM were considered as dengue positive for acute dengue virus infection (DV⁺). During the period of study a total of 878 patients were attended at the units of the public health system with probable acute DV infection. The serological results demonstrated that 249 patients were infected by DV, representing a prevalence of 28.4% of dengue infection. The most frequent symptoms and signs in DV⁺ patients were: fever, headache, myalgia and prostration. Additionally, 13% (32/249) of DV⁺ patients did not have fever, which is one of the most characteristic symptom of dengue. Regarding the sociodemographic data, the average age was significantly higher in DV⁺ versus DV⁻ patients. Moreover, monthly fluctuations of dengue cases throughout the period of study correlated with the change in precipitation (correlation of 0.53) and temperature (correlation of 0.53), three months before the epidemics. Finally, there was a predominance of dengue cases in the east part of Cambé, where it is bordered by Londrina. Interestingly, the epidemic trend in these two cities were similar and did not follow the epidemiological dengue pattern in the rest of the State. This data suggests a geographical diversity in this region which does not necessary represents the dengue epidemic in the whole State

Palavras-chaves: Cambé, Dengue, Epidemiology

Antiviral effect of the extract and isolates of Psychotria sp. for different levels of viral charge of Zika virus in Vero cell culture

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Resumo

Zika virus (ZIKV) is an emerging mosquito-borne flavivirus, and represent a significant public health concern because to the large-scale dissemination of the disease and of the infection-associated syndromes. There are not antiviral drugs or vaccines against this virus and antiviral research becomes relevant. The genus Psychotria has species for which bioactive extracts have been reported with antimicrobial and anti-inflammatory activity. Thus, the aim of this work was to evaluate the antiviral activity in the extract D4 of Psychotria sp. and in their isolates DX and TX. The cytotoxic concentration was obtained for 50% of the cells (CC₅₀). Then, the effective protective concentration was investigated in 50% of the infected cells (EC₅₀), obtaining the index of selectivity (IS). The cytotoxic activity was not detected for D4 up to the concentration of 750 µg/mL and the values for DX and TX isolates the CC₅₀ values were 119.3 µg/mL and 348.9 µg/mL, respectively. The in vitro assays, antiviral activity against ZIKV (moi of 0.1), the effective/protective concentrations for 50% of the infected cells (EC₅₀), were 26.43 µg/mL, 31.87 µg/mL and 101.4 µg/mL for D4, DX and TX, respectively. The estimate of the selectivity index (IS) for extract and isolates showed values above 3, as recommended by the literature. From the antiviral activity at moi of 0.1, its action was investigated against a higher viral charge, i.e, 1, 5 and 10 viruses per cell. It was observed that the increase in viral charge also caused an increase in the concentration required of both extract and isolates to inhibit the infection. However, this increase was not proportional to the increase in viral charge, since the infection using 100 times more virus per cell, the same effective antiviral concentration increased only 4 times (102.32 µg/mL) for the D4 extract, 3 times (97.71 µg/mL) for DX and twice for TX (231.25 µg/mL). Together, our results show that the extract of this plant has antiviral action against ZIKV, and that the isolates can be part of its active principle. The EC₅₀ values found on different moi give indications of efficiency and/or selectivity of the compounds tested. We have now started studies in our laboratory to to identify possible antiviral mechanisms.

Palavras-chaves: Antiviral, Flavivirus, Psychotria sp, Zika virus
Zika virus (ZIKV) transmission have been reported in 84 countries worldwide according to the 2017 World Health Organization situation report. In Brazil, it was reported 8.839 ZIKV confirmed cases in 2017, while in the same year, the northeast region, where the Brazilian 2015 ZIKV epidemic was first detected, have reported 5.270 ZIKV probable cases. According to Brazilian Ministry of Health, until June 2018, it has been reported in the country 5.401 probable ZIKV cases, of which 2.155 cases (39,9%) were confirmed. Due to ambiguous symptoms, molecular diagnostic by RT-PCR have been widely used as the diagnosis assay conferring specificity and sensitivity in human sample diagnostics. After ethical committee approval, we performed RT-qPCR screening on extracted RNA from 336 different body fluids samples, collected over the years of 2016 and 2017, from 184 symptomatic patients living in the city of Feira de Santana, Bahia, Brazil. Nested RT-PCR were performed on the ZIKV positive samples for posterior sequencing of a partial NS5 gene fragment by Sanger method. From those RT-qPCR testing, It was identified 10 (~3%) samples (blood (n=6), urine (n=2), saliva (n=2)) showing positive detection of ZIKV RNA, with a threshold value (Ct) median of 34,9. So far, only two partial ZIKV NS5 gene sequences from saliva of two adult female patients (one collected in Oct. 2016 and the other in Feb. 2017) have been obtained. Phylogenetic analysis of those sequences enabled the reconstruction of a Maximum-Likelihood tree showing they are coherently placed in the clade containing other ZIKV Asian genotype sequences, including other Brazilian isolates. Although only 3% of the tested samples were positive for ZIKV RNA by RT-qPCR, the ZIKV sequences obtained from two isolates may suggest a continuing transmission of the ZIKV in that city since ZIKV 2015 epidemic. Once this city has only 59,7% of its residences with adequate sanitary sewage, we argue that public health authorities should continue to provide sanitary attention due to the significant role adequate water supply and sanitation plays in the combat to Aedes spp. related infectious diseases. Finally, more efforts are necessary for sample collection during the acute phase of the ZIKV infection, so there will be more chances to isolate and sequence viral genetic material, allowing posterior bioinformatics analysis on ZIKV local transmission patterns. Financial support: Fundação de Amparo à Pesquisa do Estado da Bahia – FAPESB.

Palavras-chaves: Body fluids, Molecular diagnostics, Phylogenetics, Sequencing, Zika virus
EPIDEMIOLOGICAL ANALYSIS OF ZIKA, CHIKUNGUNYA AND DENGUE VIRUSES IN THE CITY OF LARANJAL AT THE RIO CAJARI EXTRACTIVE RESERVE, STATE OF AMAPÁ, BRAZIL

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Resumo

Introduction: The Rio Cajari Extractive Reserve covers a large forest area that lacks adequate infrastructure and has a high incidence of mosquitoes which is an important route of arboviruses transmission. The clinical symptoms of these arboviruses are similar and cross-reactivity can occur in the serological tests making it difficult to diagnose and thus requiring additional molecular tests. Our goal was to characterize the incidence of Zika, Chikungunya and Dengue virus in plasma samples collected in Laranjal do Jari, a city present in the Rio Cajari Extractive Reserve in the state of Amapá.

Material and methods: 369 plasma samples were collected between 2013 and 2016 and selected based on the presence of clinical symptoms of infection by arboviruses, such as fever, rash, headaches and muscle pain, and sent to the Retrovirology Laboratory at UNIFESP. Viral RNA was extracted and submitted to four qPCR protocols: Pan-Flavivirus, Zika, Chikungunya and Dengue. Molecular results were compared to the serological results of the Brazilian Epidemiological Bulletin of the Ministry of Health.

Results: The results obtained through the qPCR were inconsistent comparing to the serological data released by the epidemiological bulletin of Laranjal do Jari in 2016. In the present study, 20 (5.42%) of 369 samples were positive for Pan-flavi and 18 (4.88%) were positive for Zika. In contrast, the bulletin reported 800 suspected cases of Zika but confirmed just 179 (22.38%) of the cases after serology. Regarding the Dengue, 2795 cases were reported and 1351 (48.33%) were confirmed. The present study found only 2 (0.54%) positive samples for Dengue. The bulletin also reported 93 (12.04%) confirmed Chikungunya cases, but in the analyzed cohort no positive samples were found. Conclusion: The tests performed at Amapá Health Unit are serological and quantify dengue specific IgM antibodies. In contrast, our test quantified virus present in plasma. The viremia period of the Dengue and Zika lasts for approximately five days, while IgG and IgM are produced after five and seven days. It is possible that at the time of blood sampling the viral load was already very low while the production of IgM was increasing. Another possibility may be related to presence of cross-reaction in the serological test with other Flaviviruses resulting in a false positive after recent infection by another viral agent.

Financial Support: Fundação de Amparo à Pesquisa do Estado de São Paulo.

Palavras-chaves: Zika, Chikungunya, Dengue, Arbovírus, qPCR

INFLUENCE OF POLYMORPHISMS IN TNFA GENES (rs1800629) AND IL6 (rs1800795) IN CHRONIC HEPATITIS B AND C VIRUS INFECTIONS

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INFLUENCE OF POLYMORPHISMS IN TNFA GENES (rs1800629) AND IL6 (rs1800795) IN CHRONIC HEPATITIS B AND C VIRUS INFECTIONS

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In viral hepatitis, liver damage occurs through the action of the immune system, with its innate and adaptive immune responses, mediated by the influence of cytokines that will act directly or indirectly in the inflammatory process. Tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) are cytokines that act in the immune system, with a proinflammatory action on the immune response of individuals. This study aimed to determine the frequencies of the polymorphisms in the TNFA and IL6 genes in chronic hepatitis B and C carriers and to identify possible associations with the progression of these infections. The study was cross-sectional and analytical, in which were investigated populations of patients with chronic hepatitis B (74), chronic hepatitis C (101) and a control group (300) composed by blood donors. DNA was extracted from the peripheral blood samples and then submitted to polymorphisms investigation in the TNFA genes (rs1800629) and IL6 (rs1800795) by real-time PCR (qPCR). Biochemical tests were performed only for patients, and serological tests were done for all study participants. Histopathological examinations were performed only in patients with indication of biopsy, following the French classification METAVIR, punctuating the activity of the portal and periportal inflammatory infiltrate from 0 to 3 and structural alterations from 0 to 4. Statistical analysis were performed in the BioEstat 5.0v program, adopting as significance level p-values (TNFA). It was observed an association of the GA and AA genotypes with AST, ALT and GGT enzymes levels among HBV carriers. In HCV patients, a significant association of genotype frequencies was observed when the patients and the control group were compared, and the GG genotype was associated with AST. In the polymorphism rs1800795 (IL6), the association of the GG genotype with AST, ALT and GGT, and of the CG and CC genotypes with HBV viral load in patients was observed. In the HCV group, the association of GC and CC genotypes with AST and ALT was observed. The studied polymorphisms with inflammatory activity and staging of fibrosis were not observed in both groups of patients. We conclude that TNFA and IL6 gene polymorphisms appear to be associated with progression of clinical and laboratory findings of HBV and HCV infections, but studies with other population groups of different ethnicities are necessary to confirm these results.

Palavras-chaves: HBV, HCV, IL6, SNPs, TNF
The present study aims to report the epidemiological, viral and clinical aspects of Chikungunya Fever (CF) in Rio Grande do Norte (RN) state, Brazil. For this we used whole blood, serum, plasm, liquor, urine and/or blister fluid obtained from 284 suspected cases of Dengue, Zika or Chikungunya in 2016. CHIKV was detected in 44.4% (126/284) of the total studied cases by qRT-PCR. Neither Dengue nor Zika were detected. The present study detected more positive Chikungunya cases in the first 3 months of 2016. The frequency was higher in females (49.2%; 44.44% non-pregnant and 4.76% pregnant), followed by males (36.51%) and newborns (14.29%). The number of Chikungunya cases in newborns, adults aged 41 to 50 years, and adults older than 61 years old were higher compared to the other age groups. The most frequent symptoms were fever, arthralgia, myalgia and arthritis. In addition, we observed a gradual decrease of viremia being more evident after the second day of symptoms appearance. The present study is important to increase knowledge about the main clinical manifestations and the main epidemiological aspects of this predominantly symptomatic disease that has become a public health problem in Brazil.

Financial Support: CNPq, CAPES e UFRN.

Palavras-chaves: Chikungunya, Rio Grande do Norte, Brazil

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**ANTIVIRAL ACTIVITY OF SAPONINS FROM *Quillaja saponaria* AND *Quillaja brasiliensis* AGAINST MAYARO VIRUS**

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**Resumo**

The search for new molecules with antiviral activity is important, especially for viruses that cause disease and mortality in living beings, which ones do not have treatment. Among these viruses are the Mayaro virus (MAYV), an arbovirus belonging to the family Togaviridae, genus Alphavirus and causative agent Mayaro fever with arthritic characteristics and chronification potential. Described with antiviral activity and other biological actions, saponins are molecules of plant origin, present in several species of plants, which present an amphiphilic nature, emulsifying characteristics and solubilize lipids. This work aims to evaluate the anti-MAYV action of two purified fractions of saponins, called Quil-A® (extracted from the bark of *Quillaja saponaria* Molina) and Fraction B (from the leaves of *Q. brasiliensis*). Firstly, the cytotoxicity of the saponins was evaluated by the MTT cell viability assay to establish the cytotoxic concentration to 50% of the cell monolayer (CC50). For this, the VERO cell line (*Cercopithecus aethiops* kidney) was exposed to serial dilutions of Quil-A® and Fraction B for 24 hours and 48 hours. Antiviral activity was assessed by plaque reduction assay, therefore the cells were infected with the virus and after viral adsorption were treated with non-toxic concentrations of the compounds. Viral suspension and culture medium were used as viral and cellular control, respectively. After the 48 hours of incubation (5% CO2, 37°C), the cell monolayers were stained with violet crystal and then the lysis plates were counted. The CC50 in the 24 hours incubation period was 18.11 µg/mL for Quil-A® and 26.67 µg/mL for Fraction B, in the 48 hours incubation period was 13.24 µg/mL and 12.04 µg/mL, for Quil-A® and Fraction B respectively. The reduction of the MAYV lysis plates in the treatments with Quil-A® at concentrations of 6 µg/mL, 5 µg/mL and 4 µg/mL was 100%, 90.70% and 76.82%, respectively. In the treatments with Fraction B, the reduction in the same concentrations were of 100%, 97.28% and 61.64%, respectively. These results demonstrate the potential anti-MAYV activity of both saponins fractions, in a dose-dependent manner. Finally, it is important to continue this study to evaluate the interference of the compounds in the replication cycles of the virus, in order to investigate the mechanism(s) of anti-MAYV action of these saponins.

Financial support: CAPES, CNPq, DCIT - Ministério da Saúde – Brasil, Universidade Feevale.

**Palavras-chaves:** Arbovirus, Cytotoxicity, Fraction B, Plaque reduction assay, Quil-A
THE MAIN FACTORS ASSOCIATED WITH THE INCREASE OF THE HIV/AIDS INCIDENCE AMONG THE ELDERLY

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Resumo

According to data from the WHO (World Health Organization), in 2013, in Brazil, approximately 730 thousand people were contaminated with the immunodeficiency virus. The HIV seropositive diagnosis rate among the public of people over 60 years of age exceeded the index of 80% of growth in the last 12 years. According to data from the National Program of STDs/AIDS, the increase was of 47% in elderly women and of 53% in elderly men. The South region of Brazil presents the greatest index of new cases and the incidence rate in elderly is of approximately 10.39 cases for each 100,000. This work aimed to analyze the main factors associated with the increase in the incidence of HIV seropositive elderly in Brazil. This study was constituted by a literature review in which was performed a consultation on scientific articles selected through search on the databases Scielo, CAPES, Health Ministry, common domain and BVC from the Lilacs source. The database search was carried out using terminologies registered in the Descritores em Ciências da Saúde. It was possible to evidence through this study that the main path of contamination associated with the HIV among the elderly population is the sexual one in heterosexual relations, and still according to the literature, the HIV diagnosis within this age range is usually late, due to the lack of knowledge that this population presents about the disease, given the fact that most infected people presented a low educational level. In addition, with the increase in life expectancy and with the new methods of maintaining the sexual activity in an elderly age, there is an increase in the exposition of this age range to the HIV virus. Thus, as demonstrated, the increase in the incidence of elderly people contaminated with the immunodeficiency virus in Brazil has as its main associated factors the lack of information, low educational level, increase in life expectancy and the difficulty that still exists in debating sexuality in the senior age. It can be concluded from this data that more scientific productions about this theme is necessary, as well as HIV/AIDS prevention campaigns specifically directed to this population, respecting their particularities so that they can effectively reach this public and reduce the new cases of the disease among the elderly.

Financial Support by the autors.

Palavras-chaves: HIV/AIDS, ELDERLY, PUBLIC HEALTH

CHEMISTRY AND IN VITRO EVALUATION OF THE ANTI-ZIKA VIRUS ACTIVITY OF ETHANOLIC EXTRACTS FROM Lundia corymbifera AND Tanaecium pyramidatum (Bignoniaceae)

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Resumo

Zika fever is an arboviruses resulting from infection cause by Zika virus (ZIKV). ZIKV is a RNA virus and belongs to the Spondweni serotype, Flavivirus, Flaviviridae family, which presents a genetic and serological relationship with other flaviviruses of public health importance: dengue, encephalitis, yellow fever and hepatitis C viruses. Treatment of this infection is important even in asymptomatic cases,
mainly in pregnant women, since there is an association between ZIKV infection and microcephaly in newborns. There is no specific antiviral or vaccine to treat this infection. The importance of natural products as sources of new drugs is universally recognized and there is no lack of promising results regarding the antiviral potential of these products. In this context, the present work aims to evaluate in vitro antiviral activity against ZIKV of ethanolic extracts of trunks and leaves of the Bignoniaceae species: Lundia corymbifera and Tanaecium pyramidatum. Initially, the mean cytotoxic concentration (CC<sub>50</sub>) of these crude extracts was determined in the VERO cell line (n = 3) by MTT colorimetric assay. After, antiviral activity against ZIKV in VERO cells (n = 3) was performed by the MTT method, the results were expressed in terms of 50% effective concentration (EC<sub>50</sub>). In addition, in order to identify the main constituents of the extracts, chromatographic profiles of these extracts were determined by UPLC-DAD-MS. The cytotoxicity (CC<sub>50</sub>) of trunk and leaves extract of L. corymbifera was 121.0 and 181.30 µg/mL, respectively, whereas the trunk and leaves extracts of T. pyramidatum showed CC<sub>50</sub> of 190.6 and 189.9 µg/mL. In relation to the anti-Zika virus activity, L. corymbifera trunk extract presented EC<sub>50</sub> of 105.0 µg/mL and leaves extract the EC<sub>50</sub> was about 71.25 µg/mL. The EC<sub>50</sub> of the T. pyramidatum trunk extract was 130.5 µg/mL and leaves extract presented EC<sub>50</sub> of 81.63 µg/mL. UPLC-DAD-MS analyzes allowed to identify as main constituents from leaves extract of L. corymbifera and T. pyramidatum are polifenolic compounds like flavonoids and phenilpropanoid glycosides. Leaves extracts of L. corymbifera and T. pyramidatum were more active in vitro against ZIKV, showing promising sources of substances with potential anti-Zika virus activity. Financial support: FAPEMIG, CAPES, CNPq, PROPP-UFOP.

**Palavras-chaves:** Antiviral activity, Zika virus, Lundia corymbifera, Tanaecium pyramidatum, Bignoniaceae

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**VIRTUAL SCREENING AND EVALUATION OF ANTIVIRAL EFFECT OF MOLECULE QT AGAINST THE NS5 PROTEIN OF THE Zika virus**

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**Resumo**

*Zika virus* (ZIKV) is a flavivirus belonging to the arbovirus (Arthropod-borne virus) group that can be transmitted in parallel ways to vector transmission, such as vertical transmission. ZIKV presents symptoms that can be confused with Dengue, except for cases of microcephaly and Guillain-Barré syndrome. To date there aren’t specific therapies for ZIKV disease. Knowing that there are reports of antiviral activity of the QT molecule in the NS5 target of Dengue virus, and seeing the similarity between viruses of the same family, the antiviral activity of QT against ZIKV was evaluated. So, the QT was transferred to NS5 of ZIKV through Discovery Studio program, and one centered box was constructed in this binder to define the region in which QT would be anchored using the AutoDockTools program. The box was defined as a cube with dimensions of 16x16x16 Å and coordinates X (21.458), Y (4.286) and Z (11.298). The molecule QT had the charge
calculated to physiological pH and 3D structure defined by the MarvinSketch program. After preparation, the docking of NS5 was done with AutoDockVina. The value obtained for QT binding energy was -7.0kcal/mol. In addition, the in vitro assays were done. Initially, cytotoxic assays were conducted to determine the cytotoxic concentration to 50% of the cells (CC50). For this, mammalian cells (VERO) were added into 96 well microplates (5x10^4 cells/well), and after 24 hours, were treated with QT at different concentrations. The revelation was obtained after 48h by MTT (methyl thiazol tetrazolium) colorimetric technique. We have detected the CC50 concentrations higher than 1000 µg/mL for QT. For antivirals assays, the same cells pre-treated with extracts were infected with ZIKV at multiplicity of infection (moi) of 1 virus/cell, 48 hours after infection (hpi) we found the protective/effective concentration to 50% of infected cells (EC50). The results showed that QT were able to inhibit ZIKV at 70.3µg/mL concentration. Finally, the selective index (SI) was calculated which refers to the ratio between CC50 and EC50 of QT, which should be above 4.0. The SI was 14.2for QT. Thus, it was possible to prove that QT has broad spectrum activity acting as antiviral in both viruses and we are proving the possible mechanism of infection in vitro.

Financial Support: FAPEMIG, CNPq and UFSJ.

Palavras-chaves: antiviral, bioassays, Molecular Docking., ZIKV

BARRIERS OF ATTENDANCE CLINICAL FOLLOW-UP OF CONGENITAL CYTOMEGALOVIRUS INFECTION IN LOW-INCOME POPULATIONS

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Resumo

Cytomegalovirus (CMV) is the most common congenital viral infection, and a leading cause of hearing loss and neurologic disabilities in children worldwide. Clinical follow-up for congenital CMV is recommended for early intervention, better management and control the long-term associated sequelae. However, low-income populations face challenges such as lack of financial resources, weak public infrastructure, poor knowledge, and negligence, which could be impactful for intervention purposes. In this study, we have assessed factors related to the lack of attendance clinical follow-up for congenital CMV infection. The study was carried out during February 2010–December 2012 in South Bahia State, Northeast Brazil, and focused on socio-economic factors correlated with barriers of attendance clinical follow-up of congenital CMV infection. Quarterly medical visits for all newborns diagnosed with congenital CMV infection were planned. Complementary laboratory tests such as hemogram, hepatic function panel, total and fractionated bilirubin, and auditory evaluation were also included. Twenty-five newborns diagnosed with congenital CMV infection were enrolled. A total of 96% were asymptomatic. The only symptomatic individual died due to the progressive congenital CMV infection. Half of asymptomatic newborns attended to clinical follow-up, and 8,33% (n=2) presented hearing loss, the main outcome related to congenital CMV infection. However, they did not returned to Brainstem-Evoked Response Audiometry test (BERA) as recommended. The most common barriers related to the lack of attendance clinical follow-up were high urban mobility, lack of knowledge and negligence regarding CMV infection, and lack of financial support (even own or public). Our results suggest that congenital CMV infection and irreversible outcomes such as mental retardation and hearing loss, could be neglected in low-income population due to barriers and low-adherence to the clinical follow up. We believe that increasing efforts promoting public health and education goals could improve the population awareness and commitment to clinical follow up. Financial Support UESC

Palavras-chaves: CMV, congenital infection, neglected disease, clinical follow-up, low-income population
GENOTYPIC AND EPIDEMIOLOGICAL ANALYSIS OF NOROVIRUS IN NORTHERN REGION OF BRAZIL

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Resumo
NoV is an important pathogen causing outbreaks of gastroenteritis. It is a highly infectious virus, stable and easily transmissible by ingestion of contaminated food and water. This virus belongs to the family Caliciviridae, genus Norovirus, being classified into seven genogroups (GI-GVII) and 41 genotypes. The objective of this study was to perform the genotypic characterization of NoV, with description of recombinant genotypes and genetic variants. Extraction of the genetic material was performed by the QIAquick® kit according to the manufacturer's instructions. For the genotypic characterization the capsid and polymerase genes were partially amplified. The positive samples were sequenced and analyzed in the Geneious R10 program by the Neighbor-Joining method (bootstrap, 1000 replicates). The genotypic identification was performed using the Norovirus Genotyping Tool. During the years 2015 and 2016, a prevalence of 33.2% (175/528) was obtained for NoV. This virus was detected in fecal samples of all age groups, with the infection of > 6-12 months (45.4%) being the most affected by NoV infections (G test

Palavras-chaves: norovirus, genotypes, gastroenteritis

STUDY OF SUSPECTED DENGUE CASES NEGATIVES IN THE SOROLOGICAL TEST FOR DETECTION OF ANTIGEN NS1: FAILURE IN THE DIAGNOSIS OR EMERGENCY OF OTHER ARBOVIROSES?

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Resumo
Abstract
Dengue is currently considered one of the most important arboviruses. In Brazil between 2011 and 2016 were reported more than six million cases, but only a few of these millions, were confirmed laboratory. In view of the significant number of reports and negative results, the objective of this study was to investigate the presence of dengue virus in samples with "No Reagents" results for the NS1 antigen, assessing if there was a failure in the laboratory diagnosis, or the existence of other arboviruses circulating in the state of Amazonas. Using the RT-qPCR technique, the presence of dengue virus in 306 serum samples from patients with clinical suspicion of infection was investigated. The samples that remained negative were investigated for the presence of Zika (ZIKV), Chikungunya (CHIKV), Mayaro (MAYV) and Oropouche (OROV) virus by the same methodology, as well as the presence of IgG and IgM the dengue virus by ELISA. Of the 306 analyzed samples, 17 (5.5%) were positive for DENV, with three sequenced for serotype 4. Thirty-four (10.8%) were positive for ZIKV, one (0.3%)
for CHIKV, thirteen (4.2%) for MAYV and nine (2.9%) for OROV. In relation to the NS1 test, all the kits evaluated presented 100% agreement in negativity. For the anti-dengue antibodies of the IgG class, of the 306 samples tested, 134 (43.8%) had positive results. Regarding the detection of the IgM antibody, different positivities were observed for commercial kits: VIRION (n = 250) 35.6% positive; FOCUS (n = 105) 10.5% positive and PANBIO (N = 80) 20% positive. Our results confirm cases of false negative results for the NS1 tests of three commercial kits, in addition to the circulation of other arboviruses among patients from different municipalities in the state of Amazonas. Source of funding and resource: Molecular epidemiology of Dengue in the State of Amazonas: Phylogeography and factors associated with failure in the serological detection of the antigen NS1. CAMP FAPEAM // SUSAM // MS / CNPq 001/2013 - PPSUS

Palavras-chaves: Arboviroses, Dengue Virus, NS1 protein, Mayaro virus, Oropouche virus

GEOPHAPHICAL DISTRIBUTION OF HEMORIO’S BLOOD DONORS INAPTS BY POSITIVE SOROLOGY FOR HUMAN IMMUNODEFEICIENCY VIRUS (HIV) IN THE RIO DE JANEIRO CITY AND NEIGHBORS

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Resumo

Introduction: It has been known for more than 20 years that Human Immunodeficiency Virus (HIV) can be transmitted through blood transfusion and therefore it is part of the parameters tested in the serological screening of blood donors. In the year 2017, 5.23% of the cases of serological inability were due to the presence of positive serology for HIV (initial testing) at the State Institute of Hematology Arthur de Siqueira Cavalcanti - Hemorio. Objective: The main objective of the present study is to identify the geographical distribution of HIV through the blood donation candidates in Hemorio. Methods: The study was done through data collection in the Institution's Information System (sacs-sofis), using the enzyme-linked immunosorbent assay (ELISA) and the Nucleic Acid Test (NAT) between 2014 and 2017. The neighborhood and the municipality of residence of the candidate were collected and then made the statistics of these to suggest the geographical distribution of the virus in the city of Rio de Janeiro and neighbors. In no case will the candidates be identified. Results: Through sacs-sofis, 1047 numbers of donations with HIV reactivity between 2014 and 2017 were verified. Replicates and false positives were excluded. There were 22, 20, 15 and 18 reactive in the Capital in 2014, 2015, 2016 and 2017, respectively. In turn, 8, 11, 15 and 14 were reactive in the Baixada Fluminense during the same years. Thus, there were 123 candidates for donation with positive serology for HIV in these regions. Discussion: Based on the collected data, it was possible to verify that between the years 2014 and 2016 there was an increase in the candidates for the donation by positive HIV serology in the Baixada Fluminense and in the same interval, a decrease in the Rio city. However, from 2016 to 2017, there were no significant changes in the two populations. In general, it can be seen that the geographical distribution of candidates for HIV+ blood donation in the city of Rio de Janeiro and neighbors in the last four years is not homogeneous. Conclusion: In the present study, it will be possible to have an indication of the geographical distribution of HIV in part of the State of Rio de Janeiro. This indicative will enable unfolding and new questions, which may involve socioeconomic indicators, such as age, gender, level of education, among others in future studies.
SOFOSBUVIR EXHIBITS ANTIVIRAL ACTIVITY IN A MODEL OF CHIKUNGUNYA VIRUS INFECTION IN VIVO

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Resumo

Chikungunya virus (CHIKV) causes a febrile disease associated with chronic arthralgia, which may progress to neurological impairment. Chikungunya fever (CF) is a consolidated public health problem, in tropical and subtropical regions of the world, where control of CHIKV vector, mosquitos of the Aedes genus, failed. Since there is no vaccine or specific treatment against CHIKV, infected patients receive only palliative care to alleviate pain and arthralgia. Thus, drug repurposing is necessary to identify antivirals against CHIKV. Recently, the structure and activity of CHIKV RNA polymerase was partially resolved, revealing similar aspects with the enzyme counterparner on other positive sense RNA viruses, such as members of the Flaviviridae family. We then evaluated if sofosbuvir, clinically approved against hepatitis C virus RNA polymerase, which also aims to dengue, Zika and yellow fever viruses replication, would inhibit CHIKV replication. Arthralgia model was adapted for Male Swiss Webster mice (8 weeks-old) were infected with $10^4$ TCID$_{50}$ in right hind paw towards the ankle. Sofosbuvir was given orally (20 mg/kg), beginning one-hour before the first virus injection. Treatment was conducted for 6 days. Paw oedema was evaluated from the first to the sixth day after infection by hydropletismometer. Besides that, three-day-old Swiss mice were infected intraperitoneally with $2 \times 10^2$ TCID$_{50}$ of virus. Treatments with sofosbuvir were carried out with sofosbuvir at 20 mg/kg/day intraperitoneally. Treatment started one day prior to infection (pretreatment) or two days after infection (late treatment). Animals were monitored daily for survival, weight gain, and virus-induced short-term sequelae (righting in up to 60 seconds). To test the righting reflex, animals were tested daily during the course of acute infection animals were held in a supine position with all four paws facing up in the air for 5 seconds. Then, animals were released, and the time the animal took to flip over onto its stomach with all four paws touching the surface was measured. Sofosbuvir exhibited antiviral activity in vivo, by preventing CHIKV-induced paw oedeme in adult mice and mortality on neonate mice model, at 40 and 80 mg/kg/day. Our data demonstrates that a prototypic alphavirus, CHIKV, is also susceptible to sofosbuvir. Since this is a clinically approved drug, it could pave the way to become a therapeutic option against CF.

FINANCIAL SUPPORT: CNPq, FAPERJ, INI, IOC and FIOCRUZ

Palavras-chaves: ANTIVIRAL, CHIKUNGUNYA, SOFOSBUVIR, TREATMENT
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Resumo

Zika virus infection (ZIKV) is an arbovirose that is spread to humans by Aedes aegypti and/or Aedes albopictus. ZIKV is a RNA virus and belongs to the Spondweni serocomplex, Flavivirus genus, Flaviviridae family, which presents a genetic and serological relationship with other flaviviruses of public health importance: dengue, encephalitis, yellow fever and hepatitis C viruses. Treatment of this infection is important even in asymptomatic cases, mainly in pregnant women, since there is an association between ZIKV infection and microcephaly in newborns. There is currently no specific treatment or vaccine for ZIKV infection. The importance of natural products as sources of new drugs is universally recognized and there is no lack of promising results regarding the antiviral potential of these products. In this context, the present work aims to evaluate in vitro antiviral activity against the ZIKV of ethanolic extracts of trunks and leaves of the species Tecoma castaneifolia, T. garrocha, T. stans var angustata and T. stans var stans. Initially, the mean cytotoxic concentration (CC50) of these extracts was determined by the MTT colorimetric assay using the VERO cell line (n = 3). Then antiviral activity against ZIKV in VERO cells (n = 3) was performed by the MTT method, the results were expressed in terms of 50% effective concentration (EC50). In addition, the chromatographic profiles of extracts by UPLC-DAD-MS were determined. Trunk extracts were more cytotoxic with CC50 from 0.1934 to 159.0 µg/mL, whereas those from leaves were not cytotoxic at the highest concentration tested (CC50 > 200.0 µg/mL). Anti-Zika virus activity was not observed in the trunks extracts of T. stans species, however, those of T. castaneifolia and T. garrocha presented EC50 of 66.78 and 131.0 µg/mL, respectively. Leaves extracts presented EC50 ranging from 53.62 to 149.90 µg/mL. UPLC-DAD analyses allowed the identification of glycosylated phenylethanoids as the main constituents of these extracts. From these extracts a phenylethanoid was isolated and evaluated for antiviral activity, resulting in a EC50 of 21.64 µg/mL. Leaves ethanolic extracts were more active in vitro against Zika virus when compared to trunks extracts. Results obtained to date suggest that the observed antiviral activity may be related to the presence of phenylethanoids in these extracts. This work was financially supported the FAPEMIG, CAPES, CNPq, and PROPP–UFOP.

Palavras-chaves: Antiviral activity, Zika virus, Tecoma, Phenylethanoid glicoside, Bignoniaceae

GENETIC DIVERSITY OF HPyV10 AND HPyV11 DETECTED AMONG BRAZILIAN CHILDREN

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Resumo

The Human Polyomaviruses (HPyVs) belong to the Polyomaviidae family and are non-enveloped, double-stranded DNA viruses. HPyVs Malawi (MWPyV or HPyV10) and Saint Louis (StLPyV or HPyV11) are emerging viruses that have been originally detected in fecal material and tentatively associated with diarrheal disease. In this study, we analyzed stool specimens from Brazilian children
with and without acute diarrhea to investigate the excretion of HPyV10 and HPyV11 and to analyze the genetic diversity of the virus strains detected. A total of 460 stool specimens were obtained from children with acute diarrhea of unknown etiology and 106 stool specimens were obtained from healthy children under 10 years of age, and the specimens were screened for HPyV10 and HPyV11 DNA by PCR. Positive samples were sequence to identify the virus genotypes. HPyV10 and HPyV11 were detected in 7.2% and 4.7% of stool specimens from children with and without diarrhea, respectively. Of the diarrheal samples 33 (7.2%) were HPyV positive: 11 (2.4%) were positive for HPyV10, 20 (4.4%) were positive for HPyV11 and 2 (0.4%) were positive for both viruses. Among the 106 non-diarrheic samples, 5 (4.7%) were HPyV positive: 4 (3.8%) were positive for HPyV10 and 1 (0.9%) was positive for HPyV11. Both viruses were equally prevalent among children with diarrhea as healthy children (2.4% versus 3.8%, p = 0.51 and 4.4% versus 0.9%, p = 0.082, respectively). Most of the 33 positive diarrheal samples were from children ≤ 2 years of age, however, there was no significant difference in relation to age and positivity for HPyV (p = 0.137). There was also no difference between sex and degrees of disease (severe, moderate or mild). Phylogenetic analysis showed that all the genotypes described so far for HPyV10 and HPyV11 circulate among the studied population. Our result did not support an association between HPyV10 and HPyV11 in stool and pediatric gastroenteritis. Nevertheless, the excretion of HPyV10 and HPyV11 in faces indicates that fecal-oral transmission is possible. The results of this study revealed, in a pioneering way, the circulation of HPyV10 and HPyV11 in Brazil.

Financial Support: CNPq, CAPES and FAPERJ.

Palavras-chaves: Gastroenterite, Epidemiologia, HPyV10, HPyV11, Poliomavirus

IDENTIFICATION OF HUMAN MASTADENOVIRUS SPECIES IN SAMPLES OF PATIENTS WITH RESPIRATORY INFECTION IN SOUTHERN BRAZIL

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Resumo

*Human mastadenovirus* (HAdV) genus is related to several diseases, among them upper and lower respiratory tract illness. HAdV species B, C, D and E (HAdV-B, -C, -D and -E) are mainly associated with respiratory infections. The goal of the present study was to identify the HAdV species associated with respiratory infections in hospitalized patients in different cities of the state of Rio Grande do Sul – BR. The samples (nasopharyngeal secretion) were collected in periods from 1996 to 2004 and 2011 to 2017. Previous analyzes were performed to detect the influenza virus by LACEN-RS (Central Laboratory of Public Health), being throughout samples were negative. Subsequently a screening was carried out and the samples were positive for HAdV. Then, the samples were sent to the Laboratory of Molecular Microbiology – University Feevale for the identification of HAdV species. The samples were submitted to nucleic acid extraction with a commercial kit (Biopur®). For screening the presence of HAdV, a partial sequence of the DNA polymerase gene was amplified by nested PCR. Sequencing was
performed in positive samples. In a total of 57 samples evaluated, HAdV DNApol gene was detected in 54 samples. Different HAdV species were found: HAdV species B (28; 49.1%), C (15; 26.3%), D (2; 3.5%), and E (5; 8.7%). Four previously positive samples in the nested PCR, it was not possible to identify the specie due to the low quality of the DNA sequence. It should be noted that specie D was only present in 2017 and specie E only in the years 2011 and 2012. Among the 54 positive samples, it was possible to survey the history of 47 patients (18 females and 29 males). No relationship between sex and identified HAdV species was observed.

The age of the patients ranged from


Palavras-chaves: DNApol gene, HAdV-D, Human mastadenovirus, Respiratory Infections

HUMAN ALPHAHERPESVIRUS 2 (HHV-2) IN PREGNANT WOMAN WITH AND WITHOUT ZIKA VIRUS.

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Resumo

Zika virus (ZKV) infection is an acute exanthematous disease mainly transmitted by the bite of an infected Aedes species mosquito. Human Alphaherpesvirus 2 (HHV-2) is one of the most sexual transmitted pathogens worldwide that shows high incidence in women of reproducing age and infects the genital mucosa leaving to a latent and persistent infection can be reactivated during the pregnancy. Both ZIKV and HHV-2 can be transmitted from mother to her fetus through transplacental route, are neurotropic and leave some neurological deformities during embryonic development. A recent study showed that the HHV-2 infection in human trophoblasts increased the permissiveness of ZIKV in these cells, which could lead to effects teratogenic effects. The aim of this study was to evaluate the prevalence of HHV-2 in a pregnant woman infected and no-infected with ZIKV from Rio de Janeiro. A total of 167 pregnant women (94 of them were zika-positive and 73 of them were zika-negative) were included in this study, all pregnant signed the consent form and collected blood samples. The zika infection was confirmed by q-PCR. All serum samples were tested by commercial immunoassay for detect IgG against HHV-2 (Biokit® Bioelisa HSV-2 IgG, Werfen Company) and all positive serum samples for IgG were tested for detect IgM against HHV-2 (Ridascreen® HSV-2 IgM, R-Biopharm). DNA viral of samples was extracted using the commercial kit (High Pure Viral Nucleic Acid Kit, Roche®). The median age of pregnant women was 25.3 years, 9.6% (6/62) were black, 45.2% (28/62) were white and 45.2% (28/62) were brown. The majority of the population (96.5%) living in the urban area. About the gestational age, 55.4% (66/119) were in the 2nd trimester (41/69) being 59.6% of them ZIKV-positive. The overall seroprevalence of HHV-2 IgG in the pregnant woman was 18.5%, 11.7% (11/94) was ZIKV positive and 6.8% (5/73) ZIKV negative. The prevalence of HHV-2 IgM in IgG positive population with and without ZIKV were 9% (1/11) and 20% (1/5), respectively. Beside antibodies detection, no serum samples showed HHV-2 DNA. The results revealed a higher prevalence of HHV-2 among pregnant with ZIKV than without ZIKV. Beside of this, HHV-2 DNA no was found in
serum samples, suggesting there were no episodes of reactivation in the period of study among pregnant with Zika. As HHV-2 reactivation episode can occur anytime, is need the monitoring of this pregnant to prevent active coinfection and possible transplacental transmission.

**Palavras-chaves:** HHV-2, Zika, pregnant, coinfection

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**SOROPREVALENCE OF CHIKUNGUNYA VIRUS IN HEMORIO BLOOD DONORS**

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**Resumo**

**Introduction:** The Chikungunya virus (CHIKV) is a member of the Togaviridae family, the Alphavirus genus that emerged in Africa. Symptoms caused by CHIKV infection may persist for months or years. Thus, it is a serious public health problem. Transmission by transfusion is uncertain, therefore there is a need for blood donor studies. **Objective:** The main objective is to determine the soroprevalence of Chikungunya virus in Hemorio blood donors. **Methods:** Random serum samples from blood donors from July, August and December 2016 and 2017 and May 2018 were used. A total of 2317 was tested. For the detection of IgG, July 1987, August and December 2017 were used. For CHIKV IgM 330 of May 2018 and 528 of December 2017, totaling 858. Among them, 528 of December 2017 were tested for the two parameters. They were processed in automated ELISA. **Results:** From the total of 2317 samples analyzed, 1987 investigated for CHIKV IgG and 200 were reactive. Of these reactives, 116 are of the masculine gender and 84 of the feminine. The age group of 18-39 years had 139, followed by 40-59 with 55, below 18 and above 60 years we had 3 each, totaling 6. As for the place of residence of the reactive donors, 121 were from the city of Rio de with 10 in the neighborhood of Taquara, and in the neighboring cities, 79 with Nova Iguaçu with 23. Regarding the CHIKV IgM, there were 858 samples with 29 reactive, 11 in December 2017 and 18 in May 2018, 10 are male and 19 are female. The age range of 18-39 with 25, 40-59 with 3 and under 18 years only 1 reactive. In the prevalence by place of residence of the donor the city of Rio de Janeiro was highlighted with 25 and in the neighboring 4. The group of 528 samples of December 2017, tested for IgG and IgM, showed reactivity of 3, all are from the capital, 2 of the female and 1 male, 2 are 18-29 and 1 from 30-49. **Discussion:** The results show a high soroprevalence in 2016 and 2017, but when comparing the two years there was no increase in IgG, but maintenance. In contrast, the presence of IgM almost tripled compared to a period of less than 6 months. This suggests that CHIKV infection among asymptomatic individuals may have increased over the years as donors undergo clinical screening and IgM detection occurs in the acute phase of the disease. **Conclusion:** The data from this pioneering study are extremely important, since we can not predict which damage the CHIKV infection can cause the patient.

**Palavras-chaves:** Blood donors, Chikungunya virus, Rio de Janeiro

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**Association of C-reactive protein serum levels with CRP-717 T/C polymorphism and viremia in HCV and HBV carriers**

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**CONCLUSION:**

of moderate correlations (0.36-0.67) and strong correlations ($r > 0.68$), respectively, among established nodes. Robust interaction networks were inferred for the haplotype carriers compared to $F_0$; $ALT$ ($p = 0.0179$) and $GGTG$ ($p = 0.0307$) for $A_2$; and $AST$ ($p = 0.0473$) for $A_3$, and $viremia$ ($p = 0.0232$) for $F_0$. The linkage disequilibrium between the polymorphisms was calculated (Haplovie 4.2). The Hardy-Weinberg Equilibrium (HWE) was inferred; the $G$-test was used to compare the frequency of polymorphisms between histological degrees; the Mann Whitney test in comparison of the polymorphisms was quantified using the automated immunoturbidimetric method. The $TT$ genotype was the most frequent in all studied groups and was associated with higher plasma levels of the protein but not with the progression of liver disease. Low levels of C-reactive protein were associated with increased viremia and scores indicative of severe fibrosis and cirrhosis. The present results demonstrated a close relationship between the ability of the virus to replicate and cause liver damage and the low serum concentrations of C-reactive protein. Future research may determine if these results can be interpreted as a possible form of escape for the virus by decreasing its action as an opsonin and decreasing phagocytosis, which are functions of CRP in the immune response.

**Resumo**

C-reactive protein (CRP) plays an important role in the innate immune response due to its opsonization action in the capture and removal of cells by facilitating phagocytosis. There is strong evidence that changes in the production of CRP may be associated with increased susceptibility to infectious and autoimmune diseases, thereby affecting the severity and development of clinical disease. The present study investigated the association of the rs2794521 (-717 T/C) polymorphism in the CRP gene among individuals with chronic hepatitis B and C, correlating it with markers of hepatic inflammation, fibrosis scores, viral load and plasma protein levels. A total of 185 blood samples obtained from patients with HBV ($n = 74$) and HCV ($n = 111$) seen in the ambulatory of liver diseases at the Hospital of Santa Casa of Esmeraldas Foundation of Pará (FSCMPA) and the University Hospital João de Barros Barreto (HUBBB) as well as 300 samples from healthy donors were analyzed. Polymorphism screening was performed by real-time polymerase chain reaction (qPCR), and protein levels were quantified using the automated immunoturbidimetric method. The $TT$ genotype was the most frequent in all studied groups and was associated with higher plasma levels of the protein but not with the progression of liver disease. Low levels of C-reactive protein were associated with increased viremia and scores indicative of severe fibrosis and cirrhosis. The present results demonstrated a close relationship between the ability of the virus to replicate and cause liver damage and the low serum concentrations of C-reactive protein. Future research may determine if these results can be interpreted as a possible form of escape for the virus by decreasing its action as an opsonin and decreasing phagocytosis, which are functions of CRP in the immune response.

**Palavras-chaves:** C-reactive protein, SNP, HBV, HCV, viremia

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**POLYMORPHIC VARIANTS IN GENE NGFB MAY ALTER THE VIRAL LOAD LEVELS AND HEPATIC ENZYMES IN DIFFERENT HISTOLOGICAL DEGREES OF PATIENTS WITH CHRONIC VIRAL HEPATITIS**

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**Resumo**

**INTRODUCTION:** The $NGFB$ gene encodes the neural growth factor (NGF), whose function is related to cell survival and differentiation. Studies indicate that NGF can regulate liver cell proliferation. Polymorphisms in the $NGFB$ gene have been associated with different pathologies, however, there are no studies that analyze them in relation to the progression of viral hepatitis. The aim of the present study was to evaluate the influence of four polymorphisms in the $NGFB$ gene on different histological degrees of patients with chronic hepatitis B and C. **MATERIAL AND METHODS:** Peripheral blood samples from thirty one hepatitis B and sixty eight hepatitis C patients were collected for DNA extraction and genotyping of $Arg80Gln$, $Val72Met$, $Ala35Val$ and $Ala18Ala$ polymorphisms by qPCR (TaqMan® SNP Genotyping Assays). Viral load data, liver enzymes and histological profile were collected from patient's records. Histological records were classified according to the METAVIR scale for inflammatory activity ($A0$-$A1$) and hepatic fibrosis ($F0$-$F4$). The linkage disequilibrium between the polymorphisms was calculated (Haploview 4.2). The Hardy-Weinberg Equilibrium (HWE) was inferred; the $G$-test was used to compare the frequency of polymorphisms between histological degrees; the Mann Whitney test in comparison of the polymorphisms against the enzymatic data and viral load (Bioestat 5.0 and Graphpad Prism 6.1). The Pearson Linear Matrix estimated interaction networks (Bioestat 5.0 and Cytoscape 3.6) ($\alpha: 95\%, p < 0.05$). **RESULTS:** The level of inflammation $A0$-$A1$ ($66.67\%$) and $F0$-$F1$ fibrosis ($47.48\%$) prevailed. All polymorphisms were in HWE. The frequency of polymorphisms was not statistically different between histological degrees analyzed. For $A2$-$A3$ and $F0$-$F1$ profiles, viral load was higher in $GGCG$ haplotype carriers compared to $GGTG$, whereas liver enzyme levels were lower in $GGCG$ carriers [viral load (0.0056); $ALT$ (0.0193); $AST$ (0.0232) and $GTT$ (0.0553) for $A2$-$A3$, and viral load (0.0473); $ALT$ (0.0307) and $AST$ (0.0179) for $F0$-$F1$]. Robust interaction networks were inferred for the $GGCG$ and $GGTG$ haplotype data, with the prevalence of links of moderate correlations (0.360.67) and strong correlations ($r>0.68$), respectively, among established nodes.

**CONCLUSION:** The present study suggests that polymorphisms in the $NGFB$ gene may alter liver function in different
Resumo

Monocytes from HIV-patients present increased expression of activation markers and production of cytokines, which contributes to an enhanced inflammatory status that characterizes AIDS progressing. 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 activationmay be beneficial to prolong patient’s life and to reduce the use of ART. Beta-cyclodextrin (BCD) is a cholesterol-sequestering drug 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 monocytes derived from HIV+ patients 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 stimulationm. Accordingly, BCD-treated monocytes showed significant reduction of TNF-alpha and IL-10 secretion and expression of MHC-II. This response was not due to overall reduction of lipid rafts associated proteins nor to the content of cholesterol intermediates, since cholesterol and surface proteins CD45, CD59 (raft and non-raft associated proteins) and TLR-4 were already recovered at the time of stimulation. Also, no difference in the lipid profile were detected between BCD-treated or untreated monocytes at this time point. BCD treatment downregulated TNF-a and IL-10 mRNA expression, indicating that the drug acts at transcriptional levels. In addition, LPS-induced p38-phosphorylation was diminished upon BCD treatment, indicating that BCD anti-inflammatory response is mediated by abolishing p38 activation. We also investigated whether dendritic cells (DCs) activation would be affected by BCD treatment and we observed that BCD induced a decreased expression of MHC class II in LPS-stimulated myeloid DCs. 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-chaves:** HIV, Inflammation, BCD, Monocyte

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**Investigation of Dengue virus infection in yellow fever suspected cases, in 2018, Minas Gerais, Brazil**

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**Resumo**

Arboviruses as Dengue virus (DENV), Yellow fever virus (YFV) and Zika virus (ZIKV) (Flaviviridae, Flavivirus) may cause similar symptoms in humans, what points the importance of laboratory tests for a correct diagnosis. Since 2016 huge yellow fever (YF) outbreaks have been taking place in Minas Gerais, Brazil that is an endemic dengue region. Most of the patients who got YF were from rural areas what is in agreement with the sylvatic YF profile. The aim of this work was to investigate anti-DENV IgM/IgG in patients suspected to have YF, from different cities of MG. All the samples (n=318) were collected in 2018 and were previously tested for YFV by LACEN-MG using MAC-ELISA/YFV (IgM) or RT-qPCR-YFV. A total of 205 was positive in one or both tests confirming the infection by YFV (21 IgM+/PCR+, 6 IgM+, and 178 PCR+). Samples from the acute phase were then submitted to the analysis using the rapid Dengue IgG/IgM Eco Test (ECO Diagnóstica). From the 205 patients with positive tests for YF, 59 patients had antibodies anti-DENV (19 IgM+/IgG+, 12 IgM+/IgG-, and 28 IgM-/IgG+). When considering only the patients who presented IgM anti-YFV, the analysis of anti-DENV antibodies demonstrated that 04 were IgM+/IgG+, 2 were IgM+/IgG-, five were IgM-/IgG+, and 16 were IgM-/IgG-. From the 113 patients without laboratory confirmation of YFV infection, 43 presented anti-DENV antibodies: 13 IgM+/IgG+, 03 IgM+/IgG-, and 37 IgM-/IgG+. From six of YFV negative patients (IgM-/PCR-), two were anti-DENV IgM-/IgG+. The cross-reactivity in flavivirus serology can be observed, in that way, the detection of anti-DENV IgM could not be used as the only evidence of a recent of DENV infection, especially in patients with acute yellow fever. Positive IgM samples will be tested by PCR to investigate the infection by different flaviviruses. On the other hand, 97 patients presented IgG, what could indicate a previous circulation of DENV within this population. Further tests, as plaque neutralization assays should be performed to test this hypothesis. In view of the routine diagnosis, of diseases caused by flaviviruses, the use of only IgM tests should be carefully analyzed, since the risk of false positive result due to cross reactivity is matter. In that way, it is important to carry out other tests, such as PCR or more specific serological tests to confirm which virus is responsible for infection in patients, especially during epidemic periods.

**Financial Support:** CNPq, FAPEMIG, CAPES, UFMG.

**Palavras-chaves:** Arboviruses, Brazil, Dengue virus, Antibodies IgM/IgG, Yellow fever virus
NEXT GENERATION SEQUENCING OF THE HEPATITIS B VIRUS FROM CLINICAL SAMPLES: IMPACT FOR VIRUS GENOTYPING

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Resumo

Hepatitis B virus is an enveloped virus that induces liver chronic disease. The HBV virus can be classified into eight genotypes (A-H) according to genome sequence and its distribution is preferentially geographical. The HBV genotyping has been done by Sanger sequencing or reverse hybridization, but both methods present limitations. Sanger sequencing is inaccurate to detect minority genetics variants and reverse hybridization detects only known mutations. Next-generation sequencing (NGS) is a robust tool for clinical virology with different protocols available. The NGS using hepatitis B clinical samples without any enrichment method obtain low reads number, low virus genome coverage and high noise (reads from human genome). The objective of this study was to develop a new method to detect viral genetics polymorphisms or more accurate genotyping from clinical samples using full genome amplification followed by NGS. We were able to amplify a full HBV genome using three overlapping amplicons and further NGS showed to be a robust method. Coverage of the complete HBV genome was satisfactory and sufficiently informative to distinguish HBV genotypes from clinical samples. Our results show that NGS method here proposed is an appropriated technique for correct HBV genotyping, mainly in patients carrying out mixed genotypes classified according to other techniques. The agreements between reverse hybridization and NGS results are probably due to detection the majority genotypes and clashing results between techniques can be solved by sequencing. Moreover, the new NGS method can be significant for analyses of whole genome and studies for a brief summary and potentials implications. Financial Support: This study was funded by Ministry of Health, Federal Government of Brazil.

Palavras-chaves: genotyping, Hepatitis B virus, NGS, phylogeny analysis

APPLICATION OF A NOVEL MOLECULAR MARKER TO IDENTIFY HPV VIRAL TYPES IN HIV POSITIVE WOMEN IN THE STATE OF SERGIPE, BRAZIL

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Resumo

Human papillomavirus (HPV) is a group of viruses that present high tropism for the stratified squamous epithelium, infecting
keratinocytes. Over 200 viral types of HPV have been identified, and almost 40 types preferentially infect the epithelial cells of the genital tract. In women, these viruses can infect basal cells of the squamocolumnar junction, where they can settle and generate lesions. Infections caused by HPV are the most prevalent sexually transmitted infection caused by the virus in the world, affecting approximately 11% of global population. Classification of HPV types is performed based on the L1 gene. However, several studies are based on the detection of only a fragment of this gene, representing some limitations for diagnosis and genotyping. The objective of this study was to apply a novel molecular marker to identify HPV viral types from HIV positive women, using degenerate primers based in entropy (EntroA), for informative genomic regions from HPV. It was used 277 samples collected from HIV positive patients attended at the Center of Medical Specialties of Sergipe, Brazil during the year 2017. The sample DNA was extracted using Wizard Genomic DNA Purification kit (Promega), and conventional PCR was performed. For each reaction 15 µL PCR Master Mix (Promega), 9 µL of ultrapure water, 1.5 µL of each primer (10pmol) and 3 µL of DNA sample was used. The thermal cycle programming was 94°C (10 minutes), 94°C (1 minute), 55°C (1 minute), 72°C (1 minute) and 72°C (10 minutes), corresponding to initial denaturation, denaturation, annealing, extension and final extension, during 40 cycles. The PCR product was visualized in 2% agarose gel using Diamond Nucleic Acid Dye, Gel Loading Buffer and translucent. Positive samples were purified using PCR and gel clean-up kit (Promega) and sequenced by Sanger sequencer. Sequencing quality and assembly of contigs was carried out by using PreGap4 and Gap4 programs, incorporated in Staden package. The BLAST local alignment tool will be used to identify the viral types. Our analyses showed that 26.7% samples were positive through detection by the entropy primers, in comparison with 7.2% positive samples detected by MY primers. Although more studies are needed, these findings show that EntroA could be used to identify HPV isolates.

Financial Support: CNPq, CAPES and FAPITEC-SE.

Palavras-chaves: HPV infection, cervical cancer, sexually transmitted infection

EVALUATION OF EVOLUTIONARY MUTATIONS IN HUMAN RESPIRATORY SYNCYTIAL VIRUS (HRSV) F PROTEIN WITH POTENTIAL RESISTANCE IN PALIVIZUMAB (PZV) BINDING EPITOPES IN CHILDREN WITHOUT PZV.

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Resumo

Human Respiratory Syncytial Virus (HRSV) is one of the leading causes of deaths among hospitalized children and infants with acute respiratory infections worldwide. The RSV virus is an (-) ssRNA and non-segmented virus, which has a genome of 15,200 bp in the Long strain. Its genome consists of 10 genes that code for 11 proteins, and those of viral surface are the most appropriate targets for the development of immunoprophylactic treatments, as there is currently no vaccine against RSV. This has been the most widely used strategy through development and improvement of humanized monoclonal antibodies, such as Palivizumab, which was approved by the FDA in 1998, acting as an inhibitor of viral fusion of epithelial cells through its binding to the F protein. Palivizumab binds to the antigenic site I which comprises between amino acids 258 and 276 of the F protein. Mutations in this region may confer resistance to neutralization by palivizumab. A total of 283 nasopharyngeal aspirate samples that were positive for RSV, were collected from children with less of 24 months of age, with acute respiratory disease hospitalized in the Department of Pediatrics and Child Care of the Santa Casa de Misericórdia of São Paulo who did not undergo prophylactic treatment by palivizumab between March 2008 and September 2010. After processing, genetic material, PCR and sequencing using Sanger technology, and assembly of the reads using
Seqman II v.5.03 software in the Lasergene DNASTAR software package were aligned, trimmed, and after redundancy elimination and coverage analysis, some were excluded. The remaining sequences were submitted to evolutionary analysis in order to evaluate possible F protein mutations present in the region of monoclonal antibody activity, selective pressure analysis, bias analysis of codon use, intra and intergenotype divergence in this region, as well as analysis of possible glycosylation sites and a brief prediction of structural and functional changes caused by the 276 position mutation. Our results shows that the N276S mutation was introduced in 2009 in the Santa Casa samples and was present in the NA1 genotype, also the following year, suggesting that the mutation at position 276 is more frequent in subtype A than in B and as a conclusion was not directly related to palivizumab resistance, but may be related to the induction of both host and maternal neutralizing antibodies on palivizumab antigenic site II.

Financial Support: FAPESP

Palavras-chaves: F protein, HRSV, MARM, Palivizumab

HIGH FREQUENCY OF PIGEON CIRCOVIRUS IN FREE-LIVING PIGEONS (Columba livia) IN SOUTHERN BRAZIL

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Resumo

Pigeon circovirus (PiCV) is an immunossupressive agent that is thought to cause young Pigeon Disease Syndrome (YPDS). It has been reported in domesticated and urban pigeon populations worldwide. The PiCV is classified within the family Circoviridae, genus Circovirus and has a small circular ssDNA genome. In this study, we describe the frequency and viral loads of PiCV in serum of Brazilian free-living pigeons evaluated by qPCR. A total of 185 serum samples were collected from apparently health urban pigeons from seven south Brazilian cities. The PiCV genomic sequences available at GenBank were aligned and primers were designed in a conserved region to detect Rep ORF (Forward: 5’ TTTGTGGAGTTCAACGTCTCG 3’ and Reverse: 5’GCACTTCTTCTGGCTGTACC 3’; amplicon size: 78 bp). The reaction detection limit was evaluated through a standard curve obtained after amplicon cloning in competent Escherichia coli. SYBR Green based real-time PCR was applied in all samples, which were tested in duplicates with positive and negative controls and a standard curve for viral DNA quantification. The qPCR analytical sensitivity was 50 PiCV molecules/reaction in linear scale with 1.02 efficiency and ability to detect lower number of molecules in a non-quantifiable manner. The melting curve was specific and the Tm was 0.3. The frequency of PiCV in pigeons sera was 93.5% (173/185). The viral load was evaluated in 115 of these samples and ranged from 8 x 10^3 to 1.5 x 10^13 DNA molecules/mL sera. There was no significant difference regarding the viral loads and the origins of the samples (p = 0.068) as evaluated by Kruskall-wallis in SPSS v. 21. This is the first study evaluating PiCV circulation in Brazil and the data show that the virus is widely distributed in pigeons’ population of evaluated regions. As the animals were apparently healthy, future studies must be performed to determine the role of PiCV in YPDS.

Palavras-chaves: PICV, PIGEON, qPCR, SYBR GREEN, VIRAL LOAD

An experimental platform for drug discovery against Mayaro virus infection

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Introduction and objectives – Mayaro virus (MAYV) is a neglected arbovirus with a significant potential for emergence and the ability to cause a painful arthritis-like disease. There is no vaccine or specific treatment against Mayaro fever (the disease caused by MAYV in humans), and the mechanisms driving disease development are poorly understood. Here, we describe the development of a pharmaceutical industry-inspired platform of in vitro and in vivo experimental models focused on the discovery of potential treatments against Mayaro fever. Methodology and results – First, we developed a high-throughput screening (HTS) assay using the automated platform at LNBio/ CNPEM. We tested more than 1000 compounds from the NIH Clinical Collection, leading to the identification of 21 hit compounds (~2%) that protected Vero cells from deleterious effects of MAYV infection. Our in vitro assays include plaque assays and viability tests (MTT and Live/Dead assays) to study how hit compounds may reduce MAYV replication or reduce MAYV-induced cytopathic effect/cell death. Our in vivo experimental model consists of a MAYV infection model in immunocompromised ABR mice, which lack functional type I interferon responses. Inoculation with MAYV SJRP, a strain isolated from a symptomatic patient in Brazil, was highly pathogenic to ABR mice, leading to paw inflammation and evolving to systemic infection, viremia, weight loss and death. Paw inflammation was further analyzed using histology and a new microtomography technique, showing that mononuclear leukocytes are recruited to the site of infection and that recruitment precedes paw swelling and edema. Finally, we established protocols for large scale MAYV production and purification, which led to 100-fold concentrated stocks of purified virus. Concentrated stocks were still infective and suitable for advanced techniques, such as cryo electron microscopy. Conclusions – We have developed an experimental platform focused on drug discovery against MAYV, a neglected arboviral disease. We were able to identify 21 compounds with protective properties against MAYV in vitro, which will be further characterized in the near future. Our mouse model of infection resembles important characteristics of Mayaro fever in humans, and will be used for testing of our leading compounds. If successful, our research may result in the first candidate treatment for MAYV infection.

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Palavras-chaves: Drug discovery, Inflammation, Mayaro virus, Mouse model, Treatment

Standardizing of molecular strategy to identification of HPV in women of state of Sergipe, Brazil

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Resumo

The Human Papillomavirus (HPV) is considered one of the most prevalent sexually transmitted infections (STIs) worldwide and it is characterized as the main cause of cervical cancer. One of the more conventional methods of prevention is the Papanicolau that, through sample collection from the cervical region, can identify lesions associated with HPV, but its main disadvantage is the incidence of false negative results. From this, tests that guarantee more effective results are shown as reliable alternatives for the preventive treatment of these STIs. Thus, the objective of the study was to standardize a more sensitive and specific molecular test in the detection of different types of HPV in women with cervical lesions. The procedure used was the multiplex PCR technique, containing primers...
referring to eleven types of HPV, 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66 in 356 samples of cervical lesions. Of the total, 122 samples (34.3%) were identified with some viral type being type 45 (11%) the most prevalent. This is an important data because this type is considered carcinogenic. Of the total, 34 samples (9.5%) presented coinfections by two or three types of HPV. These results showed that this test presented sensitivity and specificity, as it was able to recognize and distinguish different types of HPV.

Financial Support: CNPQ, CAPES and FAPITEC/SE.

Palavras-chaves: HPV, molecular diagnostics, cervical lesions, PCR, multiplex
patients had detectable HHV-6 viral load, with one presenting persistent viremia, with high viral load in the 6th and 12th months (≥10^5 copies/mL). Conclusion: EBV, HCMV and HHV-6 infections were detected in high prevalence during the first year after renal transplant, pointing out the importance of monitoring these viral infections to avoid possible complications, such as graft loss and even patient death. Financial Support: CAPES, FAPERJ.

Palavras-chaves: Herpesvirus infection, Kidney transplant, Molecular diagnosis
Resumo

Yellow fever virus (YFV) (**Flaviviridae**, **Flavivirus**) is the etiologic agent of yellow fever (YF). The YF vaccine is the most successful prophylactic interventions for controlling the disease. The vaccine is composed of attenuated virus and may cause adverse events. Despite the existence of a YF vaccine, since 2016, Brazil is facing an unprecedented in scope YF outbreak, affecting mainly Minas Gerais (MG) State. From December/2016 up to July/2018, 1003 human cases and 339 deaths caused by the YFV have been reported in MG. A total of 302 YF patients (2017=72 and 2018=230) were admitted at Hospital Eduardo de Menezes-BH. The aim of this work was to investigate Yellow fever cases in patients with previous vaccination record, in 2017/18, Minas Gerais, Brazil. During these outbreaks, some cases were suspected to have the disease due to adverse events after YF vaccination. We investigated 42 cases of patients who got sick 1 to 30 days after vaccination. Using the available samples (the ones closest to the acute phase) we were able to detect YFV by RT-qPCR in 26 cases. The partial NS5 gene was sequenced and phylogenetic analyses indicated the infection by the wild type YFV in all cases. In that way, we did not detect cases of adverse vaccination events. Regarding the vaccination record of the patients from 2017, 35 (48.61%) reported never been vaccinated against YF. This data indicates a low vaccination coverage in populations living in risk areas, especially in rural areas once 88% of YFV patients from 2017 lived there. A total of 36 YF patients (2017/18) had vaccination cards checked and in ten cases the previous YFV vaccination was confirmed (1-19 years) before the disease. Of these 10 patients, seven had received only one dose of the vaccine (four were vaccinated in the first year of life), and three received two doses of the vaccine against YFV. We run the Yellow Fever IgG/IgM ECO Teste (ECO Diagnóstica) in four of these patients. None of them presented IgG and one presented anti-YFV IgM. These ten patients were supposed to be protected against YF, and those samples will be further investigated regarding the presence and titer of neutralizing antibodies against YFV. The vaccination is still the best way to prevent YF and although a single dose of YF vaccine is believed to confer life-long protection, some studies have shown the decay of immune response against YF, reinforcing the need of a vaccine booster after 10 years.

Financial support: FAPEMIG,CNPq,CAPES,UFMG

Palavras-chaves: Adverse events, Brazil, Flavivirus, Yellow fever vaccination, Yellow fever virus
Resumo

Yellow fever (YF) is an infectious viral hemorrhagic and vector-borne disease affecting human and non-human primates in tropical areas of Africa and South America. The symptoms of yellow fever include fever, headache, jaundice, muscle pain, nausea, vomiting and fatigue. However, the clinical picture can vary from a febrile disease 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. Recently, in 2016–2018, intense Yellow fever virus (YFV) re-emergence events have been observed in nonendemic areas and in endemic areas with historically low YFV activity, all displaying low vaccination coverage. Since July of 2016 until June 2018, the Minas Gerais State in Brazil, faced the largest outbreak of the YF with more than 1,000 cases and almost 340 deaths confirmed by the disease. Owing to the scarce studies evaluating the immune response in YF patients, the goal of this study was to evaluate the cellular immunity of adult patients who present the acute phase of the infection. Thus, 95 patients hospitalized at the Eduardo de Menezes Hospital, Belo Horizonte, Minas Gerais state, Brazil were enrolled in this study. A total of 10 healthy subjects who received previous immunization with the 17DD yellow fever vaccine were enrolled as a control group (CT). Circulating cytokines and chemokines were quantified in the serum by multiplex assays. Our data showed that YF patients presented increased levels of circulating IL-6, IL-15, IL-10, CXCL8, CXCL10, CCL3, CCL4 and CCL5 besides decreased levels of IL-1-beta and IL-4 as compared with the CT group. Interestingly, death in the YF patients group is associated with increased levels of pro-inflammatory mediators such as TNF, CXCL8 and CCL5 as well as decreased levels of IL-10, a regulatory mediator. Overall, our data indicate that may altered profiles of circulating inflammatory/regulatory mediators are associated with distinct clinical outcomes during human YF infection.

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Palavras-chaves: Yellow fever outbreak, Cytokines, Chemokines, Clinical outcome

ANTIVIRAL POTENTIAL OF IONIC LIQUIDS AGAINST ZIKA VIRUS

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Resumo
The scientific interest in ionic liquid molecules (ILs) has been growing exponentially in the last decade, mainly due to its physico-chemical properties that allow application in new technologies. These compounds have an imidazole or pyridinium
ring and a long alkyl chain, consisting of a charged hydrophilic head group and a hydrophobic tail, which give to them an amphiphilic nature and facilitate the interaction with biological membranes. In 2016, the WHO declared Zika Virus (ZIKV) infections as a global health emergency, mainly due to complications such as microcephaly and Guillain-Barré syndrome. Thus, based on the membrane interaction capacity of the ILs and the fact of ZIKV is an enveloped virus, we evaluated the antiviral potential of 11 different ILs. The majority of compounds analyzed have a imidazole cationic nucleus, with different substituents chains and anions, named: SI16S, SI10S, SI16Cl, SI18Cl, SI(16)MCl, SI(16)ICl, SI16DCl, SI(10)Cl, SI16Im, SI16PCI and SIB. For the lysis plate reduction assay, Vero cells were seeded at a density of 20000 cells/well in a 24 well-plates and maintained in standard condition (5% CO₂ atmosphere at 37°C) for 24 hours. Virus suspension (100 PFU/mL) was added to the cultures, followed by 1h incubation. Then, different concentrations of the compounds (adjusted according to CC50 previous results) were produced (0.3 to 15 µM) in a MEM solution with a 1% carboxymethylcellulose and added on the cultures. The plates were maintained in standard condition for 72 h, and subsequently stained with Violet Crystal. Percentages of inhibition of lysis plate numbers were determined in relation to the viral control. The compounds with the highest percentages of inhibition were SI10S (66% inhibition at 1.8 µM) and SI18Cl (66% and 42% at concentrations of 2.5 and 1.25 µM, respectively). The SI16S caused a decrease of 45 and 33% (2.5 and 0.3 µM), while SI(16)MCl inhibited 36% of ZIKV lysis plates at 7.5 µM and 34% at 1.8 µM. The other compounds showed an inhibition index lesser than 33%. Although the effects on the ZIKV have been little expressive, the ILs have moldable chemical structures that allow thinking about adjustments and synthesis of other ILs to improve their effect against ZIKV. In addition, it is speculated that the amphiphilic profile of these molecules may actually interfere with the replicative cycle of this virus. Financial support: CAPES/CNPq/DCIT – Ministério da Saúde - Brasil.

Palavras-chaves: Antiviral, Imidazolium salt, Lysys plate assay, Zika Virus

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CLINIC AND EPIDEMIOLOGY OF HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS IN WOMEN ATTENDED AT A SPECIALIZED PUBLIC SERVICE IN BLUMENAU – SANTA CATARINA

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Resumo

Human Papillomavirus (HPV) is a non-enveloped virus and the infection is the most common among sexually transmitted infections (STIs). Although most of the people have the infection eliminated in up to two years, a portion of individuals develops cervical carcinoma, the most relevant cause of death in young women in Brazil. The virus is found in 99% of cases of cervical cancer; thus, infection is a necessary condition, but not sufficient for its emergence. To evaluate clinical-epidemiological risk factors, it had been used the data of 49 women diagnosed with High-Grade Squamous Intraepithelial Lesion (HSIL) attended at the Center for Women's Health Care (CAISM), in Blumenau, SC, between 2014 and 2017. Variables evaluated included age, marital status, education level, neighborhood of origin, routing source ESF, the age of onset of sexual activity, time of diagnosis, periodicity of the Pap Smear after diagnosis and year of the last examination performed. The analysis of the epidemiological variables shows that women had ages between 19 and 67 years (mean of 39.79 years), with mean age of 38.51 years for diagnosis of cervical cancer and 16.27 years of age to start sexual activity - this factor related to prolonged exposure to the HPV. Regarding marital status, the majority were unmarried (28.57%), however, 51.02% had a single partner, with 26.53% consensual union. The analysis of the educational level showed that the majority presented complete high school (51.02%). Preventive examination after diagnosis was performed annually in 65.31% of the women, every 6 months in 24.49%, and 8.16% didn't follow up. The highest number of women with diagnosis coincides with the most populous locality of Blumenau (Velha with 16.33%, and Itoupavazinha with 12.24%). Thus, it is possible to conclude that in addition to the increasing incidence, most variables of the profile of women with cervical cancer in Blumenau is similar to those of the Brazilian population. Therefore, from the recognition of this profile, the importance and necessity of preventive and resolutive actions are verified through public policies that prioritize the efficacy of diagnosis,
self-care and understanding of the relevance of the prevention of STI and pathology.

Financial support: Universidade Regional de Blumenau - FURB

**Palavras-chaves:** Cervical cancer, Epidemiology, Human Papillomavirus, Pap Smear, Women’s health

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**CLASSICAL HUMAN ASTROVIRUS IN SAMPLES FROM CHILDREN ATTENDED AT A HOSPITAL IN GOIÂNIA, GOIÁS, BRAZIL**

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**Resumo**

Classical human astroviruses (HAstV) are classified into eight serotypes (HAstV 1-8). Viral transmission occurs by fecal–oral route, through person-to-person contact, ingestion of contaminated food or water, and by contact with fomites. These viruses constitute important agents of acute non-bacterial gastroenteritis and can infect individuals of all ages, predominantly children up to five years of age. The objective of this study was to investigate the occurrence and to estimate the viral load of HAstV in fecal samples obtained from children up to six years of age with or without symptoms of acute gastroenteritis (vomiting and/or diarrhea, with or without abdominal pain, accompanied or not by fever) attended at a hospital in Goiânia, Goiás. Samples are being tested and the viral load determined by real-time reverse transcription polymerase chain reaction (RT-qPCR TAqMan), using standard recombinant plasmid standard curve and specific probe and primers for the capsid region. For this, fecal samples were obtained from 251 children attending a referral hospital in Goiânia from May 2014 to April 2015. To date, 56 samples were tested. Of the total samples tested, 10.7% were positive for HAstV, with viral load ranging from $5.46 \times 10^8$ to $1.33 \times 10^9$ GC/g feces (mean of $8.46 \times 10^8$GC/g of feces). Among the positive samples, 83.3% (5/6) were from children up to two years old. One-hundred percent of samples were from children with diarrhea, and 33.3% (2/6) of them presented vomit. The results highlight that astrovirus infection is most common among younger children, and that diarrhea is the most frequent symptom associated. At the end of the study, we hope to correlate viral loads with symptoms, and also with HAstV serotypes, thus, contributing with relevant information for a better understanding of the molecular epidemiology of these agents in the pediatric population of Goiânia, Goiás.

Financial Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); Universidade Federal de Goiás (UFG).

**Palavras-chaves:** Human Astrovirus, Children, Viral gastroenteritis
IDENTIFICATION AND VALIDATION OF Zika virus SPECIFIC PEPTIDES TO COMPOSE A DIFFERENTIAL SEROLOGICAL DIAGNOSTIC TEST

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Resumo

The infection by Zika virus (ZIKV) is associated to severe neurological complications and, due to the co-circulation of other arboviruses and the different outcomes and the diseases caused by them, besides the difficulty of differentiating these infections just with their clinical aspects, the development of a specific differential serological diagnostic test for zika is crucial. So, the objectives of this project is: I) predict the B-cell epitopes from the ZIKV sequences; II) design and screen antigenic specific peptides for ZIKV, and; III) test the recognition of these peptides by human sera samples. For this, South American and African sequences of ZIKV and DENV reference sequences 1, 2, 3 and 4 were downloaded. Using a B-cell epitope predictor, 37 epitopes from all the ZIKV polyprotein and their corresponding in the DENV consensus polyprotein were identified. The epitopes were analyzed in silico about their amino acid composition and their biochemical properties. A conformational study was also done to select the best epitopes. Thus, 20 epitopes were used for design peptides that were commercially synthesized and used in a protein microarray experiment, using 41 sera samples from patients infected by ZIKV or DENV, in addition to 10 samples from healthy volunteers. In general, the peptides tested did not present a significant distinction for the 3 clinical groups of samples. However, 3 peptides of ENV, NS2-B and NS3 viral proteins were highlighted by their strong signal intensity on microarray and, consequently, they demonstrated a great recognition by the samples, especially when was evaluated the recognition of these peptides in relation to IgG, when the intensity was greater by the zika positive sera samples. Therefore, those results were a motivation to test again the peptides synthesized with new samples. In addition, new peptides were designed too for a better and promising results.

Palavras-chaves: Zika virus, Differential serological diagnostic test, B-cell epitopes prediction

CHIKUNGUNYA IN PERNAMBUCO - FROM ITS INTRODUCTION TO THE CURRENT DAYS

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Resumo
The chikungunya fever is a febrile disease, caused by an RNA virus belonging to the Alphavirus genus and Togaviridae family. The virus was isolated in Brazil in 2014, and in Pernambuco (PE), the first autochthonous cases were confirmed in 2015, in the Agreste Region, in the countryside. In that year, 105 counties reported 8,497 suspected cases, and between 2016 and 2018, 65,384 cases were reported in all counties of the state of Pernambuco. Considering its epidemiological relevance, it is necessary to analyze the Chikungunya cases in Pernambuco from its introduction to the present day 2018, at the epidemiological week 26 (SE 26). This is a descriptive study of the reported cases of chikungunya from the National System of Notifiable Diseases (SINAN, Brazil) in the period ranging from 2015 to 2018 (SE 26) in Pernambuco. Our results showed that between 2015 and SE 26 of 2018 the attack rate of chikungunya in the state of Pernambuco was less than 1%, which is considered bellow the expected for the disease, with the highest number of reported cases occurring in the metropolitan region, and the highest incidence rate occurring in Zona da Mata Norte, at the northern seacoast of the state. The predominant age group was between 20 and 59 years. The year 2016 was highlighted with a 200% increase in the number of deaths reported by arboviruses compared to 2015. From 4,450 pregnant women reported with rash in 2016, 1,057 (24%) were confirmed for Chikungunya Fever. Therefore we can assume that the attack rate of the disease is still below expected, with the counties from Sertão presenting a higher epidemic risk for chikungunya for subsequent years. An increase in the mortality rate pattern for arboviruses after the introduction of chikungunya was evident. As for pregnant women with rash, there was an increase in the number of notifications in 2016 because it was an epidemic year for Chikungunya where the majority of the population of Pernambuco was susceptible to the new virus introduced in 2015.

Palavras-chaves: Epidemiology, Arboviruses, Alphavirus

VIRAL DETECTION IN CEREBROSPINAL FLUID OF CHILDREN WITH SUSPECTED OF MENINGOECEPHALITIS

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Resumo

Viral infections are the main cause of infections in the central nervous system (CNS) in the world, overcoming bacterial, fungal and protozoa infections in frequency. This study aimed to detect viruses in the cerebrospinal fluid (CSF) of patients with meningoencephalitis. A total of 299 CSF samples were collected from children aged 0 to 12 years old with suspected infection in the CNS hospitalized at the João Paulo II Children's Hospital, Belo Horizonte (MG) between the years 2014 and 2018. All CSF samples were tested by qPCR for enterovirus (ENTV), herpes virus (HHV) 1, and 2, chikungunya virus, dengue virus (DENV), zika virus (ZIKV), Saint Louis encephalitis virus, West Nile virus and Yellow fever virus (YFV). Fourteen ENTV-positive samples (5%) were identified of which seven were sequenced and grouped with non-polio group B of ENTV. Eight samples were positive for HHV-1/2 and 15 were positive for DENV (5%). Among the DENV-positive CSF samples, DENV-3 was the most prevalent, being detected in seven samples, DENV-2 in four samples and DENV-1 in two samples. Cases of double infection with DENV-1 and 3 were identified in one sample, DENV-2 and 3 in one sample and cases of triple infection with DENV-1, 2 and 3 in two samples. None of the samples were positive for DENV-4. Two samples presented cases of coinfection between DENV and ENTV (1%). Ten samples were positive for ZIKV and six were positive for YFV. Two of the YFV positive samples, collected in the year 2017, were sequenced and the analysis confirms that the sequences were grouped together with other wild YFV sequences, including samples from the epidemic of 2017. Thus, more attention should be given to cases of viral meningoencephalitis, especially those caused by flaviviruses, associated with severe neurological manifestations, especially in children, because these cases are usually neglected.
VIROME CHARACTERIZATION IN DIFFERENT TYPES OF NON-HODGKIN LYMPHOMA

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Resumo

Non-Hodgkin's lymphoma (NHL) is a heterogeneous group of lymphoid tissue neoplasms, with varied clinical, genetic and morphological characteristics. This lymphoma is the most common form of hematological malignancy worldwide. In addition to risk factors, some infectious agents cause or are related to the risk of developing NHL. Recently, a new species of parvovirus was identified in Mycosis fungoides, a cutaneous T-cell NHL, and the infection was confirmed by in situ hybridization. However, the influence of this virus on cancer development and its prevalence has not yet been elucidated. Given the variety of mechanisms by which infections can cause NHL, this field of research deserves continued attention to understand agents' mechanisms of action and to identify novel infectious causes of NHL. Therefore, the aim of this study is to characterize the viral composition in different types of NHL. Tumor tissue samples from NHL stored at the Instituto Nacional de Câncer were processed for viral DNA and RNA extraction. The viral nucleic acids were prepared for 19 samples with the Nextera XT kit and sequenced on the Illumina MiSeq platform. Virus identification analyses were performed comparing the sequences obtained with viral sequences in the GenBank database through the BLASTX tool. Until now, 11 samples have been processed and analyzed. An average of 60% of the reads obtained mapped to the human genome. Among the remaining reads, we found five virus families that infect vertebrates: Anelloviridae, Flaviviridae, Herpesviridae, Papillomaviridae and Retroviridae. The Epstein-Barr virus, a herpervirus associated with different types of NHL, was found in one sample. Reads from the Anelloviridae were found in four samples. This virus family has a ubiquitous presence without any clinical manifestation so far described in humans. Reads of HIV were found in all samples. Most samples were from patients tested negative for HIV, and the two remaining from untested patients. This suggests that the reads could be a contaminant from HIV+ samples sequenced in the same experiment. HPV4 and HPV16 are epitheliotropic viruses that were found in one sample each. The flavivirus human pegivirus was found in one sample. Some studies associate this latter virus with risk of lymphoma. In summary, this study has described the viral diversity found in lymphomas, and the next steps will be evaluating...
the prevalence of each individual virus in the lymphoma cases.

Support: MS, CNPq, FAPERJ

Palavras-chaves: Non-Hodgkin lymphoma, viruses, Next-generation sequencing

OUTCOMES OF CERVICAL CANCER AMONG HIV-INFECTED AND HIV-UNINFECTED WOMEN TREATED AT THE BRAZILIAN NATIONAL INSTITUTE OF CANCER

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Resumo

Human immunodeficiency virus (HIV) infection increases the risk of some malignancies. The most common cancers in this population are AIDS-defining cancers: Kaposi's sarcoma, non-Hodgkin's lymphoma, and cervical cancer. From introduction of HAART in 1996, there was a significant increase in the life expectancy of people living with HIV. These individuals, who have died relatively young, are now aging and the risk of developing diseases due to the aging process has become increasingly in this population. Cervical cancer (CC) is an important cause of morbidity and mortality in HIV infected women. Many HIV infected women diagnosed with CC will not die of AIDS and therefore it is important to understand the impact of HIV on the prognosis of cancer in patients who have received treatment for it. We evaluated mortality, response to treatment and relapse among HIV-infected and HIV-uninfected women with cervical cancer in Rio de Janeiro, Brazil. Cohort study of 87 HIV-infected and 336 HIV-uninfected women with cervical cancer. Patients at the Brazilian National Institute of Cancer (2001–2013) were matched on age, calendar year of diagnosis, clinical stage, and tumor histology. Staging and treatment with surgery, radiotherapy, and/or chemotherapy followed international guidelines. We used a Cox models for mortality and relapse after complete response. We found a trend (p=0.056) in the association of HIV infection with overall survival; 35% and 49% of HIV + and HIV- women, respectively, were alive in 5 years. However, when we adjusted for clinical stage, the odds of dying associated with HIV lost significance (HR 1.29, 95% CI 0.95-1.75). We observed a strong association (p

Palavras-chaves: HIV, Câncer de colo de útero, Prognóstico

EVALUATION OF MULTIPLEX REAGENTS FOR DETECTION OF DENGUE, CHIKUNGUNYA AND ZIKA VIRUSES BY REAL-TIME RT-PCR

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Resumo

Viruses transmitted by arthropods, particularly those transmitted by mosquitoes, are increasingly important health threats. Currently, DENV is a growing health problem in the subtropical and temperate
urban areas of Argentina. The recent introduction of the CHIKV and ZIKV made diagnosis and notification of cases more complex. The objective of this work was to evaluate the analytical sensitivity and the diagnostic capacity of 3 multiplex qRT-PCR methods, comparing them with the reference "in house" singleplex molecular technique for the diagnosis of DENV, CHIKV and ZIKV. Three methods of qRT-PCR multiplex were evaluated: Light Mix Modular real-time PCR Multiplex Modular Panel Zika, Dengue and Chikungunya (TibMolBiol-Roche) (1), Dengue-Chikungunya-Zika Virus (multiplex kit) (Genesig) (2) and Trioplex Real time RT-PCR assay (CDC) (3). The strains of DENV-1(HAW#1), DENV-2(NG-C), DENV-3(H87), DENV-4(H241), ZIKV(Asian-American genotype) and CHIKV(Asian-American genotype) were titred (UFP/ml) at the same time that an aliquot was taken to perform RNA extraction with the QIAmp Viral RNA kit (Qiagen). From the extracted viral RNA, serial dilutions (factor 10) were made to evaluate the limit of detection. In addition, samples from 38 patients with suspected arbovirus infection received at the laboratory and processed by "in house" methodologies with positive (n:24) or negative (n:14) results, were processed. For the kit 1, the LC480 Thermal Cycler was used, for the kits 2 and 3 the Applied Biosystems 7500 Fast thermocycler were used. The sensitivity observed for CHIKV was similar (3 PFU/ml) to that obtained with the "in house" protocol for the 3 methods evaluated. The sensitivity for DENV was similar to the "in house" protocol for reagent 1 (0.01 PFU/ml) and lower for reagents 2 (1.1 PFU/ml) and 3 (0.1 PFU/ml). The sensitivity for ZIKV was similar to the "in house" protocol for reagent 3 (0.6 PFU/ml) and lower for reagents 1 and 2 (6 PFU/ml), which could lead to false negative results since this agent presents low viremia in patients. The similar clinical manifestations and the difficulties in the diagnostic differentiation of DENV, ZIKV and CHIKV using the laboratory techniques currently available, constitute a challenge for the surveillance system and the health team. The availability of commercial genomic detection reagents will strengthen the differential diagnosis of these agents, while providing accessible tools to a greater number of laboratories in the region.

Palavras-chaves: chikungunya, dengue, multiplex, real time RT-PCR, zika

TWO ATYPICAL POINTS OF RECOMBINATION IN GII.P7-GII.6 RECOMBINANT NOROVIRUS DETECTED IN TWO TIME PERIODS

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Resumo

Noroviruses are an important cause of acute gastroenteritis. The high incidence of norovirus is a reflection of its great genomic and antigenic variability resultant of evolutionary mechanisms, such as recombination. The most described point of recombination is the overlapping of ORF1/ORF2 that encodes polymerase and viral capsid. However, others breakpoints have been described as atypical points of recombination for noroviruses. The aim of this study was to investigate the breakpoint of GII.P7-GII.6 norovirus. The recombinant strains were previously detected in fecal samples from children collected in 2010 and from 2014 to 2015, in Goiânia, Goiás. The GII.P7-GII.6 norovirus strains were sequenced using primers targeting ORF1/ORF2 junction. The ORF1 and ORF2 genotypes were determined by the platform Norovirus Typing Tool v.2.0 and genetic recombination was confirmed by phylogenetic analysis with MEGA7 software, using amino acid and nucleotide substitution model appropriate for each one. Additionally, the platforms SimPlot v.3.5 and RDP4 v.4.94 were used to identify the recombination points. Six sequences of GII.P7-GII.6 were obtained in 2010 and four in the 2014-2015...
period. Phylogenetic analysis using nucleotide and amino acid of polymerase and capsid regions showed two distinct groups of strains, according to the sample's collection time; therefore, one consensus sequence of each group was constructed to determine the breakpoint of recombination. The SimPlot analysis showed a different recombination point for each consensus sequence, in 201 bp and 221 bp in GII.P7-GII.6_2010-consensus and GII.P7-GII.6_2014-15-consensus, respectively. Those points corresponded to norovirus genome positions 5011 and 5032. This sequence is located near the end of the RNA polymerase RNA dependent and upstream from ORF1/ORF2 overlap. Therefore, considered an atypical breakpoint of recombination, when the KU870455 sequence was used as reference. The different points of recombination may suggest different recombination events. Furthermore, breakpoints on the polymerase gene may reflect differences in strains replication; however, further analyses, such as modeling the protein, are required to confirm this hypothesis. Characterization of atypical breakpoints of recombination of norovirus is important to understand how recombinants arise and if those changes contribute to viral replication and/or dissemination.

Palavras-chaves: Norovirus Recombinant, Viral evolution, Genetic diversity, Viral Gastroenteritis

DETECTION OF HUMAN PEGIVIRUS (HPgV) IN HUMAN T LYMPHOTROPIC VIRUS POSITIVE PATIENTS AND BLOOD DONORS IN THE CITY OF BELÉM, PARÁ.

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Resumo

Human Pegivirus (HPgV) is a pan-lymphotropic RNA virus, and several studies correlate its co-infection with Human Immunodeficiency Virus (HIV) to beneficial effects to HIV-infected patients, as higher T CD4+ lymphocytes count and lower HIV load. Nevertheless, there is a lack of data regarding to HPgV among people infected with retroviruses other than HIV, as human T cell lymphotropic virus (HTLV), which is associated to clinical manifestations as adult T cell leukemia/lymphoma (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (PET/MAH). Thus, the present study aims to describe HPgV frequency among HTLV-positive individuals in the state of Pará, Brazil and evaluate the possible effects of co-infection between these infectious agents. For that purpose, blood samples from HTLV-positive and seronegative people residing in Belém, Pará, were selected. These specimens were collected between the years of 2003 and 2016 for HTLV-group, and june and september of 2012 for blood donors (control) group. For detection of HPgV, nucleic acids extraction followed by quantitative polymerase chain reaction (qPCR) were performed. Quantitative and qualitative analysis based on clinical and epidemiological data from the patients enrolled in the study were executed using GraphPad Prism 7.0 and BioEstat 5.3 softwares. A total of 158 samples were included in the study, among which 74 (46,8%) derived from HTLV-positive individuals, and 84 (53,2%) derived from control group. An overall positivity rate of 7,6% (12/158) was found to HPgV in the and, when analyzing both groups singly, frequencies of 5,4% (4/74) and 9,5% (8/84) were observed to HTLV-positive and control groups, respectively. Although no significant statistical differences have been noted regarding to HPgV-positive individuals between studied groups, as well as regarding to age distribution, gender and clinical diagnostic in HTLV mono- and co-infected patients, to our knowledge, this is the first report of HPgV circulation in a HTLV-positive population in Brazil and the primary detection of HPgV viral RNA in blood donors seronegative to HTLV, HIV and hepatitis B and C virus in the Northern region of this country.
These findings highlight the presence of HPgV in this region of Brazil and stimulate the performance of new researches with this approach in order to enhance the understanding and establish the panorama of HPgV infections in these populations.

Palavras-chaves: Blood Donors, HIV, Human Pegivirus, HTLV, LT CD4+

HUMAN POLYOMAVIRUS 1-4 DISTRIBUTION AMONG HEALTHY BRAZILIAN VOLUNTEERS

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Resumo

Human polyomaviruses (HPyVs), are members of the Polyomaviridae family, non-enveloped, double-stranded DNA viruses. The routes of transmission of HPyVs remain unknown, however oral and respiratory transmission has been proposed. HPyVs infection has been associated with disease in immunocompromised persons. However, HPyV excretion has been observed in samples from immunocompetent persons, and the significance of these infections is unknown. The objective of this work was to evaluate the excretion and distribution of HPyV (HPyV1-HPyV4 [former BKPyV, JCPyV, KIPyV, and WUPyV, respectively]) among asymptomatic individuals. Saliva samples from 889 healthy volunteers living in nine different locations in Brazil were analyzed by real-time PCR to detect HPyV1-4. Of the 889 participants, 346 (39%) had evidence of infection with one or more HPyV species: 127 (14.3%) had HPyV1 only; 70 (7.9%) HPyV3 only; 60 (6.7%) HPyV4 only, and 25 (2.8%) HPyV2 only. Coinfections were detected in 64 participants (7.3%). There was a great diversity of HPyV species detected in different geographic regions and within the same region. HPyV1 was detected in all geographic areas except Paraná and HPyV3 was detected in all geographic areas except, Santa Catarina and Rio Grande do Norte. In contrast, HPyV4 was only detected in the Southeast region (Rio de Janeiro and Minas Gerais), and HPyV2 was detected with high frequency in Pará but the low frequency in other localities. DNA viruses have proven useful as markers that both corroborate and extend the population histories inferred from human DNA. In the case of HPyV1 and HPyV2, several publications have reported that these viruses co-evolved with the human population and that they could be used as markers for human migration. Although this characteristic has only been described for HPyV1 and HPyV2, it is reasonable to speculate that other HPyV species would be similarly useful. The Brazilian population is comprised of a genetically heterogeneous group of persons with European, African, and Amerindian origins. The regional ethnic distribution of the Brazilian population has been strongly influenced by immigration throughout history. Therefore, it is possible that the great variation in the frequency and distribution of HPyV species could be attributed to the genetic diversity of this population.

Financial Support: CNPq, CAPES and FAPERJ.
Palavras-chaves: HPyV1-HPyV4, Human Polyomaviruses, Healthy Brazilian Volunteers, Saliva Samples

REMINISCENT STUDY OF WILD HUMAN RABIES OUTBREAK IN MUNICIPALITY OF MELGAÇO AT MARAJÓ ISLAND IN THE PERIOD OF JANUARY UNTIL JUNE, IN 2018.

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Resumo

Introduction: Human Rabies is a disease caused by a virus of the genus Lyssavirus, of the Family Rhabdoviridae, that affect the peripheral nervous system through the bite of the infected animal, whose saliva contains the virus is inoculated in subcutaneous tissues and muscles. The majority of cases occur through transmission of infected animals as dogs and bats. The wild cycle, which can be transmitted by bats, foxes, skanks and many other animals, is still not completely and effectively controlled by man. Due to its high level of lethality, it has become a serious public health problem mostly for the North and Northeast regions of the country. In 2018, an outbreak of Human Rabies occurred, whose the kind of transmission was through bats in the Municipality of Melgaço – PA, in the Archipelago of Marajó. Melgaço is one of the poorest Municipality of Pará.

Methodology: This study has a descriptive retrospective epidemiological character, and was carried out at Secretaria de Saúde Pública do Pará (SESPA) from a period of January to June 2018. These data were computed and analyzed by using statistical methods whose aim was to describe the expansion of the rabies cases in the region during the period the researches took place. Results: 14 cases of Human Rabies were reported, of which 5 of them were confirmed by the laboratory and 3 led the patients to death. Melgaço has the worst Índice de Desenvolvimento Humano (IDH) of the state. Lack of information, basic sanitation and an inadequate structure concerning health of the locals, contributed to the outbreak. Furthermore, the residents are accustomed to being bit by bats while sleeping at night. Another factor that is worth highlighting, is the increasing deforestation in the region, the impact of this activity have brought bats closer to communities.

Conclusion: Based on the findings, it is possible that the outbreak in Melgaço is mostly due to the low IDH of the region and the loss and fragmentation of the habitat of the species of the bats that transmit human rabies. In order to prevent a new outbreak residents are advised to put screens on their houses and mosquitoes net on their beds and the annual vaccination of domestic animals besides, measures should be taken after exposure to the virus as: washing the affected site with soap and water and immediately start the prophylactic schemes with serum 3 and 4 doses of vaccine. Financial Support: FIBRA.

Palavras-chaves: Epidemiology, Human Rabies, Rabies virus

BK VIRUS MOLECULAR DIAGNOSIS AND INFECTION MONITORING IN RENAL TRANSPLANT PATIENTS FROM RIO DE JANEIRO

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Resumo

Introduction: The BK virus (BKPvV) yields a subclinical kidney infection in immunocompetent individuals. However, viremia may occur in kidney transplant (KT) patients with ongoing...
immunosuppression and can cause graft damage. BKPyV-associated nephropathy (BKVN) has no specific treatment and is a leading cause of organ transplant loss. **Objective:** In this study, we evaluated the incidence of BKPyV replication in KT recipients as well the clinical impact of BKPyV replication during post-transplant monitoring in Rio de Janeiro State’s Transplant Center, the Hospital São Francisco de Assis (HSFA). **Materials and Methods:** In this cohort, plasma samples were collected and patients were assessed monthly during the first year post-transplant, along with demographic and clinical data. BKPyV infection was diagnosed through nested PCR, and quantification of viral infection were performed through real time PCR (TaqMan). **Results:** Eighty-four patients were included in this study, 25% (21/84) patients had at least one plasma sample with detectable BKPyV, while 24% (5/21) had persistent viremia. According with Kaplan-Meier method, the estimated probability of remaining free of viremia at 1 year post-transplant was 83% (95% IC: 73% to 93%). Regarding viral load, all patients had detectable BKPyV load in plasma, ranging from $10^{1.7}$ to $10^{5.15}$ copies/mL within different months, with the highest at 3rd month. Finally, the occurrence of viremia could be observed in all 12 months of follow-up and was observed more frequently in the 3rd and 4th months post-transplant. **Conclusion:** Early diagnosis of BKPyV in KT patients had great importance for graft survival and the follow-up is cost-effective due to complications deriving from BKPyV infection, such as graft loss and even patient death.

**Financial Support:** FAPERJ/CAPES

**Palavras-chaves:** BK virus, renal transplantation, immunosuppression, viremia, screening

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**ANTIVIRAL EFFECT OF COMPOUNDS OF Tontelea sp. AGAINST Zika virus**

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**Resumo**

The flavivirus Zika (ZIKV) belongs to the arbovirus (Arthropod-borne virus) group and its able to infect humans. The 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 compounds 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 higher than 1000 µg/mL for TGB, TGC and TGD. For antivirals assays, Vero cells pre-treated with extracts were infected with ZIKV at multiplicity of infection (moi) of 1 virus/cell. 48 hours after infection (hpi), we found the protective/effective concentration to 50% of cells (EC50) and the results showed that TGB, TGC, TGD were able to
inhibit ZIKV at 40.37, 32.72, and 55.43 µg/mL concentrations, respectively. Finally, the selective index (SI) was calculated which refers to the ratio between CC\textsubscript{50} and EC\textsubscript{50} each extract, which should be above 4.0. The SI were larger than 4 for the extracts TGB (SI=24.77), TGC (SI=30.56) and TGD (SI=18.04). The TFAE was considered toxic or non-selective. Therefore, our data indicate potential antiviral action against Zika virus in this compounds present in the plant studied that belongs to Tontelea genus. We have now started studies in our laboratory to determine the mechanism against the virus.

Financial Support: FAPEMIG, CNPq and UFSJ.

Palavras-chaves: Antiviral, Tontelea sp., ZIKV

CHIMERIC PROTEIN LVBA-recHTLV-1/2 SHOWED GREAT SENSITIVITY IN INDIREC ELISA IN HOUSE USING SERA FROM FOUR BRAZILIAN COHORTS

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Resumo

The Human T-lymphotropic virus (HTLV-1/2) is a retrovirus that can induce severe neurological and neoplastic diseases. Epidemiological data point out that 2 million people in Brazil are infected with HTLV-1/2. Once the HTLV-1/2 infection indicates heterogeneous distribution and cluster pattern and the majority of studies were conducted in donor blood banks and only few have been conducted in the general population, these numbers may be underestimated. HTLV-1/2 screening is mandatory in blood centers since 1993, but there is a need to extend it to other groups and sectors of health. Despite the available commercial diagnostic tests indicates sensitivity and specificity higher than 97%, studies reveal that in Brazilian cohort these data are not higher than 80%. Considering this, this project aimed to evaluate a chimeric protein LVBA-recHTLV-1/2 as antigen [Env, Gag and Tax epitopes of HTLV-1 and HTLV-2] in an indirect ELISA in house and in a lateral flow immunoassay (LFIA) using biological samples from four Brazilian cohorts. The development of tests based on national biotechnology for screening blood products is essential to ensure the implementation and maintenance of public health programs, bringing the perspective of reducing costs, minimizing viral transmission and improving epidemiological data. For the ELISA \textit{in house} assay, double-blinded analysis were carried out with 518 sera: 28 HTLV-1+, 7 HTLV-2+, 49 HTLV/-HIV+ and 27 HTLV- from Pará; 3 HTLV-1+, 2 HTLV/-HIV+, 3 HTLV/-HBV+ and 182 HTLV- from Maranhão; 97 HTLV-1+, 9 HTLV-2+, and 92 HTLV- from Minas Gerais (GIPH cohort); 3 HTLV-1+, 12 HTLV-2+ and 4 HTLV+ from São Paulo. Results showed 97.8% sensitivity (95% CI 94.5% to 99.4%) and 77.2% specificity (95% CI 72.46% to 81.45%). Area under the ROC curve was 0.9816 (95% CI 0.9693 to 0.9938), together with kappa coefficient of 0.681 (95% CI 0.621 to 0.740) show great accuracy and good consistency of the test. Also, LFIA has been developed \textit{in house} in an antibody-antigen system with colloidal gold of 40nm conjugated with human anti-IgG. LFIA was carried out with pools of positive or negative sera samples from GIPH cohort. Preliminary results showed 100% specificity and sensitivity for this assay. Altogether, LVBA-recHTLV-1/2 has potential to be used as antigen in diagnostic tests for HTLV in Brazil.

Palavras-chaves: HTLV/2, Diagnostic, Chimeric protein, ELISA, LFIA

HUMAN PAPILLOMAVIRUS IN HEAD AND NECK LESIONS IN BAHIA, BRAZIL.
Human papillomavirus (HPV) is a DNA virus that infects epithelial cells of the skin and mucous membranes of humans, causing various types of lesions. This virus has been found in the great majority of neoplastic lesions of the cervix. In relation to head and neck cancers, cigarette smoking and alcohol are the main risk factors. However, HPV may also be a risk factor for this pathology. The present study had as the main objective to identify HPV DNA in paraffin-embedded tissue of head and neck lesions from two Pathology Anatomy Laboratories from Itabuna and Vitoria da Conquista, Bahia. It was obtained 28 samples from August/2016 until December/2017. Clinical specimens of formalin-fixed were initially deparaffinized, following the DNA extraction, HPV detection and subtyping by PCR. The subtypes were confirmed by sequencing. The mean age of patients was 62.1 (39-86) years and 68.2% were male. Besides, the largest number of samples was from the oral cavity and the most frequent histopathological diagnosis was squamous cell carcinoma. These results are according the literature. In relation to HPV detection, two samples were positives (2/28=7%): a benign lesion, whose histopathological diagnosis was laryngeal papilloma (HPV 11), and a malignant lesion (Squamous Cell Carcinoma) from hard palate (subtype not determined). In addition, the cigarette and alcohol was the most important risk factors for the development of head and neck cancers in this study.

Financial support: UESC

Palavras-chaves: HPV, HEAD AND NECK CANCER, BAHIA

ASSOCIATION BETWEEN IL10 -1082 / -819 AND TNFA-308 HAPLOTYPES AND THE SUSCEPTIBILITY TO CERVICAL CARCINOGENESIS IN HPV INFECTION

Introduction: Human papillomavirus (HPV) is responsible for high-grade cervical lesions and cervical cancer. The inflammation plays a key role in cervical cancer progression. The objective of this work was to investigate the possible association between IL10 and TNFα haplotype analysis and high risk HPV infection in the cervical carcinogenesis risk in women from Northeastern Brazil. Metodology: A total of 654 samples was evaluated in this study. HPV detection and genotyping were performed by
PCR. Genotyping of *IL10* SNPs (rs1800871 and rs1800896) and *TNF*α SNP (rs1800629) was performed by High Resolution Melt analysis and fluorogenic allele-specific probes, respectively. **Results:** It was observed that women carrying the GTA and ATG haplotypes had 3.85 and 17.99-fold, respectively, increased cervical cancer susceptibility when infected by HPV-58. In women infected with HPV-16 and HPV-18, statistically significant results in women carrying the GTA and ATA haplotypes was observed. They had a 2.32 and 3.67-fold, respectively, increased cervical cancer susceptibility when infected by these two HPV types. **Conclusion:** Our study indicates that the association of genetic polymorphism in inflammation-related genes represents a risk to the susceptibility in the development of cervical cancer in women infected by HPVs 16, 18 and 58.

**Financial support:** FACEPE e o CNPq.

**Palavras-chaves:** Cervical cancer, *IL10*-1082 / -819 , HPV-58, *TNF*α haplotype

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**IDENTIFICATION AND CHARACTERIZATION OF SAMPLES COLLECTED FROM PATIENTS WITH SUSPECTED DENGUE IN JUIZ DE FORA, MINAS GERAIS STATE, IN 2016**

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**Instituição**  

**Resumo**

Dengue is a disease caused by *Dengue virus* (*DENV*1 – *DENV*4), a group of viruses of the *Flavivirus* genus, *Flaviviridae* family. They are transmitted by mosquitoes of the genus *Aedes* and are widely spread throughout the world. This disease can be clinically classified as dengue without warning signs, with warning signs and severe dengue. The pathogenesis of severe dengue is multifactorial, and several studies have tried to relate its pathogenesis to viral characteristics, serological status and genetic characteristics of the host. Brazil is currently facing a severe epidemiological scenario with a high incidence of arboviruses that are usually not detected due to incorrect diagnosis and lack of adequate surveillance. Dengue stands out in this scenario with an alarming number of severe cases and high mortality. In Juiz de Fora-MG (JF), Brazil, in 2016, over 27 thousand cases of severe dengue were notified, with 48 deaths. These data show the importance of an adequate diagnosis and investigation of factors related to severe dengue predisposition. The objective of this study was to do molecular analysis of clinical samples from suspicious dengue cases in JF, to identify and characterize circulating *DENV* samples. With this purpose, RNA viral extraction was conducted using QIAamp® Viral RNA Mini Kit (QIAGEN) in 50 serum samples from JF collected during 2016 outbreak, followed by RT-PCR reaction for molecular detection of *DENV*, according to Bronzoni *et al.* (2005) methodology. The PCR products were typed from *DENV*-1 to *DENV*-4, according to differential sizes of the amplicon. Twelve samples were positive and among this, ten samples were positive for *DENV*-1 and two samples were positive for *DENV*-4. The positive samples were sequencing using ABI 3730 Sanger sequencing and the results showed that in the outbreak that took place in JF in 2016 there was a vast circulation of *DENV*-1, result confirmed by epidemiological bulletins. The presence of *DENV*-4 was also detected and the circulation of this serotype in the area may be related to the 2014 *DENV*-4 outbreak occurred in Rio de Janeiro, a neighbor state. The present study allowed the understanding the circulation of *DENV* in JF and helps provide information to improve the medical conduct of patients in...
future epidemics.

Financial Support: FAPEMIG, CAPES, CNPq

Palavras-chaves: Arbovirose, Molecular characterization, Dengue, Severe dengue

URBAN ARBOVIRUS IN PERNAMBUCO: AN EMERGING CHALLENGE

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Resumo

Arboviruses are thus designated because their replication cycle occurs in insects and can be transmitted to humans and other animals through the bite of hematophagous arthropods. Recently, they have been of great concern in public health. Some viruses have lost the requirement for enzootic amplification, producing urban epidemics exclusively for man as a vertebrate amplifier. This is the case of Dengue viruses (DENV), Zika (ZIKV) and Chikungunya (CHIKV). The impact of these Arboviruses on morbidity and mortality intensifies as extensive epidemics occur. The present study had the objective of describing the incidence of urban arboviruses DENV, ZIKV and CHIKV in the state of Pernambuco between 2014 and 2017 during the occurrence of the triple epidemic based of an Epidemiological study of data collection from the State Health Department of Pernambuco - SES-PE, to analyze the incidence of cases reported as suspected dengue, zika and chikungunya in PE. Data and secondary information from the epidemiological reports of SES-PE were collected from 2014 to 2017. A total of 384,126 cases of Dengue, Zika and Chikungunya were reported in the state of PE during the period from January 1, 2014 to December 31, 2017. There were 4 DENV serotypes (1,2,3,4), in addition to circulation of the ZIKV and CHIKV in the year 2015. The annual incidence rate, the highest coefficient recorded in the year 2016 was identified, where in every 100 thousand inhabitants approximately 2,126 were notified as suspected arboviruses patients. Dengue was the most prevalent disease in the period, with a coefficient recorded in the year 2015 of 1,660 for every 100 thousand inhabitants. In the period, 738 deaths were reported, with an ascending mortality rate with identified coefficients of 0.75; 1.56; 4.70 per 100 thousand inhabitants, respectively for the years 2014, 2015 and 2016. Arboviruses are a great challenge for public health, due to a series of factors ranging from socioeconomic impact factors, diversities of infectious agents involved, similarity of clinical manifestations, lack of efficient laboratory support, lack of immunoprophylactic measures besides the difficulty in implementation and maintenance of educational and sanitary measures. Joint actions in research and vector control may have an impact on the expansion of these emerging arboviruses.

Palavras-chaves: virus, dengue, chikungunya, zika

PREVALENCIA DE RESPIRATORIO VIRUS INFECION N EN PATIENTS OF RIO GRANDE DO NORTE,
**BRAZIL**

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**Resumo**

The acute respiratory infections are usually caused mainly by viruses and are responsible for a considerable percentage of morbidity and mortality among adolescents, affecting other age groups. Several studies have reported that viral respiratory infections present seasonal patterns, with different viral circulation on the time of the year. The purposes of this study were to detect respiratory viruses in hospitalized patients and epidemiological patterns associated with these infections. The samples are received in Central Laboratory of Public Health Dr. Almino Fernandes, Natal-RN, Brazil collected by the hospital units. The sample collection was perfomed with 3 swabs of oropharynx and nasopharynx, transported in refrigerated viral transport medium. The viral RNA was extracted using the Viral RNA isolation kit by Qiagen®. Virus identification was performed by RT-qPCR using validated protocols from Instituto Evandro Chagas, Pará, Brazil. Were analyzed 257 samples from January, 1, 2018 to June, 19, 2018. A percentage of 61.8% positivity was found in the samples, with a prevalence of Influenza viruses being 42% Influenza A H1N1 Pandemic 2009, 20% of Influenza A H3 seasonal and Influenza B (11.9%). Among others viruses was found 11.3% of Respiratory Syncytial Virus followed by Human Metapneumovirus with 9.4%. A higher prevalence of infections was observed affecting females. The most affected age groups are children up to 10 years old and over 60 years old. 19,1% of patients affected by Influenza A subtype H1N1 pandemic 2009 needed invasive ventilator support, different of patients affected by others viruses subtypes, that none needed. 83% of the patients infected by Respiratory Syncytial Virus were aged under years. In relation of the presence of risk factors, approximately 40% of patients had at least one risk factor, being mainly pneumopathies and chronic cardiovascular diseases. This study demonstrated the subtypes of respiratory viruses circulating in Rio Grande do Norte in 2018, with 3 subtypes of Influenza present. The infections of Influenza A H1N1 pdm09 causing more serious cases. Patients at the extremes of age are more susceptible, especially when they have associated risk factors. The Respiratory Syncytial virus affect mainly children under 5 years. Work was funded by Health Ministry and State Secretary of Health of Rio Grande do Norte.

**Palavras-chaves:** Respiratory Agude Infections, RT-qPCR, Respiratory viruses

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**DIFFERENTIAL PROFILES OF IMMUNE RESPONSE DURING THE ACUTE PHASE AND THE CONVALESCENCE PERIOD OF HUMAN YELLOW FEVER VIRUS INFECTION**

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Resumo

Yellow fever (YF) is an infectious viral hemorrhagic disease of great importance to public health and endemic in parts of Africa and South America. YF infection is viscerotropic in humans, with viral replication in the liver critical to the establishment of disease. In this organ, yellow fever virus (YFV) induces hepatocyte apoptosis and lytic necrosis, which combined with steatosis, results in most of the liver damage observed during infection. In addition to the virus-induced cytopathic effect, several studies suggest that the immune response itself, via a systemic and unbalanced cytokine response, is a major driver of hepatotoxicity during the YF disease. Clinically, the yellow fever is considered a hemorrhagic fever with hepatitis, despite of the precise mechanisms of YFV-induced pathogenesis are poorly understood. 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 from adult patients with confirmed YF infection during the acute phase of infection and the convalescence period. Thus, 95 patients hospitalized at the Eduardo de Menezes Hospital, Belo Horizonte, Minas Gerais state, Brazil were enrolled in this study. A total of 10 healthy subjects who received previous immunization with the 17DD yellow fever vaccine were enrolled as a control group (CT). Circulating cytokines and chemokines were quantified in the serum by multiplex assays. Our data showed that Yellow Fever patients (YFP) presented increased levels of some circulating cytokines (IL-6, IL-15 and IL-10) as well as chemokines (CXCL8, CXCL10, CCL3, CCL4 and CCL5) besides decreased levels of IL-1-beta and IL-4 as compared to the CT. Moreover, during the convalescence period, part of the YFP presented a clinical condition termed as late hepatitis characterized by higher circulating levels of IL-10, TNF-α, CXCL8, CXCL9 than patients who did not present this condition. Overall, the data suggest a differential profile of immune response during the acute phase or the convalescence period of human YF virus infection and may indicate that altered profiles of circulating inflammatory/regulatory mediators are associated with distinct clinical outcomes. Financial Support: Fundação Oswaldo Cruz (FIOCRUZ), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Palavras-chaves: Yellow fever outbreak, Late hepatitis, Cytokines, Chemokines, Clinical outcome
Resumo

Arboviruses are arthropod-borne viroses, responsible for infection increasing in human in tropical and subtropical areas. While Eastern, Venezuelan, and Western Equine Encephalitis (EEE, VEE, and WEE, respectively) viruses usually cause encephalitis in humans and horses in the Americas, Dengue virus (DENV) and Zika virus (ZIKV) are arboviruses which have recently emerged as causes of neurologic illness. It is known, also, that ZIKV and Chikungunya virus (CHIKV) are associated with Guillain-Barré Syndrome and recently in Brazil a large outbreak of Zika with neurological manifestations was reported. The neurological manifestations related to Dengue are exceptional occurrences and less described in the literature. Thus, this study is part of prospective surveillance system of arbovirosis and aimed investigating the incidence of DENV, CHIKV and ZIKV in patients with neurological symptoms with previous viral infection, to establish a correlation with clinical, virological diagnosis and morbidity and/or mortality. We selected, from arboviral disease-suspected patients with neurological symptoms, 114 samples of cerebrospinal fluid (CSF) and 10 of serum were collected during 2016 and 2017. The CSF and serum were screened by Enzyme-Linked Immuno sorbent Assay (ELISA) and/or reverse transcription polymerase chain reaction (RT-PCR) for DENV, CHIKV and ZIKV. Among all samples, 9 (7.25%; 9/124) were positive to arboviruses. DENV infection was detected in 66.7% (6/9) cases and ZIKV infection in 33.3% (3/9). CHIKV was not identified. Guillain Barre Syndrome, viral meningoencephalitis, and motor syndrome with injury predominantly in lower limb were the most frequent manifestations observed. Data, even preliminary, showed the importance of virological identification of these agents in relevant clinical conditions. The association of arboviruses with neurological disorders is evident and increasing. The literature offers many case reports but few prospective analyses based on surveillance system. In a hyperendemic context of arboviruses co-circulation, the broad clinical impact of the seviruses may just be established by constant and active surveillance system of diagnosis. The present study will bring important advances for the differential diagnosis and the establishment of etiological agents profiles associated with central nervous system disorders in Brazil.

Palavras-chaves: arboviruses, dengue, neurological manifestation, zika

DENGUE AND ZIKA VIRUS MULTIEPITOPE ANTIGEN EXPRESSION USING PLANT AND BACULOVIRUS SYSTEMS FOR SEROLOGICAL DIAGNOSIS

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Resumo

Dengue virus (DENV) and Zika virus (ZIKV) are arthropod-borne virus that belongs to the genus Flavivirus of the family Flaviviridae, that causes million of infections worldwide. Due to the emergence of ZIKV in endemics areas of DENV, clinical diagnostic can not distinguish both virus diseases due to similarity of the symptoms. Furthermore, available serological methods have cross-reactions between DENV and ZIKV due to the high similarity. Therefore, the purpose of this work is to express DENV and ZIKV multiepitope antigens using plant system via Agrobacterium tumefaciens and recombinant baculovirus to obtain more specific antigens for each virus. Multiepitope antigen genes were designed using multiple alignment and epitope mapping. Then, genes were chemically synthesized and cloned in vegetable viral vector Pepper mild mottle virus (PMMoV) and in pFastBac Amino vector (pFB-A) to further infection to leaves of Nicotiana benthamiana plants and cultured cells of Trichoplusia ni TN5B, respectively. After infection, N. benthamiana plants were maintained for 10 days in proper conditions to their growth. So, the
infected leaves with PMMoV-ZIKV multiepitope antigen were collected and macerated for the antigen extraction. The bacmid transfected TN5B cells were cultivated at 28ºC for 3 days. Next, Western blotting was performed using leaf and TNB5 cells extracts to detect antigens with anti-histag monoclonal antibody. Leaf samples infected with PMMoV_ZIKV vector showed positive signals of a 19 kDa protein, which was the expected size for the ZIKV multiepitope antigen. On the other hand, proteins of 30 kDa for DENV and 41 kDa for ZIKV were detected. The size of the detected proteins were expected according to the type of vector due to the fusion protein partner expressed with the antigen. Therefore, these DENV and ZIKV antigens will be evaluated their reactivity as antigens using patients' sera previously examined for both viruses. These approaches may provide better specificity in diagnostics, with better bio-security and cost-effectiveness.

**Palavras-chaves:** DENV, ZIKV, DIAGNOSTIC, PROTEIN EXPRESSION, MULTIEPITOPE

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**DETECTION OF YELLOW FEVER VIRUS IN NON-HUMAN PRIMATES IN ESPÍRITO SANTO STATE**

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**Resumo**

Yellow fever (YF) is a disease endemic in the Brazilian Amazon Basin, with sporadic cases reported in other regions during warm/wet seasons. The greatest YF outbreak documented in the country, in the recent decades, has been occurring since 2016, mainly in the southeast region. In Brazil, the yellow fever virus (YFV) is maintained in nature through a sylvatic cycle involving non-human primates (NHPs), and wild mosquitoes (Haemagogus sp. and Sabethes sp.) and the urban cycle is considered eradicated, since 1942. New World primates are susceptible to YFV infection, developing the disease in most cases and often leading to death. Species of Alouatta genera are the most vulnerable ones to YFV infection. We analyzed samples from NHPs carcasses from different municipalities of Espírito Santo (ES). Total RNA was extracted from liver (RNAeasy Mini Kit, Qiagen) and used for 1-Step RT-qPCR (GoTaq®Probe 1-StepRT-qPCR System-Promega) using specific primers and TaqMan® probe targeting the 5'-NC region of YFV genome. From positive samples, cDNA was synthesized and used in PCR, with primers targeting CprM/envelope. After sequencing, phylogenetic analysis was performed. From 36 liver samples analyzed, 34 (94.44%) were positive for YFV. The YFV genome was detected in 31/33 Alouatta guariba, 2/2 Callitrichus personatus, and 1/2 Callithrix geoffroyi, from seven different municipalities from ES. Phylogenetic analysis, based on partial CprM/envelope gene, was performed, indicating that YFV strains from this study, clustered within Genotype South America-I, within other YFV strains from the current outbreak in Southeast Brazil. The data showed YFV infection in NHPs occurring mainly in central and southwest mountain regions of ES. These are border areas with MG state where, since early 2017, a higher number of cases (475 confirmed cases) of YFV infection in humans have been reported. For years, ES state was considered an indene area for yellow fever and from 2016 until now, an outbreak has affected this region with an impact on human and animal health. Further phylogenetic and eco-epidemiological analysis will be performed to characterize YFV dynamics and help to understand its circulation and possible maintenance in ES.

**Financial Support:** FAPEMIG, CNPq, CAPES, UFMG and FAPES

**Palavras-chaves:** Non-human primates, PCR, Yellow Fever, Yellow Fever Virus
CHIKUNGUNYA VIRUS ISOLATION IN CELL CULTURE: COMPARISON BETWEEN VERO AND C6/36 CELL LINES FOR USE WITH CLINICAL SPECIMENS

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Resumo

Chikungunya virus (CHIKV) is an emergent arbovirus in Brazil, belonging to the genus Alphavirus, Togaviridae family. Human infection by CHIKV produce disease with symptoms that resemble the dengue infection or other arbovirus diseases, such as fever, rash and arthralgia. In endemic regions for arboviruses cocirculation, the etiological diagnostic is mandatory and the viral isolation is the gold standard method of choice. In vitro, CHIKV is able to infect a wide range of lineage cells. The aim of this study was compare the two cell lineages Vero E6 (mammalian cells) and C6/C36 (mosquito cells) commonly used in the viral diagnosis for the CHIKV isolation from clinical specimens. In this study, five human serum samples with viral RNA detected by RT-PCR, collected from febrile patients presenting rash and arthralgia were used to virus isolation procedure in cell culture. First, Vero E6 and C6/36 cells were plated 24h prior to infection and then cells were washed with PBS and 500uL of serum sample at dilution 1:20 were added, incubated for 1h at 37º or 28º respectively. After virus adsorption, the supernatant was removed, the cells were washed and the Leibovitz's L-15 media supplemented with 2% FBS was added. The cells were observed daily for cytopathic effects. For CHIKV-infected C6/36 cells the required incubation time was higher than 10 days for virus collection and storage of the viral isolate and the viral cytopathic effects became evident within 5 days post-inoculation, with the appearance of elongated cells and syncytia. In contrast, CHIKV-infected Vero E6 cells showed early evidence of infection, within 2-3 days post-infection, characterized by cellular apoptosis/lysis and required a maximum 4 days for collection and storage. The CHIKV virus isolation was confirmed by both detecting the virus cellular infection by immunofluorescence and the presence of viral genome in the supernatant by RT-PCR. In conclusion, for a timely diagnosis, Vero E6 cells seems to be more suitable than C6/36 cells, since the cell lysis caused by the infection can be observed within 3 days post inoculation. In contrast, C6/36 cells can provide a more distinctive cytopathic effects by CHIKV.

Financial support: FAPEAL; PROEST/UFAL.

Key words: cell culture, Chikungunya, viral isolation.

ANTIVIRAL ACTIVITY OF DIFFERENT NATURAL COMPOUNDS AGAINST RABIES VIRUS

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In this study, the antiviral activity of two natural compounds, were investigated on rabies virus. The first preparation examined consisted of a hydroethanolic extract from *Dalbergia variabilis* Vogel, a native plant from Brazil belonging to the family Fabaceae, popularly known as Jacarandá-branco, Cipó-pau or Canela-do-Brejo. The second was bufotenine, an alkaloid isolated from the skin of some amphibians like *Rhinella jimi*, a common species in Brazil; this product also can be found in *Anadenanthera colubrine* seeds, a plant popularly known as Angico branco. Both compounds were tested against three distinct genetic lineages of RABV previously adapted on cells culture (IP4005, IP964 and IP4871). The viruses were multiplied in murine neuroblastoma cells (Neuro-2a) and the maximum tolerated concentration (MTC) was determined in Neuro-2a cells. Titration of RABV strains were performed in Neuro-2a cells with and without the addition of the natural compounds at the MTC. Preliminary results show that the two natural compounds were able to reduce infectious virus titers, suggesting a potential antiviral effect on rabies virus strains.

Financial Support: Instituto Pasteur

Palavras-chaves: Alkaloid, antiviral, natural compounds, Rabies, vegetal extract

PREVALENCE OF ROTAVIRUS AND NOROVIRUS IN CHILDREN WITH DIARRHEA DISEASE BETWEEN THE YEARS OF 2013 AND 2015 IN ARACAJU-SE

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Resumo

Acute gastroenteritis (AG) is a serious global public health problem in pediatrics. It is prevalent in childhood, especially in developing countries such as Brazil. Diarrhea is the second cause of death in children under five, causing more than 750,000 deaths per year. About 70% of cases of diarrhea are due to infection with enteric viruses, including Rotavirus (RV) and Norovirus (NoV). The implementation of the Rotarix vaccine in Brazil since 2006 have reduced morbidity and mortality due to AG. Studies have shown that this vaccine has great applicability for most RV genotypes homotypic to G1P[8], but the effect against the heterotypic G2P[4] genotype is 41%. After the implementation of the vaccine, norovirus may have become the main etiological agents of AG and it is necessary to monitor its prevalence as well as RV prevalence since heterotypic genotypes can continue to circulate even in vaccinated children. This study aims to monitor the prevalence of rotavirus A (RVA) and norovirus infections in children with acute diarrheal disease in Aracaju-SE. Retrospective
observational study based on the detection of viral RNA in previously collected fecal samples from children between 2013 and 2015 in the pediatric section of the Urgency Hospital of Sergipe. The age ranged from neonates to children under 12 years of age, both sexes, with acute diarrhea for less than 14 days. The screening of 435 samples for RVA was performed by ELISA at the Central Laboratory of Public Health of Sergipe. Of the 435 initial specimens, 368 were analyzed for NoV by quantitative RT-PCR to diagnose NoV genogroup I and II at the Oswaldo Cruz Foundation. Prevalence of 38.9% of NoV and 17% of RVA was observed in the study population. Among NoV positive samples, the genogroup II represented 94.4% of the cases. The results shows that RVA continues to contribute significantly to cases of AG, however, NoV GII was the main virus causing AG in the children participating in this study. The findings obtained here highlight the need for constant surveillance of these enteroviruses as etiological agents of AG, as well as the investigation of circulating genotypes in the state, since they collaborate for the construction of regional epidemiological data and help control morbidity and mortality.

**Palavras-chaves:** children, gastroenteritis, norovirus, rotavirus

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**IS THERE A DIFFERENCE OF THE LIPID PARAMETERS BETWEEN HIV-POSITIVE AND HIV NEGATIVE PATIENTS IN THE CITY OF PORTO ALEGRE/RS?**

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**Resumo**

The lipid profile is not defined just by our alimentary habits, but also by environmental and genetic factors. The lack of physical exercise, a diet rich in lipids, smoking and alcoholism are risk factors for the development of dyslipidemia and atherosclerosis. It’s known that the human immunodeficiency virus (HIV) itself causes lipid changes in the carrier, increasing triglycerides’ and very low-density protein’s levels; which exacerbates with the antiretroviral therapy. The aim of this review is to compare the lipid parameters of HIV-positive patients with HIV negative, both populations from the city of Porto Alegre, RS. Plasma levels of total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoproteins cholesterol (LDL-C), and triglycerides of 203 HIV-positive patients (93 women, 110 men) were analyzed and compared with data evaluated by Fiegenbaum et al. Comparison of each parameter was analyzed by T-Studant test. Values between the sexes were evaluated by Mann-Whitney Test U. The average age of females was 38.0 ± 10.7 years and 36.80 ± 9.9 years in males, 37.3 ± 10.3 years being the average for the population. There was a significant difference between the ages of the two groups evaluated (p 0.0211). The rate of total cholesterol (p 0.0019), LDL-C (p 0.0053) and triglycerides (p 0.0007) of seropositive patients was significantly higher in relation to the non-carrier population. HDL-C’ levels showed no significant difference. Our data corroborate with those found in the literature. Although the n of both populations was different, we could observe a significant difference between lipid parameters of HIV carriers in relation to the seronegative population.

**Financial Support:** Universidade Feevale

**Palavras-chaves:** HIV, HIV-NEGATIVE, HIV-POSITIVE, LIPID PARAMETERS

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**INVESTIGATION OF YELLOW FEVER VIRUS IN BRUMADINHO, METROPOLITAN REGION OF MINAS GERAIS, DURING THE 2018 OUTBREAK**

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Resumo

The yellow fever is a disease of great importance for the public health, caused by Yellow Fever virus (YFV) (Flaviviridae, Flavivirus). The YFV is responsible for sporadic outbreaks, although there are no reports of urban transmission of YFV in Brazil, since 1942. The main vectors of YFV in the sylvatic environment are Haemagogus and Sabethes and in the urban environment, the main vector is Aedes aegypti. Since 2016, the Brazilian Southeast faces a large outbreak of YF, affecting human and non-human primates (NHP). The aim of this study is the molecular investigation of YFV in mosquitoes and NHPs samples, both collected in January/2018, in Brumadinho, a city in the metropolitan region of Belo Horizonte (Minas Gerais, Brazil). Seventeen mosquitoes were collected, seven in a rural area and 10 in a periurban area (including Aedes sp., A. albopictus, A. scapularis, Haemagogus sp., Limatus sp., Sabethes sp. and Trichoprosopon sp.). Six liver samples of NHPs were also collected, five collected in a rural area and one in an urban area (including four female and two male of species Callithrix penicillata). Total RNAs were extracted (RNAeasy Mini Kit, Qiagen®) followed by RT-qPCR (GoTaq®Probe 1-StepRT-qPCR System-Promega), with primers and probe specific to 5’-NC region. From 17 analyzed mosquitoes, one (Haemagogus sp., collected in the periurban area) was positive to YFV, as well as all NHPs tested, from urban and rural areas. Partial nucleotide sequences of YFV were generated, confirming the detection of YFV. The results showed the infection of Haemagogus sp. in periurban area and of NHP in rural but also in urban areas, demonstrating the circulation of YFV within and near urban areas, what poses a risk to human and non-human primates. Only one specimen of Haemagogus sp. was infected with YFV, but a greater number of mosquitoes from different species and from different areas of the city should be analyzed to monitor the risk of YFV transmission in the urban area.

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Financial support: CAPES, CNPq, UFMG, FAPEMIG.

Palavras-chaves: Yellow Fever virus, Non-human primates, vectors.

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Resumo

A widespread epidemic of Zika virus (ZIKV) infection was reported in 2015 and its association with fetal abnormalities,
including microcephaly, represented a global health emergency. Both animal models and in vitro studies are being used to uncover the pathogenesis of this emerging disease and to develop vaccine and therapeutic strategies. Our group has shown that chicken embryos are susceptible to infection and revealed that virus replicates in skeletal muscle tissue. Aiming to characterize the infected cells, skeletal muscle cells from embryos on the eleventh day of development were harvested from legs and breasts, plated on coverslip coated plates and infected with the Brazilian strain, RIO U1 ZIKV in the 0.1, 0.01 and 0.001 MOI. After 72 hours, cell cultures were analysed by immunofluorescence using anti-Flavivirus and anti-Myosin antibodies. Confocal microscopy revealed virus positivity on muscular cells, myocytes and myoblasts and, in some wells, we observed reactivity to viral proteins in fibroblasts. Infection was more evident on 0.1 MOI samples, although in 0.01 MOI wells the pattern repeated itself in a smaller number of cells. Our in vitro results show that fibroblasts and skeletal muscular cells are susceptible to ZIKV infection, mimicking the in vivo infection. This data contribute for understanding of virus infection mechanisms and the possibility of viral attenuation, through serial passages in this culture system.

Financial Support: FAPERJ/CNPq/FIOCRUZ

Palavras-chaves: ZIKA, ZIKV, Chicken Embryos, Muscle Cell, Cell Culture

THE YELLOW FEVER COLLECTION: AN IMPORTANT HERITAGE FOR VIROLOGIC AND SCIENTIFIC PURPOSES

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Resumo

The Yellow Fever Collection of the Oswaldo Cruz Institute (CFA), Fiocruz, presents 498 thousand cases (liver samples collected by viscerotomy between 1930 and 1970). Each case has a piece preserved in formaldehyde, one or more stained histological sections and a paraffin block, being of great importance for the diagnosis of several diseases including Yellow Fever. The absence of techniques that allow precision for a given diagnosis has led to many cases being given with indefinite etiology. However, with the technological advances and the expansion of the use of immunohistological and molecular techniques, it is possible to confirm positive cases and to clarify the doubtful ones, together with the control of material from experimental infection. The immunohistological labelling is done through the indirect immunofluorescence technique, evaluating histopathological characteristics from protocols already standardized by the Laboratory of Pathology of the Oswaldo Cruz Institute. At the same time, the molecular techniques aimed at investigating and confirming the diagnosis of CFA documented cases using nucleic acid extraction kits and the accumulated knowledge in the literature about the biomolecular characteristics of Flavivirus. Regarding the conservation of nucleic acids and antigens during processing, the methodology tested seems to be promising since, we have been able to efficiently perform immunostaining techniques without requiring previous antigenic recovery. The application of the immunostaining technique was successful, we were able to detect viral antigens in histological sections of the freshly cut CFA from the paraffin blocks. Thus, it is expected that the results contribute to the knowledge of histopathological peculiarities of yellow fever, in addition to developing and standardizing methodology more effective for the conservation of nucleic acids which allows subsequent extraction and purification of RNAs amplifiable from FFPE materials without altering the morphological preservation. In addition, we are also doing the digitalization of slides from the CFA cases and we are gradually making these images available online. Thus, it will be possible to consult online anywhere for a second diagnostic opinion, for example, without the need for physical transport of material or the specialist, adding information to the collection as well as preserving it from dispersion, damage or loss secondary to transport or handling.
OUTBREAKS' PREVALENCE OF Aedes aegypti IN NOVO HAMBURGO CITY/RS FROM 2014 TO 2017

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Resumo

Introduction: Nowadays, there has been a significant increase in the number of epidemiological outbreaks of the Aedes aegypti mosquito since reported cases, by the Sanitary Surveillance, of diseases such as Dengue fever, Chikungunya fever and Zika virus have increased. The dengue virus is an arbovirus belonging to the Flaviviridae family since it has a single-envelope and a single-stranded RNA which has four serotypes: DEN-1, DEN-2, DEN-3 and DEN-4. The virus can be transmitted by two mosquito’s species, Aedes aegypti and Aedes albopictus, but in Brazil there are only reports of transmission by the first one. Its transmission occurs through the female’s bite which acquires the virus by feeding on blood of an infected person. Symptoms, in most cases, are high fever, severe headaches, pain behind the eyes, loss of appetite and red spots throughout the body. Until now, there is no specific treatment for this virus.

Objective: To report the main outbreaks of A. aegypti in Novo Hamburgo city (NH), comparing data obtained between the years 2014 and 2017 in relation to foci and infestation index.

Methodology: The data obtained come from activities developed by an agreement of the “Projeto de Prevenção e Combate à Dengue” of the Feevale University with the NH City Hall, using reports from 2014 to 2017.

Results: In NH city, 222 epidemiological outbreaks were found in 2014, 448 in 2015, 1735 in 2016 and, 5389 in 2017. During this period there was an average prevalence of 76,75% in households, 12,5% in companies and schools, 7,25% in special vector research and 1% in both trade and vacant lands. Infestation rate was low (< 1) in 2014, average (1,8) in 2015 and high (5 and 4,6, respectively) in 2016 and 2017; where low indices vary from 0,1 to 0,9, average from 1 to 3,9 and high, > 4. Conclusion: After analyzes, it is noticeable that between 2014 and 2017 there was a 1217% increase in the epidemiological outbreaks of the vector. Between the years 2015 and 2016 there was an increase of approximately 288% of vector outbreaks, which can be related to the Zika virus epidemic state’s decree by the Brazilian Ministry of Health in November of 2015. Hence it can be concluded that outbreak foci increased significantly, indicating that the city’s population is not practicing the information passed on by dengue’s combat and prevention agents.

Financial Support: None.

Palavras-chaves: Dengue, Novo Hamburgo, Epidemiology, Outbreaks, Vector

SEVERE CASES OF YELLOW FEVER OUTBREAK ARE RELATED TO SYLVATIC TRANSMISSION AND NOT WITH VACCINATION ADVERSE EFFECTS

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Resumo

Yellow fever (YF) is a viral disease caused by the prototype member of the genus Flavivirus, the Yellow Fever Virus (YFV), an enveloped virus containing a positive-single-strand RNA. Yellow fever is
endemic in tropical regions of Africa and South America, where is maintained in nature by transmission between non-human primates and blood-feeding mosquitoes. Humans are infected sporadically when bitten by sylvatic mosquitoes that previously fed on a viremic monkey (sylvatic cycle), but may also serve as the viremic host for inter-human transmission (urban cycle). The YFV that spreaded from Africa to the Americas generated two genotypes from the Western African ancestor (South America I and II). South American genotype I is the most disseminated and frequently detected during epizootic and epidemic waves in Brazil. At the end of 2016, a large outbreak of YF started in Southeast Brazil. Until July 2017, 777 YF human cases, and 261 deaths were registered. In 2018 the epidemic continued, where 1,127 human cases and 331 deaths have been confirmed. YF vaccination is strongly recommended using the live attenuated 17DD strain. Adverse events (approximately 1 in 250,000 vaccinees) may occur following vaccination, such as yellow fever vaccine associated viscerotropic disease or neurotropic adverse events. Here we evaluate the genetic diversity and dispersion of YFV last outbreak in Brazil, and the virus population associated with severe cases and vaccine adverse event suspect cases. Whole YFV genome of 12 humans and 14 non-humans primate from Rio de Janeiro, Minas Gerais and Espirito Santo were sequenced using short amplicons method covering the viral genome. The amplicons generated (~500pb) were sequenced by two different NGS platforms, Ion Torrent and MiSeq. All sequences generated clustered with genotype I. None of the human severe cases showed polymorphisms associated with vaccine attenuation, suggesting no cases of adverse effects. The phylogenetic analysis supports the sylvatic cycle of YFV transmission with sequences of humans and NHP from the same geographical region grouping at the same branch. No humans to humans transmissions were observed. Our results reinforced the safety and efficiency of YFV vaccination to prevent new outbreaks. Financial Support: CNPQ and FAPERJ.

Palavras-chaves: Yellow Fever, NGS, vaccine, phylogenetic, Flavivirus

SEROLOGICAL PROFILE POST VACCINATION AGAINST YELLOW FEVER IN LABORATORY WORKERS

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Resumo

Yellow fever (YF) is an acute febrile viral disease caused by the Yellow fever virus (YFV) which belongs to the genus Flavivirus, family Flaviviridae. The natural reservoirs of the virus are non-human primates which live in forests and tropical areas. The YFV is transmitted by the bite of infected mosquitos of the Aedes, Haemagogus and Sabethes genus. The disease is endemic in Africa and Latin America, with the major registered outbreak occurring in southwest Brazil between 2016-2018. The symptoms of the disease are easily mistaken by other arboviruses infections of clinical relevance, like dengue and zika, with the diagnosis being made by association of the clinical-epidemiological data and laboratory testing. The disease doesn’t have treatment and the most effective form of prevention to date is vaccination, with the current vaccinal scheme being 1 dose for a lifetime of protection. The current study aims to evaluate the serological profile post-vaccination
against YFV in laboratory workers. For this, 53 samples were collected from individuals that work in virology labs in Belo Horizonte. The samples were submitted to the plaque reduction neutralization test to verify the presence of neutralizing antibodies against YFV. Tests to verify the presence of antibodies against other flaviviruses like dengue were also performed. The results showed the detection of YFV neutralizing antibodies in 97% of the participants. Association between the titer of neutralizing antibodies and the collected data was not observed. Those results reinforce the literature in the face of the efficacy of the current YFV vaccine.

Financial Support: CAPES, CNPq and FAPEMIG.

**Palavras-chaves:** Yellow Fever, Flavivirus, PRNT, Neutralizing antibodies, yellow fever vaccine

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**IMPACT OF EPSTEIN-BARR VIRUS INFECTION ON TCD4+/CD8+ LYMPHOCYTES AND THE PLASOMATIC VIRAL LOAD OF PATIENTS INFECTED BY HIV-1 ANTIRETROVIRAL- THERAPY-NAIVE**

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**Resumo**

**Introduction:** HIV infection can lead to the depletion of the components of the immune system, especially CD4⁺ T lymphocytes, allowing the development of immunodeficiency. EBV infects of latent form more than 90% of the population and can reactivate, allowing a process of acute or chronic inflammation mainly in immunosuppressed. This virus is known to be immunomodulatory, and may influence the pathaway of the immune response in co-infection. **Objective:** Evaluate the impact of EBV infection on HIV viral load and CD4⁺ and CD8⁺ cell counts. **Material and methods:** 10 mL of whole blood were collected from 100 HIV infected patients older than 18 years and antiretroviral-therapy-naive, attended at the Center for Attention in Acquired Infectious Diseases (CASA DIA) from January to March, 2018. The presence of IgM against EBV was evaluated by ELISA (Kit-RIDESCREEN) definig the groups G1 (HIV⁺/EBV⁺) and G2 (HIV⁺/EBV⁻). TCD8⁺ and TCD4⁺ lymphocyte counts were determined by flow cytometry (FACSCount REAGENTS BD Biosciences) and the viral load of HIV was quantified by qPCR (Kit mSample Preparation System-ABBOTT). The analyzes were done by descriptive statistics and Mann-Witney test using BioEstat.v.5.3 (α = 95; p<0.05). **Results:** The participants had an age range of 18-64 years (mean = 29.4, median = 26 and SD = 9.5) and 88% were male. Regarding sexual orientation, they declared to be homosexual 52%, heterosexual 35% and bisexual 13%. Of the samples, 33 were positive for EBV. The co-infected group (G1) had higher HIV viral loads than G2, showing an association with EBV reactivation (mean G1 = 424,631 copies/mL, mean G2 = 163,206 copies/mL), (p = 0, 0017). EBV reactivation was also related to the highest number of CD8⁺ T lymphocytes (mean G1 = 1541 cells/mL; Mean G2 = 1110 cells/mL), (p = 0.0306). EBV reactivation can cause the stimulation of more CD8⁺ T lymphocytes that can be stimulated by HLA class I molecules for lysis of these viral agents. There was no statistically significant difference in TCD4⁺ lymphocyte counts between the groups (mean G1 = 334 cells/mL, mean G2 = 387cells/mL) (p = 0.5001). **Conclusion:** The highest HIV viral load in the G1 group may be related to depletion of the immune system due to co-infection, which leads to increased HIV replication. The CD8⁺ T lymphocyte count was related to the expected immune response mechanism. Thus, reactivation of EBV modifies the immune scenario and progression of HIV infection.

**Financial Support:** IEC/SVS/MS.CAPES.
Chikungunya virus (CHIKV) is an enveloped, positive sense, single stranded RNA arbovirus. This arthritogenic alphavirus causes the chikungunya fever (CHIKF) characterized by symptoms as high fever, rash and moderate to severe polyarthralgia. This disease was first reported in 1952 in Tanzania, East Africa. In the year 2014, the first Brazilian CHIKV cases were reported in the states of Amapá and Bahia. Previous official records attested that first cases of CHIKF in the Alagoas state begun in October 2015, however, we provided here a single serological evidence of early community transmission of this arbovirus since June 2014 in this state. In this study, we obtained serum samples from patients attended in a reference public hospital for molecular diagnostic of arboviruses during a Dengue virus outbreak in Maceio city, from January to September 2014. Then, we analyzed the presence of IgM and IgG anti-CHIKV by ELISA (Euroimmun®) in the samples obtained after 7 days post-illness onset (n=16). Interestingly, one sample (6.25%) showed positivity for anti-CHIKV IgG (67.7 relative units / mL) and borderline levels for IgM. This sample was collected from a patient 8 days post-illness onset, in June 2014 which had the confirmed acute dengue fever by detection of anti-DENV IgM. Nevertheless, this positivity of anti-CHIKV IgG was an unexpected and surprising finding suggesting that CHIKV could be circulating in the Alagoas state since June 2014, and not only in 2015. The CHIKV outbreak detected in Alagoas in 2016 belongs to the East-Central South African (ECSA) genotype, whose first detection in Brazil was reported in Feira de Santana in June 2014. From this, it was estimated by specialists a prediction of very high risk of CHIKV spread and establishment in other Brazilian states starting in November/December 2014 for the northeast coast (such as Alagoas). However, our results suggests that CHIKV could be circulating in the Alagoas state earlier than expected. Importantly, we cannot discard a possibility of cross-reactivity occurrence with antibodies for other alphaviruses, such as Mayaro. However, this virus was not yet been reported in Alagoas state. Therefore, serum neutralization tests will be necessary to further demonstrate the specificity of the IgG detected in this preliminary study to confirming the early transmission of CHIKV in Alagoas state.

Financial support: FAPEAL.

Palavras-chaves: Alagoas state, Chikungunya, IgG
FREQUENCY OF RESPIRATORY VIRUS IN THE CITY OF NOVO HAMBURGO (RS-BRAZIL)

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**Resumo**

**Introduction**: Acute respiratory infections are frequent, accounting for high rates of medical visits, hospitalizations, and deaths. Influenza affects approximately 5 -15% of the population annually, severely affecting from 3 to 5 million people worldwide. It is estimated that 650,000 deaths per year occur, especially with children under 5 years old and elderly over 75 years. **Objective**: To evaluate the frequency of respiratory virus identified by molecular biology in samples from patients seen at a laboratory in the city of Novo Hamburgo (RS-Brazil). **Material and methods**: We performed a retrospective cross-sectional study by evaluating the results of simultaneous detection of multiple respiratory viruses using Polymerase Chain Reaction technique (Multiplex-PCR). The data refer to the detection of Respiratory Syncytial Virus (RSV), Mastadenovirus (AdV), Influenza A (INFA), Influenza B (INFB), Parainfluenza Virus 1 (PIV 1), Parainfluenza virus 2 (PIV 2), Parainfluenza 3 (PIV 3) Bocavirus (BoV), Rhinovirus (RNV) and Metapneumovirus (MPV) in nasopharyngeal aspirate samples processed between June 2017 and July 2018. **Results**: A total of 1380 Multiplex PCR were performed to detect the respiratory virus mentioned previously. Of these, 35.5% (476/1380) of the samples were positive for at least one of the screened virus. RSV was detected in 54% (257/476) of the samples. Other viruses were found in different frequencies: PIV 3 12.4% (59/476), INFA 8.6% (41/476), PIV 1 5.9% (28/476), PIV 2 5% (24/476). Each of the remaining respiratory viruses (AdV, INFB, BoV, RNV, and MPV) was detected in less than 10 samples. In summary, there were 47 positive results. Some samples had coinfection with more than one virus. **Conclusion**: More than one-third of the samples were positive for some respiratory virus. RSV was the most prevalent virus. Similar to RSV, PIV 3 can induce pneumonia and bronchiolitis in infants, although they are often less severe infections than RSV. The INFA, which was detected in less than 10% of the samples, was not identified in the subtype level; therefore it is impossible to know the occurrence of relevant agents such as H3N2 and H1N1. PCR-Multiplex contributes to the reduction of unnecessary prescription of antibiotics, besides preventing the transmission of respiratory virus. Thus, the use of this technique in clinical diagnosis is not only beneficial for the patients but also for the public health in general.

**Palavras-chaves**: Molecular biology, Multiplex-PCR, Nasopharyngeal aspirate, Respiratory Syncytial Virus, Respiratory viruses

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METAGENOMIC STUDY FOR MONITORING VIRUSES IN BAT SAMPLES FROM STATE PROGRAM OF RABIES CONTROL

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Resumo

Bats are natural reservoirs for a wide variety of viruses of public health concern, including important zoonotic viruses. Epidemiological data revealed that bats are responsible for most cases of human rabies in Brazil in the last two decades. This change in the rabies epidemiological profile, which was previously predominantly transmitted by dogs, has stimulated surveillance of rabies virus in bat samples. In the present study, bat samples from the species Brazilian free-tailed bat were collected in urban areas of Rio Grande do Sul, under the State Program of Rabies Control. Such samples were analyzed by a metagenomic approach aiming at the identification of other viruses, providing a better understanding of the viral diversity of this species. Samples were collected as two pools of feces and of urine at bat colonies previously mapped at municipalities of São Gabriel and Tavares. Next, DNA was extracted and enriched with Phi29 DNA polymerase. The quality was checked in an agarose gel and quantified with QUBIT. Samples were sequenced on an Illumina MiSeq platform. The de novo assembled contigs were obtained with SPADES program and overhanging pairs of mapped reads for each contig edge were assembled using PRICE. Next, the obtained contigs were submitted to Blast against NCBI database to compare with reference genomes. The best-matched sequence was retrieved to perform the phylogenetic analysis. Nucleotide and protein editing was performed in a conservatively way and the sequences were aligned using the MUSCLE alignment tool. For each analysis, the best-fitted model was selected and the trees were inferred using the Bayesian method. The highest viral diversity was obtained from urine samples. Sequences related to 4 viral families that infect a wide variety of hosts have been found: Anelloviridae, Circoviridae, Papillomaviridae and Poxviridae. The analysis of the sequences with identity to the L1 gene of the papillomavirus suggest that a new species of the genus Nupapillomavirus has been identified. In addition, a papillomavirus not yet classified, described at insectivorous bats from French Guiana, was found. These findings add to the knowledge about the viruses carried by these animals and demonstrate that the use of metagenomics as a surveillance tool expand the range of viruses that can be of public health concern investigated in these samples.

Palavras-chaves: Bats, Metagenomics, Rabies, Surveillance

NOROVIRUS CIRCULATING IN GASTROENTERITIS CASES DURING 2015 AND 2016 IN ASUNCION, PARAGUAY: IDENTIFICATION OF EMERGING GENOTYPES

Autores

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Resumo

Norovirus (NoV), one of the main causes of viral Acute Gastroenteritis (AGE) in all ages, is considered the major causing agent in children < 5 years old, after the decrease of rotavirus (RVA) due to vaccination. NoV is classified into 6 genogroups (GI to GVI) and 38 genotypes. Through genetic drift and recombination, genetic variants emerge, modulating human infections. In a first study about NoV diversity in pediatric population from Asuncion, we reported the co-circulation of 10 different genotypes (GI.3 and GI.8; GII.3 to GII.8, GII.16 and GII.17) in AGE cases negative for bacteria and RVA. Here we present the frequency and genetic diversity of NoV from non-bacterial AGE children ≤5 years that attended to the Hospital General de Barrio Obrero, in Asunción - Paraguay, from October 2015 to October 2016. By using RT-PCR, NoV was detected in 26, 5% (56/211) of samples, 17% out of them were positive for GII, 7,5% for GI and in 1,3% GI/GII co-infections were detected. Partial gene sequencing was performed to the viral polymerase and the major capsid protein, VP1. Three genotypes were identified: the recombinants GII.P7-GII.6 and GII.P16-GII.4-Sydney - which reported as emergent
worldwide - prevailed in 50% and 33% of the NoV samples, respectively; and in a minor proportion (16%) GII.P7-GII.7 was detected. The continuous molecular monitoring of NoV will allow detecting emerging strains, understand its evolving dynamic, contribute to vaccine development and, diminish the disease burden. This is important because AGE is still one of the main causes of childhood mortality, especially in developing countries such as Paraguay.

Financial support: CONACYT, Prociencia, IICS-UNA.

Palavras-chaves: norovirus, gastroenteritis, emerging genotypes

**PROPILIS AS A SOURCE OF ANTIVIRAL COMPOUNDS: ACTION OF HYDROMETHANOLIC EXTRACT OF GEOPROPILIS FROM SCAPTOTRIGONA POSTICA AGAINST RUBELLA VIRUS REPLICATION**

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Resumo

There are many researches on the chemical composition and biological activities of propolis from Apis mellifera (Apidae specie). The antiviral activity of propolis from different geographic regions against different virus species, in different steps of viral replication were demonstrated by many authors. Propolis exhibited antiviral activity against different types of 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. The present study evaluated the effect of the hydromethanolic extract of geopropolis from Scaptotrigona postica on Rubella virus infected Statens Serum Institut Rabbit Cornea (SIRC) cells. The results on cell viability and cell proliferation assays indicated that geopropolis was non-toxic to cultured SIRC cells. In order to evaluate if this geopropolis would be able to inhibit the infection caused by Rubella virus particles were carried out the following experiments, viral binding, penetration assay, antiviral assay, real-time PCR, and transmission electron microscopy. The chemical profiles of hydromethanolic extract of geopropolis from S. postica exhibited the presence of flavones-C-glycosides, as the main constituents, together with pyrrolizidine alkaloids and catechin derivatives as reported previously. This geopropolis exhibited antiviral activity against herpes type I virus. The inhibition of Rubella replication observed in this study can be corroborated by our previous study and studies carried out by others authors. As far as we known, this is the first report about the antiviral activity of geopropolis from Scaptotrigona postica against a Togaviridae virus.

Palavras-chaves: antiviral activity, geopropolis, rubella virus, Scaptotrigona postica,

**EVALUATION OF MAYARO VIRUS ENVELOPE PROTEIN 2 FOR SEROLOGIC DIAGNOSIS OF MAYARO VIRUS INFECTION**

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Resumo

Mayaro virus (MAYV) is a neglected arthropod-borne virus (arbovirus) maintained in an unclear zoonotic cycle involving mosquitoes from Haemagogus genus as the main vector of transmission. It is classified into Alphavirus genus (Togaviridae family), antigenically clustered into the Semliki Forest complex group. Its genome is constituted of a positive single-stranded RNA of 11.5kb in length, which contains two genes that encode four non-structural (nsP1 - 4) and five structural (C, E3, E2, 6K, and E1) proteins. MAYV is responsible to cause the Mayaro fever disease in humans, which includes symptoms as fever, exanthema rash, myalgia, retro-orbital pain, headache, diarrhea and a severe arthralgia that can be recurrent and persist for months or even years. In the present study, we have developed an ELISA using as antigen the recombinant envelope protein 2 (E2) of MAYV (rE2-MAYV) produced in E. coli system. A panel of 68 human serum samples from arboviral suspected cases were analyzed for IgG and IgM antibodies detection and titration using rE2-MAYV ELISAs. Positivities of 33.8% (23/68) for IgG and 45.6% (31/68) for IgM determined 100% sensibility and 78.95% specificity when compared to the results of a MAYV-specific PRNT50. Additionally, the positive MAYV neutralizing samples demonstrated high titers of detection by rE2-MAYV ELISA, suggesting a highly sensitive test. Low levels of cross-reactivity were observed to CHIKV-specific human and murine antibody samples, but not to other Alphavirus antibodies. In short, we have developed a rapid, simple, specific and sensitive IgG and IgM MAYV rE2-ELISAs and our preliminary results show their potential applicabilities on diagnosis of infections by this virus.

Financial Support: Fundação de Amparo à pesquisa do Estado de São Paulo (FAPESP)

Palavras-chaves: Mayaro, ELISA, Diagnosis, Envelope protein 2

SERO-PREVALENCE TO ARBOVIRUSES IN A REGION WITH LOW OCCURRENCE OF MOSQUITO-BORNE DISEASES

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Resumo

There is no information on the current seroprevalence for the rates of seropositive individuals to Dengue virus (DENV), Zika (ZIKV) and Chikungunya (CHIKV), among other arboviruses in regions of low occurrence of mosquito-borne diseases, such as Rio Grande do Sul. It is now important to have a better picture of serological status for these viruses in a given region, specially regarding the indication of vaccination or not against DENV, which in turn presents a risk of predisposing prior seronegative individuals to develop a more severe disease. On the other hand, this may also allow infer about the circulation of these viruses in subclinical infections. The aim of this work is to evaluate the seroprevalence of individuals living in a region with low incidence of arboviral diseases (Vale do Rio dos Sinos, RS), despite high levels of Aedes aegypti infestation. In total, 160 human serum samples from healthy subjects were tested by enzyme-linked immunosorbent assay (ELISA) for detection of specific IgG against DENV, ZIKV and CHIKV and read on SpectraMax® M Series spectrophotometer. The samples were transferred from the inventory of routine exams of both Clinical Analysis Laboratory Exame and Feevale University's Integrated Center of Health Specialties. Initially 12 samples (7%) were anti-DENV positive IgG, 1 sample demonstrated IgG levels within the borderline range, as well as 5 samples tested for CHIKV. All samples were negative for anti-ZIKAV IgG. There was no relationship between DENV IgG levels with gender or age. The results indicate low seroprevalence for DENV despite high rates of Aedes aegypti vector infestation in the region. Yet it is in
agreement with the comparatively small number of Dengue cases in the region. The absence of antibodies against ZIKV and possibly reduced to CHIKV in the studied population points to a still reduced circulation of these arboviruses in the state. Nevertheless, the study continues with the addition of more sera to the sampling, contributing both to assessing the risk of epidemics and to improving public policies enrolled to prevent these diseases.

Financial Support: FAPERGS, CNPq, CAPES, MCTIC

Palavras-chaves: ARBOVIRUSES, CHIKUNGUNYA, DENGUE, SEROPREVALENCE, ZIKA

DURATION OF HUMORAL AND CELLULAR IMMUNITY INDUCED BY LOWER DOSES OF YELLOW FEVER VACCINE (17DD-YF) IN VOLUNTEERS EIGHT YEARS AFTER A DOSE-RESPONSE STUDY

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Resumo

Yellow Fever is an endemic disease in tropical and subtropical regions of South America and Africa. Annually, there are approximately 80,000–200,000 cases worldwide. The live attenuated 17DD Yellow Fever vaccine is the mainstream countermeasure for controlling disease expansion. However, a combination of problems – vaccine stockpile depletion, limited production capacity, plus an enhance on confirmed cases of yellow fever and consequently requirement to increase vaccine doses production – induced Bio-Manguinhos to conduct a dose-response study with the Yellow Fever vaccine (17DD-YF) in 2009. Was administrated the vaccine in the usual dose of 27,476 IU (full dose, reference) and in five alternative formulations with decreasing numbers of viral particles (10,447 IU, 3013 IU, 587 IU, 158 IU, and 31 IU) by the subcutaneous route and usual volume (0.5 mL). Therefore, the ongoing research aims to determine whether seropositivity for Yellow Fever and memory effector CD8+ T-cells was maintained eight years after YF vaccination of subdoses in non-revaccinated participants in the referred study. Seroneutralization serology will be performed for yellow fever of all participants, with methodology validated by the Viral Technology Laboratory of Bio-Manguinhos/Fiocruz (LATEV/FIOCRUZ) and for phenotypic-functional analysis, peripheral blood mononuclear cells (PBMCs) will be obtained and cultured in presence and absence of the 17DD vaccine antigen. Our results demonstrated that all subdoses maintained CD8+ T-cells effector memory levels and PRNT titres above the levels of non-vaccinated individuals, after eight years. Nevertheless, PRNT titres of doses higher than 587 IU are corresponding to those found in the reference group 5–9 years after primary vaccination. In addition, CD8+ effector memory showed the same pattern when compared to the 1-9 years levels after primary vaccination of the reference group. These findings are crucial for the recommendation to use 17DD-YF vaccines in subdoses (at least 587 IU) in adults’ vaccination campaigns, mainly in outbreaks – a topic of high interest to the Word Health Organization (WHO) and Brazilian National Immunization Program. This study received financial support of Wellcome Trust and Bio-Manguinhos/FIOCRUZ.

Palavras-chaves: Yellow Fever, Vaccination, lower doses, PRNT, Cell Immunology
17DD-YF RE-VACCINATION HEIGHTEN THE LONG-TERM MEMORY COMPONENTS IN POPULATIONS OF ENDEMIC AREAS

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Resumo

The recent outbreaks of yellow fever in Africa and Brazil required extensive vaccination campaigns that impacted the international YF vaccine stockpile and brought to light the discussion about the need of booster doses to guarantee the long-term memory in populations living in endemic countries. This study aimed to evaluate the impact of 17DD-YF re-vaccination regimens on the long-term persistence of correlates of protection in a Brazilian cohort. We have evaluated the duration of neutralizing antibodies and the status of 17DD-specific T and B-cell memory following primary, secondary and multiple vaccination regimens. The results demonstrated that primary vaccination is accompanied by a progressive decline of PRNT-seropositivity, decreased levels of effector memory CD4+ and CD8+ T-cells (EMCD4, EMCD8) and IFN+CD8+ T-cells (IFNCD8) after 10 years. The secondary vaccination was able to promptly restore the PRNT-positivity to 100% as well as the levels of EMCD4, EMCD8 and IFNCD8. PRNT-seronegativity at baseline prior to revaccination led to higher response to 17DD-YF booster dose. Moreover, both secondary and multiple vaccination regimens guarantee the long-term persistence of higher PRNT-positivity (89% and 100%, respectively) and cell-mediated memory correlates (100%) even after 10-years following booster vaccination. Together, these findings underscored the relevance of booster doses to heighten the 17DD-YF specific immune response to guarantee the long-term persistence of memory components. Secondary or multiple vaccinations improved the correlates of protection triggered by 17DD-YF primary vaccination, suggesting that booster regimens may be needed to achieve efficient immunity in areas with high risk of yellow fever transmission. Financial Support: FAPEMIG, Bio-Manguinhos/FIOCRUZ, PROEP/IRR/FIOCRUZ, CNPq, PNI and Secretaria de Vigilância em Saúde (SVS) – Ministério da Saúde, Brazil.

Palavras-chaves: Yellow Fever, 17DD vaccine, neutralizing antibodies, memory CD8+ T-cells

EFFECTS OF THE DIFFERENT CONCENTRATIONS OF E2 GENE OF HUMAN PAPILLOMAVIRUS 31

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Resumo

The recent outbreaks of yellow fever in Africa and Brazil required extensive vaccination campaigns that impacted the international YF vaccine stockpile and brought to light the discussion about the need of booster doses to guarantee the long-term memory in populations living in endemic countries. This study aimed to evaluate the impact of 17DD-YF re-vaccination regimens on the long-term persistence of correlates of protection in a Brazilian cohort. We have evaluated the duration of neutralizing antibodies and the status of 17DD-specific T and B-cell memory following primary, secondary and multiple vaccination regimens. The results demonstrated that primary vaccination is accompanied by a progressive decline of PRNT-seropositivity, decreased levels of effector memory CD4+ and CD8+ T-cells (EMCD4, EMCD8) and IFN+CD8+ T-cells (IFNCD8) after 10 years. The secondary vaccination was able to promptly restore the PRNT-positivity to 100% as well as the levels of EMCD4, EMCD8 and IFNCD8. PRNT-seronegativity at baseline prior to revaccination led to higher response to 17DD-YF booster dose. Moreover, both secondary and multiple vaccination regimens guarantee the long-term persistence of higher PRNT-positivity (89% and 100%, respectively) and cell-mediated memory correlates (100%) even after 10-years following booster vaccination. Together, these findings underscored the relevance of booster doses to heighten the 17DD-YF specific immune response to guarantee the long-term persistence of memory components. Secondary or multiple vaccinations improved the correlates of protection triggered by 17DD-YF primary vaccination, suggesting that booster regimens may be needed to achieve efficient immunity in areas with high risk of yellow fever transmission. Financial Support: FAPEMIG, Bio-Manguinhos/FIOCRUZ, PROEP/IRR/FIOCRUZ, CNPq, PNI and Secretaria de Vigilância em Saúde (SVS) – Ministério da Saúde, Brazil.

Palavras-chaves: Yellow Fever, 17DD vaccine, neutralizing antibodies, memory CD8+ T-cells
EFFECTS OF THE DIFFERENT CONCENTRATIONS OF E2 GENE OF HUMAN PAPILLOMAVIRUS 31


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Introduction: Cervical cancer represents the fourth type of neoplasia more frequent among women around the world. Although on cervical lesions and tumors, HPV-16 is more frequent worldwide, HPV-31 is found in different countries, being also an important agent for the carcinogenic process. Persistent infections caused by Human Papillomavirus with high oncogenic risk (HR HPVs) is a necessary condition, however it is not enough for the development of cervical cancer. Environmental and genetic factors are involved on the cervical carcinogenesis. Therefore it is important to evaluate the E2 gene of HPV-31, due to the fact that the E2 protein, when bound to LCR (Long Control Region) sites can act as a repressor or a transcriptional activator, aiding with the increase or decrease of viral oncogenic expression. Methodology: A sample of E2 gene HPV-31 and a sample containing LCR of HPV-31, both with no alterations, were used. The samples were cloned and then subcloned in an expression vector of mammal cell. The recombined were then sequenced in order to obtain confirmation. HeLa cell groups were co-transfected in the presence of expression vector containing the LCR of HPV-31, the E2 gene in different concentrations (10ng, 25ng, 100ng and 300ng) and the Renilla plasmid. After forty eight hours the cells were lysed and the cell extracts were prepared for the reading of luciferase of NanoLuc and renilla. Results: In low concentrations (25ng) the E2 transcriptional regulator activated the expression of Nluc reporter gene (its expression is conditioned by LCR) and in high concentrations (300ng) the expression of this gene was decreased. Conclusion: E2 gene concentration can influence the viral carcinogenesis potential.

Financial Support: Fundação de Amparo à Ciência e a Tecnologia (FACEPE) e o Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Palavras-chaves: CARCINOGENESIS, E2 GENE, HUMAN PAPILLOMAVIRUS 31

RAPID ANTIGEN DETECTION TEST FOR INFLUENZA VIRUS DIAGNOSIS AS A DIAGNOSTIC TOOL.

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Resumo

Viruses are the predominant cause of acute respiratory illness worldwide and are responsible for substantial morbidity and mortality in children between 1 and 5 years of age. Seasonal influenza is a major contributor to respiratory infections in children. The disease causes high morbidity among children, and annual vaccination is the most effective infection prevention strategy. In particular, children under 6 years of age are reportedly more vulnerable to influenza infection than children from other age groups, and the infected children pose a significant burden to their families. Influenza virus also causes severe illness, including pneumonia and death in transplant recipients, as well as nosocomial infections and outbreaks. In Brazil, rapid antigen detection tests for Influenza virus are not routinely used as a diagnostic tool, although there are tests licensed by ANVISA. Our objective was to evaluate the FLU Test Kit (QUIDEL Corp, CA, USA) as a screening tool for Influenza virus in children with acute respiratory disease in comparison with the indirect immunofluorescence assay (IFA) as gold standard. In this work, 75 nasopharyngeal aspirates samples from pediatric patients with IFA results were used: 26 positive for Influenza, 14 positive for other respiratory viruses, 3 co-infections (Influenza and other respiratory virus) and 32 non-reactive samples. All of these samples were then tested by QuickVue Influenza A + B Biomerieux® rapid test, and we obtained 8 samples with divergent results between the two assays; 7 negative for the rapid test but positive by IFA and 1 positive sample for the rapid test but positive for another respiratory virus by IFA. Real-time PCR was performed for the 8 divergent samples: 7 positive for Influenza and 1 negative (for both real-time PCR and rapid test). These results show that the rapid test has a sensitivity of 75% (63.5 - 86.5) and a specificity of 98% (60.4 - 100). Therefore, the rapid test shows a good sensitivity and a high specificity.

Finantial support: Funding was granted by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

Palavras-chaves: Diagnostic, Influenza Virus, Test Rapid, Specificity

Genomic diversity changes of Influenza A (H3N2) strains circulating in Brazil during 2017-2018 Seasons: What to expect in the coming winter?

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Resumo

Influenza A(H3N2) virus was dominant during the 2017 influenza epidemic season and it caused a regular influenza activity in Brazil. In contrast, it caused an intense influenza season in other parts of the world, as in northern hemisphere (2017/2018). This virus has a high rate of genetic and antigenic variability and the vaccine strain needs to be revised/changed constantly.

The aim of this study was evaluate vaccine match, virulence and antiviral resistance, observing the viral polymorphisms and reconstructing the phylogeny of influenza H3N2 viruses circulating in Brazil through 2017-2018 epidemic seasons.

Genomic sequencing of the influenza A(H3N2) were performed, followed by a phylogenetic reconstruction of each gene segment.

In this study, was observed a large diversity of H3N2 genetic clusters, including 3C.3a, 3C.2a and their subgroups, with the
dominance of 3C.2a1 (2016-2017 interepidemic and 2017 epidemic period), a distinct group related to the 2017 vaccine strain A/HongKong/4801/2014-like. However, the genetic profile changed in the interepidemic season 2017-2018, the dominant genetic group was 3C.2a2, bearing antigenic substitutions (T131K, R142K and R261Q). This dominance was similar to observed in some parts of the world; however, it had a minor impact in Brazil. For the 2018, a new strain was chosen to compose the Southern Hemisphere vaccine and it has 5-6 antigenic changes in comparison to the dominant 3C2a2 circulating in South America since September 2017; however, the antigenic analysis revealed a vaccine match among this group. Furthermore, the phylogenetic reconstruction of viral genome presented evidences for the interlineage reassortment among the genetic group 3C.3a, but it seemed to be geographically restricted to South America and no evidences of extensive spread or virulence was found among the samples detected in this group. Regarding to antiviral resistance, all analyzed samples were sensitive to the oseltamivir. Comparing the influenza clinical outcome with the polymorphisms and genetic groups detected we could not find a virulence pattern. In Brazil, around 80% of deceased influenza cases had risk factors for influenza severity. It indicates the importance of underlying conditions for patient clinical outcome.

This study showed the genetic and antigenic change of H3N2 between two seasons and highlights the necessity of the optimization in influenza vaccine field (universal vaccine) against the rapid evolution of this virus.

Palavras-chaves: Influenza A (H3N2), Viral surveillance, interlineage reassortment, viral evolution, hemagglutinin

ANALYSIS OF COINFECTION OF DENGUE VIRUS AND ZIKA VIRUS IN A MAMMALIAN CELL MODEL

Autores

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Resumo

Co-circulation of arboviruses such as Dengue virus (DENV), Zika virus (ZIKV) and Yellow Fever virus (YFV) 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 infections. DENV is the most important arbovirus posing threat to public health worldwide. World Health Organization estimates that almost a hundred million people are infected every year. The disease is caused by infection with one of the four different serotypes (DENV 1-4). Zika virus caused, between 2015 and 2016, epidemics in America, Africa, Pacific and southeast of Asia, and has been declared a world public health concern by WHO due the disease association with neurological problems and severe newborn sequelae as microcephaly, reported in Brazil. Thus, the first aim of this project was to investigate the in vitro co-infection with different DENV serotypes.. Accordingly, VERO cells were co-infected with combinations of DENV serotypes and overlay media and co-infected monolayers were processed qPCR with specific primers. The strategy employed here successfully amplified genomic segments of different DENV serotypes during co-infections in VERO cells and also co-infections with YFV and ZIKV. We were able to detect genome replication during co-infections between all DENV serotypes, only observing viral interference between DENV serotypes 1 and 4, and DENV serotypes 1 and 3. 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 coinfectected samples, and the results indicate that are low interference in the genome replication between the two serotypes under this conditions. We also analysed coinfection between DENV-4 and ZIKV, using the previous quantification strategy and had
similar results with no viral interference detected. At least we also started to analyse the coinfection between DENV-4/ YFV, and ZIKV/ YFV with initial results being the same as the previous ones. Additional data is necessary for confirmation of both results, especially regarding the production of viable particles. We foresee that this strategy will provide insights on arboviruses-host interplay, intrahost competition and ultimately therapy strategies that can be employed to counteract arboviruses.

Financial support: CNPq, CAPES e FAPEMIG

Palavras-chaves: Coinfection, Dengue virus, Vero cells, Zika virus

MOLECULAR EPIDEMIOLOGY OF ZIKA, CHIKUNGUNYA AND DENGUE VIRUSES IN THE CITY OF JUAZEIRO DO NORTE, CEARÁ.

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Resumo

Introduction: Currently the most relevant arboviruses are Chikugunya (CHIKV) and Zika (ZIKV), which represent a public health problem in Brazil and in the world, along with the Dengue virus. Clinical symptoms are very similar between arbovirus infections and cross-reactivity occurs in serological tests, making diagnosis difficult. In addition, there are still regions in Brazil where the circulation of other arboviruses other than dengue has not yet been studied, as is the case of the city of Juazeiro do Norte in the state of Ceará. The objective of this work was to study the molecular epidemiology of Zika (ZIKV), Dengue (DENV) and Chikungunya (CHIKV) viruses in the city of Juazeiro do Norte, Cariri Region, Ceará. Material and Methods: 115 sera from LACEN / Juazeiro do Norte collected between January and July / 2016 and stored in the Laboratory of Retrovirology of UNIFESP were studied (CEP / UNIFESP # 1,717,485). The sera were previously tested in LACEN by IgM and IgG serologies for DENV, CHIKV and ZIKV. All samples were tested by RT-qPCR using hydrolysis probes to Chikungunya, Zika and Dengue and by ZDC Multiplex RT PCR Assay (Bio-rad Laboratories). Results: The mean age of the patients was 38.3 (range 2-80); 74% were female; the mean between onset symptoms and sample collection was 10.9 days (range 1-34). Of the 41 patients who performed CHIKV IgM serology, 30 were reactives (RT-qPCR = 10 positive), 10 were non-reactive (RT-qPCR = 3) and 1 were inconclusive (RT-qPCR = negative). Of the patients who performed DENV IgM serology, 72 were non-reactive (RT-qPCR = 23 ZIKV positive; 2 coinfections with ZIKV / CHIKV; 1 CHIKV and all negative DENV); 2 IgM reactives (RT-qPCR = 1 ZIKV positive and all CHIKV and DENV negatives). All other samples were negative with RT-qPCR. Conclusion: Most of the samples were collected during an inadequate period of infection for a reliable diagnosis, most of the time due to the delay in search of medical service. Most of the serologies requested were for DENV showing the difficulty in clinical diagnosis and adequate testing. The RT-qPCR presented discordant results regarding the serologies
and the two coinfections ZIKV / CHIKV demonstrating an urgent need of using molecular testing.

**Palavras-chaves:** Epidemiology, Zika, Chikungunya, Dengue, Juazeiro do Norte

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**EFFECT OF EMODIN ASSOCIATED WITH PHOTODYNAMIC THERAPY IN HPV-16 POSITIVE CERVICAL CARCINOMA CELLS**

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**Resumo**

The Human Papillomavirus (HPV) is a family of DNA viruses with more than 200 types, and can be classified in low and high risk HPVs. The most important high risk HPVs are the HPV-16 and HPV-18, that together are responsible for more than 70% cervical carcinoma cases. Current treatment modalities for cervical cancer are the combination of cisplatin based chemotherapy with radiation, however they present severe adverse effects. Therefore, ongoing efforts are necessary to developed new effective therapeutic strategies to enhance chemotherapeutic efficacy and decrease these side effects. The emodin has attracted extensive attention due to its anti-inflammatory, antineoplastic, and proapoptotic effects in recent years. Moreover, the interest in the photodynamic therapy in the treatment of cancer has grown exponentially, due to the efficiency of the tumor cells destruction. So, the aim of this study was to analyze the effect of emodin associated with photodynamic therapy in cervical carcinoma cell lines infected with HPV-16. Until now we evaluated the cellular viability, cellular uptake and phototoxicity of emodin. The SiHa and CasKi (HPV-16 positive) and HaCaT (immortalized human keratinocytes) were plated in 96-well culture plates with 10⁴ cells/well. After 24 hours, the cells were treated with different concentrations of emodin (0.47; 0.94; 1.87; 3.75; 7.5; 15; 30; 60; 120µM) for 24, 48 and 72h. The cellular viability was measured by MTT assay. The cellular uptake of emodin was performed by fluorescence microscopy and evaluated for 15’, 30’, 1, 3, 6 and 24 hours. The phototoxicity assay was performed with concentration previously determinate by MTT assay. In 30 minutes and 1 hour of incubation with emodin the cells were irradiated with laser (50J/cm²) for 4 minutes. After 24 hours was evaluated the viability cellular through MTT assay. The cellular viability assay showed increased of cytotoxicity in concentration and time-dependent manner. The fluorescence microscopy images showed that the emodin were able to internalize cells in 30 minutes, with marked decrease in intracellular uptake after 1 hour. Besides, we observed significant phototoxic effect for both cervical carcinoma cells, as well as the HaCaT line.

**Palavras-chaves:** Emodin, photodynamic therapy, cervical carcinoma, high-risk HPV

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**ACTION OF POLYMORPHISMS IN TNFA GENES (rs1800629) AND IL6 (rs1800795) IN CHRONIC HEPATITIS B and C VIRUS INFECTIONS.**

**Autores**
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In viral hepatitis, liver damage occurs through the action of the immune system, with its innate and adaptive immune responses, mediated by the influence of cytokines that will act directly or indirectly in the inflammatory process. Tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) are cytokines that act in the immune system, with a proinflammatory action on the immune response of individuals. This study aimed to determine the frequencies of the polymorphisms in the TNFA and IL6 genes in chronic hepatitis B and C carriers and to identify possible associations with the progression of these infections. The study was cross-sectional and analytical, in which were investigated populations of patients with chronic hepatitis B (74), chronic hepatitis C (101) and a control group (300) composed by blood donors. DNA was extracted from the peripheral blood samples and then submitted to polymorphisms investigation in the TNFA genes (rs1800629) and IL6 (rs1800795) by real-time PCR (qPCR). Biochemical tests were performed only for patients, and serological tests were done for all study participants. Histopathological examinations were performed only in patients with indication of biopsy, following the French classification METAVIR, punctuating the activity of the portal and periportal inflammatory infiltrate from 0 to 3 and structural alterations from 0 to 4. Statistical analysis were performed in the BioEstat 5.0v program, adopting as significance level p-values TNFA), it was observed an association of the GA and AA genotypes with AST, ALT and GGT enzymes levels among HBV carriers. In HCV patients, a significant association of genotype frequencies was observed when the patients and the control group were compared, and the GG genotype was associated with AST. In the polymorphism rs1800795 (IL6), the association of the GG genotype with AST, ALT and GGT, and of the CG and CC genotypes with HBV viral load in patients was observed. In the HCV group, the association of GC and CC genotypes with AST and ALT was observed. Correlations of the studied polymorphisms with inflammatory activity and staging of fibrosis were not observed in both groups of patients. We conclude that TNFA and IL6 gene polymorphisms appear to be associated with progression of clinical and laboratory findings of HBV and HCV infections, but studies with other population groups of different ethnicities are necessary to confirm these results.

Financial support: CNPQ

Palavras-chaves: HBV, HCV, IL6, TNF
Resumo

In Brazil, the most affected regions by the Zika virus (ZIKV) are concentrated in the Southeast and Northeast. Although Paraná does not belong to such regions, more than 63,000 probable cases of ZIKV have been reported in the study period. Notification data are important tools to better understand the regional epidemiological profile and assist in the development of public policies aimed at disease control. The aim of the study was to conduct a descriptive epidemiological analysis of suspected and confirmed cases of ZIKV in patients attended at the 15th Regional of Health of the state of Paraná (15º RH). The study was conducted according to the Ethics Committee of the State University of Maringá (Nº. 2.364.256). Between January 2015 and May 2017, 4,262 cases were reported, of which 92 (2.2%) in 2015, 3,942 (92.5%) in 2016 and 228 (5.3%) in 2017. The highest reported frequency of the first symptoms occurred in March 2016 with 1,380 cases (32.4%). Of the suspected ZIKV cases, 73.9% were classified by laboratory criteria and 17.3% by clinical-epidemiology. Of the total, 2,515 (59%) were females and 456 (10.7%) were pregnant, of which 126 (27.63%) were in the first trimester, 176 (38.6%) in the second trimester and 154 (33.77%) in the third gestational trimester. The mean age among the evaluated cases was 32 years. It is worth mentioning that 922 (21.6%) were underage and 3,272 (76.8%) were self-declared belonging to the white race. With regard to schooling, 815 (19.1%) had completed high school. The majority of the individuals were urban residents (3,862, 90.6%). Data analysis showed that the patients seen in the 15º RS came from 214 municipalities, of which 207 were from Paraná. Cascavel was the city with the highest number of suspected cases (412, 9.7%). Regarding to the cities belonging to 15º RS, 379 suspected and/or confirmed cases were observed, distributed in 14 cities and Maringá was the most affected city with 294 (77.6%) cases. Of the total cases evaluated, 3,768 (%) evolved to cure and 400 (9.4%) of the reports were confirmed as positive for ZIKV. Although the state of Paraná is not among the most affected regions of Brazil by the ZIKV, the numbers of suspected cases call attention and evidence the need for combat and prevention measures at national level. Financial Support: CAPES, CNPq, FA, LIG-UEM.

Palavras-chaves: Epidemiologic Studies, Health Information Systems, Zika Virus

Genomic characterization by NGS of HIV-1 proviral quasispecies of patients undergoing first-line therapeutic success

Autores

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Resumo

Increased access to highly active antiretroviral therapy (HAART) by individuals infected with the human immunodeficiency virus (HIV) has become a reality worldwide. In this context, several regions established free access to antiretroviral therapy (ART) to all HIV+ individuals, such as Brazil which currently covers more than half of the infected individuals. HIV-1 has a remarkable genetic variability resulted from several mutational events, the lack of a proofreading function by reverse transcriptase enzyme and high rate of replication.
In such context of continuous intra-host variability, several highly related viral variants, called quasispecies, are generated. The HIV quasispecies generated during infection may influence viral persistence and pathogenicity, representing a challenge for treatment; however, the clinical relevance of minority variants is still uncertain. Thus, in this study we determined the archived proviral sequences, the viral subtype and the ART-resistance mutations of a Brazilian cohort of HIV+ patients with undetectable viral load submitted to HAART as first-line treatment option through next generation sequencing (NGS) to obtain the near full lenght proviral genome (NFLG).

This study included 32 patients from Hospital de Ipanema, RJ and 12 from HUCFF-UFRJ, RJ. For all samples, the genomic DNA was extracted from PBMCs for PCR amplification of proviral DNA, construction of genomic libraries and sequencing on Illumina MiSeq platform. The analyzes were carried out in Geneious software, where the reads were used in the assembly with reference. From the consensus sequence extracted from each sample, it was possible to define the infecting HIV subtype through phylogenetic analyzes. The presence/frequency of variants resistant to ARVs was determined based on IAS consensus and Stanford database. In total, 32 samples were successfully sequenced (11 NFLG consensus sequences were obtained). Phylogenetic analyzes indicated the predominance of subtype B. Of these, 23 patients have at least one mutation able to confer resistance to ARVs. Together, these data highlights the importance to monitoring minority variants associated with antiretroviral resistance and its clinical impact, in order to assist future therapeutic switches, to complement the studies of proviral archived diversity and to contribute to the success of ART.

Financial Support: Ministério da Saúde

Palavras-chaves: HIV-1, Quasispecies, NGS, Antiretroviral drug resistance, Diversity

The Use of a Pan-Flavivirus RT-qPCR Assay for Differential Diagnosis and Active Surveillance in Brazil

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Resumo

Flaviviruses are considered a serious threat to public health in many parts of the world, as many are highly pathogenic to humans and animals, such as Yellow Fever virus, West Nile virus, Japanese encephalitis virus and Dengue virus. Many of them have spread to different geographic regions where their circulation had not been detected previously, causing new outbreaks. Diagnosis of these infections is often difficult, due to the large number of symptoms presented, which can be confused with other diseases of different etiological causes. The main direct methods currently used for detecting these viruses are intracerebral inoculation in neonatal mice, inoculation in cell cultures and specific RT-PCR. The present work aims to evaluate the sensitivity of a RT-qPCR for genus Flavivirus circulating in Brazil, where multiple flavivirus co-circulate, and its use for surveillance, dengue differential diagnosis and screening, since there`s a lack of specific diagnosis for most of them. Samples of the standard flaviviruses Yellow Fever, Bussuquara, Iguape, Ilheus, Saint Louis Encephalitis, Cacipacore and Zika were quantified by titration by plaque forming units (UFP) or TCID50 to evaluate the detection limits for each of them. The limits found ranged from 0.01 PFU for Ilheus virus to 1 PFU for Yellow Fever and Iguape viruses and 1x10¹⁶ TCID50 / 100µL for the Bussuquara virus. In addition, the present work was able to identify, after cDNA sequencing, the first human case of Zika virus, isolated from a febrile
patient, and both Ilheus and Iguape viruses, isolated from different species of Culicidae mosquitoes, and a possible new insect-specific flavivirus from Aedes mosquitoes. The Alphaviruses Mayaro and Chikungunya were not amplified. The present protocol showed high sensitivity and specificity, and therefore it may be used for the differential diagnosis of the different flaviviruses that occur in Brazil, as well as for viral monitoring studies in sentinel animals and vectors, thus collaborating with public health. It is also possible to detect new flavivirus that are arthropode-specific.

Palavras-chaves: Flavivirus, RT-qPCR, Surveillance

ISOLATION AND CHARACTERIZATION OF KLEBSIELLA PNEUMONIAE LYTIC BACTERIOPHAGES FROM HOSPITAL SEWAGE SAMPLES

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Resumo

Introduction: Klebsiella pneumoniae have emerged as an increasingly important cause of community-acquired nosocomial infections, mostly due to extensive use of broad-spectrum antibiotics in hospitalised patients, which has led to both increased colonization by Klebsiella and the development of multidrug-resistant strains that frequently produce extended-spectrum β-lactamases and/or other defences against antibiotics. Many of these bacterial strains are highly virulent and exhibit a strong propensity to spread. In the research effort entertained herein, strictly lytic bacteriophage particles were isolated from Hospital-sewage in Sorocaba (Brazil) and characterised as phage therapy candidates against Klebsiella pneumoniae strains isolated from thirty eight human clinical samples. Methods: Physicochemical characterization of the isolated bacteriophage particles included verification of bacterial lysis, phage morphology, amplification of the bacteriophages, titration of the concentrated bacteriophage suspensions, SDS-PAGE electrophoresis, XRD and UV-Vis scannings, whereas biological characterization encompassed evaluation of their lytic spectra, host range, efficiency of plating (EOP) assays, determination of the one-step growth curve (OSGC) for latent period and burst size determinations, and determination of the adsorption curve for calculation of the bacteriophage adsorption rate onto its bacterial host cell. Results and Discussion: The bacteriophages isolated and characterized produced clear (and different) plaques of bacterial lysis and exhibited a broad lytic spectrum against several Klebsiella pneumoniae strains isolated from (human) clinical samples. Conclusions: The bacteriophage particles were capable of efficiently lysing the majority of Klebsiella pneumoniae strains comprising a collection of 38 clinical isolates from Sorocaba (Brazil), thus exhibiting a number of properties indicative of potential utility in phage
Acknowledgements: FAPESP (São Paulo, Brazil: Refs. No. 2016/08884-3 and 2016/12234-4) and CNPq (São Paulo, Brazil: Research Productivity (PQ) fellowship granted to Victor M. Balcão, Refs. No. 306113/2014-7 and 308208/2017-0), is hereby gratefully acknowledged. Funding for Fernanda Morelli, Lilian Harada, Erica Silva and Welida Campos by UNISO, in the form of scholarships, is hereby also gratefully acknowledged.

Palavras-chaves: Bacteriophages, Isolation and characterization, Klebsiella pneumoniae, Phage therapy, Bacterial pneumonia

ISOLATION AND CHARACTERIZATION OF ACINETOBACTER BAUMANNII LYTIC BACTERIOPHAGES

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Resumo

Introduction:Acinetobacter baumanniiis the second most frequently (non-fermenting) bacterium isolated in healthy human beings. However, it often exhibits pathogenicity, generally affecting immunocompromised individuals, being often isolated in infections acquired in a hospital environment, usually by antibiotic-resistant strains. Resistant strains of this species are especially prevalent in intensive care units, where sporadic cases of infection are common, and in some more virulent cases may become epidemic. Given its ability to infect the human respiratory tract, A. baumanniioften causes nosocomial pneumonia, especially pneumonia associated with mechanical ventilation. It can also cause several other infections, including skin, wound and bacteremia infections. In the research effort entertained herein, strictly lytic bacteriophage particles were isolated from Hospital-sewage in Sorocaba (Brazil) and characterised as phage therapy candidates against a Acinetobacter baumannii strain ATCC 19606. Methods:Physicochemical characterization of the isolated bacteriophage particles included verification of bacterial lysis, phage morphology, amplification of the bacteriophages, titration of the concentrated bacteriophage suspensions, SDS-PAGE electrophoresis, XRD and UV-Vis scannings, whereas biological characterization encompassed evaluation of their lytic spectra, host range, efficiency of plating (EOP) assays, determination of the one-step growth curve (OSGC) for latent period and burst size determinations, and determination of the adsorption curve for calculation of the bacteriophage adsorption rate onto its bacterial host cell. Results and Discussion: The bacteriophages isolated and characterized produced clear plaques of bacterial lysis and exhibited a lytic spectrum against Acinetobacter baumannnii strain
Conclusions: The bacteriophage particles were capable of efficiently lysing the *Acinetobacter baumannii* strain ATCC 19606, thus exhibiting a number of properties indicative of potential utility in phage therapy.

Acknowledgements: FAPESP (São Paulo, Brazil: Refs. No. 2016/08884-3 and 2016/12234-4) and CNPq (São Paulo, Brazil: Research Productivity (PQ) fellowship granted to Víctor M. Balcão, Refs. No. 306113/2014-7 and 308208/2017-0), is hereby gratefully acknowledged. Funding for Welida Campos, Liliam Harada, Fernanda Morelli and Erica Silva by UNISO, in the form of scholarships, is hereby also gratefully acknowledged.

Palavras-chaves: Bacteriophages, Acinetobacter baumannii, Phage therapy, Isolation and characterisation

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CAN HIV INFECTION SUSCEPTIBILITY BE INFLUENCED BY A POLYMORPHISM IN REGULATORY T CELLS?

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Resumo

Susceptibility to an infection could be linked to several factors such as genetic diversity. It has been associated with the success of the establishment of infections, as well as contributing to the advancement of the disease. Polymorphisms in immunoregulatory cells, such as in the *FOXP3* gene of regulatory T cells, can be involved in susceptibility to HIV infection. The study aim was to investigate influence *FOXP3* rs2280883 C/T polymorphism on HIV infection susceptibility. 356 HIV-positive subjects were selected and 325 HIV-negative, among those 472 are Euro-descendant and 154 are Afro-descendent, 360 subjects are females and 269 are males, the mean age for HIV- is 36,9 ± 12,2 and for HIV+ 40,8 ± 10,2. The polymorphism was genotyped using TaqMan probes by qPCR StepOne® - Applied Biosystems®. Allelic and genotypical frequencies were estimated between groups by 2x2 contingency table for Fisher's exact test. For significance analyze chi-square was used, odds ratio using Woolf's approximation. These frequencies showed a significant difference between HIV-positive and controls (pp = 0,0312) and it can be associated with susceptibility to this infection. Moreover, in Afro-descendent, the allele could be a protection factor. Our preliminaries results indicate a possible associated between rs2280883 SNP and HIV infection susceptibility.

Financial support: FAPERGS funding agencies, Capes, Feevale University.

Palavras-chaves: FOXP3, HIV, SNP, Susceptibility, Tregs

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POSSIBLE ASSOCIATION BETWEEN HLA CLASS I AND ZIKA VIRUS

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Resumo

The immunopathogenesis of Zika virus (ZIKV) is not fully elucidated. It is known that the virus compromises the physical integrity of the humans, and is related to the development of diseases, such as microcephaly. Human leucocyte antigens (HLA) are involved in the immune response to virus presenting endogenous antigens to T CD8 lymphocytes, contributing to activation of the cytotoxic function of these cells and death of the infected cells. Studies of genetic polymorphisms are important, as mutations may cause changes in the immune response, contributing to the susceptibility or protection to diseases. This study evaluated a possible association between HLA class I and ZIKV, according to the Ethics Committee of the State University of Maringá (Nº 2.364.256). Seventeen patients with ZIKV diagnosed by laboratory exams and 34 controls apparently without symptoms related to ZIKV participated in the study. HLA-A, -B and -C genotyping was performed by the PCR-SSO method, Luminex technology (LABType® SSO, One Lambda, Inc.). The results were evaluated by HLA Fusion 3.0 software. Comparisons among the allele frequencies were performed using the chi-square with Yate’s correction test. The risk was assessed by calculating the Odds Ratio (OR) with a 95% confidence interval obtained by OpenEpi software. The mean age was 38 years (SD ± 15.4) for patients and 39 (SD ± 14.6) for controls. The analyzed sample was in Hardy-Weinberg equilibrium. The highest allele frequencies observed for patients and controls were HLA-A*02 (0.23 vs 0.29), HLA-B*35 (0.15 vs 0.13) and HLA-C*07 (0.23 vs 0.31). A comparison of the distribution of alleles between the groups indicated an increased frequency in patients than in controls for HLA-A*11 (0.18 vs 0.03, P: 0.03; Pc: 0.33; OR: 7.07; CI: 1.34-37.2) indicating a possible association. However, the significance was lost after the Bonferroni correction. In conclusion, no difference was observed in the distribution of the allelic groups in patients with Zika virus in this preliminary study. Finally, more samples will be used to confirm these findings and their relation to killer cell immunoglobulin-like receptors (KIRs), since HLA class I antigens are ligands for KIR molecules. Financial Support: CAPES, CNPq, FA, LIG-UEM.

Palavras-chaves: Genetic Association Studies, Genes, MHC class I, HLA-A Antigens, Zika Virus
Parvovirus B19 (B19V) infection is prevalent worldwide, commonly affects children and can cause various clinical syndromes from infectious erythema to non-immune fetal hydrops. In addition, B19V may cause atypical clinical manifestations due to B19V persistence in non-precursor erythroid tissues. Studies show that liver infection by B19V can range from an increase in liver transaminases to acute liver failure (ALF). Thus, it has been proposed that B19V can be considered as an etiologic agent of viral hepatitis, especially in children, although this occurrence is rare. The aim of this study is report a case of a 9-years-old female immunocompetent child who developed ALF of unknown etiology (non-A-E hepatitis). The patient was hospitalized with ALF and underwent a liver transplant, fifteen days after hospitalization. Twenty days after the surgical intervention, the patient died. At the time of death, the patient presented thrombocytopenia, severe anemia and an increase in transaminases levels, alkaline phosphatase and gamma glutamyl transferase. Infections with other hepatotropic viruses and autoimmune diseases were excluded. B19V infection was investigated by ELISA for detection of anti-B19 IgM and IgG, and by real-time PCR for detection and quantification of the B19-DNA viral load in samples collected before and after hepatic transplantation (HT). In order to evaluate the active replication of the virus in the hepatic tissue, transcripts (mRNA) were investigated in this sample. The serum samples collected before and after HT were positive for anti-B19V IgG and B19-DNA with viral load of 6.03x10^7 copies/mL and 8.52x10^7 copies/mL, respectively. In the hepatic tissue it was possible to detect DNA-B19V with viral load of 1.65x10^8 copies/mL (or 2.64x10^5 copies/mg) and transcripts of VP1 of 326bp. Phylogenetic analysis revealed that B19V belongs to genotype 1A. Detection of B19V-DNA in serum and in hepatic tissue with high viral load, associated with anti-B19V IgG positivity, in the absence of IgM, indicates the occurrence of a persistent infection. Together, these laboratory and clinical findings (severe anemia and thrombocytopenia) suggest the diagnosis of B19V infection associated with ALF. These data highlight the importance of considering the differential diagnosis for B19V in cases of ALF.

Palavras-chaves: Acute liver failure, Diagnostic, Human Parvovirus B19, Real-time PCR, Serology

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**Resumo**

Yellow fever (YF) is endemic in the Brazilian Amazon Basin, but outside this region, the reemergence of Yellow Fever virus is occasionally reported, especially during epidemic season. The greatest YF outbreak documented in Brazil in the recent decades has been occurring since 2016 up to 2018, especially in Southeast Brazil, with thousands of human cases (more than 1,900) and deaths of non-human primates (NHPs) reported so far. From January/2017 to March/2018, we received NHPs samples from urban, periurban and rural areas of MG state to be analyzed. Total RNA was extracted from liver (RNAeasy Mini Kit, Qiagen) and used for 1-Step RT-qPCR (GoTaq®Probe 1-StepRT-qPCR System-Promega) with specific primers probe targeting the 5'-NC region of YFV genome. cDNA of positive samples was synthesized and used in PCR, with primers targeting CprM/envelope. Amplicons were sequenced and sequences used for phylogenetic analysis. Out of 268 liver samples, 147 (54.8%) were positive. YFV genome was detected in *Callithrix* sp. (n=17), *Alouatta* sp. (n=22), *Callithrix* sp. (n=99), and *Sapajus* sp. (n=01). YFV was detected in NHPs from 83 municipalities of MG, in rural (n=65), urban (n=66), and from periurban (n=13) areas. Phylogenetic analysis indicated that YFV strains clustered within Genotype South America-I. 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, located in Metropolitan mesoregion. Positive animals have been collected up to July 2017,

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**YELLOW FEVER IN NON-HUMAN PRIMATES FROM URBAN AND PERIURBAN AREAS: BRAZIL, 2016/2018**

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**Resumo**

Yellow fever (YF) is endemic in the Brazilian Amazon Basin, but outside this region, the reemergence of Yellow Fever virus is occasionally reported, especially during epidemic season. The greatest YF outbreak documented in Brazil in the recent decades has been occurring since 2016 up to 2018, especially in Southeast Brazil, with thousands of human cases (more than 1,900) and deaths of non-human primates (NHPs) reported so far. From January/2017 to March/2018, we received NHPs samples from urban, periurban and rural areas of MG state to be analyzed. Total RNA was extracted from liver (RNAeasy Mini Kit, Qiagen) and used for 1-Step RT-qPCR (GoTaq®Probe 1-StepRT-qPCR System-Promega) with specific primers probe targeting the 5'-NC region of YFV genome. cDNA of positive samples was synthesized and used in PCR, with primers targeting CprM/envelope. Amplicons were sequenced and sequences used for phylogenetic analysis. Out of 268 liver samples, 147 (54.8%) were positive. YFV genome was detected in *Callithrix* sp. (n=17), *Alouatta* sp. (n=22), *Callithrix* sp. (n=99), and *Sapajus* sp. (n=01). YFV was detected in NHPs from 83 municipalities of MG, in rural (n=65), urban (n=66), and from periurban (n=13) areas. Phylogenetic analysis indicated that YFV strains clustered within Genotype South America-I. 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, located in Metropolitan mesoregion. Positive animals have been collected up to July 2017,
indicating a prolonged circulation of YFV, including in urban areas, what could be a risk to the reurbanization of YF. Further studies are needed to further investigate YFV circulation in vertebrate hosts and vectors within urban areas in Southeast Brazil to better predict the risk of urban YF outbreaks. * Grupo de Estudos de Febre amarela 1,2,3,4: Mello, Érica Munhoz 2; Prado, Alaine Isabela A 1; Stump, Rodolfo GAV 1; Massara, Rodrigo L 1; Pascoal, Ana MO 1; Perini, Fernando 1; Paglia, Adriano P 1; Teixeira, Erika P 3; Barreto, Cecília 3; Vilela, Daniel AR 3; Matos, Laerciana SS 3; Alves, Pedro Augusto 3; Kroon, Erna G 1. IMR and NIOS contributed equally to this work.

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Palavras-chaves: Yellow Fever virus, Yellow fever, Non-human primates, Surveillance, Brazil

FUNCTIONAL ANALYSIS OF VARIANTS FROM THE LONG CONTROL REGION (LCR) OF HUMAN PAPILLOMAVIRUS 31

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Resumo

FUNCTIONAL ANALYSIS OF VARIANTS FROM THE LONG CONTROL REGION (LCR) OF HUMAN PAPILLOMAVIRUS 31

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Introduction: The Human Papillomavirus (HPV) infects the epitelial tissue and sexual contact is the main form of transmission. Nowadays, over 200 types have been described. With approximately 530 thousand new cases each year around the world, the cervical cancer is the fourth most common type of cancer amongst women. HPV’s genome is composed by the LCR (Long Control Region), the genes Early and Late. Variations on the LCR region can influence the progression of cervical cancer and the loss of binding capacity of the transcriptional factors E2, YY1, NF-1, AP1. Methodology: In this study, three samples containing molecular variants of LCR from the HPV-31 (belonging to the A and C variants) were used, along with the prototype (HPV-31 – J04353 1) and the transcriptional regulator E2.
The samples were cloned and, afterwards, subcloned in expression vectors from mammal cells. The recombinant were sequenced in order to confirm it. HeLa cell groups were co-transfected with the polymorphic LCR of HPV-31, or with the prototype LCR of HPV-31, the Renilla plasmodium containing the transcriptional regulator E2. Forty eight hours after the transfection, the cells were lysed and the cellular extracts were prepared for the reading of luciferase of NanoLuc and Renilla. Two experimental replicas were done and all happened in triplicate. Results: The studied isolates showed that the polymorphisms on the LCR of HPV-31 helped on changing the expression levels of the Nluc reporter gene (its expression is conditioned by the LCR). Statistical analysis showed a significant difference between the polymorphic LCRs and the control with a p

Conclusion: A more refined profile of the HPV-31 begins to be observed as well as its importance to the prognosis of cervical lesions.

Financial support: Fundação de Amparo à Ciência e a Tecnologia (FACEPE) e o Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Palavras-chaves: HUMAN PAPILLOMAVIRUS 31, LONG CONTROL REGION (LCR) , Luciferase

Detection of the ZIKA Virus in Peripheral Blood Mononuclear Cells (PBMC) and Platelets after in vitro exposition

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Resumo

The ZIKA virus (ZIKV) is an arbovirus of the Flaviviridae family that is currently relevant because of its association with congenital defects, such as microcephaly, and severe neurological effects such as Guillain-Barré syndrome. Despite its relevance, the mechanism of infection and development of the disease has not yet been elucidated when compared to other virus in its family. Previous studies have demonstrated that Dengue Virus (DENV) and Hepatitis C Virus (HCV), other Flavivirus, are able to associate with Peripheral blood mononuclear cells (PBMC) and platelets. It has also been demonstrated that the human platelet antigen (HPA) polymorphism of the integrin family (HPA-1, -3 and -5) exerts an influence on the binding of DENV and HCV to platelets. In this context, the present study evaluated the ability of ZIKV to interact with platelets and PBMC in vitro, and the association of HPA-1, -3 and -5 polymorphisms to virus interaction with platelets. Cells obtained from non-infected blood donors were incubated with the ZIKV at 37°C for 48 hours. After the incubation the supernatant was removed, and the cell pellets were obtained. The all supernatant and the cell pellets were analyzed for ZIKV presence by the value of CT determined by RT-qPCR. The HPA-1 and -3 polymorphisms were genotyped from donors’ DNA by PCR-SSP and HPA-5 by PCR-RFLP. The results
indicated the virus presence in PBMC and platelets pellets (CT < 40) after incubation suggesting, for the first time, that ZIKV interacts with PBMC and platelets and these cells could be a biological reservoir to the virus in infected patients. There were no association between HPA polymorphism and CT obtained after platelets-virus interaction, suggesting that other molecules from platelet surface can be associated with the virus-platelet ligation.

Financial Support: FAPESP

Palavras-chaves: PBMC, Platelets, ZIKV

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**IMMUNE RESPONSE TO S AND PRE-S1-S2/S HEPATITIS B ANTIGENS IN HIII AND LIII OUTBRED GENETICALLY SELECTED MICE AND BALB/c**

**Autores**

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**Resumo**

Hepatitis B vaccination is still the best tool to prevent HBV infection and its complications. However, after vaccination schedule with S antigen (HBsAg) approximately 5% of vaccinated adults have an insufficient response. To overcome the non-responsiveness vaccination effect a new generation vaccine using yeast expressing the S protein and other surface components, pre-S1 and pre-S2, was developed. In this work we analyze the antibody production and CD4/CD8/CD19 cells profile of animals immunized with the conventional Hepatitis B vaccine and the new Pre-S1-S2/S one. Two outbred mouse lines obtained by genetic selection according to the high (HIII) or low (LIII) antibody responsiveness and BALB/c inbred mice were used for immunizations in order to analyze different patterns of response to the hepatitis B antigens. Mice groups received 2 doses of the S or Pre-S1-S2/S antigens adsorbed in aluminum hydroxide by the s.c. route with 30 day-interval. Individual serum was periodically collected and tested by ELISA for specific IgG antibody and its subclasses titration. In addition, 30 days after the last booster mice group received an i.p. antigen injection and after 7 days its spleen cells were isolated and CD4, CD8 and CD19 cells subsets were analyzed by cytometry. The LIII mice immunized with Pre-S1-S2/S antigen showed higher specific IgG titles at 15 days (p

Supported by Fundação Butantan and PAP/SES

**Palavras-chaves:** animal model, hepatitis B, immune response, vaccine

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**EXPRESION OF CHIKUNGUNYA VIRUS GLYCOPROTEIN IN INSECT CELLS FOR DIAGNOSIS AND VACCINE DEVELOPMENT**
Neutralizing antibodies generated by a DNA vaccine encoding the ectodomain of DENV2 envelope protein: the influence of domains I and II versus domain III in neutralization

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Instituição

Resumo

Dengue represents a global public health problem with epidemics in tropical and subtropical regions. The etiologic agent of this disease is the dengue virus (DENV) that comprises four antigenically distinct serotypes: DENV1-4. Its viral genome encodes three structural and seven non-structural proteins. The envelope (E) protein has three domains (domains I, II and III) and interacts with receptors present on the surface of the host cell via domain III (DIII), resulting in the endocytosis of the viral particle. This protein is the main target for the production of neutralizing antibodies (NAb), which play a critical role in the host protective immune response. Thus, induction of NAb represents an important aspect for vaccine development. Neutralizing antibodies against each of the three domains of E protein have already been described. However, the contribution of these domains individually for production of strong and broadly NAb is not fully understood. Our group has developed several DNA vaccines against DENV, including the plasmid pE1D2, which encodes the ectodomain (domains I, II and III) of
DENV2 E protein. This plasmid induced a protective immune response against DENV2 in BALB/c mice, with NAb production. Therefore, in the present work, we aimed to better characterize the neutralizing antibody response generated by the pE1D2 vaccine. For this reason, antibodies against DIII were depleted from pE1D2-immunized mouse serum by passing through an affinity column in which the DENV2 DIII recombinant protein was fixed. Antibody titers were quantified by ELISA with DIII or the E ectodomain as solid-phase bound antigen, before and after the affinity column step, confirming the efficiency of the employed depletion protocol. Subsequently, NAb levels were assessed in VERO cells by plaque reduction assays using DENV2. Results showed that antibodies targeting epitopes contained in DI/II presented more neutralizing activity than those directed only to DIII. In addition, anti-DIII antibodies interfered negatively in neutralization mediated by antibodies directed to DI/II. Thus, our results suggest that the development of a vaccine based on the induction of antibodies with neutralizing activity should encompass the response directed to the immunogenic epitopes present not only in DIII but also in domains I and II.

Financial Support: IOC-Fiocruz, CNPq, INCTV, FAPERJ

Palavras-chaves: Dengue, DNA vaccine, Neutralizing antibodies, envelope protein

DEVELOPMENT OF A PEPTIDE-BASED SEROLOGIC NANODEVICES TO DIFFERENTIALLY IDENTIFY DENGUE AND ZIKA INFECTIONS

Autores

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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 and two that are DV-specific. These peptides were synthesized for use in two alternative diagnostic strategies. The first was an ELISA-like IgG/IgM assay in a flexible vinyl plate. We tested our peptide ELISA assay with 170 human samples from patients who had known a diagnosis of DV and/or ZV infection. The pepELISA DV-specific showed a sensitivity of 97% and a specificity of 96.3%. The pepELISA ZV-specific showed a sensitivity of 77.9% and a specificity of 97.7%. With the success of pepELISA, we evaluated a lateral flow-based assay using gold nanoparticles (GNP) as an alternative strategy, in two immobilization variation. For the first, biotinylated peptides were conjugated with streptavidin and spotted onto a nitrocellulose membrane and GNP conjugated with anti-human IgG were ran with the human samples. For the second test, peptides synthesized covalently linked to lipoic acid were conjugated to the surface of GNP. 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. These 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, FAPESP, INCT Dengue, NIH AI100190.

**Palavras-chaves:** Dengue, Diagnostic, Nanotechnology, Rapid Test, Zika

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**Development of a mouse model of Ilhéus virus infection and disease for testing of potential treatments**

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**Resumo**

**Introduction and objectives** – Ilhéus Virus (ILHV) is a neglected arthropod-borne flavivirus, together with other important pathogens such as Dengue, Saint Louis encephalitis and Zika viruses. ILHV infections occur in South America, such as in the Pantanal region. Infection with ILHV may lead to an unspecific febrile disease, which may evolve to neurological disease and encephalitis. Although its circulation in Brazil and the risks posed by infection, the mechanisms of disease development are largely unknown and no treatments or vaccines are available. Thus, we aimed to develop a mouse model of ILHV infection to expand our understanding of ILHV infection and disease, also delineating possible therapeutic strategies against this neglected arbovirus. **Methodology and results** – Lethality and inoculation route for ILHV were determined in vivo through survival assays with daily monitoring of development of symptoms, in both immunodeficient (A129) and wild-type
ILHV caused disease via intracranial injection in both models, leading to death within 3 days p.i. in A129 and 7 days in WT mice, but only A129 developed disease via subcutaneous (s.c.) injection. Lethality was dose-dependent in A129 mice, with a LD$_{50}$ of $10^{1}$ PFU via s.c. Disease symptoms were apparent 1 day before death and included neurological alterations, ocular inflammation and loss of movements. ILHV replicates to high titers in Vero and SH-SY5Y cells, a human neuroblastoma cell line, as measured using a plaque assay. Cell viability is reduced after 48h-72h post infection, as assessed using the LIVE/DEAD® Viability/Cytotoxicity Kit (ThermoFisher). We tested galanin, 2'-C-methylciclidin (2’CMC) and itaconic acid (IA) as possible treatments for ILHV infection. Our results show that galanin protected cells from ILHV-induced death in vitro. 2’CMC, but not IA, significantly reduced viral load in cell cultures. **Conclusion and perspectives** – In our model, ILHV infection was pathogenic to adult WT mice only when injected in the brain. The susceptibility of A129 to peripheral infection corroborates the literature on other flaviviruses and indicates that ILHV replication is likely regulated by type I IFNs. Galanin and 2’CMC treatment in vitro showed that reducing ILHV-induced CPE or ILHV replication may be beneficial for the host, but further experiments are necessary to confirm efficacy and elucidate the involved mechanisms.

**Funding:** FAPESP (Grant n° 2018/02993-0), CNPq Chamada 14/2016 (440379/2016-4)

**Palavras-chaves:** Ilhéus virus, mouse model, therapeutic strategies

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**ANALYSIS BY DIFFERENT METHODS OF CELL VIABILITY OF NANOVACCINE FOR THE PREVENTION OF NEWCASTLE DISEASE IN POULTRY**

**Autores**  
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**Resumo**

Newcastle disease is one of the highly pathogenic viral diseases of avian species. The lipid envelope of Newcastle disease virus (NDV) contains two surface glycoproteins, fusion protein, and hemagglutinin-neuraminidase (HA-NA). Commercial vaccines are based on attenuated viral strains, which may cause respiratory symptoms in immunocompromised birds. A disadvantage in the use of live attenuated vaccines is that the induction of antibody reactive to the virus interferes with the serological surveillance of the birds in active surveillance programs. This disease is a notifiable disease and one of the main sanitary barriers. Therefore, vaccination strategies are essential in both commercial and backyard poultry production. This study aimed to evaluate the immunogenicity of the NDV protein subunit by in vitro assays. A nonpathogenic NDV virus sample (No. 209/04) was used, kindly provided by the Ministry of Agriculture. For the virosomes preparation, the viral suspension was diluted in Triton X-100 (1%) to dissolve the viral envelope, followed by ultracentrifugation (1h/100000xg/4°C) to remove the nucleocapsid. Then, it was added a solution of phospholipids, and the surfactant was removed with the aid of a hydrophobic resin. The virosomes were characterized by the Zeta potential values between $-2.3\pm0.2$ mV, and an average size of $109\pm11$ nm demonstrated good electrostatic suspension stability. The HA assay of the viral suspension from the pre and post treatment remained similar. No virus replication was observed when treated NDV was inoculated into embryonated chicken eggs. The in situ transmission electron microscopy showed a concentration of nanostructures in the membrane of the nanoparticles. Immortalized macrophages lines, RAW 264.7, were used to evaluate the virosome and...
its influence on cytotoxicity and cell growth. Analyzes of MTT, cytotoxicity and cell counting at dilutions of 1:2-1:256, in 24, 48 and 72h were performed. The rate of cellular apoptosis at different concentrations of virosomes through was evaluated using the LIVE/DEAD®Viability/Cytotoxicity Kit and APO-DIREC assays. The results obtained were satisfactory, with endocytosis of virosomes by macrophages and low cytotoxicity (less than 5%), especially at the dilution of 1:16-1:32. All these results are an indicator of a promising NDV nanovaccine, which will be further evaluated in vivo in order to prevent the Newcastle disease in poultry.

Financial support: Embrapa and FAPESC

Palavras-chaves: Newcastle disease virus, Virosome, Nanovaccine, Cell viability, Poultry

EXPRESSION AND PURIFICATION OF AN IMMUNODOMINANT REGION OF THE NON-
STRUCTURAL PROTEIN NS3 OF BOVINE VIRAL DIARRHEA VIRUS FOR USE IN
IMMUNODIAGNOSTIC ASSAYS

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Resumo

Bovine Viral Diarrhoea virus (BVDV) is an important pathogen of cattle that causes reproductive, respiratory and enteric disease, leading to significant economic losses to dairy and beef cattle worldwide. Cattle naturally infected with BVDV develop humoral immune response predominantly directed to glycoprotein E2 (gp53), yet the non-structural protein 3 (NS3) is also a potent inducer of antibodies. Anti-NS3 antibodies are only produced in response to infection (or vaccination with live vaccines), and, thus, may be used to differentiate natural infection from vaccination with inactivated vaccines. Thus, the objective of this work was to express and characterize BVDV NS3 for use in an indirect enzyme-linked immunosassay test able to differentiate immunized from naturally infected animals. A fragment of 344 aminoacids corresponding to residues 205 to 549 of the non-structural protein NS3 of BVDV was chosen based on its conservation and immunogenicity/immunodominance. A corresponding 1050 nt synthetic DNA oligonucleotide was synthesized and optimized for expression in E. coli, containing the coding sequence flanked by 5’ and 3’ multiple cloning sites. This fragment was cloned, using the 6xHis-tag fusion protein, into the pRSETA expression vector, and its expression was performed in E. coli. An insoluble protein of approximately 41 kDa was purified from transformed E. coli lysates. The pellets were subjected to sonication and protein solubilizing was performed using 8M urea solution. Purification was performed by the affinity chromatography. The recombinant protein was detected by Western blot (WB) using monoclonal antibodies anti-histidine and anti-NS3. The recombinant protein was recognized by convalescent sera of calves experimentally infected with BVDV, but not by sera of animals immunized with inactivated vaccines. These results showed that BVDV NS3 expression can be performed on a large scale in E. coli, and its properties enable its use in immunoenzymatic assays for BVDV diagnosis.
CASE REPORT: DELAYED DIAGNOSIS OF DENGUE IN A PATIENT WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Resumo

Dengue, caused by dengue virus (DENV), is among the most important arboviral diseases worldwide, and in Paraguay, it represents a major public health problem. Studies indicate that patients with autoimmune diseases and acute dengue may present a complicated clinical picture. This report describes the case of a 43-year-old woman with a diagnosis of systemic lupus erythematosus (SLE) who presented with severe dengue in 2018. The patient was diagnosed with SLE in 2011 due to articular manifestations and Kikuchi syndrome, and she was on stable treatment with mycophenolate, hydroxychloroquine and low-dose prednisone. Ten days before presentation, the patient developed a subjective fever with arthralgias, myalgias and loose stools that improved with over-the-counter treatment. On the day of the presentation and admission to the hospital, the patient reported a high fever (40°C), headache, arthralgias, myalgias, nausea and vomiting. Laboratory tests revealed thrombocytopenia (128,000/uL) but negative results for DENV NS1 (rapid test) and anti-DENV IgM (capture ELISA). Two days later, testing for anti-Chikungunya virus IgM was performed and was negative. Due to high clinical suspicion, a diagnosis of dengue was again entertained. Testing was negative for DENV NS1 (rapid test) and DENV RNA by real-time RT-PCR. However, the patient had positive results for anti-DENV IgM and IgG (rapid test). After two days, the presence of anti-DENV IgM was confirmed by capture ELISA. A chest X-ray and computed tomography scan showed mild bilateral pulmonary infiltrates, bilateral pleural effusions and a small pericardial effusion, consistent with a diagnosis of severe dengue. One week after admission, the patient showed improvement in her symptoms and thrombocytopenia. There was no evidence of an exacerbation of her SLE symptoms during the acute illness. In the literature, cases have been described where the diagnosis of SLE was delayed due to diagnostic confusion created by an acute DENV infection. However, the opposite situation is described here, where the diagnosis of acute dengue was complicated by a preexisting diagnosis of SLE. The detection of specific antibodies could have been delayed by immunosuppressive treatment and initial symptoms may have been attributed to an SLE flare. This case reveals the importance of a high clinical suspicion and follow-up testing for dengue in patients with autoimmune diseases and those on immunosuppressive treatment.

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CORRELATION OF TRADITIONAL AND MOLECULAR METHODS TO QUANTIFY MEASLES VIRUS

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Resumo

Measles is a highly contagious viral disease, which affects susceptible individuals of all ages and remains a cause of death among young children globally. A recent increase in the number of measles cases in Americas has worried the Pan-American Health Organization. Vaccination campaigns have been conducted to combat this disease with MMR vaccine that also immunizes against rubella and mumps. The potency of vaccine viruses is determined using the conventional assays plaque forming unit (PFU) or median cell culture infective dose (CCID50). Although these tests are considered gold standard, they take a long time to be completed. Thus, the virological technology development laboratory of Bio-Manguinhos is committed to understanding the replicative profile of these viruses, to establish a faster method for viral titration. For this purpose, we compared qPCR and plaque assay methods. Measles strain Schwartz batch AMESBVA030 (6.28 Log10 CCID50) were cultured in Vero cells (65.000 cel/cm²) with 0.01 and 0.001 multiplicity of infection (MOI), in four independent experiments. Supernatants were harvested every 24 hours after incubation at 37 ° C with 5% CO2, for seven days. Virus titer was determined at different time points by plaque assay using Vero cell monolayers seeded in 24-well plates and by qPCR Taqman system designed to nucleoprotein, (690-877 genome position). Results of viral titer in PFU/mL and RNA copies/mL were plotted to 24 – 168 h, in order to compare viral fitness. The major grow peak was observed at 120 h and 144 h to plaque assay and qPCR respectively, for both MOI. Correlation between these methods using software R, revealed $r = 0.94$ and $r = 0.93$ to MOI 0.01 and 0.001 respectively. A difference of approximately 3.8 (± 0.5) Log10 was observed to results in RNA copies/mL to PFU/mL. The time point with the smaller difference between these units may define the best infection condition, where there are fewer defective particles. Previously, this comparative analyze was made for Yellow Fever vaccine virus (YFV) using qPCR method to evaluate viremia in clinical or preclinical trials. These results indicated that it is possible to use linear regression to estimate viral PFU/mL titers based on qPCR values, in order to implement new protocols.


Palavras-chaves: measles, vaccine, potency, qPCR, virus
ASSESSMENT OF THE CONTRIBUTION OF HIV PATIENT SERUM ANTIBODIES THAT RECOGNIZE LYMPHOCYTE ANTIGENS TO THE INHIBITION OF THE HIV-1 ENVELOPE-DEPENDENT MEMBRANE FUSION

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Resumo

Pathogenicity of HIV-1 strains is related to their efficiency to induce fusion of CD4+ T cells. Membrane fusion depends on the interaction of the HIV-envelope (Env) with CD4 and coreceptor molecules on human T lymphocytes. In addition to virus receptors, other adhesion/signaling molecules on infected and target cells and virus particles can enhance fusion. The presence of anti-lymphocyte autoantibodies (ALAs) in HIV patients' serum suggests their possible contribution to the inhibition of Env-mediated membrane fusion. We initially determined the binding of antibodies from the sera of 38 HIV-1 infected individuals, to human CD4+ Jurkat T cells. Seventy-four and 60% of sera were positive for binding of IgG and IgM to Jurkat cells, respectively. We evaluated the contribution of ALAs to the patient's serum activity against HIV-envelope dependent cell-cell fusion. The effect of sera on the fusion of CD4+ with Env+ Jurkat cells was tested before and after adsorption on CD4- negative Jurkat cells to remove ALAs. Analysis of the effect of sera on the fusion of CD4+ with HIV-1 Env-expressing Jurkat cells showed a variable degree of inhibition by serum samples. Inhibition of fusion decreased in 58% of serum samples after adsorption, indicating that ALAs contributed to fusion inhibition in these sera. Fusion increased in 31.6% and did not change in 10.5% of other serum samples after adsorption. The extent of the contribution of ALAs to the effect of sera on fusion was highly variable, with an average of 33%. Thus, fusion inhibitory ALAs other than anti-CD4 antibodies may contribute significantly to the inhibition of Env-mediated cell-cell fusion. Only detection of fusion inhibitory ALAs, but not total ALA levels, associated with the patients' low plasma viral loads, suggesting that they may participate in virus containment during HIV-1 infection. Fusion inhibitory ALAs may be relevant to the antiviral humoral immune response in a substantial fraction of HIV-infected patients.

Palavras-chaves: autoantibodies, membrane fusion, Lymphocyte, VIH-1

CHARACTERIZATION OF A DELIVERY SYSTEM BASED ON NANO-MULTILAMELLAR LIPID VESICLES (NMVs) FOR A FRAGMENT OF NS1 PROTEIN OF ZIKA VIRUS

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Resumo

Zika virus (ZIKV) is an arbovirus transmitted to humans by the bite of mosquitoes of the genus Aedes, but studies show its transmission also by blood transfusion, maternal-fetal and sexual transmission. Infection is associated with cases of microcephaly and Guillain-Barre syndrome and so far there are no effective treatments or vaccines available to the population, despite the critical demand for approaches capable of preventing the occurrence of infections, especially in endemic areas of Brazil. The search for safe vaccines capable of conferring protection is a priority, and in this sense we have developed and characterized a delivery system based on a unique combination of phospholipids, nano-multilayer lipid vesicles (NMVs). NMVs are composed of neutral and anionic lipids and are bilamellar in shape, allowing the incorporation of proteins in their hydrophilic portion, as well as the binding to histidine-tagged recombinant proteins, thus promoting the controlled release of the antigen. When combined with a fragment of the non-structural protein of ZIKV (ΔNS1-ZIKV), the resulting nanoparticle was analyzed regarding the physicochemical characteristics. The recombinant ΔNS1-ZIKV protein was expressed in prokaryotic system and obtained from the soluble fraction of the cell extract, yielding 5 mg/L of culture after purification and dialysis processes. The NMV-ΔNS1-ZIKV presented negative residual charge and approximate diameter of 180 nm. The percentage of protein release was analyzed in vitro at three different temperatures, as well as the percentage of protein incorporated. C57/BL6 mice were inoculated intramuscularly with NMV-ΔNS1-ZIKV and the biodistribution of these nanoparticles was observed. Our results demonstrated that the combination of NMVs to protein-based antigens may confer characteristics that can promote immunogenicity, and can be considered a promising strategy to immunize murine models.

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Palavras-chaves: Delivery System, Vaccine, NS1, ZIKV, Nanoparticles

AVIAN INFLUENZA VIRUS: THE ESTABLISHMENT OF AN INTERNATIONAL DIAGNOSIS STANDARD SYSTEM

Autores

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Resumo

Influenza A virus (IAV) infection is one of the largest public health problems worldwide. The establishment of a robust laboratory diagnostic system is essential to control outbreaks of avian influenza virus (AIV) in domestic bird populations. This study aimed to describe the AIV laboratory diagnosis in Brazilian avian species. A total of 2,230 samples were collected from January 2017 to September 2018 from different species and geographic regions. The diagnostic methods included virus isolation, reverse transcription polymerase chain reaction (RT-PCR) and real-time RT-PCR. The results showed that the main species affected were turkeys, chickens and quails, with detection rates of 1.93%, 0.48% and 0.27%, respectively. The virus isolation method had the highest sensitivity, while real-time RT-PCR had the highest specificity. The establishment of an international diagnosis standard system is crucial for the control and prevention of the spread of AIV in Brazil.
Avian Influenza virus (AIV) can cause one the most relevant poultry infirmity. The AIV transmission from birds to human becoming a threat of the influenza pandemic. The ease of AIV dissemination across the countries has required that an international standard diagnosis would be settled down. The Brazilian AI Reference Laboratory has established a cooperation with the World Organization for Animal Health to develop international standard reagents for AIV subtypes diagnosis consisting AIV Master Seed (MS), inactive AIV antigens, AIV hyperimmune sera all under high quality control. Firstly, the MS from NVSL and seventeen Brazilian AIV samples were replicated into allantoic cavity of chickens eggs (SPF). The positive allantoic amniotic fluid (AAF) harvested, cleared and distributed as MS for storage, at -80°C, and alive antigen was inactivated using β-propiolactone (0.05%). The AIV hyperimmune sera has performed by AIV antigen inoculation into 10-30 SPF White Leghorn chickens with two-three months of age, maintained in isolators 14 days in conditions BSL-3. The quality control of the AIV assays efficiency and products have included the sterility, titer-using HI by antiserum or HA by MS stock tests. The AIV subtype identification from H and N was performed using a panel of AI antisera (H1-H16), and (N1-N9) respectively. Seventeen of AI MS lots and fifteen of inactivated antigens lots produced reached HA titers of 1: 512 and 1: 256, respectively. However, AIV MS before and after inactivation process showed five viruses similarly HA/IA titer, two have dropped 1log2, four 2log2, one 3log2, and two 4log2 in the HA/AI titer. The HI titer of fifteen AIV antiseras lots reaching 1:4,096. The AIV booster of five subtypes have performed to enhance the antiseras titer. All antiser scars data submitted to Mantel-Haenszel chi-square test to correlate the quantities of antigens inoculated in each chicken and HI titer attained. However, it did not figure out a correlation about them (P=0.8888), suggesting the titers are independent of the amounts of antigen used. Finally, the establishment of an international system of diagnostic standards aimed at its expansion to South American countries should increase the production of all 16 AIV subtypes concerning of AIV genetic variation. The influenza reference reagents are produced successfully, but is necessary to increase the HA titer, consequently new lots for each AI subtypes will be produced. **Financial support**: MAPA

**Palavras-chaves**: Avian Influenza virus, reference reagent , subtypes, quality control, Brazil

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**EVALUATION OF EXPERIMENTAL VACCINE PLATFORM AGAINST Zika virus USING BOVINE SERUM ALBUMIN NANOPARTICLES**

**Autores**

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**Resumo**

The Zika virus (ZIKV) is a virus belonging to Flavivirus genus. It is currently considered an emerging virus that has caused epidemics on islands in the Pacific Ocean region and in several countries in the Americas. Associated with this increase in the number of reported cases, the possible association between this infection cases microcephaly and other neurological syndromes in Brazil (e.g., Guillain-Barré syndrome) has contributed to the establishment of an emergency state by the World Health Organization (WHO). Thus, finding an effective vaccine to prevent infections caused by ZIKV has become one of the main objectives of the WHO, since there are no vaccines for ZIKV in advanced stages of development. Nanoparticles containing associated antigens are being investigated in order to develop better vaccines. Preliminary studies made by our group have shown that bovine serum albumin nanoparticles (NPs) are promising vaccine delivery systems for Dengue virus and other
microbial antigens. This study aims to evaluate the ability of albumin nanoparticles associated with ZIKV antigens to induce the production of neutralizing antibodies that are able to combat and prevent ZIKV infection. Three formulations of NPs with different amounts of inactivated ZIKV were produced and they were characterized for size, charge, stability at different temperatures and morphology. In vitro assays were performed to evaluate the cytotoxicity of this formulation and also to measure the nanoparticle uptake by ZIKV permissive cells. Finally, animals were immunized with the nanoparticles and total anti-ZIKV IgG antibodies were detected by ELISA. The results indicate a stable nanometric platform of 251.7 ± 14.3 nm in diameter and -23.6 ± 6.5 mV of surface charge that presents an irregular spherical morphology. Viral genome detection on nanoparticles indicates the presence of ZIKV in the formulation. The nanoparticles are internalized by VERO cells, without any significant cytotoxicity. Immunized mice showed significant production of total anti-ZIKV IgG. However, the immunization was not able to prevent the development of morphometric changes in the offspring of the animals immunized after infection. Further studies should be performed to evaluate the neutralizing activity of these antibodies and to evaluate the potential protective effect of this vaccine platform in a murine model of microcephaly.

Palavras-chaves: Zika virus, bovine serum albumin, vaccine, nanoparticles

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**Pichia pastoris as a vaccinal platform for the Zika virus**

**Autores**

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**Resumo**

In view of the wide dissemination of the Zika virus worldwide and the severity of clinical manifestations related to infection, several studies have been developed to establish effective vaccine approaches for this virus. Yeasts such as *Pichia pastoris* have been used not only to produce heterologous proteins but also as carriers of vaccine antigens due to the immunostimulatory properties of components of their cell wall. Besides the intracellular carrying, it is possible to use recombinant protein anchorage systems on the yeast surface as a strategy to enhance the presentation of the antigens. The present work aims to develop a vaccine platform based on the production and anchoring of ZIKV proteins on the surface of *P. pastoris*. The expression cassettes used are composed of sets of epitopes relating to viral proteins E (Envelope) and NS1 (non-structural 1), predicted in silico as to their binding capacity to B cells, and MHC class I and II molecules. The genes were fused to the α-Agglutinin anchor protein gene, cloned into the expression vector and used to transform *P. pastoris*. The constructs obtained were used in the expression induction experiments. Dot blot, western blot, and immunofluorescence were performed to confirm the production of the proteins. After obtaining the recombinants, an initial analysis of the immune response profile induced by *P. pastoris* was performed *in vitro*. Recombinant and non-recombinant yeasts were incubated with splenocytes isolated from the BALB/c mice. The cytokines IL-10, IL-17, TNF-α, IFN-γ, IL-6 and IL-4 were measured by flow cytometry from culture supernatants collected at the time of 24h, 48h and 72h of culture. Non-recombinant *P. pastoris* induced an increase in IL-10 and TNF-α production, while recombinant yeasts exhibited, in addition, an increase in IL-4 and IL-6 expression. The increased production of the cytokines reported here is indicative of activation of immune system cells such as macrophages and T cells, proving the immunogenicity of the proposed vaccine constructs. In summary, these results indicate *P. pastoris* as an interesting system for production and delivery of the target antigens, besides presenting adjuvant activities, evidencing this platform as a promising vaccinal strategy to be tested *in vivo*.

Financial Support: CAPES, FACEPE.

**Palavras-chaves:** Cell surface display, ZIKV, Pichia pastoris, Vaccine
QUANTIFICATION OF NEWCASTLE DISEASE VIRUS IN VACCINE BY REAL-TIME QUANTITATIVE REVERSE TRANSCRIPTASE-PCR (qPCR) AND THE RELATION WITH TITRATION IN EMBRYONATED EGGS

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Resumo
The prophylaxis of Newcastle disease (ND) in Brazil is based on the active immunization using live vaccines and this procedure must be evaluated by the official quality control. The titration is required to verify the minimum quantity of the infectious virus in a vaccine’ dose. The Brazilian legislation required $10^{6.2}$ EID$_{50}$/dose of vaccine using the conventional titration, conducted in embryonated eggs. In this study, we examined qRT-PCR data of ND vaccine and compared the results with titration in embryonated eggs, determining if both methodologies for the evaluation of the number of virus in vaccine by dose are comparable. The Qiaamp viral RNA mini kit and the Quantinova Probe RT-PCR Kit (Qiagen) were used to perform the RNA extraction and qRT-PCR assays respectively. M+4100 5’primer, M-4220 3’primer and M+4169 probe, were used to amplify and detect a 121-bp fragment of the Matrix gene. For this, ten commercial vaccines against ND prepared using the strains C2,Clone 30, CL, B1, VH, Ulster and LaSota, from six main producers, manufactured in Brazil or imported, were tested using three batches of vaccines, according to the manufacturer instruction. Vaccines were submitted to titration, and an resuspended aliquot was harvested and keep at -80°C until extraction of viral RNA to perform the qPCR. The statistical analyses of the titer/dose of vaccines were obtained by three titration of each vaccine strains (a,b,c). Two runs were made by the fresh air method, and compared with the results obtained with qPCR. The relation between the titers obtained in embryonated eggs with the respective number of copies of DNA /dose obtained by qPCR (R) was analyzed by the hierarchical ANOVA that explained 69% of the total variation of the R's. The effects between runs by type of vaccine strain were similar, with the exception of the vaccine containing the strain LaSota-L5 and B1-L6. However, the means of this relationship showed that the titre in embryonated eggs was 56% in the number of viral particles/dose (with limits of 99.5% of confidence between 47% and 65%) and obtained a coefficient of variation of 6.3%, a standard deviation of 0.0353 and an expanded uncertainty of 0.0903. In conclusion, the titre/dose vaccines by the conventional method shows a relation of around 56% to the molecular method, it is suggested a risk analysis to use these molecular
Production, characterization, and immunological analysis of a HSV-1 nanoparticle vaccine in murine dendritic cells.

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**Resumo**

There are no effective vaccines for many human viruses, including Herpes Simplex Virus-1 (HSV-1). This virus can cause serious damage in neonates and immunocompromised individuals. The major immunodominant epitope of HSV-1, and which has great potential for vaccine development is the SSIEFARL, a peptide sequence from glycoprotein B, a viral envelope protein. This peptide is very unstable for direct application in vaccine formulations and is not well phagocytized by antigen presenting cells, such as dendritic cells. Polymeric nanocapsules (NCP) are candidates to produce optimized systems for delivery of small or unstable molecules to the immune system. The objective of this work is to produce NCP loaded with SSIEFARL (Nano/SSIEFARL), to evaluate the físico-chemical parameters, encapsulation releasing, as well as to determine Nano/SSIEFARL phagocytosis and activation in murine dendritic cells (JAWS II) in vitro. Empty NCP or FITC-labeled peptide SSIEFARL were produced by the interfacial polymerization technique. The physicochemical characterization was performed on Zetasizer® equipment, the release of FITC-labeled peptide SSIEFARL was performed by diffusion dialysis in saline. Analysis of Nano/SSIEFARL phagocytosis in JAWS II was analyzed by confocal microscopy. Cellular activation was performed by cytokine dosing through ELISA assays and by surface marker by flow cytometry. The NCP produced presented the polydispersity index 0.453; size 363.3 d.m.; zeta potential -28.3. The peptide was slowly released from the nanoparticles in 24-hour period. The confocal microscopy analysis demonstrated that after 3h of Jaws II 1x10^5 cells / mL with 100 µL of Nano/ SSIEFARL the nanoparticles were located in the cytoplasm. The cytokines IL-1β and TNF-α increased 5 fold and 2.5 fold, respectively, in Jaws II cultured with Nano/SSIEFARLS, compared to cells cultured with empty NCPs. These results indicated that Nano/SSIEFARL was efficiently produced, the peptide is maintained into the nanoparticle enough time to be phagocyted by dendritic cells and after phagocytosis, the cells produce inflammatory cytokines.

**Palavras-chaves:** nanoparticle, vaccine, HSV-1, SSIEFARL
Resumo

Introduction: Bovine papillomavirus (BPV) is the etiological agent of bovine papillomatosis, infectious disease characterized by the presence of benign tumors that can progress to malignancy. The phylogenetic classification of the PVs is performed based on the sequence homology of the Open Reading Frame L1, the most conserved among different viral serotypes. Given the importance of L1 protein and the immunogenicity of saponins, these emerge as a promising candidate as adjuvant for veterinary use. Objectives: This study aimed to evaluate the mutagenic and genotoxic potential of the isolated and purified protein as well its effects when associated with saponins and a comparison with the adjuvant widely used aluminum hydroxide. Methods: Genomic lesions, which after processed without repair can result in mutations, were detected by comet assay. Possible damages to genetic material caused by structural chromosomal changes (clastogenesis), as well as chromosomal losses (aneugenesis) were evaluated by the micronucleus test. Both tests were done on polychromatic erythrocytes and Vero cells. The evaluation of apoptosis and necrosis of treated Vero cells was made by Annexin V / PI staining and flow cytometry. Results and Discussion: The results with the two vaccine products (L1 + Saponin and L1 + Aluminum Hydroxide) showed damages compatible with the positive control in the comet assay and both slightly elevated the micronucleus levels, in the Cell Viability Assay the results with Aluminum Hydroxide were satisfactory, characterizing Aluminum Hydroxide as a safer adjuvant according to the proposed tests, better than the saponins.

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Palavras-chaves: vaccine, adjuvant, bovine papillomavirus, saponins, genotoxic tests

Quillaja brasiliensis saponins induce robust humoral responses in an experimental zika virus vaccine in mice

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Resumo

Zika virus (ZIKV) is an arbovirus that belongs to the Flaviviridae family, which includes dengue and yellow fever viruses. Recent ZIKV outbreaks have linked ZIKV to the development of severe fetal abnormalities that include microcephaly and Guillain-Barre' syndrome in adults. Over 1.5 million people were infected with ZIKV in Brazil in 2015 alone, and tens of millions more could be infected in the Americas in the coming years. Despite a number of research efforts, to date, there no approved vaccine available to prevent ZIKV infection. In the present study, the humoral immune response of mice immunized with an experimental ZIKV vaccine formulated with a natural saponin adjuvant was evaluated following inoculation by two routes of immunization. Two different adjuvants: QB-80, a
Saponin-enriched fraction extracted from *Quillaja brasiliensis*, and nanoparticulate QB-80 immunostimulating complexes (IMXQB-80) were evaluated and compared to a standard aluminum adjuvant. *Five week old* female C57BL/6J mice (n= 9/group) were subcutaneously immunized on days 0 and 14 with antigen plus either QB-80; IMXQB-80; aluminum or antigen without adjuvant. In addition, two groups were immunized by intravaginal route with either antigen plus QB-80 or only antigen. Mice sera were collected 14 and 28 days after the first immunization and antibodies were measured by ELISA. Subcutaneously inoculated QB-80 and IMXQB-80 induced strong serum antibody responses encompassing specific anti-Zika IgM, IgG, IgG1, IgG2b and IgG2c. However, the intravaginally delivered antigen adjuvanted with QB-80 seemed not to stimulate antibody production. The IMXQB-80 stimulated an immune response to levels comparable to those attained with the QB-80 adjuvanted vaccine, despite containing only a quarter of antigen included in the former. These findings reveal that QB-80 is an adjuvant capable of stimulating a strong humoral immune response in immunized mice. The intravaginal route was not as effective as the other evaluated routes in stimulating immune responses with the preparation containing QB-80 as adjuvant.

**Palavras-chaves:** adjuvant, immunization, immune response

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**Antiviral activity of Podalia hemolymph against Rubella virus**

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**Resumo**

Viral infections have become a serious public health problem leading to death of thousands of people annually. For this reason, the search for new antiviral agents has become an urgent necessity. Therefore, bioprospecting from insect exudates has become an increasingly frequent option. The present work aimed to identify, isolate, and chemically characterize molecules with antiviral activity against the Rubella virus in the hemolymph of *Podalia* sp, caterpillars of the *Megalopygidae* family (Lepidoptera:). Rubella virus (genus *Rubivirus*, family *Togaviridae*) is a single-stranded, positive sense, RNA virus that may cause fetuses injuries, mainly when susceptible women are infected during the first trimester of pregnancy. Potent antiviral activity against rubella virus (RV) has been observed in the hemolymph of *Podalia* sp. Furthermore, we evaluated the effect of hemolymph on Rabbit Cornea (SIRC) cells infected with rubella virus. The results of both cell viability and cell proliferation assays indicated that hemolymph was not toxic to cultured SIRC cells. Viral binding assay, antiviral assay, PCR, real-time PCR, and transmission electron microscopy were used to demonstrate that hemolymph in posttreatment could inhibit the production of infectious RV particles.

**Palavras-chaves:** antiviral activity, Congenital Rubella Syndrome, Hemolymph, Megalopygidae, Rubella virus
DESIGN AND EVALUATION OF RECOMBINANT CHIKUNGUNYA VIRUS E1 AND E2 ENVELOPE PROTEINS FOR SERODIAGNOSIS

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Resumo
Chikungunya (CHIKV), an arbovirus that belongs to the Alphavirus genus of the Togaviridae family, causes a disease characterized by acute onset of fever accompanied by arthralgia. CHIKV also has been associated with cases of meningoencephalitis (primarily in neonates), Guillain–Barré syndrome and hemorrhagic disease. Similar to Dengue virus (DENV) and Zika virus (ZIKV), CHIKV is also transmitted to humans mainly by Aedes (Ae.) aegypti and Ae.albopictus mosquitoes. The clinical similarities, cross-reactivity and cocirculation of these arboviruses in Brazil have complicated their differentiation, highlighting the need for new diagnostic tools. A serologic test can be useful for acute detections as well as for surveillance and epidemiological studies. Two envelope proteins of CHIKV (E1 and E2) have been shown to be immunodominant and associated with the generation of antibodies, thus, are strategically important for the serological diagnosis of this infection. The aim of this work was to design synthetic genes coding for E1 and E2 proteins of CHIKV to be used as antigens in diagnostic assays. The nucleotide coding sequences of CHIKV-E1 and CHIKV-E2 were analyzed in silico, commercially synthesized, cloned in pET-21 and expressed in Escherichia coli BL21 (DE3). Computational methods were used to predict the structure and antigenic potential of the recombinant proteins. The overall antigenic prediction score for E1 and E2 in VAXIJEN_v2.0 were 0.50 and 0.53 (probable antigen) respectively. To confirm predictions, recombinant proteins were purified by affinity chromatography and evaluated for their antigenic potential. Antigenicity of the proteins was initially confirmed by western-blot using sera from CHIKV infected infected mice. Additionally, the seroreactivity of recombinant CHIKV E1 and E2 envelope proteins was evaluated using a panel of sera samples from human patients, CHIKV seropositive or not, by indirect IgG ELISA. The recombinant CHIKV E1 and E2 envelope proteins showed sensitivity of 95% and 82%, and specificity of 76% and 100%, respectively. In conclusion, the results indicated that these proteins maybe useful antigens to detect CHIKV infection.

Financial Support: CNPQ and CAPES

Palavras-chaves: Chikungunya, ELISA, diagnosis, arbovirus, recombinant protein

Recombinant Influenza viruses encoding a murine cytokine as tools to evaluate the role of that cytokine during influenza virus infection.

Autores
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Resumo
It is known that cytokines, such as IL-12, IFN-γ, IL-4, IL-10, TNF-a, IL-17 and IL-6 play important roles during influenza A virus infection, either improving or worsening its outcome. However, their role is still far from being completely elucidated. Recombinant influenza viruses are interesting platforms to deliver heterologous molecules. Therefore, aiming to better understand the role of an inflammatory cytokine during the infection with influenza A virus, we used reverse genetics techniques to generate two defective influenza viruses. The first one encodes the inflammatory cytokine (herein named X). The second one encodes an unrelated (control: CT) sequence. FLU-X has been found genetically stable and able to express the heterologous sequence in cell cultures as well as in lungs of infected mice, at different time-points after infection and seems to be harmless to mice. Remarkably, when compared to the group inoculated with FLU-CT, the mice inoculated with FLU-X displayed reduced mortality when challenged with an influenza virus of another subtype. Taking together, our preliminary results suggest that recombinant influenza viruses encoding cytokines are useful tools to study the role of those proteins during influenza infection. Likewise, it could represent a novel strategy to improvement of the immunological response to influenza vaccination. Financial Support: CAPES, CNPq, FAPEMIG e INCTV.

**Palavras-chaves:** cytokine, Influenza, recombinant flu, reverse genetics, virus infection

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**Immunodominant T-cell epitopes in mice inoculated with DNA vaccines based on the dengue E and NS1 proteins**

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**Resumo**

Dengue fever is an arthropod-borne viral disease that poses a major public health threat to tropical and subtropical countries worldwide. Its etiological agent, the dengue virus (DENV), is comprised by four antigenic serotypes (DENV1-4), and its viral genome encodes three structural (capsid, membrane and envelope) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). Induction of neutralizing antibodies has been traditionally associated with protection, but the importance of the cellular immune response against the virus has been increasingly highlighted in the recent years. Our group has constructed two DNA vaccines against DENV2 based on the envelope (E) and the NS1 proteins, identified as plasmids pE1D2 and pCTANS1, respectively. Both plasmids were able to induce protection in BALB/c mice challenged with DENV2 by eliciting both humoral and cellular immune responses. In the present work, we investigate the immunodominant epitopes located in the E and NS1 proteins that are recognized by CD8+ or CD4+ T cells in mice immunized with these DNA vaccines. Splenocytes isolated from immunized mice were stimulated with two peptide libraries (containing 98 and 86 peptides for E and NS1 proteins, respectively) and production of IFN-γ was evaluated by ELISPOT assay. A total of 4 peptides in the E protein and 3 peptides in the NS1 were identified after immunization of BALB/c mice. The cellular immune response was also evaluated after DENV2 challenge, revealing further 3 and 4 epitopes recognized by T cells in animals vaccinated with pE1D2 or pCTANS1, respectively. In addition, recognition of E and NS1 immunodominant epitopes was also assessed in C57BL/6 mice in regard to their different MHC haplotype expression. Different epitopes were recognized by C57BL/6 isolated cells using the same peptide libraries, although one NS1-derived peptide was strongly positive in the assays performed with cells from both animal strains. Overall, we identified relevant E and NS1 T-cell epitopes recognized in immunized-BALB/c mice involved with the production of IFN-γ elicited by the pE1D2 and pCTANS1 vaccines, revealing different immunodominance patterns before and after DENV2 challenge. By comparing these results with those obtained from C57BL/6-immunized mice we were also able to evaluate whether the identified epitopes were MHC-restricted.

Financial Support: PAPES-Fiocruz, IOC-Fiocruz, CNPq, INCTV, FAPERJ

**Palavras-chaves:** Dengue, T-cell epitopes, DNA vaccine, envelope protein, NS1 protein

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**Immunodominant T-cell epitopes in mice inoculated with DNA vaccines based on the dengue E and NS1 proteins**

Maysa Leandro de Assis 1, Paolla Beatriz Almeida Pinto 1, Agatha Resende Pacheco 1, Lauro Lima Miranda 1, Katia Regina F. L. Quaresma 1, Simone Morais da Costa 1, Ada Maria Barcelos Alves 1

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**Resumo**

Dengue fever is an arthropod-borne viral disease that poses a major public health threat to tropical and subtropical countries worldwide. Its etiological agent, the dengue virus (DENV), is comprised by four antigenic serotypes (DENV1-4), and its viral genome encodes three structural (capsid, membrane and envelope) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). Induction of neutralizing antibodies has been traditionally associated with protection, but the importance of the cellular immune response against the virus has been increasingly highlighted in the recent years. Our group has constructed two DNA vaccines against DENV2 based on the envelope (E) and the NS1 proteins, identified as plasmids pE1D2 and pCTANS1, respectively. Both plasmids were able to induce protection in BALB/c mice challenged with DENV2 by eliciting both humoral and cellular immune responses. In the present work, we investigate the immunodominant epitopes located in the E and NS1 proteins that are recognized by CD8+ or CD4+ T cells in mice immunized with these DNA vaccines. Splenocytes isolated from immunized mice were stimulated with two peptide libraries (containing 98 and 86 peptides for E and NS1 proteins, respectively) and production of IFN-γ was evaluated by ELISPOT assay. A total of 4 peptides in the E protein and 3 peptides in the NS1 were identified after immunization of BALB/c mice. The cellular immune response was also evaluated after DENV2 challenge, revealing further 3 and 4 epitopes recognized by T cells in animals vaccinated with pE1D2 or pCTANS1, respectively. In addition, recognition of E and NS1 immunodominant epitopes was also assessed in C57BL/6 mice in regard to their different MHC haplotype expression. Different epitopes were recognized by C57BL/6 isolated cells using the same peptide libraries, although one NS1-derived peptide was strongly positive in the assays performed with cells from both animal strains. Overall, we identified relevant E and NS1 T-cell epitopes recognized in immunized-BALB/c mice involved with the production of IFN-γ elicited by the pE1D2 and pCTANS1 vaccines, revealing different immunodominance patterns before and after DENV2 challenge. By comparing these results with those obtained from C57BL/6-immunized mice we were also able to evaluate whether the identified epitopes were MHC-restricted.

Financial Support: PAPES-Fiocruz, IOC-Fiocruz, CNPq, INCTV, FAPERJ

**Palavras-chaves:** Dengue, T-cell epitopes, DNA vaccine, envelope protein, NS1 protein
ZIKA VIRUS NON-STRUCTURAL RECOMBINANT PROTEIN 1 (NS1 ZIKV) EXPRESSION, PURIFICATION AND EVALUATION OF VACCINAL POTENTIAL.

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Resumo

Zika virus (ZIKV) is an arbovirus that belongs to the Flavivirus genus and is transmitted to humans by Aedes mosquito bites. ZIKV has been responsible for outbreaks in several countries, including Brazil. The disease caused by ZIKV is usually acute and self-limited, however, some cases may lead to more severe disease forms, such as microcephaly and Guillaum-Barré syndrome. Several studies are underway to develop alternatives to prevent the infection by ZIKV, since there are no current commercial vaccines available. In this context, the search of antigens with potential to induce protective immunity is fundamental. Thus, the present study aimed to express and purify the ZIKV non-structural protein 1 (NS1 ZIKV) in a prokaryotic system, with preserved antigenicity and immunogenicity aiming its use as an antigen in murine model. The expression of NS1 ZIKV protein was performed on recombinant E. coli BL21 (DE3)-RIL strain followed by its purification by nickel affinity chromatography. NS1 ZIKV recombinant protein was expressed only in the insoluble pellet fraction of the bacterial lysate and was submitted to an optimized renaturation stage (refolding). The refolded protein was tested on different buffers (HEPES and sodium phosphate) and protein stability was evaluated after the dialysis. The final protein yield was approximately 1 mg per liter of culture. The antigenicity of the recombinant protein was measured in ELISA after testing the protein reactivity with antibodies generated after ZIKV infection. NS1 ZIKV protein immunogenicity was evaluated after immunization of C57BL/6 and AG129 mice (three subcutaneous doses), associated or not with two different adjuvants: Poly (I: C) and LT-B adjuvants. In both mouse lines, high IgG antigen-specific antibody responses were achieved particularly with the C57BL/6 mice. Thus, the recombinant protein was obtained with good stability and preserved its antigenicity and immunogenicity. After the optimization process, we concluded that the recombinant protein had the required quality of a model antigen for vaccine studies of potential ZIKV vaccines based on non-structural recombinant proteins.

Financial support: FAPESP, CAPES.

Palavras-chaves: FLAVIVIRUS, NS1 PROTEIN, ZIKA, VACCINE, RECOMBINANT PROTEIN

SEED TREATMENTS ON WHEAT STRIPE MOSAIC VIRUS MANAGEMENT AND MOLECULAR CHARACTERIZATION OF PLASMODEIOPHORID VECTOR

Autores  Fernando Sartori Pereira 1, Lucas Antonio Stempkowski 1, Juliana Borba Valente 1, Monica Farias 1, Matheus Correa Borba 1, Douglas Lau 2, Paulo Kuhnem 3, Ricardo Trezzi Casa 1, Fábio Nascimento da Silva 1
Instituição 1 UDESC - Universidade do Estado de Santa Catarina (Lages/SC), 2 Embrapa Trigo - Empresa
The Wheat stripe mosaic virus (WhSMV) is a new characterized viral species associated with soil-borne wheat mosaic disease (SBWMD) in Brazil. The SBWMD management is difficulty because the plasmodiophorid vector of the virus forms resistance spores and remains viable in the soil for several years. The aim of this study were test seed treatments for vector control and plant development in the field with a history of this disease and characterize molecularly the plasmodiophorid associated with wheat samples infected by WhSMV. The trial was conducted in the field during the winter in 2017 season, in Passo Fundo, Rio Grande do Sul State, southern Brazil. The experimental design was a randomized block, with five replications and two cultivars (TBIO Toruk and BRS Guamirim). Thirteen seed treatments were used, including seeds treated with water + polymer (control); *Bacillus subtilis*; *Bacillus amyloliquefaciens*; *Trichoderma asperellum*; acibenzolar-s-methyl; azoxystrobin; pyraclostrobin + thiophanate methyl + Fipronil; thiophanate methyl + fluazinam; phenamidone; fludioxonil + metalaxyl + thiabendazole + azoxystrobin; fluxapyroxade; metalaxyl; and dimetomorph. The effectiveness of seed treatment was evaluated by incidence of SBWMD at wheat flowering stage and grain yield. Azoxystrobin showed the lowest incidence, when compared with control, other fungicides, biological control agents and resistance inducer. Seed treatment using thiophanate methyl + fluazinam showed higher grain yield. Wheat roots of the cultivar TBIO Toruk and BRS Guamirim were collected and the molecular characterization of partial nuclear 5.8S and internal transcribed spacer 1 (ITS 1) of the *P. graminis* ribosomal DNA sequences was performed. Additionally, the presence of WhSMV was confirmed by RT-PCR using specific primers and sequencing. Nucleotide identities and phylogenetic analysis supported the classification of plasmodiophorid found in the wheat roots as *P. graminis*. This is the first molecular characterization of *P. graminis* in Brazil. The results presented here indicated the association of *P. graminis* with roots of wheat plants infected by WhSMV in Brasil and suggests that the new virus WhSMV could be transmitted by plasmodiophorid. Financial Support: EMBRAPA, UDESC, CAPES.

Palavras-chaves: Triticum aestivum, Polymyxa graminis, Virus, Incidence, Detection

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**EVOLUTIONARY DYNAMICS OF BIPARTITE BEGOMOVIRUSES: ONE GENOME, TWO HISTORIES**

**Autores**
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**Resumo**
An intriguing aspect of virus evolution is the emergence of viruses with segmented genomes. A special case of genome segregation are viruses which have their genomes segments packed into separate
particles. Given the trade-offs between functional complementation and independence among the distinct components in viruses with divided genomes, it would be expected that the different components would be in an intimate process of co-evolution. The genus **Begomovirus** (family *Geminiviridae*) is comprised of viral species with one or two genomic components of circular, ssDNA. Thus, begomoviruses include viruses with non-segmented as well as divided (bipartite) genomes. The bipartite nature of these viruses has been little explored in evolutionary studies, and little is known about the evolutionary dynamics of the different components. Using a population genetics approach, we performed a parallel evolutionary analysis of the two components (DNA-A and DNA-B) of five well sampled begomoviruses that infect cultivated and non-cultivated plants. Our results demonstrate that the DNA-B, as well as the DNA-A, segregates based on geographical origin. In most datasets analyzed, the DNA-B was more variable than the DNA-A. The exception was *Macroptilium yellow spot virus* (MaYSV), for which the DNA-A was more variable than the DNA-B due to a recombination event at the interface between the Rep gene 5' region and the intergenic region. The DNA-B was more prone to recombination than the DNA-A, with a higher number of events. Interestingly, we detected small ORFs in the complementary-sense strand of the DNA-B of several MaYSV isolates. These ORFs are homologous to the Rep gene located in the DNA-A, indicating occurrence of intercomponent recombination events. Together, our results indicate the two components evolve under different selection pressures, and thus display distinct evolutionary histories. The higher degree of genetic variability of the DNA-B may reflect weaker selection pressures due to the fact the functions encoded by its proteins can, to some extent, be provided by the proteins encoded by the DNA-A.

Financial Support: CAPES, CNPq and FAPEMIG.

**Palavras-chaves:** geminivirus, begomovirus, molecular evolution, reassortment, recombination

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**CULTURAL PRACTICES EFFECTS ON A SOIL-BORNE WHEAT MOSAIC DISEASE IN SOUTHERN BRAZIL**

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**Resumo**

There are several diseases affecting wheat crops, including viral diseases such as the Soil-borne wheat mosaic disease (SBWMD), responsible for damages that can reach up to 85%. The viruses associated with SBWMD are transmitted by *Polymyxa graminis*, a soil microorganism known to infect grasses roots. The use of resistant cultivars is the main management strategy recommended for this disease. However, in Brazil, there are no virus-immune cultivars and given the constant evolution of plant viruses, genetic-only management becomes uncertain in the long run. In this way, we investigated the effect of nitrogen (N) fertilization and crop rotation on SBWMD incidence and wheat grain yield in two different field experiments conducted at Embrapa Wheat in 2017. Wheat cultivars Embrapa 16 (resistant) and BRS Guamirim (susceptible) were submitted to four nitrogen doses in the form of urea (0, 30, 60 and 90 kg ha⁻¹) divided into two ontogenetic stages: double-ring and terminal
As environmental conditions influence viral infection, the experiment was sown in three periods. The study of the effects of crop rotation was performed in an experiment established in 1980 by Embrapa Wheat. The crop rotation schemes were monoculture of wheat, oats-wheat, vetch-oats-wheat and oats-barley-oats-turnip-wheat. Parcels (120 m²) were divided into three sub-plots, and sown with BRS Parrudo (resistant), BRS Reponte (moderately susceptible) and BRS Guamirim (susceptible) cultivars. Experimental areas has a history of the presence of the vector and the virus and was confirmed by molecular test. The effect of N on the incidence of SBWMD was not evident. The incidence ranged from 0.5 to 17% for cultivar Embrapa 16 and 1 to 95% for cultivar BRS Guamirim. Productivity for cultivar BRS Guamirim was positively influenced by N doses when the incidence were below the 50% threshold. The sowing period with higher rainfall showed higher incidence of the virus. The incidence in the crop rotation experiment was very low (0-0.12%). In wheat monoculture, the incidence was significantly higher in relation to crop rotation, although it showed a weak correlation with grain yield (-0.37) for BRS Reponte cultivar. Cultural practices may be used as complementary tools for managing soil-borne viruses in wheat in southern Brazil. Financial Support: EMBRAPA, UDESC, CAPES.

Palavras-chaves: Polymyxa graminis, Crop rotation, Fertilization, Grain yield

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Deforming mosaic disease caused by Begomovirus species (Family Geminiviridae) transmitted by whitefly (Bemisia tabaci biotype B) is an emerging disease affecting potato crop (Solanum tuberosum L.) that has been more often detected over the last ten years in Brazil. Despite of been detected at low incidence, table potatoes and potatoes for the processing industry of different varieties, 45-50 days after planting of tubers and showing yellow mosaic, and leaf deformation symptoms. High whitefly populations were observed in the production fields considered for sampling. Leaf samples were submitted to total DNA extraction using CTAB method, Rolling Circle Amplification (RCA) with Phi-29 DNA Polymerase, followed by polymerase chain reaction (PCR)-based methods for begomovirus detection. The first PCR was performed using degenerate primers (PAL1v1978/PAR1c496; Plant Dis. 77:340-347, 1993) that amplify a 1.1 kbp fragment of DNA-A component. The second PCR was done with ToSRV-specific primers (Phytopathol. 103:436-444, 2013) that amplify 820 pb amplicon also of the DNA-A component. Cloning and sequencing of 15 isolates was performed. Begomovirus detection occurred in 150 samples out of 200 [DF: 89; Goiás: 61 (Cristalina-20; Luziânia-41)] using the universal primers indicating that begomovirus presence is associated with leaf deformation and yellow mosaic symptoms observed in diseased plants in the field. ToSRV-detection was also identified in the same number of samples. 150. Sequencing data confirmed data obtained from PCR using specific primers, and ToSRV was the only Begomovirus species identified in all 15 isolates sequenced. These data indicate the prevalence of this Begomovirus species infecting potato in the Central region of Brazil.

Palavras-chaves: Begomovirus, potato, detection, PCR, ToSRV

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Palavras-chaves: Begomovirus, potato, detection, PCR, ToSRV
Resumo

PPWSP aims to act on introducing an early and precautionous conscience toward protecting Brazil's agriculture from within or outside movement of exotic-quarantine plant viruses. It was motivated by learning from economic-social-environmental damages due to introduction, establishment, and spread of quarantine potato viruses (*Solanum tuberosum*) on seed-potato production (ex.: *Potato virus Y* - PVY, from Canadian imported seed-potato lots, turning unmarketable a then major potato cvs: 'Monalisa'). Considering these matter as a need of "hygiene" practices pro agriculture, the PPWSP methodology focus on teaching plant health science while building hygiene conscience about the danger of virus spreading via movement of not certified (sanitary unknown) plant propagating materials: seed, cuttings, stem, tubers, sprouts etc. Students (9-11yrs), at fundamental schools, are guided to put "hands and minds" on basic plant health science concepts. Potato sprouts detached from seed-potato tubers, pre-identified as healthy or perpetuating common virus (PVY and/or *Potato leafroll virus* - PLRV), are used as "seed" (propagating material). Each student watch (take notes) of two or four plants growing healthy x one virus infected. As potato are only 80-day life cycle (allowing 2 PPWSP/year) and the viruses causes fast and easy visible symptoms, each students is taught to observe, compare and measure plant germination (growth) foliage/tuber development, as well recognize (draw) diseased plants and insects (aphids, whiteflies, etc) as virus transmitters (vector). PPWSP activities are performed in scientific-like greenhouse; currently it has been carried out successfully in two municipal schools in the cities of Limeira and Cordeirópolis (SP). In conclusion, we consider that PPWSP plays a fundamental contribution toward plant protection, pro Brazilian agriculture, by building an early plant protection conscience on preventing plant virus movements such as human brought into home garden and winged insect vectors taking into farm plantations. It is considered an innovative, precocious, learning to be carried-applied-spread for a life span; perpetuated from child to adulthood. We hope the PPWSP will continue to work along these lines on the basis of a precautious and science-based approach. Lets spread the PPWSP to fundamental schools for the sake of a continued Brazilian agro-prosperity. (FUNDAG/MICROGEO, GRUPO TREVISAN supported).

Palavras-chaves: Fundamental Plant Protection, Potato Viruses, Solanum tuberosum, Potato virus Y (PVY), Science Municipal Schools

Genetic analyses of a new alphabaculovirus isolated from the winter pest Mythimna sequax
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Resumo

The Baculoviridae is a viral family that comprises viruses with double-stranded supercoiled DNA. The genome size varies between 80-180 kbp with 90-180 genes; from those, only 38 were identified in all sequenced genomes. Baculoviruses are insects-specific pathogens and largely used for biological control and substitute efficiently agrochemicals. Currently, the family is divided into four genera, Alpha- and Betabaculovirus are infectious to lepidopterans, Gammabaculovirus are infectious to hymenopterans, and Deltabaculovirus are infectious to dipterans. In a previous work, we characterized the genome of a new baculovirus isolated from the winter pest Mythimna sequax. This insect causes impact on rice and wheat crops during the larval stage. The virus was tentatively named by Mythimna sequax nucleopolyhedrovirus (MyseNPV). The complete genome was found to present 148.403 bp, G+C content of 40.3%, 169 genes with 13 being unique to baculoviruses. Moreover, the virus was classified into Alphabaculovirus and was closely related to other seven noctuid-infesting baculoviruses including viruses isolated from Mamestra spp. and Helicoverpa armigera. In this work, we aimed to expand the understanding of this new virus by means of carrying out other in silico analyses. Based on the partial sequence of lef-9, lef-8, and polyhedrin, we found that MyseNPV fulfill all the criteria to be considered a new species. The cluster formed by the seven viruses plus MyseNPV contains only three true species, which are MacoNPV-A, MacoNPV-B, and MyseNPV. The other five viruses are considered only isolates. We also performed a selection analysis for each gene shared by the MyseNPV-related viruses through an individual dN/dS analysis. We found a total of 221 different genes with only 152 shared by the eight genomes. Solely two genes are suffering positive selection in this group, hoar and MyseNPV-127, which are considered not essential genes. The other genes were under negative pressure with fp25k, p28, cathepsin, and vlf-1 being the most pressured. Overall, MyseNPV is indeed a new species of baculovirus, and the selection analysis showed that most of the genes are under purifying pressure, which suggests a high level of gene conservation. This is of importance for a better understanding of the genetic evolution of baculovirus and may points out clues to understand virus diversification during evolution.

Financial support: CNPq, CAPES, UFSM, FAPDF

Palavras-chaves: baculovirus, MyseNPV, genome, virus diversification, selection analysis

Molecular and ultrastructural analyses of Biston suppressaria nucleopolyhedrovirus (BisuNPV), a new viral isolate that infects the tea looper caterpillar

Autors  Lucas Boeni de Oliveira ¹, Daniel Ricardo Sosa-Gomez ², Bergmann Morais Ribeiro ³, Daniel Mendes Pereira Ardisson de Araújo ¹


Resumo

The Baculoviridae is a viral family with double-stranded DNA varying from 80 to 180 kbp. The viruses
are orally infectious to insects and an interesting alternative as biological control agents of agriculture. Baculoviruses are currently divided into four genera, one of them is *Alphabaculovirus* that infects lepidopterans such as *Biston suppressaria* (Guinée, 1858; synonymous of *Buzura suppressaria*). This insect is polyphagous and causes damage to tea crops in India, China, and Nepal. In this work, we sequenced the complete genome and characterized the ultrastructure of a new viral isolate from *B. suppressaria*, and evaluated the main differences considering the novel genome and other two previously sequenced Chinese isolates (Hubei and Guangxi). The sample was provided by Embrapa-Soja and the information such as date and place of collection is unknown. We used the 454 pyrosequencing method to sequencing the viral DNA and the obtained reads were mapped to a reference genome (isolate Hubei) using the software Geneious R.9. The genome was annotated based on the other genomes by full genome alignment and gene comparison. The new genome contained about 121 kbp (G+C of 36.7%) with 132 ORFs with more than 150 bp. The nucleotide identities based on the core genes alignments using the new isolate and both Guangxi and Hubei were 99.5 and 99% respectively. Moreover, the new isolate was found to be closer to the ancestor of Hubei and Guangxi. Transmission electron microscopy analyses of the BisuNPV occlusion bodies revealed the presence of single nucleocapsid within each occluded virion. We performed a full genome MAFFT alignment to search for intragenic variants among the isolates, setting the novel genome as reference. 132 ORFs were found considering the three complete genomes; 92 ORFs presented at least one nucleotide variation with a total of 363 single nucleotide variants (SNVs). 145 SNVs were found to be non-synonymous and 218 synonymous. Moreover, P74 (PIF-0) presented the highest number of SNVs. P74 is a *per os infectivity factor* (*pif*) required for primary infection of the insect midgut epithelium cells and conserved in all baculovirus genomes sequenced to date. Overall, the characterization of new baculovirus isolates is of importance to the field since it allows for a better understanding of baculovirus evolution and diversity.

**Financial support:** CNPq, UFSM, Capes

**Palavras-chaves:** baculovirus, Biston suppressaria, genomics, evolution, single nucleotide variants

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**DESCRIPTION OF TWO NEW NONCULTIVATED HOSTS OF BEGOMOVIROSES**

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**Resumo**

Begomoviruses are part of the Geminivirus family of single-stranded DNA viruses, with members of economic importance to crops such as tomato and beans. *Begomovirus* is a genus of one or two ssDNA components, with twined icosahedral particles, transmitted by the whitefly *Bemisia tabaci*. There are more than 14 begomoviruses described infecting tomatoes in Brazil. In common beans, the begomovirus *Bean golden mosaic virus* (BGMV) is responsible for losses that can reach up to 100% under field conditions. Although noncultivated plants are not important economically, they may serve as sources of inoculum when they harbor a virus of an economically important host. Besides this, the noncultivated plants are considered as a source of high mutation and recombination rates. Two noncultivated and unrelated plant species were collected, *Neonotonia wightii* and *Parthenium hysterophorus*, displaying characteristic symptoms of infection by *Geminivirus*, in Santo Antonio de Goiás-GO, Brazil. To examine the possible presence of geminiviruses as the cause of the symptoms, total DNA of the plants was extracted by the CTAB (4%) method, and carried out specific polymerase chain reaction to verify the presence of Geminivirus. PCR fragments compatible with the expected masses were obtained.
with degenerated primers described in the literature (Rojas, 1993), specific for Geminivirus. These PCR fragments were cloned using the commercial vectors pGEM T-easy (Promega) and CloneJet (Termofisher). The recombinant vectors were used to transform Escherichia coli by heat shock. Selected clones, with the expected size fragments, were subject to sequencing by the dideoxinucleotide chain terminator technique at Macrogen, South Korea. The sequences obtained were analyzed for quality and submitted to BLASTx on NCBI. Both hosts were infected by geminiviruses as observed by the high identity to known geminiviruses. N. wightii was infected by BGMV and P. hysterophorus was infected by Euphorbia Yellow Mosaic Virus (EuYMV), described in Euphorbia spp. These plants have never been described hosting these viruses. No one begomovirus have been described in N. wightii until now, but in P. hysterophorus it was described an infection by a begomovirus in India. It's known that EuYMV populations have high degree of genetic variability unlike BGMV that has low genetic variability. In spite of this characteristics, the BGMV has been associated with higher losses than EuYMV.

Financial Support: Embrapa.

Palavras-chaves: Geminivirus, Weeds, Plant viruses

AN ALPHASATELLITE INTERACTS WITH BIPARTITE BEGOMOVIRUSES AND INCREASES SYMPTOM SEVERITY IN TOMATO PLANTS

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Resumo

Begomoviruses (family Geminiviridae) are whitefly-transmitted viruses with circular single-stranded DNA genomes that are frequently associated with DNA satellites. Alphasatellites are circular, single stranded DNA molecules, capable of autonomous replication, but dependent on a helper begomovirus for cell-to-cell and systemic movement in the plant and for insect-mediated plant-to-plant transmission. Begomoviruses constitute a serious constraint to crop production worldwide. The presence of Euphorbia yellow mosaic alphasatellite (EuYMA) increases the severity of symptoms induced by Euphorbia yellow mosaic virus (EuYMV) in Euphorbia heterophylla, Nicotiana benthamiana and Arabidopsis thaliana. The present work investigates whether EuYMA is capable of interacting with two tomato-infecting begomoviruses, Tomato yellow spot virus (ToYSV) and Tomato severe rugose virus (ToSRV) in tomato and N. benthamiana. The plants were biolistically inoculated with infectious clones of each virus and of the alphasatellite in different combinations. Infection was confirmed by PCR with specific primers 21 days after inoculation. Symptoms of ToYSV and ToSRV in the presence or absence of the alphasatellite were evaluated weekly. Interaction between the two begomoviruses and EuYMA was demonstrated by detection of ToYSV, ToSRV and EuYMA in apical leaves of both tomato and N. benthamiana plants. This indicates that begomovirus-mediated systemic movement of EuYMA occurs in these hosts. Furthermore, the presence of the alphasatellite increased the severity of symptoms induced by ToYSV and ToSRV in both hosts. However, the association of EuYMA with the two begomoviruses was less efficient in tomato than in N. benthamiana (as measured by a lower percentage of plants in which the presence of the alphasatellite was detected). This could be due to difference in DNA accumulation of the satellite in each host, or to less efficient movement of the satellite in tomato compared to N. benthamiana. Together, our results suggest that the association of
EuYMA with begomoviruses is not species-specific. Further studies are needed to determine the relevance of alphasatellites in natural infections by bipartite begomoviruses.

Financial Support: This work was funded by grants from CNPq and FAPEMIG.

Palavras-chaves: geminivirus, Euphorbia, DNA satellite, EuYMA, ToSRV

MIXED INFECTION OF CUCURBIT APHID-BORNE YELLOWS VIRUS AND COWPEA APHID-BORNE MOSAIC VIRUS IN PASSIFLORA SPP IN BRAZIL

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Resumo

In 2015, a high incidence of virus-like symptoms such as blistering, mosaic and leaf deformation was reported in passion fruit plants by growers in passion fruit producing areas of Bahia, currently the main passionfruit producing state in Brazil. Symptomatic Passiflora spp. leaves were collected in Lençóis (n=21) and Jussiape (n=9), Bahia State. dsRNA was extracted from all samples, pooled, and sequenced by high throughput sequencing (HTS) in an Illumina HiSeq 2500 platform. De novo assembly of the short reads with ABYSS 1.9 (K-mer=64) resulted in several contigs related to Cucurbit aphid-borne yellows virus (CABYV, Genus Polerovirus, Family Luteoviridae), and Cowpea aphid-borne mosaic virus (CABMV, Genus Potyvirus, Family Potyviridae). A CABYV sequence isolate from Spain (JF939814) was used as a reference to map the reads using Geneious 9.1. A consensus contig of 5,643 nt covered by 33,290 reads was recovered and named CABYV-PF (MH257573). CABYV-PF encodes ORFs P0 to P5, and P3a, characteristic of poleroviruses and has 87-94% nt identity with several CABYV isolates from different countries. Similarities of protein sequences range from 92-99% when compared with protein sequences from the reference CABYV from France (NC_003688). Using the sequence of CABMV isolate MG-Avr from Brazil (HQ880243) as reference to map the reads, a consensus contig of 9,643 nt was assembled from 3,469,335. To evaluate the incidence of CABYV in all samples, RT-PCR was done with the RNA extracted from the individual plants, followed by Southern blot analysis with a CABYV-derived probe. The incidence of CABMV was also evaluated by RT-PCR. CABYV was detected in 52% (11/21) of the samples from Lençóis, CABMV in 91% (19/21) of the samples, and 48% (10/21) of the plants were infected by both viruses. In the samples collected in Jussiape, the frequencies of CABYV and CABMV infection were 11% (1/9) and 55% (5/9), respectively, and no mixed infection was detected. CABYV has a wide range of hosts and infects cultivated and uncultivated plants of several botanical families, however, this is the first record of CABYV in passion fruit.

Financial Support: Embrapa, CNPq, and FapDF

Palavras-chaves: passionfruit, polerovirus, CABYV, potyvirus, CABMV

PERFORMANCE OF Bemisia tabaci ON Datura stramonium AND ITS POTENTIAL AS AN
Begomoviruses are the most important viruses affecting tomato (*Solanum lycopersicum*) crops in Brazil, and *Tomato severe rugose virus* (ToSRV) is the major important species in Brazil. ToSRV is transmitted by *Bemisia tabaci* mediterranean (MED or Q biotype) and Middle east asia minor 1 (MEAM1 or B biotype) whiteflies, and it can be found in several hosts, among them weeds, that can serve as alternative hosts for this virus and whitefly species. This study aimed to evaluate the potential of the weed datura (*Datura stramonium*) as an alternative host to ToSRV and the effect of the viral infection on the performance of two different species of *B. tabaci*: MED and MEAM1. For transmission assay, only MEAM1 whitefly vectors were used in order to transmit ToSRV from tomato to datura plants and among datura plants. Additionally, the capacity of ToSRV to be transmitted back from datura to tomato plants was evaluated. For ToSRV detection, DNA extraction followed by PCR was performed. To estimate ToSRV effects in performance assays of *B. tabaci*, the number of eggs, nymphs, and adults produced by 100 couples of both MED and MEAM1 on infected and healthy datura plants was evaluated. The results revealed that the transmission efficiency of ToSRV from tomato to datura plants was 33%. However, ToSRV was not transmitted among datura plants, and the efficiency of transmission from datura to tomato plants was ineffective, being only 3%. In ToSRV infected plants, MEAM1 whiteflies had a significantly higher rate of reproduction compared with MED whiteflies. Although not being a good alternative host in ToSRV transmission, datura plants are good hosts for whiteflies performance.

**Financial Support:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)

**Palavras-chaves:** Whitefly, Begomovirus, Weeds
Cape gooseberry (Physalis peruviana L.) stands out at the small fruits by the appearance and fruit quality and is a great cultivation option for small and medium producers. For still being an emerging crop in Brazil there are few studies, especially those related to diseases. Cape gooseberry plants were found in the Santa Catarina Highlands, showing viral symptoms of mosaic, yellowing, leaf crinkle and deformed fruits. This work aimed to perform biological and molecular characterization of cape gooseberry associated virus, as well as to quantify the damage caused due to viral infection. In biological tests, 23 indicator plants belonging to Chenopodiaceae, Cucurbitaceae, Fabaceae e Solanaceae families were inoculated using buffered plant extract, while negative control was performed in duplicate consisting of inoculations without inoculum. Total DNA and RNA were extracted of the samples for virus molecular characterization using universal primers for the Begomovirus, Potyvirus, Orthotospovirus and Sobemovirus genus. Serological tests were performed to evaluate the presence of Potato leafroll virus (PLRV, Polerovirus), Potato virus X (PVX, Potexvirus) and Potato virus Y (PVY, Potyvirus). Damage quantification were performed by healthy and infected plants comparison using the following parameters: photosynthesis; plant height; leaf size; specific leaf area; SPAD index; mass of fruits per plant; average fruit mass; total soluble solids content (° Brix); titratable total acidity; and the pH of the fruits. In biological characterization, only some developed symptoms were similar to those reported in the literature for the viruses already described infecting cape gooseberry, suggesting the occurrence of a not yet reported virus in that crop. Serological and molecular tests confirmed the presence of a Sobemovirus, and the absence of co-infection. Molecular tests also suggest the Velvet tobacco mottle virus (VTMoV, Sobemovirus) was the causal agent of symptoms observed in P. peruviana in the Santa Catarina state. Viral infection caused a delay in growth, reduction in leaf area, specific leaf area, and smaller SPAD index. Inoculated plants showed a reduction of 70% in fruit production, and in all fruit quality parameters evaluated, except for pH, in which there was no significant difference.

Financial Support: FAPESC, UDESC and CAPES.

Palavras-chaves: Physalis peruviana, Small Fruits, Diagnostics, Virus, Damage

RESISTANCE EVALUATION OF CAPSICUM GENOTYPES FOR BEMISIA TABACI MEDITERRANEAN SPECIES

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Resumo

The Solanaceae family is important in Brazilian agriculture, because comprise economically relevant cultures such as tomato, eggplant and Capsicum sp., that have been observed under infection by tomato severe rugose virus – ToSRV. ToSRV is transmitted by the whitefly Bemisia tabaci cryptic species MEAM1 and MED, this last one well adapted to sweetpepper. Here we evaluated the B. tabaci MED performance on different Capsicum sp genotypes from IAC (1357, 1579, 1544, 1551 and 1549). Clip cages with 10 couples were used to infest 10 leaves of each genotype, representing 10 repetitions in the experiment. After 1 day, the adults were removed, and the eggs and nymphs were counted until the total emergence of the adults. The performance cycle of whiteflies interacting with the genotypes lasted 30 days, in which it was observed, on average, for the IAC 1357, 1544, 1549, 1551 e 1579 genotypes, respectively 127, 121, 46, 88 and 92 eggs, 108, 43, 34, 40 and 88 nymphs and 121, 3, 0.5, 0.6 and 36 adults emerged. The 1357 genotype was highly susceptible to B. tabaci MED, as well as previously observed for MEAM1 whiteflies. The 1579 genotype, described as resistant to MEAM1, was
highly susceptible to MED. Antibiosis to MED, as previously observed for MEAM1, was observed for the *Capsicum* genotypes 1554, 1549 and 1551 since low number of adults emerged for these plants. These genotypes might have defense mechanisms such as physical surface factors and volatile substances to the most invasive *B. tabaci* species.

**Palavras-chaves:** performance, Solanaceae, whitefly

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**Turnip mosaic virus NATURALLY INFECTION LETTUCE, CHARD AND ROCKET SALAD IN BRAZIL**

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**Resumo**

Vegetable leaves are an important part of daily diet in Brazil. Abnormalities have been observed in lettuce (*Lactuca sativa*), chard (*Beta vulgaris* subsp. *vulgaris*) and rocket salad (*Eruca sativa*) fields in Sao Paulo state – Brazil, during 2016-2017. Typical symptoms of virus infection were observed in this plants, and associated with them we also found weeds, such as raphanus (*Raphanus raphanistrum*) and forage turnip (*Brassica rapa*) with mosaic symptoms and the presence of high populations of aphids. Initially, the collected symptomatic leaves were submitted to indirect ELISA test, using a polyclonal potyvirus antiserum (Agdia, Inc), and all the plants were found positive. Total RNA was extracted (Total RNA Purification Kit, Norgen), followed by RT-PCR reaction using the universal primers W-CIEN (5‌‘-ATG GTT TGG TGY ATY GAR AAT-3′) and PV-1 (5′-GAT TTA GGT GAC ACTATA GT[17] 3′) that amplify part of the potyvirus capsid protein (CP) gene, and a fragment of the expected size (~850 bp) was obtained. These amplicons were sequenced, and all showed nucleotide identity with *Turnip mosaic virus* (TuMV). Further, the complete sequence of the CP gene (1109 bp) from all isolates were amplified using a specific primers pair (TuMV 8698 Fwd: 5′- TAC CTA GCA ATC TTT G - 3′ and TuMV 9807 Rev: 5′- GGC AAT CGA GAT ACT ATC TC - 3′). These amplicons were purified (Gel Extraction Kit, Qiagen) and directly sequenced in forward and reverse directions using the PCR primers, confirming the presence of TuMV in the symptomatic plants. Commonly, the TuMV isolates can be grouped into two pathotypes: B (mainly infecting plants of the genus *Brassica*) and BR (infecting plants of the both genera *Brassica* and *Raphanus*), and all isolates detected in this work clustered in the basal-BR clade using Bayesian analysis. We conclude that TuMV is becoming frequent on vegetable leaves in Brazil.

**Palavras-chaves:** basal-BR, Potyvirus, TuMV

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**MOLECULAR CHARACTERIZATION OF GENETICALLY DIFFERENTIATED BEGOMOVIRUS**
COAT PROTEINS

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Resumo

Begomoviruses are single-stranded DNA plant viruses (genus Begomovirus, family Geminiviridae) encapsidated into twinned quasi-isometric (“geminate”) particles and transmitted by a cryptic species complex of whiteflies referred as Bemisia tabaci. A number of phylogenetic studies based on full-length DNA-A (or DNA-A-like) sequences indicate that begomovirus populations are geographically structured possibly due to the short-range migration/dispersal of whitefly populations. In fact, model-based population genetic approaches indicate the existence of well-defined subpopulations within the global begomovirus meta-population. In this study, we applied a repertoire of phylogenetic and population genetic tools for clustering begomovirus isolates into genetically differentiated subpopulations based on their coat protein (CP) sequences. To investigate details on the genetic segregation at amino acid level, we mapped the subpopulation-specific profiles into CP consensus sequences. In addition, amino acid replacements were mapped into CP three-dimensional models built in SWISS-MODEL. All analyses were applied on a data set composed of 3,220 CP sequences downloaded from Genbank database on April 2018. Residue replacements contributing to the segregation into New and Old World begomoviruses were predominately located within the CP N-terminal region, which potentially mediates the intercapsomer contact. Two amino acid positions within the six-residue segment of the βD/βE-loop, identified as essential for controlling insect transmission, also contributed to the segregation into New and World begomoviruses. Both positions comprised replacements of amino acids with distinct biochemical properties and might be involved in the viral adaptation to local whiteflies populations. Interestingly, several amino acid replacements defining the highly divergent swepovirus subpopulation were unique, including those in the βD/βE-loop. These results might indicate the existence of distinct transmission properties in swepoviruses or, alternatively, a more relaxed negative selection, which does not impose severe selective constraints to protein structure or function. Together, our results indicate that several polymorphisms defining the main begomovirus subpopulations lead to significant changes in the amino acid composition of the begomovirus coat protein.

Financial Support: CNPq; UFU; ICIAG.

Palavras-chaves: Evolution, Population genetics, Bioinformatics, Geminivirus

CHARACTERIZATION OF PEPPER RINGSPOT VIRUS (PepRSV) INFECTIOUS CLONE AND USE AS VIRAL VECTOR

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Resumo

The genus *Tobravirus* comprises three species *Tobacco rattle virus* (TRV), *Pea early-browning virus* (PEBV) and *Pepper ringspot virus* (PepRSV). The genome of tobraviruses consists of two single-stranded RNA segments (RNA1 and RNA2) in positive sense. Infectious clones of TRV is extensively used as virus-induced gene-silencing (VIGS) vector for studies on virus-host interactions and plant gene functions. However, the complete infectious clones of PepRSV, the only tobravirus present in Brazil, are not reported yet. The RNA2 sequences of PepRSV isolates possess unique molecular features, different from TRV and PEBV isolates and the construction of own infectious clone was required to study such differences as well as to obtain the molecular tool. In this study, PepRSV infectious clones of two isolates, CAM (RNA1 and RNA2) and LAV (RNA2) were constructed, then the RNA2 construct was modified for characterization of the molecular features of the virus and for use as viral vectors. The cDNA constructs for both homologous (RNA1 and RNA2 of CAM isolate) and heterologous (RNA1/CAM and RNA2/LAV) combinations were infectious in *Nicotiana benthamiana* plants. After 7 days post inoculation (dpi), the agroinfiltrated *N. benthamiana* with RNA1/RNA2-CAM/CAM constructs presented symptoms of mosaic in leaves and top distortion in *N. benthamiana* plant, similar to those observed in plants infected with the original PepRSV isolate. The constructs of the heterologous combination, RNA1/RNA2-CAM/LAV after 7 dpi, the agroinfiltrated plants showed almost identical symptoms confirming that both combinations (homologous and heterologous) were infectious. VIGS constructions with GFP or PDS inserted in RNA2 induced silencing of target genes. The results the reduced GFP signals compared to the control transgenic 16c *N. benthamiana* plants was observed initially on young leaves after 7 dpi and the GFP reduction spread to some older leaves at 10 dpi. For PDS construction, the photobleaching effect was initially observed at 7 dpi at 15 dpi the PDS gene silencing effect was amplifying up to 21 dpi. All the inoculated plants presented the photobleaching effect, with high efficiency of gene silencing. Systemic translocation of PepRSV RNA1 only construct (NM infection) was also confirmed in *N. benthamiana* plant. The development of the infectious clones and vectors based on PepRSV is significant importance in Brazil to substitute TRV vector for plant science.

Financial Support: CNPq

Palavras-chaves: Virgaviridae, Infectious clone, VIGS

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HIGH SEED TRANSMISSION RATES OF COWPEA MILD MOTTLE VIRUS IN COMMON BEAN

**Autores**

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**Resumo**
Common bean (*Phaseolus vulgaris*) is an important staple crop in Brazil and is affected by several viruses, including Cowpea mild mottle virus – CPMMV (genus *Carlavirus*, family *Betaflexiviridae*). CPMMV is transmitted by the whitefly *Bemisia tabaci* and has been found infecting beans in high incidences in Central and Northeastern regions of the country. African strains of CPMMV were reported to be seed-transmitted in cowpea, soybean and bean cultivars. For the Brazilian CPMMV from bean and soybean isolates seed transmission assays using visualization of symptoms and ELISA as evaluation methods were negative. In this work, we used the highly sensitive qPCR and RT-PCR techniques to assess seed transmission of CPMMV in common bean. Common bean ‘BRS FC 401 RMD’ seedlings were mechanically inoculated with CPMMV. Infection was confirmed by RT-PCR. Twenty seeds set by the ‘BRS FC 401 RMD’ infected plants were planted and maintained in a growth chamber protected from insects. Total RNA was extracted from young leaves 30 days after inoculation by Trizol Reagent®, according to the manufacturer’s manual. The cDNA was prepared using oligodT and random primers with SuperScript III Reverse Transcriptase. qPCR reactions were performed in triplicate using Platinum® SYBR® Green qPCR SuperMix-UDG with ROX (Invitrogen) and CPMMV specific primers. Actin 11 gene was used as an internal control and cDNA from tomato and arabdopsis plants as negative controls. Melting curve analysis confirmed the specificity of the amplifications. Seed-borne CPMMV was detected in 100% of the plants by qPCR. In contrast, only 20% of the plants were positive by end-point RT-PCR using the same cDNA, followed by gel electrophoresis, and visualization of virus-derived bands. The results show that CPMMV isolate from central Brazil could be seed transmitted in ‘BRS FC 401 RMD’ beans in high rates though it was more efficiently detected by qPCR than RT-PCR. More studies are necessary to verify the effects of the seed-born CPMMV in common bean crops and the behavior of seed-borne infected plants as inoculum sources for whitefly transmission.

Financial Support: Embrapa, CNPq, and FapDF.

Palavras-chaves: Phaseolus vulgaris, seed transmission, Carlavirus, Cowpea mild mottle virus, common bean

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**OCCURRENCE OF Orthotospovirus INFECTING SCARLET EGGPLANT IN BRAZIL**

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Resumo

Viruses belonging to the *Orthotospovirus* genre stand out as one of the major plant virus, which affects vegetables in general. Symptomatic scarlet eggplants or gilo (*Solanum aethiopicum* L.) were collected in the field during the summer of 2017 - 2018 in the city of Itapolis (Brazil). Leaves and fruits displayed necrotic and concentric ringspot suggesting infection by orthotospoviruses. Total RNA was extracted followed by RT-PCR reaction using the degenerate primers BR60 (5’ AGA GCA ATC GTG TCA 3’) and BR65 (5’ ATC AAG CCT TCT GAA AGT CAT 3’), that amplify ~ 453 bp of the nucleocapsid gene. The amplicons obtained were sequenced and showed identity of 99% with *Groundnut ringspot virus* – GRSV (GenBank accession number AY380780.1). In this work we used symptomatic leaves of scarlet eggplant macerated in 0.05M pH 8.0 potassium phosphate buffer as inoculum source for sap transmission to five plants in each range of Solanaceae family hosts, pre-dusted with carborundum (600 mesh). Necrotic local lesions and leaf deformation were observed in *S. melongena* cv. Napolitana (5/5), *S. melongena* cv. Napoli (5/5), *S. melongena* cv. Roma (5/5). *S. aethiopicum* cv. Morro Grande (5/5), *S. aethiopicum* cv. Comprido Verde Claro (5/5), *Datura stramonium* (3/5). *S. lycopersicum* hybrid Mariana (5/5) presented concentric necrotic rings on leaves, while *Nicotiana tabacum* "TNN" (4/5) and *N. tabacum* cv. Virginia (5/5) local lesions followed by systemic infection. Leaf deformation and mosaic was observed in *Capsicum annuum* hybrid Magali R (3/5) and *S americanum* (3/5). As far as we known this is the first natural occurrence of GRSV infecting scarlet eggplants in Brazil and this plant can serve as inoculum source of the virus for the different hosts tested.

Palavras-chaves: Plant virus, Groundnut ringspot virus, Solanum aethiopicum L.
EFFECT OF THE SILENCING OF THE GmERD4 AND GmIMT GENES ON THE ACCUMULATION OF COWPEA MILD MOTTLE VIRUS IN SOYBEAN PLANTS

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Resumo

Cowpea mild mottle virus (CPMMV, family Betaflexiviridae e genus Carlavirus), the causal agent of soybean stem necrosis, is an emergent virus in Brazil. To date, very few studies have addressed the replication of betaflexiviruses, to the extent that little else is known besides that replication takes place in the cytoplasm. Two soybean proteins (GmERD4, associated with responses to drought, and GmIMT, an inositol methyltransferase), have been previously shown to interact with the RNA-dependent RNA polymerase (RdRp) domain of the CPMMV replicase protein. Expression analysis of both genes in CPMMV-infected soybean plants demonstrated that they are induced at 3 and 7 days after inoculation (dai). Furthermore, overexpression of GmERD4 in soybean protoplasts increase viral accumulation. In this context, this work was conducted to better characterize the CPMMV-soybean interaction. We used virus-induced gene silencing (VIGS) with a Bean pod mottle virus (BPMV)-based VIGS vector to silence GmERD4 and GmIMT. For the evaluation of the experiment we performed the extraction of the total RNA of the silenced plants as well as the plants used as control: healthy and containing empty vector (BPMV) plants. All plants were evaluated before and after CPMMV inoculation. Subsequently, we performed the relative quantification for the target genes and absolute quantification for the CPMMV by RT-qPCR. We observed the reduction of the expression of 40% GmERD4 target genes in plants inoculated with the GmERD4-BPMV construct and 60% GmIMT in plants inoculated with the GmIM-BPMV construct. Strikingly, silencing of GmERD4 and GmIMT drastically reduced CPMMV accumulation at 5 dai, up to 14 dai in the case of GmIMT-silenced plants. These results indicate that GmERD4 is essential during the early stages of CPMMV infection, while GmIMT is essential during the entire infection process. Therefore, the interaction between CPMMV and these two proteins constitutes a potential target of antiviral measures for the control of soybean stem necrosis. Financial Support: CNPq.

Palavras-chaves: Plant-virus interaction, Carlavirus, Soybean, VIGS

CHARACTERIZATION OF CLRDV INFECTION IN ARABIDOPSIS THALIANA PLANTS

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Resumo
The Cotton Leafroll Dwarf Virus (CLRDV) is a ssRNA+ virus and a member of genus Polerovirus, family Luteoviridae. When cotton plants are infected by this virus they acquire the Cotton Blue Disease (CBD) that affects cotton plants causing a drop in productivity, as well dwarfism, yellowing of the veins, darkening of the leaves and winding of the leaf edges are observed. In order to initiate research about viral infection, it is important to establish a study model that can reproduce favorable conditions for infection. In this work, we used plants of the species Arabidopsis thaliana, that has a smaller growing phase than cotton plants and has a vast genetic database. The goal of this project is to establish and characterize CLRDV infection in Arabidopsis thaliana plants from Columbia ecotype. Wild-type Arabidopsis were inoculated with viruliferous Aphis gossypii, Glover aphids. the biological vector of CLRDV and evaluate by observing the presence or absence of symptoms and by NESTED RT-PCR diagnostic assays for CLRDV. Six weeks old Arabidopsis plants were used for inoculation in plant growth chambers conditions with 14:10 hours dark: light photoperiod. Thirty aphids were inoculated per plant. Three infection experiments were performed until now. Arabidopsis could be successfully infected by CLRDV and the virus was able to spread systemically. In the last experiment, twenty-six Arabidopsis plants were inoculated. Of these twenty were inoculated with CLRDV and six were used as negative controls. To inoculate the CLRDV were placed in each plant about thirty viruliferous aphids and after 5 days the leaves were collected and stored in freezers -80. After total RNA extraction using the reagent Trizol, we do RT reactions to obtain cDNA, which will serve as a template for the NESTED-PCR using primer sets that amplify the genes encoding viral capsid and replicase proteins.

Palavras-chaves: Virus, Arabidopsis thaliana , Infeção, CLRDV, PCR

 ANALYSYS OF THE INFECTIVITY OF PHYTOPATHOGENIC VIRUS ISOLATES USED FOR QUARANTINE DIAGNOSIS

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Resumo

For plant quarantine purposes, maintenance of collection of phytopathogenic virus isolates is essential to be used as positive controls (reference) for both greenhouse and laboratory diagnose quarantine symptomatology, serological and molecular assays. Over time samples from the Embrapa phytopathogenic viral collection – cataloged in our database as from 2009 – may have lost infectivity (virulence). The aim of the present work was to evaluate the infectivity the phytopathogenic virus isolates belonging the collection from the Embrapa Laboratory of Plant Quarantine. For that reason, the virus isolates were mechanically inoculated onto various plants from different botanical families under greenhouse conditions. In case mechanically inoculated plants developed symptoms, as compared to mock inoculated plants, ELISA serological tests were performed for confirmation of the viral identity. This collection is composed of commercial virus isolates from various origins and maintained in different plant species tissue pieces (mostly symptomatic leaf tissue), which were preserved and stored under ultra freeze conditions over the last nine years (2009-2018). Eight viral species from the above mentioned collection were herein analyzed in terms of infectivity after storage under ultra freeze conditions of infected plant material: Cucumber mosaic cucumovirus (CMV), Papaya ringspot potyvirus (PRSV), Zucchini yellow mosaic potyvirus (ZYMV), Groundnut ringspot tospovirus (GRSV), Tomato chlorotic spot tospovirus (TCSV), Tomato spotted wilt tospovirus (TSWV), Potato potexvirus X (PVX) and Pepper mild mottle tobamovirus (PMMoV). While PRSV, ZYMV, PVX, CMV, GRSV, e PMMoV isolates were still virulent/infectious over years time span, TCSV e TSWV isolates lost the infectivity. Previous studies demonstrated by plant mechanical inoculation followed by ELISA serological tests of symptomatic tissues that storage of virus-infected plant material under similar ultra freeze conditions over two years preserved the infectivity of all the ten referred isolates, what demonstrates the high efficiency of the preservation method. We confirmed the efficiency of the ultra freeze preservation method over nine years for six virus isolates belonging the Potyvirus, Potexvirus, Cucumovirus, Tospovirus and Tobamovirus genera, except for one sample the Tospoviral TCSV
species and three samples of the *Tospoviral* TSWV species, which lost infectivity.

**Financial support:** Embrapa

**Palavras-chaves:** plant quarantine, phytovirus, infectivity, virulence, ultra freeze preservation

**TRANSMISSION OF BIPARTITE BEGOMOVIRUSES IN SINGLE AND MIXED INFECTIONS BY TWO SPECIES OF THE Bemisia tabaci CRYPTIC SPECIES COMPLEX**

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**Resumo**

The genus *Begomovirus* (family *Geminiviridae*) includes viruses that infect dicotyledonous plants, have a single-stranded, circular DNA genome and are transmitted by insects of the *Bemisia tabaci* cryptic species complex. Begomoviruses are one of the most economically important groups of plant viruses, causing diseases in several economically important crops around the world. *Tomato severe rugose virus* (ToSRV) and *Tomato rugose mosaic virus* (ToRMV) belong to a complex of begomoviruses that infect tomato. In mixed infection with ToRMV and ToSRV, the accumulation of ToSRV is reduced, suggesting a preferential replication of the ToRMV genomic components in relation to those of ToSRV. Interestingly, ToRMV is not widely disseminated in the field, unlike ToSRV, which predominates in tomato fields in southeastern Brazil. Few studies have been conducted with the objective of studying the interaction between begomoviruses and their insect vector in mixed infections. This work was conducted to verify the efficiency of transmission of ToSRV and ToRMV by *B. tabaci* Middle East-Asia Minor 1 (BtMEAM1) and Mediterranean (BtMED) in single and mixed infections. Following biolistically inoculation with infectious clones of each virus in different combinations, plants with each virus in single infection were confirmed by PCR, and plants with mixed infection were confirmed by RCA following digestion with restriction enzymes that cleave each component specifically. The rate of transmission of ToSRV and ToRMV in single inoculations by BtMEAM1 were similar. However, ToSRV was more efficiently transmitted by BtMED than ToRMV. In mixed infections, the genomic components of ToRMV were always detected, unlike the ToSRV components that were rarely detected. This is consistent with previous results indicating that ToRMV exerts a negative interference on the accumulation of ToSRV at the early stages of the infection cycle. Our results indicate that ToRMV does not interfere with the acquisition of ToSRV by the insect vector. Although ToSRV is transmitted with the same efficiency in relation to ToRMV by BtMEAM1 and even at higher rates by BtMED in single infections, efficiency or success in the transmission of two (or more) begomoviruses in mixed infection may be more related to the viral concentration in the source host and the interference that one virus may exert in the replication of the other.
VARIAIBILITY AND GENETIC STRUCTURE OF A BEGOMOVIRUS OVER A SEVEN YEARS TIME SPAN

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Resumo

The genus Begomovirus (family Geminiviridae) is composed of viruses that infect dicotyledonous plants, have a genome comprised of one or two single-strand circular DNA (ssDNA) molecules encapsidated in twinned icosahedral particles, and are transmitted by insects of the Bemisia tabaci cryptic species complex. Begomoviruses are one of the most economically important groups of plant viruses causing diseases in several economically important crops around the world. A large diversity of begomoviruses has been sampled in non-cultivated hosts, especially in the Malvaceae and Fabaceae families. Begomovirus populations have a high degree of genetic variability, especially those associated with non-cultivated plants. Studying the structure and genetic variability of begomovirus populations in non-cultivated plants is necessary, considering the potential risk of host jumps and subsequent epidemics in crop plants. Although studies on the variability and genetic structure of begomovirus populations in noncultivated plants have been conducted, little is known about the temporal evolutionary dynamics of these populations. The objective of this work was to evaluate the variability and genetic structure of a population of Oxalis yellow vein virus (OxYVV) in plants of Sida acuta (Malvaceae) over a long period of time. Samples of Sida acuta were collected from 2011 to 2017 in an area of approximately 0.1 ha in the municipality of Viçosa, MG. Total DNA was extracted and full-length viral genomes were cloned and sequenced. The population of OxYVV displayed high diversity, with the coexistence of three variants. The absence of a temporal signal can be explained by a lower perturbation in the non-cultivated environment, which causes that population to evolve at a slower rate when compared to viral populations associated with cultivated plants. Nevertheless, there is a change in population composition over time, with the prevalence of variant I and the disappearance of the other two. This may be due to the differential adaptation between isolates of each variant. No recombination events were detected among isolates, although phylogenies suggest that variant II is the result of recombination between individuals of variants I and III. Additional studies on the evolutionary dynamics of viruses in natural ecosystems should be conducted to unravel the forces that guide the evolution of these organisms in their natural environment.

Financial Support: CAPES, CNPq and FAPEMIG.

Palavras-chaves: geminivirus, temporal analysis, molecular evolution, Sida, recombination
Plants have several mechanisms of defense against pathogens and different types of abiotic stresses, some of these mechanisms involve the activation of signaling cascades that are initiated with the increase of circulation of certain hormones and/or pathogen recognition. Ethylene Response Factors (ERFs) are transcriptional factors known to be involved in different response pathways against abiotic stresses. ERFVII specifically are the target of the Argynil-t-RNA transferase (ATE) that is an N-end rule protein which leads the transcription factor to the proteolytical pathway. By other side, ERFVII induces ATE trancription. ERFs are regulated by miRNA172 during Arabidopsis flower development. The objective of this work is try to check if miR172 may be silencing ERF and consequently leading to ATE repression during a viral infection. Previous studies of our group shown that during the infection of cotton plants by the Cotton Leafroll Dwarf Virus (CLRDV), a member of the genus Polerovirus, a drastic increase of the miR172 expression is observed in early infection times in susceptible plants. The CLRDV is the causative agent of Cotton Blue Disease (CBD), which is transmitted by the aphid Aphis gossypii Glover. The symptoms are: dwarfism caused by a shortening of the internodal region, bending of the leaves, intense green coloring, and yellowing of the veins. To understand how ATE modulation can influence virus resistance, we used the model plant Arabidopsis thaliana. Wild-type and ATE overexpressing 35S:ATE transgenic Arabidopsis thaliana seeds were germinated in MS medium (Murashige and Skoog 1962), transplanted to land and divided into three groups: control, aphid inoculate, where the aphid group received aphids without the virus, and virus-infected plants, which were inoculated with viruliferous aphids. The systemic leaves were collected in 12hpi, 24hpi, and 5dpi, total RNA was extracted and followed by NESTED RT-PCR for diagnosis of infection. Gene expression evaluation was performed by real time PCR. It has been observed that while expression levels of the gene for miRNA172 increase by 12 hr and decay by 24 hr and 5 dpi, the levels of ATE and ERF expression levels decrease dramatically by 12 hr and 24 hr and are expressed again by 5 dpi in WT plants. These results suggest that miR172 may be silencing arabidopsis ERF and in consequence leading to ATE expression decay, along the virus replication and spread.

Financial support: CAPES, CNPq and FAPERJ

Palavras-chaves: Virus, Vegetal, miRNA172, CBD, ERF VII

Physalis peruviana: A New Host Species of Groundnut Ringspot Orthotospovirus in the Cerrado Region of Central Brazil

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Resumo

The golden berry (Physalis peruviana L.) is an herbaceous species that belongs to the Solanaceae family, grown for fruit production, which also has medicinal and ornamental uses. The commercial cultivation of golden berry just started under Brazilian conditions. During 2017, plants showing orthotospovirus-like symptoms were observed at the Experimental Station of Embrapa Vegetables, Brasilia-DF. Deformation, mosaic and chlorotic and necrotic ringspots on leaves as well as necrotic ringspots on fruits and on the husk that enclose the fruit were observed on plants in the field. The incidence of symptomatic plants was estimated at 70%. Leaf samples were collected from symptomatic plants and tested by serology (Enzyme-linked immunosorbent assay) using polyclonal antibodies (produced at Embrapa Vegetables) against Tomato spotted wilt orthotospovirus (TSWV), Groundnut ringspot orthotospovirus (GRSV) and, Tomato chlorotic spot orthotospovirus (TCSV). Total RNA was extracted from ten leaf samples positive by serology, using Trizol reagent followed by 2 steps RT-PCR for orthotospovirus detection. Complementary DNA was synthesized with J13 primer and used for PCR reactions for GRSV, TSWV and TCSV using specific primers. Extracts obtained from symptomatic golden berry samples were mechanically
inoculated onto Datura stramonium, Nicotiana tabacum cv. TNN, N. rustica, Nicandra physaloides, Solanum lycopersicum cv. Santa Clara and, Capsicum chinense 'PI159236' that has a dominant gene (Tsw). To fulfill Koch’s postulates, plants of golden berry at the two-true-leaf stage were rubbing inoculated with extract prepared from D. stramonium infected plants. From serology tests, 15 samples out of 20 were found to be infected solely with GRSV. Results obtained by PCR revealed 594 bp amplicon when using GRSV-specific primers for all samples. No PCR-amplification was obtained for TSWV and TCSV. Typical symptoms induced by ortothospovirus infection (i.e. mosaic, leaf deformation, chlorotic and necrotic ringspots) were observed 10-15 days after inoculation on leaves of golden cherry as well as in the indicator plants. GRSV and TCSV have been previously reported infecting P. peruviana in São Paulo State. However, the present work is the first report of GRSV infecting P. peruviana in the Cerrado region of central Brazil. Because GRSV is transmitted by trips and is able to naturally infect a broad host range, this virus species could represent a threat to golden cherry cultivation.

Palavras-chaves: Orthotospovirus, Physalis, Detection, GRSV, RT-PCR

DETECTION OF Tobacco streak virus IN Nicotiana tabacum AND THRIPS BY REAL TIME RT-PCR

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Resumo

Tobacco crop, Nicotiana tabacum L. (Solanaceae) has a great importance in the Southern Brazil, due to a high commercial value of the product, sustaining significant economic-social benefit results from agro-industrial employments. Tobacco streak virus (TSV), genera Ilarvirus, is the causal agent of the necrotic streak in tobacco plants and it has been recorded in Paraná causing losses of productivity and quality in tobacco crops. Concerning TSV detection, conventional methods (ELISA and RT-PCR) may present identification flaws because of their low sensitivity and specificity. In addition, conventional PCR templates require purification of the nucleic acid, which is laborious and time consuming; increasing the risk of contamination. Therefore, this work aimed to develop and validate a protocol for direct sample preparation and real-time RT-PCR amplification, with TaqMan probe, to detect TSV in tobacco plants and thrips. The protocol using purified RNA targets by commercial kit (Promega) was compared with crude plant extracts for direct real time RT-PCR. The real time RT-PCR designed, amplified both purified RNA and crude plant extracts. Using serial dilutions of plant extracts, TaqMan real-time RT-PCR was the most sensitive when compared with SYBR-Green and ELISA techniques. The developed protocol has been allowing detection of TSV in crude vegetal extract and in individual thrips in a sensitive, specific, economic and accurate way.

Palavras-chaves: virus, vector, detection

NON-CONSERVED RECOMBINATION PATTERNS AMONGST POTYVIRUSES

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Potyviruses are widespread single-stranded RNA plant viruses (genus Potyvirus, family Potyviridae) encapsidated into flexuous and rod-shaped particles. They are able to infect a wide range of mono- and dicotyledonous plant species leading to significant losses for agricultural production. The rapid evolution and emergence of RNA viruses are consequences of their mutation and recombination-prone nature. Studies indicate that intra- and interspecific recombination may be involved in the emergence of novel potyvirus species and strains. However, whether there is or not an evolutionary conserved recombination pattern amongst potyviruses remains to be determined. In this study, we evaluated the existence of well-supported recombination hotspots across genomes of potyviruses. Nine species data sets totaling more than 1,500 complete genomes of isolates collected from around the world were retrieved from Genbank database on April 2018. Statistical evidence of breakpoint clustering was tested in Recombination Detection Program v.4 (RDP4). The sequence context at recombination hotspots was further characterized by nucleotide composition analyses and prediction of RNA secondary structures. Statistically supported recombination hotspots were detected into two out of nine potyvirus species data sets. The hotspots were centred at nucleotide positions 4,800 and 2,000 in Sugarcane mosaic virus (SCMV) and Turnip mosaic virus (TuMV) species data sets, respectively. The RNA sequences surrounding both positions showed similar profiles of nucleotide composition and were highly structured in all potyvirus genomes analysed in this study. The even distribution of recombination breakpoints in most species data sets and the detection of hotspots at distinct positions in the SCMV and TuMV genomes indicate that the recombination patterns are not conserved amongst potyviruses. In addition, neither sequence composition nor RNA secondary structures seem to explain the breakpoint clustering in the SCMV and TuMV genomes.

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Palavras-chaves: Evolution, bioinformatics, genetic diversity, selection, population genetics

Genetic diversity among Brazilian isolates of Papaya ringspot virus type Watermelon

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Resumo

Papaya ringspot virus type Watermelon (PRSV-W) belongs to the genus Potyvirus, family Potyviridae and it is the most important virus for the cucurbits in Northeast Brazil which causes losses of up to 100%. The availability of coat protein (CP), and full genome sequences has helped to clarify significantly the taxonomy status of various virus species of the genus Potyvirus. It has also help to establish criteria that can be used to distinguish sequences representing closely-related virus species from those that are strains of the same species. The knowledge of the virus genetic diversity and the mapping of virus populations enable to understand the epidemiological complexity of the viruses and their related virus. Therefore, this study aimed to characterize the genetic diversity of PRSV-W isolates collected in Brazil. The phylogenetic analysis was performed by comparing the nucleotide sequences of the region that encode the coat protein gene of three isolates. They were from different locations in the
Brazilian Northeast, namely Bahia (PRSV-W-BA), Rio Grande do Norte (PRSV-W-RN) and Ceará (PRSV-W-CE), and besides these locations isolates from other Brazilian locations reported in the GenBank were used. The phylogenetic tree of maximum likelihood estimation of the PRSV-W protein coat gene sequences from Northeast Brazil showed the existence of distinct phylogenetic groups that did not correlate with the geographic distribution, host plant and strain. PRSV-W isolates formed two large distinct groups based on their genetic distances. The isolates PRSV-W-BA, PRSV-W-RN and PRSV-W-CE have made cluster with a single group (Group I), differing from other Brazilian isolates obtained from distinct geographical regions and different hosts, with relatively low divergence. It was found that the isolates PRSV-W-BA (Juazeiro, watermelon) and PRSV-W-RN (Mossoró, squash) were more phylogenetically related than PRSV-W-CE (Mauriti, watermelon). The second group was divided into subgroups A and B, while the isolates from different strains belonged to subgroup A. These results indicate little possibility of clustering of PRSV isolates according to their geographical origin, their host plant and their strains. Knowledge of the nucleotide sequence and genetic diversity of the PRSV-W isolates is necessary for the development of efficient control strategies.

Financial Support: CAPES and CNPq

Palavras-chaves: Coat protein, Cucurbitaceae, Phylogenetic analysis, Potyvirus, PRSV-W

A NEW BIPARTITE BEGOMOVIRUS INFECTING TOMATOES IN BRAZIL

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Resumo
Brazil is a center of diversity of begomoviruses, presenting a large number of species infecting cultivated and non-cultivated plant hosts. Currently, members of seventeen species are known to infect tomato (Solanum lycopersicum) in Brazil. However, there is great variation in distribution and predominance, with only a few species being widely disseminated in the field. In this study we report a new begomovirus found in tomato samples collected in fields located near the city of Altamira, state of Pará. Total DNA was extracted from the samples and used as a template for rolling circle amplification (RCA). Amplification products were cleaved with restriction enzymes and transformed into Escherichia coli DH5α. Viral inserts were sequenced commercially by primer walking. Full-length genomes were assembled using Geneious v. 8.1. Sequences were initially analyzed using BLASTn, and identities with the closest begomoviruses were calculated with Species Demarcation Tool v.1.2 (SDT). Full-length sequences corresponding to DNA-A and DNA-B components of begomoviruses were aligned with MUSCLE implemented in MEGA v. 7.0. Phylogenetic trees based on DNA-A and DNA-B alignments were generated by Bayesian inference using MrBayes v. 3.2.6, with the nucleotide substitution model selected by MrModeltest v. 2.2. The DNA-A sequences from the two samples showed 99,62% sequence identity amongst themselves and a maximum sequence identity of 84% with the DNA-A sequence of Abutilon mosaic Brazil virus (AbMBV; FN434438). In phylogenetic analysis based on DNA-A and DNA-B alignments, the isolates clustered with AbMBV and corchorus mottle virus
Based on the International Committee on Taxonomy of Viruses (ICTV) species demarcation criterion for the genus *Begomovirus*, a new species was thus identified, for which the name *Tomato chlorotic leaf curl virus* (ToCLCV) is suggested. This is the first report of a begomovirus in tomatoes in the state of Pará. Strikingly, despite the large number of begomoviruses already reported in tomato in Brazil, new species can still be detected in this host. In reality, considering the small number of samples from the Northern region of Brazil that have been analyzed in this and in previous studies, it is possible that the true extent of the begomovirus species diversity in tomatoes in this vast region is actually much higher. **Financial Support:** CNPq, Fapemig, Norte Energia S.A.

Palavras-chaves: RCA, Solanum lycopersicum, ToCLCV

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**Papaya meleira virus (PMeV) can survive in undifferentiated papaya cells.**

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**Resumo**

*Carica papaya* L. has been extensively cultivated in tropical and subtropical regions. However, several orchards are destroyed by papaya sticky disease ‘PSD’ in worldwide. PSD is associated with a complex formed between a toti-like virus, papaya meleira virus (PMeV) and an umbra-like virus, papaya meleira virus 2 (PMeV2). Multiple evidence has been suggesting that PMeV can adopt a persistent lifestyle in papaya, as this virus does not induce symptoms and is transmitted vertically through seeds in cv. Maradol. Also, PMeV lacks a movement protein which leads the hypothesis that like persistent viruses, PMeV may be found in every tissue, including undifferentiated meristematic cells and moves when cell division occurs. To prove this point we reprogrammed papaya cells to the undifferentiated state through the induction of callogenesis in PMeV-infected papaya tissues. After 3 months, papaya callus was removed from the induction media and submitted to PMeV detection through RT-PCR. Molecular diagnosis assay showed that 50% of the 3-month-old callus tissue were infected with PMeV. The absence of PMeV in 50% of the callus samples may be explained by the faster division in the callus compared to the ability of the virus to multiply, which results in a competition between virus and host by molecules of the host itself. On the other hand, the detection of PMeV in the other 50% samples shows this virus can survive and replicate in undifferentiated cells. Moreover, as vascular connections are disrupted in callus tissue, the detection of PMeV in callus samples reinforces the idea that this virus could move simultaneously with cell division. PMeV2 was not detected in callus samples. In conclusion, this work provides insights into PMeV survival and movement through cells and reinforces the persistent lifestyle of this virus in papaya plants.

Financial Support: FAPES, CAPES, CNPq.

Palavras-chaves: Persistent Plant virus, PMeV complex, Plant tissue culture, Carica papaya

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**A NEW BEGOMOVIRUS INFECTING Hibiscus sp. IN BRAZIL**

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A high diversity of begomoviruses can be found in non-cultivated plants in Brazil, particularly in plant species of the Malvaceae, Fabaceae and Solanaceae families. These plants may act as natural begomovirus reservoirs and as sources of genetic variability, fulfilling an important ecological role. In this study we identified a new begomovirus infecting a *Hibiscus* sp. (Malvaceae) plant collected in 2016 near the city of Igarapé Miri, state of Pará, in the Northern region of Brazil. Total DNA from the sample was extracted and used as a template for rolling circle amplification (RCA). Amplification products were cleaved with restriction enzymes and transformed into *Escherichia coli* DH5α. The viral inserts corresponding to DNA-A and DNA-B begomovirus components were completely sequenced by primer walking and the full-length sequences were assembled using Geneious v. 8.1. The sequences were initially analyzed using BLASTn, and identities with the closest begomoviruses were calculated with Species Demarcation Tool v.1.2 (SDT). Full-length sequences were aligned with MUSCLE implemented in MEGA v. 7.0. Phylogenetic trees based on DNA-A and DNA-B alignments were generated by Bayesian inference using MrBayes v. 3.2.6 with the nucleotide substitution model selected by MrModeltest v. 2.2. The sequences showed a maximum DNA-A nucleotide identity of 79% with *Sida yellow mosaic Yucatan virus* (SiYMYuV). In the DNA-A-based phylogenetic tree, the isolate clustered with *Tomato golden mosaic virus* (TGMV), *Sida mosaic Bolivia virus* 2 (SiMBoV 2) and *Cleome leaf crumple virus* (CILCV). The DNA-B was most closely related to *Tomato chlorotic mottle virus* (ToCMoV), *Tomato common mosaic virus* (ToCmMV) and *Blainvillea yellow spot virus* (BIYSV). According to the current taxonomic criteria established for the genus *Begomovirus*, the virus corresponds to a new species, for which the name *Hibiscus golden mosaic virus* (HGMV) is proposed. Non-cultivated plants have an important ecological role, since they are reservoirs of viruses for cultivated plants, especially between growing seasons. In addition, the great biodiversity of begomoviruses in these plants may contribute to the emergence of new, better adapted viruses in cultivated plants (which is highlighted by the close relation between HGMV and viruses found in cultivated plants). This is the first report of a begomovirus in *Hibiscus* sp. in the Northern region of Brazil. **Financial Support:** CNPq, Fapemig.

**Palavras-chaves:** begomoviruses, non-cultivated plants, RCA
Resumo

The study of gene functions in non-model plants is often limited by the difficulty of obtaining genetically stable strains through genetic transformation in some species, including cotton. Alternative techniques, such as Virus Induced Gene Silence (VIGS), become an ally in the advancement of non-model plant genetic studies. VIGS is a technique that allows the silencing of an endogenous plant gene by spreading a viral vector generating transient expression of this gene, without the need to stably transform the plant, reducing among other things the time needed to generate null phenotypes. VIGS is a tool used for biotechnology purposes as well as studies of gene functions. In this work, VIGS is used to silence two *Gossypium hirsutum* genes associated with the resistance to cotton blue disease, mediated by GhCBD1 and GhCBD2 genes, and the GhCLA1 gene, used as endogenous control of silencing inducing a chlorosis phenotype with the objective of evaluating the role of these putative genes during Cotton Leafroll Dwarf Virus (CLRDV) infection, in resistant and susceptible cultivars. Fragments of approximately 200-300 bp of the GhCBD1 and GhCBD2 genes and approximately 400 bp GhCLA1 were inserted into the VIGS viral vector Tobacco Rattle Virus (TRV), pTRV, which is cloned into a plasmid under control of the 35S promoter, using the Gateway recombination system. Once obtained, the recombinant plasmids are inserted into *Agrobacterium tumefaciens* and agroinfiltrated into cotton plants. The CBD2 fragment was cloned into the TRV-RNA2 viral expression vector and inserted into *Agrobacterium tumefaciens*, and the CBD1 fragment was cloned into the pDNOR entry plasmid of the first step of Gateway recombination and inserted into *E. coli*. CLA1 is in the first phase of recombination. The silencing are being analyzed through gene expression by quantitative PCR and plant phenotype. Cotton plants were agroinfiltrated with *A. tumefaciens* containing the expression vectors TRV-RNA1 and TRV-RNA2-GFP to evaluate the efficiency of the system. Virus replication and scattering were evaluated over time through ultraviolet light-excited flowering. Any symptoms due to the TVR infection was observed in agroinfiltrad plants, however, systemic spread of the virus was observed in all plants that received TRV vectors. Cotton showed to be compatible with the VIGS system using TRV as vector, and can be applied as a strategy for studies of functional genomics and biotechnological application.

**Palavras-chaves:** VIGS, Blue Disease, CLRDV, Cotton

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**EVALUATION OF THE PRESENCE OF BEGOMOVIRUS INFECTING SWEET POTATOES IN THE REGION OF PELOTAS-RS**

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Resumo

Among the problems that affect sweet potato cultivation in Rio Grande do Sul (RS), viruses are the main cause of the degeneration of the crop. The characterization of these pathogens has been done almost exclusively by serology, which is why the diagnosis has been mainly associated with RNA viruses. The aim of this study was to evaluate the occurrence and frequency of Sweet potato leaf curl virus (SPLCV) with genome composed of DNA in sweet potato by PCR in the main sweet potato producing regions of Pelotas, RS. For this, 303 leaf samples were collected in different properties of the 3rd, 6th and 9th districts of the city of Pelotas, and subjected to the extraction of total DNA by the CTAB method. Total DNA was tested by PCR-based methods using pair specific primers for begomovirus detection, which amplified a fragment of 1100 bp in 20 samples. In addition, the viral genome of five samples selected was amplified by Rolling Circle Amplification (RCA) using Phi-29 DNA polymerase, and afterwards, RCA products were digested with restriction enzymes for cloning purposes. Digested RCA products were analyzed on 1% agarose gel and Sal I enzyme was selected for cloning viral genome. A DNA fragment of 2800 pb purified from agarose gel was cloned into pBluescript KS vector and sequenced by Macrogen Inc. (Seoul, Korea). The five partial clones obtained for Begomovirus showed 94% sequence identity with the isolated SPLCV-PE [BR: MP4: 09] and SPLCV-PE [BR: MP3: 09]. With the results obtained, the need for constant monitoring is reinforced, besides the adoption of propagating material of sanitary quality and control of vectors. Financial support: CAPES.

Palavras-chaves: Ipomoea batatas, SPLCV, RCA

COMPLETE NUCLEOTIDE SEQUENCE OF TWO COLE LATENT VIRUS ISOLATES INFECTING BRASSICAS IN BRAZIL

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Resumo

Cole latent virus (CoLV) has filamentous particles of 650 nm in length and a (+)ssRNA molecule of aprox. 8,300 nucleotides (nt) as genome. The virus is transmitted by aphids in a non-persistent manner and, up to date, it has been described only infecting cultivated brassicas in Brazil. Usually, CoLV-infected kales (Brassica oleracea) remain asymptomatic, or sometimes show mild mosaic. CoLV is a definitive member of the genus Carlavirus, but it genome has not been completely revealed. To address this, in the current work we have obtained the complete genomes of two CoLV isolates: T-25, an isolate from a horseradish (Armoracia rusticana) plant, Divinolândia, State of São Paulo; and T-90, collected from a kale in Arapiraca, State of Alagoas, Brazil. Total RNA from the infected leaves were extracted using Trizol® reagent (Invitrogen), and the viral sequences were obtained by NGS (Illumina HiSeq 2500 platform). Sequence assembly was carried out using the Trinity software and Geneious Package (version 10.2.2). 5’ end sequences were determined by Rapid Amplification of cDNA Ends (5'-RACE). In silico analyses demonstrated that the two isolates exhibit typical carlavirus genomic organization with six ORFs (replicase, triple gene block, CP, 11 kDa protein) and a poly(A) tail. Both isolates share low percentage of sequence identity with known species of the genus Carlavirus, whose values are below the threshold used for demarcation of new species inside this genus. Clustal analysis
of replicase and CP indicated that CoLV shares the highest sequence identity [replicase - 64% (nt),
58% (aa); CP - 54% (nt), 58% (aa)] with potato virus M. The genomes of isolates T-25 and T-90 share
78% nucleotide sequence identity between them and their CP sequences show relatively high identity
values: 78% and 81.5% (nt) and 94 and 96% (aa), respectively, when compared to the cognate
sequence of the CoLV isolate (AY340584) previously identified in Brazil. Based on CP sequences,
phylogenetic analysis of carlavirus group the CoLV isolates in a well-supported (bootstrap value 96%)
separate clade. Current work provides by the first time the complete genome sequence of the
carlavirus species CoLV.

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Eiras, Juliana Freitas-Astúa e Elliot W. Kitajima are supported by a CNPq fellowship.

Palavras-chaves: CoLV, Carlavirus, Phylogeny, Next-generation-sequencing

MICOVYRUS IN Colletotrichum lindemuthianum ISOLATES FROM RIO GRANDE DO SUL, BRAZIL

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Resumo

Mycovirus are virus that naturally infects fungi. Fungal plant pathogens infected with mycovirus can be
altered in some biological threats such a morphology, grown, sporulation rate, and virulence.
Reductions of fungal virulence by virus are being investigated and represent a potential biological
control of fungal plant pathogens. C lindemuthianum is the main pathogen in common bean (Phaseolus
vulgaris) and its control represents a serious exchange. This research aimed to evaluated the presence
of double-strand RNAs virus (dsRNAv) in 14 C. lindemuthianum isolates from different regions of the
state of Rio Grande do Sul, Brazil. For this purpose, Total nucleic acids of the fungal isolates were
extracted, and the presence of dsRNA was verified by digestion of total nucleic acids with DNase I and
S1 nucleases and electrophoresis. Three of the 14 isolates were dsRNA positive, all of them with two
segments of dsRNA ≈1.5- 2.5 kpb. The presence of virus particles was verified by density gradient
ultracentrifugation and electron microscopy observation. Virus particles were isometric with ≈30nm in
length. To demonstrate the viral effects in the host, chemical curing was carried out (Cycloheximide 2,
5 and 10µg). This results suggest that the observed virus may belong to the Partitiviridae family,
however, genomic data is necessary to confirm. Financial Support: CAPES, CNPq.

Palavras-chaves: biological control, common bean , dsRNA

THE COMPLETE GENOME OF RACHIPLUSIA NU NUCLEOPOLYHEDROVIRUS (RanuNPV) AND
**THE IDENTIFICATION OF A BACULOVIRAL CPD-PHOTOLYASE CLASS II HOMOLOG**

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**Resumo**

The looper Rachiplusia nu is a polyphagous leaf-feeder species widely distributed in South America. The main strategy for controlling the insect pest is based on chemical pesticides that can cause selection of resistant insects and pollute the environment. Therefore, biological control agents arise as an efficient approach to control insect pests in a green and safe fashion. In this report, we described the genome of a novel baculovirus isolated from the polyphagous insect pest R. nu. The genome is 128,587 bp long with a G+C content of 37.9%. Based on the concatenated sequence of the 38 baculovirus core genes, we found that the virus is an alphabaculovirus closely related to the plusiinae-infecting alphabaculoviruses, which include Trichoplusia ni single nucleopolyhedrovirus (TnSNPV), Chrysodeixis chalcites nucleopolyhedrovirus (ChchNPV), and Chrysodeixis includens nucleopolyhedrovirus (ChinNPV). The virus may constitute a new alphabaculovirus species tentatively named by Rachiplasia nu nucleopolyhedrovirus (RanuNPV). After gene content analysis, nine open reading frames (ORFs) were found to be unique to RanuNPV and several auxiliary genes with homologs in other baculoviruses, including four baculovirus repeat ORFs, two inhibitor of apoptosis, a CPD-photolyase (phr), a late expression factor 12, the hydrolases chitinase and cathepsin, and the RNA-ligase he65. We then looked at the evolutionary history of the CPD-photolyase gene and found a single event of horizontal transfer from lepidopterans to alphbaculovirus, followed by a transference from alpha to betabaculovirus. The predicted amino acid sequence of the RanuNPV photolyase homolog appears to retain its active sites, based on the homology model and comparison to other active enzymes. We suggest that the presence of these conserved domains could be related to the activity of repairing CPD lesions in the DNA that likely ensures DNA protection from sunlight damage in the environment. **Financial Support:** CAPES.

**Palavras-chaves:** Alphabaculovirus, evolution, photolyase, plusiinae, rachiplusia nu nucleopolyhedrovirus

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**FILAMENTOUS PHAGE MODULATES BIOFILM PRODUCTION BY Ralstonia solanacearum**

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**Resumo**

Viruses from genus Inovirus (Family Inoviridae) contain a single strand DNA circular genome with flexible filaments particles. Inoviruses can integrate their genome within the host genome or let it free on cytoplasm like epissomal. *Ralstonia solanacearum* is a gram-negative bacterial soil-born with large host range. This bacteria is able to infect more than 250 plant species among 50 botanical families, including several important crops. *R. solanacearum* infects plants across roots from wounds or at secondary emergency roots. From the root, *R. solanacearum* colonizes the xylem vessels spreading rapidly to aerial parts blocking the transport of water, and consequently killing the plants. We found the isolate of *R. solanacearum* infected by an Inovirus that was previously characterized and named as the *Ralstonia* virus RS1BR1 (RS1BR1) and convert the *R. solanacearum* GMI1000 in a commensal bacterium. In this study, we are investigating how RS1BR1 changes the pathogenic phenotype of GMI1000 for a non-pathogenic. Since biofilm production is one of the main virulence factor of *R. solanacearum*, we compared biofilm production in GMI1000 infected and non-infected by RSBR1. The analysis of biofilm production was performed in 96-well polystyrene microliter plates. For this, GMI1000 infected and non-infected RSBR1 by isolates was grown on static conditions and the biofilm production was measured using the violet crystal method. We observed that infected bacterial with RS1BR1 shows a reduction of biofilm production in 20%. We also investigated how RS1BR1 reduced biofilm formation by analyzing the production of small diffusible signaling (SDSM) molecules, also involved in biofilm formation in *R. solanacearum*. Preliminary results showed that the reduction of biofilm production is not connected to SDSM production. A better understanding of how viruses can modulate the pathogenicity of
bacteria can lead to a development of new methods to control the disease caused by R. solanacearum.

**Palavras-chaves:** Inovirus, Biofilm, Small diffusible signaling molecules, Ralstonia virus RS1BR1

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**BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF BRAZILIAN ISOLATES OF TURNIP MOSAIC VIRUS FROM WEEDS AND BRASSICAS CROPS**

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**Resumo**

In Brazil, four virus species have been reported infecting Brassicaceae, including Turnip mosaic virus (TuMV), the only potyvirus that infects plants of this family. There is a classification of TuMV isolates based on their interaction with resistance genes present in lines of *Brassica napus*. Phylogenetic analyses have classified TuMV in groups including World B (infecting only *Brassica*), Basal [B] (European isolates infecting only *Brassica*), BR (Asian and European isolates infecting *Brassica* and *Raphanus*) and OMs (infecting orchids). There is a knowledge gap in terms of the diversity of TuMV infecting brassicas in Brazil. The goals of our work were to characterize Brazilian TuMV isolates by sequencing their genomes and pathotyping them using the *B. napus* differentials. Five isolates were selected according to their host and geographical origins, T06 (*Armoracia rusticana* [horseradish], Divinolândia/SP), T22 (*Raphanus raphanistrum* [wild radish], Munhoz/MG), T31 (*Brassica oleracea* var. *italica* [broccoli], Santo Antônio do Pinhal/SP), T48 (*Brassica pekinensis* [Chinese cabbage], Biritiba Mirim/SP) and T61 (*R. raphanistrum*, Mogi das Cruzes/SP). Pathotyping and host range was carried out by mechanical inoculation of the isolates to four *B. napus* plant lines (S6, 165, R4, 22S) and *Brassica* and *Raphanus* species. The plants were monitored for symptom expression and tested using PTA-ELISA. Isolates T06, T22, T48 and T61 were classified as pathotype 1, whereas T31 did not fit into the previously 12 described pathotypes. It can be considered as a new pathotype, or a virus recombinant capable of overcoming the resistance in *B. napus*. TuMV-infected plants were subjected to total RNA extractions and sequencing. Phylogenetic analyses of full-length genomes using the maximum likelihood method and Mega (version 7.0) revealed that T61, T22 and T48 isolates formed a clade with isolates of the BR group, whilst T31 and T06 were clustered with World [B]. However, there was a divergence of results, T31 and T06 were able to infect *Raphanus* and belong to the World [B] group, comprised of isolates that infect only *Brassica* species. The inclusion of Brazilian TuMV isolates indicates the classification may need to be reconsidered.

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**Palavras-chaves:** TuMV, Potyviridae, pathotyping, complete sequencing, phylogeny

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**REAL TIME RT-PCR FOR DETECTION OF Cowpea aphid-borne mosaic virus AND INCIDENCE OF THE VIRUS IN PASSION FRUIT ORCHARDS IN SOUTH OF BRAZIL**

**Autores** Neemias da Silva Santos, Francis Zanini, Daniel Moritz, Henrique Petry, Edson Bertolini
Brazil is the world’s largest passion fruit grower and consumer, with a production of 695,000 tons per year. The passion fruit occupies an outstanding role in the Brazilian economic scenario, although its productivity is limited by phytosanitary factors. The viruses are the most important among the plant diseases, becoming a limiting factor to its cultivation expansion. The passion fruit woodiness disease is the main viral disease and in Brazil is caused by *Cowpea aphid-borne mosaic virus* (CABMV) which belongs to the *Potyvirus* genus and is transmitted in a non-persistent manner by several aphid species. The CABMV is endemic in the producing regions of Brazilian northeast and is responsible for the reduction of cultivated areas in the country; it has been reported in the states of Santa Catarina (SC) in 2008 and Rio Grande do Sul (RS) in 2016. Detection methods have the greatest importance in establishing control strategies allowing multiply health plant material. The objective of this study was to develop and validate a real time RT-PCR protocol to detect CABMV and the virus survey in the passion fruit’s orchards throughout Santa Catarina and Rio Grande do Sul. The real time RT-PCR has proved to be a hundred times more sensitive than the serological technique. In order to understand the virus incidence, fifty plants were monthly analyzed by the real time RT-PCR protocol during at least five months at three different regions. At São João do Sul, SC, the virus was detected with 6% prevalence at the implementation of the orchard that happened in September 2017, reaching 100% incidence in January 2018. At Torres, RS, the CABMV was detected only after ninety days of cultivation with a 10% incidence, reaching 100% in April 2018. At Araquari, SC, orchards no virus were detected. The sample preparation protocol and the real time RT-PCR are fast, economic and simple tools used to detect the CABMV. The use of infected seedlings during the implementation of the orchards contributed to the virus spread.

**Palavras-chaves:** virus, epidemiology, CABMV

THE PREFERENCE OF *Aphis gossypii* IN RESPONSE TO VOLATILES OF PASSION FRUIT INFECTED AND NON-INFECT WITH *Cowpea aphid-borne mosaic virus*.

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Resumo

The woodiness disease caused by *Cowpea aphid-borne mosaic virus* (CABMV) is the main viral disease of the passion fruit, being the virus transmitted by aphids in a non-persistent manner. The main objective of this research was characterize the preference of the aphid *Aphis gossypii*, in response to volatiles issued by passion fruits plants infected and non-infected by CABMV. Two bioassays were performed in the Etologia de Insetos Laboratory of UFRGS, in November of 2017, using *A. gossypii* and SCS Catarina cultivar of *Passiflora edulis* plants with 50 cm of height, infected and non-infected by CABMV. Colonies of adults and apterae aphids were collected in *Solanum americanum*, obtained from a passion fruit orchard localized in Santa Rosa do Sul-SC. The infected passion fruit plants were obtained in an orchard localized in São João do Sul-SC. The healthy plants were obtained on an EPAGRI's protected nursery in Urussanga-SC. The confirmation of CABMV infection was performed in the Virologia Vegetal laboratory of UFRGS, through real time RT-PCR technique. The behavioral bioassays were performed in an olfactometer type Y with pressurized air at 26°C. In the first one, non-infected versus plant absence (control) were contrasted. In the second bioassay non-infected versus CABMV infected passion fruit plants
were contrasted. The evaluated responses were the displacement of the aphid in a gap of 300 seconds and the permanence in one of the chosen arms for 30 seconds (residential time). Repetitions with 20 aphids by bioassay were performed. The results were submitted to chi-squared test through the “R” software, at a significance level of 5%. In the first bioassay no significant differences were observed: 68% of the aphids choose passion fruit plants and 32% choose the control. These results confirm that *A. gossypii* not colonize neither is attracted by passion fruit plants. In the second bioassay, 89% of the aphids chose the CABMV infected plants in detriment of the non-infected ones, confirming the preference of the *A. gossypii* for diseased plants. *A. gossypii* could have a chemical perception of volatiles compounds issued by infected passion fruit plants. New researches need to be performed to discover which volatiles issued by CABMV infected passion fruit plants affect the behaviour of the aphids.

**Palavras-chaves:** Aphid, virus, woodiness disease

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**VARIABILITY OF THE MOVEMENT PROTEIN GENES OF APPLE CHLOROTIC LEAF SPOT VIRUS ISOLATES FROM APPLE AND PLUM**

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**Resumo**

Infections by *Apple chlorotic leaf spot virus* (ACLSV) occur, generally, latently in the majority of commercial apple and plum cultivars. In some cvs. the virus causes severe symptoms with substantial economic losses. ACLSV is a single stranded positive sense RNA virus in the genus *Trichovirus*, family *Betaflexiviridae*. Movement proteins (MP) exert an important role in cell-to-cell spread of viruses away from initial infection sites. Genetic variability and diversity in plant RNA viruses are determined by many viral and host factors, including the error-prone characteristic of polymerases, the absence of 3'-proof-reading capacity of polymerases of plant RNA viruses, and as result of a viral adaptation strategy. Sequence comparisons reveal high variability between ACLSV isolates. A comprehensive molecular knowledge of regional virus isolates of ACLSV is a fundamental support to improve virus detection and characterization. Total RNA was extracted by adsorption to SiO₂ from leaf samples of apple cvs. Cripps Pink, Golden Delicious, Red Delicious, and plum cv. Polli Rosa. Primers CL 5717s 5' GAT GGC GAT GAT GAT AAG GGG TCA C -3' and CL 7103as 5' GCC TCA CAC ACC TGG CGG -3' (NC_001409) were based on _in silico_ analysis of GenBank data using CLC Sequence Viewer. Multiple alignment (ClustalX 2.1) and phylogenetic analysis of nt sequences showed that the MP of isolate M176, cv. Red Delicious (MH101991) clusters in a group with apple isolates (Japan and Germany) that lack three nucleotides at positions 951-953 (NC_001409). MPs of isolates M177 (MH521129) and M075 (MH521130), respectively from cvs. Golden Delicious and Cripps Pink, cluster with South Korean apple isolates and MP of plum isolate PR1 (MH101992) clusters with a group of peach isolates (China and South Korea). Genetic variability and selection analysis of viral sequences obtained in this study and sequences available in GenBank indicate that MPs are under purifying selection pressure, and that recombination events occurred in Brazilian, Japanese and South Korean isolates. The MP of Brazilian and South Korean isolates showed lower genetic variability than Chinese and Japanese isolates. Based on mean pairwise number of nucleotide differences per site, the 5' and 3' terminal regions of
The tomato DnaJ protein SIDj1 co-localizes with potyvirus replication vesicles in plants infected by Turnip mosaic virus

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Resumo

Potyvirus is a large and important genus of plant viruses. Together, its members infect a great number of crops and wild plants around the world, causing serious economic losses. Potyviruses have a positive sense ssRNA genome that expresses eleven proteins which interact with host factors to manipulate the plant cell in favor of virus establishment and multiplication. Virus infection induces the remodeling of cellular membranes resulting in the formation of vesicles and other structures related to virus replication and cell-to-cell movement. The viral membrane-associated protein 6K2 is implicated in vesicle formation and can be used as a marker to visualize virus induced structures by microscopy. Many proteins containing the DnaJ domain, also known as Hsp40 proteins, are present in plant genomes. They act as Hsp70 co-chaperones and as chaperones themselves. The involvement of proteins from both families in infection by different viruses was described, acting in processes as viral encapsidation, movement and replication. A tomato (Solanum lycopersicum) DnaJ protein (SIDj1) was previously identified as induced during the infection by the potyvirus Pepper yellow mosaic virus (PepYMV). The downregulation of nine homologs to SIDj1 in Nicotiana benthamiana, results in phenotypic alterations that resemble PepYMV symptoms. The infection is reduced in silenced plants, suggesting that these proteins are involved in virus infection. Aiming to understand the role of SIDj1 in potyvirus infection, SIDj1 was fused to the fluorescent protein GFP and its subcellular localization was analyzed by confocal microscopy in N. benthamiana plants infected by an infectious clone of the potyvirus Turnip mosaic virus (TuMV) expressing the 6K2 protein fused to mCherry. SIDj1 co-localizes with 6K2 induced vesicles and with a perinuclear globular structure typically observed in TuMV infected cells. Since DnaJ proteins are Hsp70 co-chaperones, and the Hsp70 cognate protein Hsc70.3 from Arabidopsis thaliana was previously described as a component of TuMV replication vesicles, SIDj1-RFP was co-expressed with AtHsc70.3-GFP in plants infected by TuMV and mock inoculated. The proteins co-localizes in both situations. The results suggest that SIDj1 may be present in replication vesicles and act together with Hsc70.3 in virus infection. Further studies are needed to confirm that hypothesis and to better understand the SIDj1 role in potyvirus infection. Financial support: CAPES, CNPq, FAPEMIG.

Palavras-chaves: Plant-virus interaction, Chaperones, Potyvirus
MOLECULAR CHARACTERIZATION OF WHEAT STRIPE MOSAIC VIRUS: A NOVEL VIRUS-ASSOCIATED WITH SOIL-BORNE WHEAT MOSAIC DISEASE IN BRAZIL

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Autors

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Resumo

Diseases caused by fungi, bacteria, nematodes and viruses affect wheat (Triticum aestivum). The soil-borne wheat mosaic disease (SBWMD) is one of the most common viral disease that causes economic losses in wheat in Brazil. The hypothesis that this disease is caused by the Soil-borne wheat mosaic virus or Wheat spindle streak mosaic virus in Brazil is based on biological characteristics, in vitro physical properties, particles morphology, as well as the ultrastructure of the affected cells. However, use of antiseras or primers produced in other countries, resulting unsatisfactory reactions in diagnostic tests. Two possibilities may explain the unsatisfactory results, first: the virus-associated with SBWMD present high genetic variability; second: another viral species, not yet characterized, is infecting the wheat. The aim of this study was to characterize molecularly the viral species associated with wheat plants presenting SBWMD in Brazil. Wheat leaves and stems displaying mosaic symptoms were collected from different wheat cultivars in Passo Fundo, Rio Grande do Sul State, southern Brazil. Double-stranded RNA was extracted and submitted to next generation sequencing. The nucleotide (nt) sequence of a putative new member of the Benyviridae family was determined, and the name Wheat stripe mosaic virus (WhSMV) was proposed. Five primer pairs were designed to confirm the new virus infection from samples used to NGS. All primer pairs used in the PCR reactions resulted in amplifications of expected size fragments. WhSMV has a bipartite genome similar to RNA1 and RNA2 of the viruses belonging to the Benyviridae family. WhSMV RNA1 with size from 6583 to 6600 nts, contains a single open reading frame (ORF) encoding a 231.7 kDa polyprotein with putative viral replicase function. WhSMV RNA 2 with size from 4879 to 4901 nts, contains six ORFs: putative coat protein (CP); putative the major protein - readthrough (RT); putative triple gene block (TGB1, 2 and 3), possibly associated with viral movement; and a hypothetical protein of 2.8 kDa. In addition to the genomic organization and nt and amino acid sequence identities, phylogenetic analyzes showed that WhSMV is related to viral species of the Benyviridae family and distinkted from the other species of the Benyvirus genus. Financial Support: BIOTRIGO Genética, UDESC, EMBRAPA, CAPES.

Palavras-chaves: Triticum aestivum, mosaic, NGS, virus, Benyviridae
DETECTION AND IDENTIFICATION BY NEXT GENERATION SEQUENCING OF VIRUSES IN TOMATO

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Resumo

The tomato is the Solanaceae of most importance in the world and in Brazil. However, this crop is the target of several phytopathogens that affect production, the viruses is one of the major limiting factors for the tomato. Among the viruses that cause the most damages to the tomato culture in Brazil stand out the genus Begomovirus, Potyvirus, Tospovirus and Crinivirus. The identification of viruses and undescribed viral species generally requires application large selection of traditional and modern techniques. However, the improvement of large-scale sequencing enabled the development of new tool known as next generation sequencing (NGS) contributing to a revolution in detection and discovery of plant viruses and viroids. In this work aimed to know a diversity of viruses and viroids presents in tomato plants of from municipality of Pelotas, Rio Grande do Sul, Brazil using NGS. Samples of tomatoes presenting symptoms of chlorosis and foliar necrosis were collected. The samples was subjected to RNA extract ion using Trizol Reagent, as recommend by the manufacture, were quantified and sequencing on the Illumina HiSeq 2500 platform. A total of 29,883,224 reads were obtained. Adapters and reads with low quality were removed from the sequences obtained using Trimmomatic and the contigs were constructed using the Trinity Assembler. Diamond was to align de novo assembled transcripts to the trEMBL database and then those contigs that mapped to Virus proteins using the annotation data provided by UniProt were selected. Based on these methods, we have detected hits with nucleotide identity mainly with the viral species Potato virus Y (PVY) and Tobacco vein clearing virus (TVCV), beyond detection of one viroid Citrus exocortis Yucatan viroid (CEVd). The NGS technique was efficient for the detection of RNA virus, as Potyvirus was expected due to reports of its occurrence in this region, but it was also possible identification of virus and viroid not yet reported in tomato (like TVCV and CEVd), respectively. Additional work is required to confirm the presence of these virus and viroid. Financial Support: CAPES.

Palavras-chaves: NGS, tomato, virus

NOVEL VIRUSES NATURALLY FOUND CO-INFECTIONING LEGUMINOUS FORAGE PLANTS IN BRAZIL, BELONG TO TWO NEW GENUS OF THE POTYVIRIDAE FAMILY

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Resumo

In Brazil forage crops represent large areas of tropical pastures for cattle feeding. These pastures can be either natural or planted and usually comprises forage grasses and legumes. The use of *Stylosanthes* sp. (legume) has increased in Brazil due to its nutritional value, and for presenting great potential in nitrogen fixation. Recently, mosaic-like symptoms have been observed in the field, indicating that this pasture legume is susceptible to virus-like pathogens. Since these symptoms strongly suggested viruses as the pathogenic agents, we have performed RNA high-throughput sequencing of virus-enriched fractions isolated from symptomatic stylosanthes leaves. Sequence
analysis blasted against virus data bases revealed complete virus genomes representing novel members of the family Potyviridae co-infecting stylosanthes plants in Brazil. These new viruses were readily recoved from infected plants by RT-PCR amplification using designed virus-specific primers. These putative new three viruses have been tentatively named “Stylosanthes mosaic-associated virus 1” (StyMaV-1) and “Stylosanthes mosaic-associated virus 2” (StyMaV-2) and “Stylosanthes mosaic-associated virus 3” (StyMaV-3). Based on the taxonomic rules for species demarcation, these viruses should be considered members of new genus within the family Potyviridae. Phylogenetic analysis showed that StyMaV-1 and StyMaV-2 are most closely related to Blackberry virus Y (genus Brambyvirus) with capsid protein (CP) amino acid (aa) identity of only 46% and 44%, (respectively). For StyMaV-3 a comparative analysis of the full genome showed 58% nucleotide (nt) sequence identity to a Rose yellow mosaic virus belonging to the new proposed genus Roymovirus. Host range assays by mechanical inoculation revealed that StyMaV-1 and StyMaV-2 were able to infect soybean plants that pose a threat to soybean cultivars. Transmissions assays are currently being carried out to determine the vectors involved in the spread of these new viruses. Overall, here we report for the first time, the complete genome sequences of two novel viruses (StyMaV-1 and StyMaV-2) infecting S. guianensis plants in Brazil, that should be considered members of a new genus tentatively denoted “Stylomovirus” within the family Potyviridae and a third new virus (StyMaV-3) belonging to the genus Roymovirus.

Palavras-chaves: legumes, livestock, Potyviridae, Stylomovirus, Stylosanthes

PRELIMINARY SCREENING FOR RESISTANCE TO Sugarcane yellow leaf virus (SCYLV) IN A PANEL OF SUGARCANE GENOTYPES (Saccharum sp.)

Autores

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Resumo

Yellow leaf disease (YLD) of sugarcane caused by Sugarcane yellow leaf virus (SCYLV) is one of the main viruses of this crop. The characterization of genotypes in response to SCYLV is fundamental to the development of resistant cultivars. Ninety eight sugarcane genotypes from an experimental field nursery were evaluated to determine resistance and susceptibility to SCYLV using foliar symptoms severity scores (Top Visible Dewlap leaf: TVD0, TVD1, TVD2 and TVD3) and quantification of the virus by DAS-ELISA. Aiming to guarantee inoculum pressure, Melanaphis sacchari, Zenith aphids' colonies were reared in lab conditions on SCYLV-infected leaves and released in the nursery. To measure SCYLV severity, a diagrammatic symptom scale was elaborated from one to four scores, where one means no symptoms (leaf completely green) and four leaf blade and midrib totally yellow. The Pearson correlation coefficient (r) was used to measure the correlation between symptom notes and virus titer. In general there was a low correlation between virus titer estimated by ELISA and the symptoms observed in the field. The highest correlation (r = 0.81) between symptoms and virus titer values was found on the TVD0 leaf. Cultivar SP-716163 showed high values in both, symptoms severity (score 4) and serological data (159 ng/100 µL), confirming the high degree of susceptibility of this genotype. All of ten genotypes IACBIO plus cultivars NG5712, CTC15, IN8488, IN8458, IJ76-293, US571415, IACCTC05-3616, IACCTC06-1050, IACCTC05-6518 and KRAKATAU showed no symptoms (score 1) and low virus titer therefore were considered resistant to SCYLV. Genotypes IACCTC06-9708, IACCTC06-9767, IACCTC05-9634 and IACSP02-3025 exhibited virus concentrations between 52 and 95 ng/100uL with symptoms (score 2 to 3) and were classified as susceptible. A group of seven genotypes (IACSP04-5065, IACSP97-6680, IACSP97-2084, IACSP01-8082, IACSP04-3150, IACSP04-3259, and
IACSP96-3069 showed intermediate symptoms (score 2) however, had low virus titer by DAS-ELISA being classified as susceptible to viral infection. On the other hand, cultivars IACSP97-6628, IACCTC05-9552 and IACSP95-6114 presented low scores of symptoms (score 1) but high virus titer, being classified as resistant to YLD. All of the sixty three remaining genotypes showed low score symptoms (1 to 2) and intermediate virus titer, corresponding to tolerant genotypes.

Financial support: R.C. Burbano is recipient of a PhD fellowship from AU/IP/PAEDEx

Palavras-chaves: SCYLV resistance, Melanaphis sacchari, virus titer, symptoms scale

INSIGHTS INTO THE TRANSMISSION AND EPIDEMIOLOGY OF MAIZE YELLOW MOSAIC VIRUS: AN EMERGING POLEROVIRUS.

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Resumo

Maize yellow mosaic virus, provisionally referred as MaYMV, is a novel Polerovirus reported in China, Brazil, Ecuador, and more recently in Africa. The broad distribution suggests the virus is potentially emerging, and along with known viruses may consist in a new bottleneck to maize production worldwide. In Brazil, we examined a variety of symptomatic maize samples from different locations of the state of São Paulo. Along with leaves and whole plants, we also collected aphids from these locations in order to test for virus transmission. Considering that this emerging virus is a Polerovirus, we hypothesized a circulative transmission mode and the possible presence of virus particles in the aphids’ bodies. Our research then aimed toward better understanding the incidence, transmission and the epidemiology of this virus. Plant and aphids samples were submitted to total RNA extraction, reverse transcription with random primers and oligodT, PCR with specific primers for the novel Polerovirus, Sugarcane mosaic virus (SCMV), Maize rayado fino virus (MRFV), and Maize chlorotic mottle virus (MCMV). We have detected MaYMV widely distributed in several samples from the state of São Paulo. Moreover, in all cases mixed infections with other viruses were always present, e.g., SCMV and/or MRFV. Confirming our hypothesis of circulative transmission, we also detected MaYMV in the aphids’ bodies extracts. Sanger sequencing of this 1800 bp MaYMV amplified fragment showed that its nucleotide sequence is 99% identical to that of our fully sequenced isolate MaYMV-SP1 (KY940544.1). In order to isolate this new polerovirus and describe the specific symptoms caused in maize, work with transmission tests is in progress with different aphids’ species. These findings contribute to a comprehensive understanding of the transmission and epidemiology of MaYMV and other viruses found in coinfection, what is crucial for the development of optimal disease management recommendations.

Financial support: A. Ramos and T. Nascimento are recipients of PhD and under-graduation fellowships from CNPQ, respectively.

Palavras-chaves: maize, polerovirus, vector transmission

Survey of tomato chlorosis virus and tomato severe rugose virus in weed plants collected in
Resumo

Tomato chlorosis virus (ToCV, genus *Crinivirus*, family *Closteroviridae*) and tomato severe rugose virus (ToSRV, genus *Begomovirus*, family *Geminiviridae*) cause the main viral diseases (yellowing and golden mosaic, respectively) in tomatoes in Brazil. The golden mosaic disease was first reported in 1960 and since the 1990's it has been causing severe losses in tomato crops in Brazil. The yellowing disease was first reported in tomato plants in São Paulo state in 2006, and was rapidly disseminated to several other regions. High incidence of both diseases is very common in tomato productions areas. The objective of this study was to determine the alternative inoculum source of ToSRV and ToCV in the field. Then, a survey was conducted on weed plants found around tomato production areas in Goiás state and the Federal District. A total of 212 weed samples were collected and subjected to PCR and RT-PCR, respectively, for ToSRV and ToCV detection. Only 5 and 8 out of 212 weed samples were positive for ToSRV and ToCV detection, respectively. Three weed species were infected, *Nicandra physaloides*, *Sida* sp., with both viruses, and *Solanum americanum* only with ToCV. The low incidence of weed plants potentially infected with these viruses suggested that these plants may not play an important role in the epidemics of golden mosaic and yellowing diseases. Therefore, it is likely that the major inoculum sources for these viruses in the fields are the tomato crops or other cultivated plants susceptible to these viruses.

Palavras-chaves: ToCV, ToSRV, weeds
The reads were subjected to pre-processing, with filtering according to quality criteria using PrinSeq. Single-nucleotide polymorphisms (SNPs) analysis was done using Geneious 9.1.2 software with the following parameters: minimum coverage of 100, frequency of 0.001 and a quality value of 30. We obtained 13 WSMV whole-genome sequencing resulting in average coding depth between 404 and 1654 reads per base across the coding region. This deep coverage creates a high-resolution view of resulting in the distribution and frequency of mutations within viral population. We obtained low SNPs frequency in 10 of the 13 viral populations. We found different number of SNPs according to the sequences of the protein. However, three isolates of WSMV from wheat were distinguished by their high SNPs frequency and distribution throughout all of the coding sequencing. Comparison of the SNPs patterns between the different host (Wheat, triticale, Avena fatua and Digitaria sanguinalis) not presented difference as it was reported for other virus. The knowledge of the spectrum of spontaneous mutations of WSMV is important given that small changes have implications in the pathogenicity, epidemiology and viral resistance-breaking.

Financial Support: INTA Project No. PNPV-1135024, PNCYO-1127034.

**Palavras-chaves:** SNPs, whole-genome sequencing , intra-host genetic diversity , pyrosequencing, wheat

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**Exploring virus diversity in grapevines**

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**Resumo**

The knowledge about diversity and ecology of viruses has increased in recent years. Consequently, this increase has shown that viruses set up several relationships with other organism beyond of parasitism. The study of viral diversity has been expanded in last years, special due to improvements in sequencing technology. In this work we analyzed viral diversity in an RNA-Seq data from grapevine plants from several different places. Twelve grapevines samples were collected from experimental filed in Brazil. The dsRNA total was extract and cDNA library and sequenced using Illumina Hiseq 2000 platform. The reads obtained was analyze for FastQC, and Trimmomatic was use for clear and trimming. Trinity was use for de novo assemble. The identiti of contigs from de novo assemble was analyze through NCBI Blastn against the RefSeq and EMBL databases. We found representatives of families Tymoviridae, Betaflexiviridae, Peribunyaviridae, Phycodnaviridae, Siphoviridae, Baculoviridae, Podoviridae, Myoviridae, Phycodnaviridae, Siphoviridae, Reoviridae, Phasmaviridae, Tospoviridae, Iridoviridae, Peribunyaviridae, Closteroviridae, Luteoviridae, Caulimoviridae, Chrysoviridae, Partitiviridae and Totiviridae. The families Tymoviridae, Betaflexiviridae, Podoviridae, Myoviridae and Partitiviridae shows more prevalence than the other families. Interestingly we also found some Nucleocyttoplasmic large DNA viruses and viroids classified as Pospiviroidae. We identified 25 species of mycoviruses from which 12 species were Chysoviridae, followed by eight species of Partitiviridae and three species of Totiviridae. In addition, we identified three species of unclassified mycoviruses. Additionally, seven mycoviruses infected fungus phytopathogenic of which three species infected fungal which infected grapevine plants. The methodology initially applied in this study demonstrated demonstrate that there is a broad diversity of viruses on grapevine plants, further studies are needed in order to identify the host of these viruses. Financial Support: FAPEMIG, EMBRAPA and UFV.

**Palavras-chaves:** Virus diversity , RNA-Seq, Mycoviruses

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THE EVOLUTIONARY HISTORY OF A VIRAL GLYCOSYLTRANSFERASE IN
BETABACULOVIRUS

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Resumo

Viruses are cell-obligate toxic agents that can alter the host behavior during infection. Caterpillars infected by several baculoviruses migrate to the upper foliage of the host plant where they die and liquefy as a sign of infection. This climbing up behavior is associated with the expression of a glucosyltransferase (egt) gene whose product inactivates insect molting and keeps it into the larval stage. Baculoviruses are divided into four genera, two of them Alphabaculovirus and Betabaculovirus (subdivided into clade-A and –B) are infectious to moths and butterflies. Importantly, the evolutionary history of the egt in these groups is not clear. In this work, we reconstructed the virus phylogeny using a set of conserved genes and prospected for the presence and absence of the egt in baculovirus completely sequenced. The egt sequences found were aligned based on a PDB-crystal structure to perform a phylogenetic reconstruction. As previously observed, we found that egt is a viral gene acquired from an insect host. All alphabaculoviruses presented egt whereas betabaculovirus varied. Remarkably, we found that alphabaculovirus possibly transferred the gene to a betabaculovirus ancestor then the virus lineages underwent three independent losses: an ancestral loss in clade-A and two independent losses in viruses from clade-B i.e. Diatraea saccharalis granulovirus (DisaGV) and Cnaphalocrocis medinalis granulovirus (CnmeGV). The influence of egt on the climbing up behavior is unclear and its evolutionary history in betabaculovirus has never been described. The egt might have been product of a horizontal transfer from alphabaculovirus during a co-infection scenario followed by three independent losses. Interestingly, in the case of DisaGV and CnmeGV that lack an egt homolog, both of them infect caterpillars that belongs to the lepidopteran family Crambidae and present tunnels-living habits inside the plant that could potentially impair the climbing up behavior.

Financial Support: CAPES

Palavras-chaves: betabaculovirus, glycosyltransferase, tree-top disease

Emergence of a recombinant virus between Squash vein yellowing virus and new potyvirus species in cucurbit crops in California, USA

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Resumo

Squash vein yellowing virus (SqYVV), a whitefly-transmitted ipomovirus, causes economic losses to cucurbits in Florida and Central America. In 2014, SqYVV was detected infecting cucurbit plants in Southern California (SqYVV-CA), and a putative full-length clone was generated with Gibson Assembly strategy. The full nucleotide sequence of SqYVV-CA was more similar to an isolate from Israel (SqYVV-IL, ~93%) compared with an isolate from Florida (SqYVV-FL, ~81%). A full-length
clone of SqVYV was agroinoculated into pumpkin plants and induced symptoms of mild vein clearing and yellowing ~21 dpi. This confirmed the infectivity of the clone, but the symptoms were relatively mild. We identified a recombination event of ~1000 bp in the first gene (P1a) of SqVYV-CA. The identity of this recombinant fragment was substantially lower (45%) than other SqVYV isolates, and was more closely related (70%) to a potyvirus species, *Papaya ringspot virus* (PRSV). Phylogenetic analysis with the nucleotide sequence of the capsid protein (CP) placed SqVYV-CA and SqVYV-IL on the same branch, and SqVYV-FL was placed on a different but adjacent branch; whereas the P1a analysis placed the SqVYV-CA on a branch with PRSV. SqVYV-CA may have been introduced from the Middle East, and underwent a recombination event of ~1000 nucleotides.

**Palavras-chaves:** SqVYV, Ipomovirus, recombinant

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**Insecticidal activity of granulovirus and nucleopolyhedrovirus isolated from a natural coinfection in Spodoptera ornithogalli larvae**

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**Resumo**

The yellow-striped armyworm, *Spodoptera ornithogalli* (Guenée) (Lepidoptera: Noctuidae) is a polyphagous pest widely distributed from Canada to Argentina, including the Caribbean islands. It has been reported on a variety of crops including alfalfa, sorghum and soybean. In Colombia, *S. ornithogalli* has been described mainly as a pest of cotton, citrus and cut-flowers. Although the damage caused to crops is mainly due to leaf herbivory, damage to tomato fruits and cotton bolls has also been reported. Here, we studied the insecticidal activity of a Granulovirus (GV) and a Nucleopolyhedrovirus (NPV) isolated previously from a naturally occurring co-infection of a *S. ornithogalli* larvae. For the separation of GV and NPV the viral suspensions were centrifuged in sucrose gradients and the obtained fractions were used to infect second instar larvae. The process was repeated three times until pure suspensions were obtained, which were then verified through transmission electron microscopy. The median lethal concentration (LC₅₀) of each isolate was calculated on neonate *S. ornithogalli* larvae using concentrations between 1×10⁴ and 1×10⁸ occlusion bodies (OB)/mL. The LC₅₀ for SporNPV was 2.3 x 10⁵ OB/mL, which was higher than the obtained using naturally mixed viruses (1.4 x 10⁵). Both the LC₅₀ and the median mortality time in second instar larvae of SporGV was significantly higher than those of SporNPV. Our results suggest a positive effect in the insecticidal activity of the co-infection of alpha and betabaculovirus, similar to reported in other noctuids. The baculoviruses co-infection could be taken advantage in the development of more efficient biopesticides. The research was financed by AGROSAVIA.

**Palavras-chaves:** Alphabaculovirus, Betabaculovirus, Biological control, Coinfection, Spodoptera ornithogalli

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**q-PCR-based method for the quantification of the Erinnyis ello baculovirus in a biopesticide formulation**

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**Resumo**
The hornworm, *Erinnys ello* Linnaeus 1758 (Lepidoptera:Sphingidae) is a widely distributed, polyphagous pest in the Americas. In Colombia it is considered a pest of rubber trees and cassava, and also can attack carica, tomato and cotton crops. The Colombian Corporation for Agricultural Research Agrosavia developed a biopesticide based on an *E. ello* Granulovirus (ErelGV isolate VG010), demonstrating the potential of this Betabaculovirus for the control of the pest. However, quality control for the product requires a method to quantify the amount of active ingredient (occlusion bodies of ErelGV) in prototype batches. To validate this methodology the biopesticide formulation, the excipients without virus and a purified viral suspension previously quantified through spectrophotometry using a calibration curve were used. The procedure was carried out using the supernatant of the dilutions (1:50 w/v) of the powders in sterile water which were heat-treated (100 °C, 5 min). The results show that the occlusion bodies concentration determined by q-PCR (1.78 x 10^9 OB/g) was very close to that expected in the manufacturing procedure (1.0 x 10^9 OB/g). The concentration of purified viral suspension was also similar to that obtained by spectrophotometry. The minimum detection limit was determined as well as the reproducibility of the technique. Our results show that the method is reproducible, sensitive and specific for the quantification of ErelGV OB on a biopesticide. This technique could be used as a quality control methodology in further development of betabaculoviruses based biopesticides. The research was financed by AGROSAVIA.

**Palavras-chaves:** Betabaculovirus, Biopesticide, Erinnyis ello, Real time PCR , Taqman Probes

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**GENOME CHARACTERIZATION OF A TENTATIVE NEW SPECIES OF CITRUS LEPROSIS-CAUSING DICHORHAVIRUS: Citrus bright spot virus**

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**Resumo**

Citrus leprosis (CL) is the main viral disease affecting the Brazilian citriculture. Endemic in the Americas, the disease is caused by a heterogenic group of *Brevipalpus*-transmitted viruses assigned to the genera *Cilevirus*, (+)ssRNA (floating genus), and *Dichorthavirus*, (-)ssRNA, family *Rhabdoviridae*, order *Mononegavirales*. At present, three CL-causing dichorthaviruses have been described: orchid fleck virus (OFV), citrus leprosis virus N (CiLV-N) and citrus chlorotic spot virus (CiCSV). Recently, sweet orange trees (*Citrus sinensis*) showing leaves with characteristic necrotic and bright chlorotic symptoms of CL were identified in the states of Santa Catarina and Rio Grande do Sul, Southern region of Brazil. Transmission electron microscopy analyses of symptomatic tissues confirmed the presence of bacilliform particles with ~40x100 nm and viroplasm in the nucleus of infected cells. RNA extracts from two of the sampled plants but that proved negative by RT-PCR tests for the detection of all known leprosis-causing viruses, were analyzed by NGS. After sequencing, the two viral genomes were assembled using Trinity program and Geneious software package. The complete genomes revealed viruses composed ~13000 nts each one, split into two molecules and with a typical organization of that found in known dichorthaviruses. Their RNA1 molecules harbor five ORFs: 3′-N-P-MP-M-G-5′, whereas the RNA2 segments encode only one ORF, the L gene. Globally, the genomes of the two new isolates share 98-99% nucleotide sequence identity between them. In the comparison with the definitive and tentative members of the genus *Dichorthavirus* CiLV-N, OFV, CiCSV, coffee ring spot virus, and *Clerodendrum* chlorotic spot virus, the new viruses showed 72%, 57%, 60%, 59%, and 60% nucleotide sequence identity, respectively. These identity values are below the criteria for demarcation of new species inside the genus *Dichorthavirus* (< 80% RNA1 and L). Therefore, according to the symptoms observed in the infected plants, the morphology of the virions, the structure of the genome, and the nucleotide sequence identity with other dichorthaviruses, the two isolates were considered as members of a tentative new species of the genus *Dichorthavirus*, and the name Citrus bright spot virus is suggested.
IDENTIFICATION OF Groundnut ringspot virus (GRSV) ASSOCIATED WITH WATERMELON THRIPS IN CENTRAL BRAZIL

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Resumo

Thrips (Thysanoptera: Thripidae) are insects of major relevance in the watermelon cultivation, since they are vectors of different plant viruses. Thus, the aim of this work was to evaluate the presence of Groundnut ringspot virus (GRSV) carried by thrips in watermelon cultivation in Central Brazil. Twentyfour samples of thrips (6 per municipality) were collected in watermelon flowers, totaling 1,569 specimens, in the commercial fields of Formoso do Araguaia (430), Lagoa da Confusão (488), Porto Nacional (379), and Gurupi (272) in the Tocantins state. The identification of the insects was performed according to taxonomic characters of each species. Total RNA was extracted with Trizol reagent and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) was performed to confirm the presence of GRSV in the thrips samples. Next, viral RNA samples were sent for sequencing the GRSV genome, using NGS (Illumina MiSeq platform). The sequence of the S fragment (3074 bp) was used to perform an alignment with other sequences deposited in GenBank. The gene sequence encoding the non-structural protein and the GRSV nucleocapsid protein showed 96.1% to 96.8% identity with the virus isolate sequences from South Africa (AF487516 and AF487517) and from Brazil (AF513219 and AF251271). GRSV presence was confirmed in all thrips samples demonstrating that it is widely distributed in the watermelon cultivation in the Tocantins state. In addition, the most species of thrips identified in watermelon crops were Frankliniella schultzei (22%), Frankliniella tritici (75%) and Frankliniella insularis (0.38%). This is the first report of GRSV presence in thrips associated with watermelon cultivation in the Tocantins state, in Central Brazil.

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LIFESTYLES OF BACTERIOPHAGES PRESENT IN FECES OF HEALTHY AND DIARRHEIC CALVES

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Resumo

Nowadays, the diversity and abundance of bacteriophages has gained prominence in the context of ecological relations in the different ecosystems of the earth. Specifically in the gut ecosystem, several studies have been developed in humans aiming to establish a correlation between phage diversity, its interactions with the microbiota and host, and health status versus disease. As an example, the ratio between phages preferentially performing lytic or lysogenic cycle in healthy or diarrheic humans. Although promising, this subject has not been vastly studied in the veterinary field which is an important field where the etiology of this disease must be identified. Neonatal bovine diarrhea is the disease with the greatest impact on bovine production and, because it has a multifactorial cause, its treatment in most cases is nonspecific and ineffective. Therefore, the objective of this work is to offer an initial prospection of the proportion of lytic and lysogenic viruses in healthy and diarrheic calf samples as well as their host spectrum. For this purpose, stool samples from healthy and diarrheic calves were collected from the Setor de Gado de Leite of Universidade Federal de Viçosa. Samples were taken to the laboratory in sterile falcon tubes conditioned at 4 °C. 1g of feces were processed for enrichment of virus lytic or lysogenic detection and subsequently subjected to lysis plate assay using various bait enteric bacteria such as Escherichia coli, Lactobacillus spp, Salmonella spp and Enterococcus faecalis. In this initial study it was possible to observe that, similar to what has already been reported in humans, viruses predominate by performing lysogenic cycle in healthy animals and virus performing lytic cycle in diarrheic animals. Future work should be performed for the morphological and molecular characterization of these viruses aiming to elucidate the ecological relations between these and the intestinal environment and the microbial dynamics in the emergence of bovine neonatal diarrhea.

Palavras-chaves: Virus, gut, dairy, calf, diarrhea

DETECTION OF BOVINE PESTIVIRUSES IN SERA OF BEEF CALVES BY A RT-PCR BASED ON A NEWLY DESIGNED SET OF PAN-BOVINE PESTIVIRUSES PRIMERS

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Resumo

Bovine pestiviruses comprise two well recognized viral species, bovine viral diarrhea virus 1 (BVDV-1) and 2 (BVDV-2) and the recently classified HoBi-like pestivirus (HoBiPeV). Reliable diagnostic tests are pivotal for pestivirus detection and control, in addition to biosecurity measures, monitoring for BVDV exposure and, vaccination in some cases. Molecular detection through reverse-transcription
polymerase chain reaction (RT-PCR) and its variations has been widely used for pestivirus diagnosis. Most RT-PCR assays for pestivirus detection target the highly conserved 5’ UTR. In this study, we employed a newly designed set of primers (BP189-389) to screen the sera of beef calves for pestiviruses in a gel-based RT-PCR. Serum samples positive for BVDV antigens in an antigen-ELISA (n=135) were submitted to RT-PCR using different sets of primers targeting the 5’UTR of the viral genome. RT-PCR with pestivirus primers 324-326 detected 110 positive samples, being BVDV-1 (n=62), BVDV-2 (n=38) and HoBi-like (n=10). A PCR using primers HCV90-368 detected 97 positive samples (64 BVDV-1; 33 BVDV-2). An additional RT-PCR round using BVDV-2-specific primers (2F-2R), detected 45 positive samples (including 38 detected by primers 324-326 and 33 by HCV90-368); whereas a RT-PCR using HoBi-like-specific primers (N2-R5) detected 26 positive samples (including 10 detected by primers 324-326). Lastly, the assay using the lab made primers BP189-389 detected all 135 ELISA positive samples, including the 26 HoBi-like detected by primers N2-R5. These results demonstrated that primers BP189-389 compare favorably against other primer sets in the detection of bovine pestiviruses, especially HoBi-like. Thus, the primers may be useful for efficient detection of pestiviruses in bovine sera and other specimens as well.

Financial support: CNPq.

Palavras-chaves: bovine, detection, diagnostic, pestivirus, serum

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ROTAVIRUS A, C, H AND AICHIVIRUS C AMONG DOMESTIC PIGS IN BARRA DO PIRAÍ, RIO DE JANEIRO

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Resumo

Diarrhea is a common illness in pigs and is one of the leading cause of death among these animals having significant economic impact in the pig industry. The aim of this study was the detection of rotavirus species A (RVA), C (RVC) and H (RVH), and Aichivirus C (AiV C) infections among pigs at different breeding stages, with and without diarrhea, in a commercial pig farm, located in the municipality of Barra do Piraí, RJ, Brazil. A total of 329 swine fecal samples from animal between 4 and 150 days of age were analyzed; 15 samples were obtained from animals with diarrhea and 314 from asymptomatic animals; 15 animals were presenting diarrhea at the time of sample collection. Samples were analyzed by RT-PCR using primers specific for each of the viruses screened. The presence of virus was evidenced in 117 (35.6%) samples. Among the positive samples, 21 (6.4%; n=329) contained only RVA, 29 (8.8%) only RVC, 27 (8.2%) only RVH and 15 (4.6%) only AiV C. Cocirculations, that is, the presence of two or more viruses in the sample were detected in 25 (7.6%) samples. RVA was found in 30 (9.1%), RVC in 43 (13.1%), RVH in 44 (13.4%) and AiV C in 28 (8.5%) samples, considering the strains detected in cocirculations. Viral detection was more frequent among animals with diarrhea (p = 0.0002). The frequency of viral detection was similar between pre- and post-weaning animals (p = 0.7135). However, RVA and AiV C were significantly more frequent among younger animals (p = 0.004 and p = 0.0088, respectively). The results demonstrate the wide circulation of RVA, RVH, RVC and AiV C among the studied herd, even among asymptomatic animals. An association was observed between excretion of AiV C and diarrhea (P> 0.0001). Enteric infections among pigs are associated with high morbidity and mortality rates in addition to changes in feed conversion rates and weight gain. These alterations result in the delay of the slaughter of the animal and, consequently, prolong the stay of the animal in the farm, generating economic losses to the producer. Moreover, the longer the stay of the infected animal, the higher the risk of spreading the infection on the farm, especially in the case of animals carrying asymptomatic infections. In addition, since RVA and RVC are considered to be potentially zoonotic viruses, the excretion of these viruses into the environment can also result in transmission to farm workers, causing interspecies infections. Financial Support: CNPq, CAPES and FAPERJ.
ACTIVITY OF GANCICLOVIR IN VITRO AND IN RABBITS EXPERIMENTALLY INFECTED WITH EQUID ALPHAHERPESVIRUS TYPE 1

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Resumo

Equid alphaherpesvirus type 1 (EHV-1) is an important pathogen of horses associated with respiratory, reproductive and neurological disease. No specific treatment is available for EHV-1 and the control strategies are based on vaccination, not always effective. Then, the aim of this work was to investigate the activity in vitro and in vivo of ganciclovir (GCV), an anti-herpetic human drug, against EHV-1, since the average effective dose was low (EC₅₀: 1.9 µg/mL) and selective index was high (IS: 490). Next, the GCV efficacy against EHV-1 was investigated in rabbits, an animal model for EHV-1 studies. For this, 30 days-old New Zealand rabbits were grouped in three groups of six animals each and submitted to different treatments: G1 (non-infected control), G2 (EHV-1 inoculated animals) and G3 (inoculated with EHV-1 and treated with GCV). Animals of G2 and G3 were inoculated by intranasal route with an EHV-1 suspension containing 10⁷ tissue culture infectious doses (TCID₅₀). Rabbits of G3 group were treated intravenous with 2.5mg/kg of GCV every 12 h for 7 days, starting on the day of virus inoculation. All animals of G2 developed apathy, inappetence, nasal discharge (serous to mucopurulent), mild to severe respiratory distress, and death (n=2). The weight gain in these animals was lower than G1 from day 4 until 14 post inoculation (pi). They shed virus in nasal secretions between days 2-3 until 10-11 pi, and seroconverted to EHV-1 at day 15 pi (antibodies titers ranged of 8 to 128). Infectious virus was recovered from lung samples of one animal that died at day 4 post-inoculation. On the other hand, rabbits of G3 remained healthy and gained weight similarly to control group (G1) during all monitoring, showing only mild serous nasal discharge (between days 5 and 12 pi). All animals of G3 shed virus in nasal discharge and seroconverted to EHV-1, indicating efficient virus replication. Virus titer in nasal discharged was statistically similar between the groups. These results indicate that GCV was effective in attenuating the clinical disease produced by EHV-1 in rabbits and, thus, should be more studied towards a potential use for the treatment of EHV-1 respiratory disease in horses.

Financial Support
Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES).

Palavras-chaves: GCV, Therapy, EHV-1, animal model

ENTERIC VIRUSES IN CANINE FEED SAMPLES MARKETED IN BULK RETAIL

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Resumo

Feeds sold in bulk retail are often purchased by dog owners in Brazil because of practicity and price. Because they are exposed to the environment, to the handling and to the possible vectors, like rats and cockroaches, feeds can end up being contaminated by microorganisms, like enteric viruses of human and animals that attend the place. The objective of this work was to standardize a technique for the preparation of feeds for genome extraction and with that to analyze the presence of...
enteric viruses like *Mastadenovirus* (AdV) of different species, *Canine Mastadenovirus A* (CAV) and *Carnivore Protoparvovirus 1* (CPV) through viral nucleic acid detection in canine feeds' samples marketed in bulk retail. For the standardization of the technique, a control feed sample was inoculated with *Human Mastadenovirus 5* (HadV) prototype strain Ad5 at different 10-fold dilution and tested with Nested polymerase chain reaction (PCR) for AdV, using pan-specific primers that amplify the conserved region of the AdV DNA polymerase gene from adenovirus of a wide range of vertebrate hosts. After, twenty samples were obtained from stores at the municipalities of Ivoti and Estância Velha, RS, Brazil, weighed into 10 g, macerated and mixed with 90 mL of Minimum Essential Medium (MEM). The samples were then incubated in Shaker-type homogenizer for 1 h and then extracted with BioPur® commercial kit following the manufacturer's instructions. Detection of viral genome was performed by PCR-Nested for AdV, as previously described. For CAV and CPV, conventional PCR was performed using primers that amplify the region of the CAV hexon protein gene and the region of CPV capsid protein gene. Then samples were sent for sequencing for viral characterization. Of the twenty samples analyzed, one was positive for *Human mastadenovirus C* and none sample was positive for CAV or CPV. The absence of these can be related to the frequent vaccination of the dogs for these pathogens. In this way, we perceive that there is contamination of human origin in the feeds commercialized in bulk, probably caused by lack of hygiene from people handling these feeds at the stores.

Financial Support: CAPES; CNPq; Fapergs; DCIT – Ministério da Saúde – Brasil.

**Palavras-chaves:** HAdV, Animal food, Nested-PCR, Standardization
important to understand which diseases are circulating in these animals (since some are migratory and can transmit a lot of diseases in every country they pass to) to monitorize the mutation of the virus and to protect captivity animals, and even humans, since some of those viruses are zoonotic. Financial Support: Capes n. 1747578.

Palavras-chaves: Birds, Paramyxoviridae, Veterinary

An overview of the serum virome of dogs from Paraíba state, Northwest Brazil

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Resumo

Domestic dogs (Canis lupus familiaris) are the most popular pets worldwide and have extensive contact with humans. Dogs share habitats with human, other domestic animals and wildlife which make them a potential risk factor for zoonotic viruses, as rabies virus and rotavirus. Moreover, the knowledge of possible bloodborne pathogens is important due the increasing application of blood transfusion in dogs. The improved availability and application of high throughput sequencing (HTS) technologies has improved the detection of known and unknown viruses. Thereby, the present study aimed to evaluate and characterize the serum virome of healthy dogs using HTS. A total of 520 serum samples of dogs from five urban centers of Paraíba state were pooled and submitted to protocols for viral enrichment. DNA libraries were prepared using the Nextera DNA sample preparation kit and sequenced using an Illumina MiSeq platform. The assembled contigs were examined to search for similarities with known sequences using BLASTX software. Contigs related to viruses belonging to Paroviridae, Circoviridae, Genomoviridae and Anelloviridae families were observed. Paroviridae family-related contigs were observed closely-related to Carnivore protoparvovirus 1 (previously known as canine parvovirus 2; CPV2), Carnivore bocaparvovirus 1 (previously known as canine minute virus; CMV) and an unclassified member defined as sesavirus previously reported in sea lion feces. The Circoviridae family-related contigs presented high identity with canine circovirus (CaCV), the Genomoviridae family-related contigs with Human associated gemykibivirus 1 (GmKV1) and the Anelloviridae family-related contigs with Torque teno canis virus (TTCV). CPV2 is the most prevalent enteric pathogen in dogs worldwide and must be tested in blood submitted to transfusion in dogs. There is low information available in the literature about CaCV and CMV in Brazilian dogs and the confirmation of its presence can increase the knowledge of their epidemiology in Brazil. Moreover, more studies are necessary to understand the GmKV1, TTCV and sesavirus importance in dogs. The virome of the dog population performed in the present work can increase the knowledge about the viral population in domestic animals. Moreover, the knowledge of viral agents resident in blood of dogs can help to prevent the dissemination of pathogens by blood transfusion in pets.

Palavras-chaves: Dog, High throughput sequencing, Metagenomic, Virome

AN OVERVIEW IN VIROME OF COMMERCIAL BATCHES OF HORSE SERUM

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Resumo

The commercial horse serum is an supplement for cell culture being an option for fetal calf serum. Among the contaminants that can be present, viruses are particularly problematic since they are difficult to detect and are not removed by the filtration process. With the advent of high throughput sequencing (HTS) it has promoted changes in virus detection, since unlike other methods that often depend on the presence of known sequences, this technology proves to be unbiased. Thus, the present study aimed to detect virus that are present in batches of commercial horse sera using HTS.

We obtained a total of five batches of commercial horse serum from three different manufacturers from Brazil, United States and New Zealand. The samples were processed and submitted to viral enrichment protocols. DNA libraries were prepared using the Nextera DNA sample preparation kit and sequenced using an Illumina MiSeq platform. The assembled contigs were examined for similarities to viral sequences with the BLASTX software. Contigs related to viruses belonging to the Flaviviridae, Herpesviridae and Parvoviridae families were observed. In sequences related to the Flaviviridae family, sequences closely related to non-primate hepacivirus (Hepacivirus A, HAV) and a pegivirus known as the Theiler’s disease-associated virus (Pegivirus D, PVD) were found. HAV has been found in horses and appears to be more genetically related to human hepatitis C virus (HCV) than any other animal hepacivirus. PVD is apparently associated with acute hepatitis in horses. Horse parvovirus was previously reported in cerebrospinal fluid (CSF) of horses with neurological signs. Commercial horse serum is important for diagnostic and vaccine production purposes and its innocuity is important to biosecurity of biological. Moreover, HTS is an useful, embracing technology for the detection of adventitious viruses in commercial biologicals. Financial Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) supported this work.

Palavras-chaves: Contamination, Diagnosis, High throughput sequencing, Horse, Virus

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Resumo

Co-evolution of Avian Coronavirus (AvCoV) and diverse avian hosts based on Codon Usage Bias

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Resumo

Co-evolution of Avian Coronavirus (AvCoV) and diverse avian hosts based on Codon Usage Bias

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Abstract

Avian Coronavirus (Nidovirales: Coronaviridae: Gammacoronavirus) has a great number of genotypes, infecting mainly the upper respiratory tract, but can also infect kidneys, intestine and reproductive tract in Gallus gallus; this virus has been detected in others species of birds like: A. cygnoides, A. platyrhynchus, C. japonica, F. peregrinus and M. gallopavo. AvCoV has a +ssRNA genome with 27.6 kb which encodes for 23 proteins (15 non-structural and 8 structural). Codon usage bias can be measured by frequency of occurrence of each codon, indicating preference of a genome for one or more codons for one amino acid. For this study sequences of AvCoV proteins nsp12 and S from different countries around the world (58 different AvCoV) were included, representing all 6 genotypes, and for the hosts mRNAs of high expression proteins in each organ in the tissues in which virus has replication were included (lungs, kidneys, intestine, brain and ovary); as a negative control; homologous mRNA sequences from two reptiles and one virus of the order Nidovirales were also included. The relative synonymous codon usage (RSCU) was noted in a binary matrix to generated a NJ tree with the software PAUP*4, which represent a preferential use of codons with both viruses and hosts in a single tree. The normalized codon adaptation index (CAI) was also calculated for all AvCoV sequences, for which values AvCoV specificity or different avian hosts species.

Keywords: AvCoV, RpRd, Spike Protein, CUB, RSCU, nCAI, ENC

Financial support: CAPES Proex

Palavras-chaves: AvCoV, RpRd, Spike Protein, CUB, RSCU
specific for BVDV-2 (BVDV-2#3) and HobiPeV (N2-R5). PCR products were subsequently submitted to nucleotide sequencing for virus identification. ELISA-positive samples were also submitted to virus isolation in cell culture. A total of 135 samples were found positive by consolidated results of the three RT-PCRs. Nucleotide sequencing and phylogenetic analysis of the 5'UTR revealed 65 BVDV-1 (48.1%), 45 BVDV-2 (33.3%) and 26 HoBiPeV (19.2%). In addition, 69 virus isolates – all non-cytopathic – were obtained upon three passages in MDBK cells. These results demonstrate a discrepancy between immunoenzymatic and molecular detection of pestiviruses in cattle sera and point out for the need of a standardization of diagnostic tests used for certification of cattle destined to export. In addition, these results indicate the circulation of the three bovine pestivirus species in beef cattle of RS.

Suporte financeiro: CNPq.

Palavras-chaves: BVDV, HoBi-like, pestiviruses, Molecular Detection, Elisa

SAMPLES OF BATS EXHIBITED CLOSE PHYLOGENY WITH HUMAN HERPESVIRUS 1 AND 2

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Resumo

Human intervention on natural environments promotes numerous changes in ecosystems, facilitating the spread of diseases. In this sense, bats stand out as important agents transmitting infectious pathogens. Characteristics of these animals, such as high geographical diffusion, species diversity, good spatial mobility and interaction with other populations can promote the transmission of infectious pathogens, including viruses. The viruses of this family can cause from mucosal lesions to more serious conditions, such as encephalitis, meningitis or disseminated infections. Information related to the distribution of Herpesviridae viruses in bats in Brazil is still poorly characterized. Thus, this study aims to identify herpesvirus in oral and anal swab samples of 12 species of bats belonging to three families and to characterize these samples in order to identify relationships with circulating strains in nature. A total of 320 samples from bat populations of the municipalities of Campinas and Rio Claro in the state of São Paulo were analyzed by Pan-herpesvirus PCR. Degenerate primers targeted to amplify a conserved region of the DNA polymerase gene herpesvirus were used. A nested-PCR format capable of amplifying fragments of 21 herpesvirus species (13 animal and eight human viruses), from the subfamilies Alphherpesvirinae, Betaherpesvirinae and Gammaherpesvirinae was used. Samples identified as positive were submitted to Sanger sequencing technique, followed by phylogenetic analysis. In total, 80 samples were positive in Pan-herpesvirus PCR and 51 (15.93%) herpesvirus compatible sequences were obtained after sequencing. Of these samples, 20 (25%) were from oral swab and 60 (75%) from oral swab. Nine bats exhibited positive results in both the oral and anal swab samples. A sample of bat Artibeus lituratus swab anal exhibited close phylogeny with human herpesvirus 1 (HHV-1), an alpha herpesvirus of the genus Simplexvirus. In addition, bat specimen of the Molossus molossus demonstrated close phylogeny with the human herpesvirus 2 (HHV-2) sequence of the genus Simplexvirus, subfamily Alphaherpesvirinae. The social, feeding, sexual behavior in different animal species has close relation as the mechanism of transmission and prevalence of virus. Thereby, know the viruses that different species of mammals and other animals host can help prevent possible
outbreaks of diseases and direct actions of preventive measures. **Financial Support:** Capes and Funcamp.

**Palavras-chaves:** Human intervention, natural environments, ecosystems, Herpesviridae, PHYLOGENY WITH HUMAN

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**DETECTION OF CHICKEN ANEMIA VIRUS (CAV) AND AVIAN GIROVÍRUS 2 (AGV2) IN FREE-LIVING PIGEON USING REAL-TIME DUPLEX PCR**

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**Resumo**

Chicken anemia virus (CAV) and Avian gyrovirosis 2 (AGV2) are members of Anelloviridae family, genus *Gyrovirus*. Both viruses can infect poultry; however, whereas CAV is a recognized avian pathogen, the role for AGV2 as disease causative agent remains to be determined. Although these viruses are widely distributed, the epidemiological factors involved in their spreading are not fully elucidated. In this sense, the investigation of such viruses in free-living pigeons may be of interest. To date, there is no data on this subject in Brazilian samples; thus, the aim of this study was to investigate CAV and AGV2 in free-living pigeons from different Brazilian cities. A total of 183 serum samples were collected from apparently healthy urban pigeons from seven south Brazilian cities. 183 serum samples were collected from apparently healthy urban pigeons from seven south Brazilian cities. TaqMan based duplex real-time PCR (dqPCR) were applied in all samples with positive and negative controls for simultaneous detection of AGV2 and CAV genomes. The reactions were performed at StepOne™ Real-Time PCR system (Life Technologies) with the following conditions: 50 °C for 2 min, 95 °C for 2 min, 40 cycles of amplification (95 °C for 15 s and 60 °C for 30 s). CAV genomes were not detected in the evaluated samples. Regarding AGV2, such genomes were detected in two pigeons, one from São Leopoldo, Rio Grande do Sul state and the other from Criciúma, Santa Catarina state. This is the first study to report AGV2 occurrence in free-living pigeons. This result opens the possibility that these animals may influence the epidemiology of poultry diseases in Brazil. However, further studies are necessary in order to conclude a causal association between pigeon’s viruses and their transmission to poultry.

**Financial support:** CAPES, CNPq, FINEP.

**Palavras-chaves:** CAV, AGV2, PIGEON, PCR, FREE-LIVING
DETECTION OF HEPATITIS B VIRUS IN AVIAN SAMPLES FROM ATOL DAS ROCAS ISLAND IN RIO GRANDE DO NORTE, BRAZIL

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Resumo

Hepatitis B virus (HBV) belongs to the family Hepadnaviridae which are a family of enveloped viruses divided into two genera: Avihepadnaviruses, that have been isolated from duck, heron, wild geese, stork and crane; while orthohepadnaviruses have been isolated from humans, apes and squirrels. Recently, was also found evidences of what is likely to be a third genus, with a new fish hepadnavirus. Those viruses have partially double-stranded DNA with a small circular genome (≈3 kb). All known hepadnaviruses are hepatotropic and cause transient and persistent infections with variable degrees of pathogenesis. In ducks, Avihepadnaviruses infection varies by the host's ability to elicit an immune response and is age dependent, mimicking the pathogenesis of human hepatitis B virus infections and providing an excellent animal model for human HBV. Natural infections occur by vertical transmission through the bloodstream and has been shown that only one genome copy of the virus is needed to successfully infect a duckling. Therefore, this study aims to detect Avihepadnavirus in 188 orotracheal and cloacal swab samples from health adults and newborns of long-tailed tyrants (Colonia colonus) and brown booby (Sula leucogaster) from Atol das Rocas which showed clinical signs as eye infections and sudden death. This small island localized 267km from the coast of Natal city, in Rio Grande do Norte, Brazil. This Island is a very important integral protection site since it is the only South American atoll, used as shelter, feeding and reproduction site for numerous avian species. The samples were collected and placed in cryotubes containing 500µl of lysis buffer and kept in ice until taken to freezer (-20°C). The extraction was automatically done in “MagMAX™ Express” (Applied Biosystems®) using the “MagMAX™-96 Total Nucleic Acid Isolation Kit” according to the manufacturer’s instructions. SYBR Green Real-Time PCR was then performed using a very specific van der Vries et. al. (2017) protocol. Approximately 14% (27/188) of the samples were positive for Avihepadnaviruses found and will be further sequenced. Even though it is hard to compare the incidence, since this virus is still poorly studied in wild birds and no similar study has been done in Brazil yet, the analysis of Beijing ducks in 2013 showed a positive rate of 63.8%, highlighting the possibility that the incidence of these viruses in aquatic wild birds may be high.

Financial Support: CAPES/ Newton Foundation Birth Consul

Palavras-chaves: Avihepadnavirus, Avian Hepatitis B, Wild Birds

PHYLOGENETIC ANALYSES OF THE SEGMENT-7 OF BLUETONGUE VIRUS ISOLATES FROM DEER, BRAZIL

Bárbara Chrispin Longo ¹, Ana Carolina Diniz Matos ¹, Zélia Inês Portela Lobato ¹, Mariana Andrioli

Autores ¹
Development of an immunohistochemical assay for detection of Equine Infectious Anemia Virus

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Resumo

Hemorrhagic disorders affecting native Brazilian deer and associated to Bluetongue virus (BTV) have been reported in captive in Brazil, hindering the management and conservation of native species. BTV is an arbovirus transmitted by biting midges from genus Culicoides. The virus genome consists of 10 segments of dsRNA that encodes 7 structural and 5 nonstructural proteins. BTV presents great genetic and antigenic variability, with at least 27 serotypes and a distinct distribution worldwide. Segment 7 (Seg-7) is the third most variable genome segment of the virus and encodes the structural protein VP7, which is considered the most immuno-dominant serogroup-specific BTV antigens, showing no cross-reaction within the members of other orbivirus species. In addition, phylogenetic analyses of Seg-7 have been correlated to the geographical origins and the vectors populations for different strains. To study the molecular epidemiology of BT viruses circulating in Brazil, the Brazilian BTV isolates was performed comparing to GenBank sequences from worldwide, by the p-distance and maximum likelihood methods. The Brazilian samples were divided into four clusters, but all there grouped with the western strains. BTV-3 and BTV-14 showed 95% similarity with samples from the USA and the Republic of Trinidad and Tobago; BTV-18, BTV-22 and BTV-24 presented 97% similarity with specimens from Argentina and other Brazilian samples, while BTV-19 grouped with strains from the USA and South Africa (SA). The non-typed BTV-Y was close related to the BTV-15 isolated from an Alpaca in SA, indicating 89% similarity. Except for BTV-Y, the Seg-7 of the Brazilian isolates clustered together and showed to be close related to samples from the American continent. This association indicates a geographical link between them, and present a relation to distribution of vector species. Further studies regarding the BTV-Y isolate should be performed to better understand potential routes of introduction in the country.

FS: Itaipu Binacional, CAPES, CNPq

Palavras-chaves: Arbovirus, Deer, Phylogenetic, Segment-7, Orbivirus
Resumo

Equine infectious anemia (EIA), a disease caused by Equine infectious anemia virus (EIAV), is considered an obstacle to the development of horse industry. EIAV is transmitted by hematophagous insects of the Tabanidae family and also iatrogenically. EIA has no treatment or vaccine and pathogenesis and immune response against the virus are not fully understood. Also, the performance of laboratorial diagnosis by serological tests can be poor or limited. In this sense, the immunohistochemistry method has been used to enhance the knowledge about the tropism and the pathogenicity of several infectious agents. Therefore, the objective of this work was the development of an immunohistochemical assay for detection of EIAV antigens in naturally infected equids tissues and organs. Fragments of organs of equids (n=9) from Apodi – RN, Brazil, diagnosed as positive for EIA were fixed in 10% buffered formalin and paraffin-embedded. Histological sections were collected on polarized slides and antigenicity recovered by heat, using citrate buffer (pH=6). These sections were incubated with polyclonal antibody for 18h, and later, with peroxidase-conjugated secondary antibody (Sigma – Aldrich) for 40 minutes. The antigen/antibody reaction was revealed with the ImmPACT™ DAB Peroxidase Substrate (Vector Laboratories) kit counterstained with hematoxylin (Sigma-Aldrich). EIAV antigens were observed in intracytoplasmic region of spleen, liver, kidneys and lungs cells. Immunohistochemical marks were present mainly in the marginal zone of the spleen (white pulp), a region with plenty of macrophages and dendritic cells, and in the hepatic sinusoids, where the Kupffer cells (liver macrophages) lies. The lung was the only organ showing histopathological alteration, with epithelial bronchiolar hyperplasia. The marks were found in the bronchiolar epithelium and the alveolar septum, as well as in the epithelium of distal and proximal tubule of kidneys. The highest amount of EIAV was observed in spleen and liver, sites normally full of macrophages, confirming the viral tropism to the mononuclear phagocytes. However, the presence of EIAV in lungs and kidneys epithelial cells indicates that the virus may also infect other types of cells besides macrophages, making possible the elimination of the virus in the urine and in oronasal secretions, and facilitating new transmission mechanisms that must be investigated. Financial support: CNPq, FAPEMIG, Capes, INCT-Pecuária, UFERSA, UFMG.

Palavras-chaves: detection, EIAV, immunohistochemical assay, naturally infected equids, tissues and organs

DISSECTING ESSENTIAL ROLES OF CHICKEN INTERFERON STIMULATED GENES IN THE PATHOBIOLOGY OF POULTRY VIRUSES

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Resumo

Poultry farming continues to expand rapidly throughout the world. As environmental impacts advance, the emergence and reemergence of viral diseases that affect both humans and animals are a serious threat to food safety around the world and a problem for sustainable production in poultry farming. The
understanding of complex host-pathogen interactions is crucial for the development of control strategies, in determining the role of poultry in the propagation of zoonotic diseases and may also benefit segments of agroindustry. Recent technological advances such as genome-wide and high throughput screening platforms that aid in the understanding of the pathology of pathogens in mammals are still rudimentary for bird studies. Viral infections induce the production of pro-inflammatory mediators and cytokines that peak during the replication phase of the virus. These mediators are interferon-stimulated gene products (ISGs) but little is known about their antiviral activity, target specificity and their mechanisms of action in birds. Using an overexpression screening approach, a library of lentivirus-based chicken ISGs was constructed and GFP positive chicken (DF-1) cells were transduced to investigate ISGs that positively and/or negatively regulate virus replication. We applied the Flow Cytometry (FACS) technique to quantify cytopathic effect through the diminishment of GFP intensity. Tools such as ELISA and qRT-PCR are being used to dose cytokines and viral load. The objective is to obtain a gene expression profile that can be correlated to the pathology of poultry viruses both in vitro and in natural infections, thereby launching a base for further studies in avian immunology. In the future, the information obtained could be used in the determination of viral resistance markers and in the modification of previously characterized avian cell lines to increase the kinetics of virus multiplication/vaccines that would otherwise not grow in cell culture or grow in lower titers.

Financial support: FAPESP, LNBio/CNPEM

Palavras-chaves: environmental, PATHOBIOLOGY, POULTRY VIRUSES, Virology, pathology

GENOTYPING OF HEPATITIS E VIRUS STRAINS IDENTIFIED IN LIVERS FROM COMMERCIAL PIG HERDS FROM MATO GROSSO STATE

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Resumo

Hepatitis E virus (HEV) is a causative agent of acute hepatitis in humans worldwide, being classified in the Hepeviridae family within the genus Orthohepevir. Among the four species classified in this genus (Orthohepevirus A to D), species Orthohepevir A groups genotypes that infect humans (HEV-1, -2, -3, -4, and -7) and pigs (HEV-3 and -4). The aim of this study was to detect infection by HEV in pigs from commercial farms with intensive production systems and culled at abattoirs submitted to federal sanitary inspection. Liver samples from 72 pigs aging from 21 to 25 weeks old, belonging to five distinct municipalities from Mato Grosso state, were evaluated for presence of HEV RNA by reverse transcription followed by nested PCR assays to amplify partial fragments of ORFs 1 and 2. In order to genotype the strains of HEV, direct sequencing and phylogenetic analyses were employed. Two out of 72 liver samples (2.8%) had HEV genome amplified by nested-PCR assays. Direct sequencing of the amplicons followed by phylogenetic analyses revealed the two strains to belong to genotype 3. The identities shared between their nucleotide sequences were 98.5% in ORF1 and 100% in ORF2. Distance estimation by nucleotide p-distance among HEV-3 subtypes employing ORF1 sequences revealed lower distance (0.087) with subtype 3j (strain Arkell; Genbank access number AY115488), while pairwise distance analysis with ORF2 sequences demonstrated lower nucleotide p-distance of 0.134 with subtype 3b representative (strain JRA1; Genbank access number AP003430). In the phylogenetic trees reconstructed by Maximum likelihood model, partial ORF1 sequences
obtained grouped together with the reference strain for subtype 3j. Differently, partial ORF2 sequences did not cluster closely to any reference sequences proposed for classification in HEV-3 subtypes. Our results confirm the circulation of HEV-3 strains within commercial pig herds from highly productive areas of Midwestern region of Brazil. Since phylogenetic analyses involving ORFs 1 and 2 yielded discordant results, occurrence of intragenotypic HEV recombination might explain these findings, extending HEV-3 diversity observed in Brazilian commercial pig herds.

Financial support: University of Cuiabá (UNIC).

Palavras-chaves: swine, HEV, PCR, sequencing, subtype

Virucidal activity of tetra cationic porphyrins against bovine herpesvirus type 1 and adenovirus

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Resumo

Porphyrins are photosensitizers, compounds that absorb the energy of light to produce reactive oxygen species that can react with lipid, amino acids and nucleic acids. Virucidal activity of porphyrins has been investigated and may provide an alternative to replace and/or to improve current methods of virus inactivation in the environment, equipments, biologicals, vaccine virus inactivation and, eventually, for use as topical therapy in cutaneous diseases associated with epitheliotropic viruses. The objective of this work was to investigate the virucidal activity of four tetra cationic porphyrins (H2TMeP, ZnTMeP, CoClTMeP and MnClTMeP) against enveloped (bovine herpesvirus type 1, BoHV-1) and non-enveloped bovine viruses (adenovirus, BAV). Initially, all porphyrins were submitted to cytotoxicity assay for MDBK cells using MTT assay. The maximal non-cytotoxic drug concentrations were then used in virucidal assays. For this, a fixed virus concentration of each virus was mixed with each porphyrin and exposed to light for 0, 60, 120, 180 min (BAV) or 0, 30, 60, 90 and 120 min (BoHV-1). After light exposure, the remaining virus titer was determined by limiting dilution and compared with virus control titers. All virucidal assays were performed at triplicate, and virus controls (without porphyrin) and dark controls (without light exposition) were included in all experiments. Two porphyrins (H2TMeP and ZnTMeP) presented virucidal activity against both viruses. BoHV-1, an enveloped virus, was completely inactivated by ZnTMeP and H2TMeP after 30 and 60 min of light exposure. The inactivation of non-enveloped virus (BAV) was complete after 180 min of virus exposure to ZnTMeP and light. After the same period, H2TMeP porphyrin produced a 10-fold reduction of BAV titer in comparison with virus control. No porphyrin was able to inactivate enveloped and/or non-enveloped virus in the absence of light, indicating that light activation is fundamental to the mechanism of virus inactivation by all tested porphyrins. The virucidal effect of other analyzed porphyrins (CoClTMeP and MnClTMeP) in enveloped and non-enveloped viruses was not significant. These results indicate that ZnTMeP and H2TMeP porphyrins have light-dependent virucidal activity on enveloped and non-enveloped viruses (that is very resistant to inactivation), showing the potential of these compounds to inactivate viruses in premises and substrates.

Financial support: FATEC

Palavras-chaves: BAV, BoHV-1, photosensitizers, virus inactivation

Porcine Circovirus type 2 and Porcine Parvovirus type 1 to 6 recovered from swine affected with post-weaning multisystemic wasting syndrome through metagenomic approach
Resumo

Porcine circovirus associated disease (PCVAD) is one of the causes of negative economic impact on pig farming systems described worldwide. Losses include expenditures with treatment, increased mortality rates, and decreased productivity. One of the most relevant manifestations of PCVAD is the post-weaning multisystemic wasting syndrome (PMWS). The main pathogen present in PMWS is porcine circovirus type 2 (PCV2). However, observational and experimental studies have shown that other agents may be involved in the pathogenesis and clinical manifestation. High-throughput sequencing combined with metagenomics analyses make it possible to identify the total microbiota in a given sample, regardless of microorganism culture. In order to contribute to the knowledge of the viruses involved in PMWS, the present study carried out the high-throughput sequencing of swine sera and subsequent analysis of the resulting metagenome. Sixteen serum samples collected on a farm in Rio Grande do Sul, from 80 and 100 days old pigs with clinical signs of PMWS, were examined. Data revealed that in addition to PCV2 sequences, porcine parvovirus type 1 through 6 (PPV1 to 6) were recovered from samples of pigs affected by PMWS. The PPV1 capsid protein sequence identified in this study presented one of the mutations found only in the pathogenic strains of this virus, suggesting PPV1 involvement in the disease. The conserved motifs found on parvovirus capsid proteins have catalytic properties and are related to virus entry into host cells inducing viral infectivity. In this study, PPV2, 3, 5 and 6 were shown to have these conserved domains, indicating that these viruses may be involved in the development of PMWS. The occurrences of PCV2 and PPV1 to 5 have already been described in pigs with PMWS, so this study reinforces previous results. PPV6 was recently described in China, Europe and the United States, and the studies did not correlate the virus to any specific disease. The present study is the first report of PPV6 in pigs presenting PMWS signals. However, further studies are necessary to be able to attribute the relationship between PPV6 in the development of SMDS.

Palavras-chaves: High-throughput sequencing, metavirome, PCVAD, PCV2, PMWS
from allantoic fluids of infected eggs 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. Influenza genomes were assembled using Newbler V 2.9 with high coverage (180x). Nucleotide alignments of the hemagglutinin (HA) and neuraminidase (NA) gene 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. The phylogenetic analysis showed that all genes of the three viruses, with the exception of the neuraminidase (NA), belonged to the H1N1/2009 cluster. The HA sequence of 28/15-1, 28/15-2 and 65/15-2 influenza viruses shared 98 to 99% of nucleotide identities. The NA segment of H1N2 viruses was closely related to an H3N2 virus that was introduced in swine in Brazil in the late 1990’s. Interestingly, H1N2 viruses (28/15-1 and 28/15-2) were isolated from pigs of the same farm in which H1N1/2009 and H3N2 IAVs were also circulating. This study highlights the importance of performing full genome sequencing of influenza virus isolated from swine in order to detect novel reassortant viruses that might represent a threat for humans.

Palavras-chaves: Influenza A virus, Pandemic H1N1/2009 influenza virus, Reassortant, Swine
identification of BVDV infection through serological and molecular surveys involving cattle herds from Mato Grosso state as well as neighboring states, more comprehensive studies should be conducted in cattle herds from this region to elucidate the importance and risk of transmission to susceptible animals via the semen.

Financial Support: Fundação de Amparo a Pesquisa do Estado de Mato Grosso - FAPEMAT

Palavras-chaves: bovine, pestivirus, BVDV, venereal transmission

ACTIVITY OF GANCICLOVIR, LACTOFERRIN AND PEPTIDE P34 IN MICE EXPERIMENTALLY INFECTED WITH FELINE HERPESVIRUS TYPE 1

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Resumo

Feline herpesvirus type 1 (FHV-1) is the major pathogen of feline upper respiratory tract disease and ocular disease among cats of all ages worldwide. Some antiviral drugs commonly used for the treatment of human herpesvirus were already tested in vitro and in vivo against feline herpesvirus type 1, but so far no antiviral drug has been developed specifically against FHV-1. Ganciclovir, a drug commonly used to treat human herpesviruses, was effective against FHV-1 in vitro, as well as lactoferrin and the peptide P34. The objective of this work was to evaluate for the first time the antiviral activity of ganciclovir, lactoferrin and the peptide P34 against FHV-1 in vivo, using BALB/c mice as experimental models. Mice were infected intranasally with FHV-1 and treated with ganciclovir, lactoferrin or the peptide P34 24 h after infection. One group was infected and not treated as the positive control group and another group was not infected nor treated, as the negative control group. After ten days of treatment, the animals were euthanized and the organs were collected to perform real-time PCR. The animals of the treated groups had markedly less clinical signs than the animals of the positive control group. qPCR analysis did not reveal a decrease of the viral load in the organs of the treated animals, suggesting that these antiviral compounds, in this dosage and frequence had no antiviral effect against FHV-1 in vivo, in this proposed experimental model.

Palavras-chaves: feline rhinotracheitis, FHV-1, antiviral therapy,

METAGENOMIC ANALYSES REVEAL LACK OF ASSOCIATION BETWEEN ENTERIC VIRUSES AND THE OCCURRENCE OF MALABSORPTION SYNDROME IN CHICKENS

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**Resumo**

Malabsorption syndrome (MAS) is an economically important disease of young broilers characterized by growth retardation, defective feather development and diarrhea. Studies have implicated several viruses in the etiology of MAS; however, the limited knowledge on the microbial community in the poultry digestive tract has hindered the identification of a causative agent(s) for MAS. To investigate the potential role of enteric viruses in this syndrome, metagenomic analyses were performed on 35 stool samples collected in seven chicken flocks with broilers displaying clinical signs of MAS, as well as 35 from clinically healthy broilers, at the same age and from the same flocks. The overall fractions of eukaryotic viral reads were 22.1% in the MAS-affected group and 14.5% in the healthy group. Genome sequences of several previously reported poultry viruses, as well as novel uncharacterized CRÉSS-DNA viruses, were identified. Genomes representative of the following families were detected: Adenoviridae, Anelloviridae, Astroviridae, Caliciviridae, Circoviridae, Paroviridae, Picornaviridae, and Reoviridae, including some putative novel genotypes. However, the comparisons between the distribution of viruses identified in diseased and healthy birds did not reach statistically significant differences. These results suggest that MAS seems not to be caused by enteric viruses. Future studies are necessary to elucidate the aetiology of such condition. Financial Support: CNPq, CAPES, FINEP.

**Palavras-chaves:** Broilers, High-Throughput Sequencing, Virome

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**ANALYSIS OF SWINE VIRUSES IN BRAZILIAN WILD BOARS (Sus scrofa) THOROUGH REAL-TIME PCR**

**Autores**  Juliana da Silva Andrade


**Resumo**

The presence of wild boars, as well as their rapid growth and dispersal in Brazil, is cause for concern due to their negative impact on nature. These animals have been considered potential reservoirs of pathogens and may be related to the dissemination and maintenance of these agents to other animals species. The objective of this study was determine the frequency of swine viruses in wild boar (Sus scrofa) in Rio Grande do Sul. For this purpose 80 lymph node pools (retropharyngeal, mesenteric, thoracic and inguinal) were evaluated. A series of SYBR Green®-based real-time polymerase chain reactions were applied to identify Torque-teno virus Sus 1a (TTSuV1a) and 1b (TTSuV1b); Porcine circovirus 2 (PCV2) and 3 (PCV3); and Porcine parvovirus (PPV). The viruses with higher frequency were TTSuV1a (54%) and PCV3 (36%). The positive frequency of TTSuV1a, PCV2 and PPV were 5%, 3% and 9% respectively. The result of this work show the occurrence of viruses important for swine health in Brazilian free-living wild boars. In addition, this is the first to study investigate the presence of PCV3 in these animals. There results may serve as a warning for preventive measures to be taken to avoid the contact of wild boars with domestic pigs.

**Palavras-chaves:** Wild boar, TTSuV1a and 1b, PCV2, PCV3, PPV
DETECTION OF A ZIKV-LIKE FLAVIVIRUS IN NON-HUMAN PRIMATES IN SOUTHERN BRAZIL

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Resumo

Flaviviruses are among the major threats in public health worldwide. It is important to investigate sylvatic circulation of these viruses in different parts of the world in order to prevent epidemics in human populations following epizootic events in interface areas. The pathogenicity of these viruses to hosts from different taxa and geographic ranges also causes direct impact in naïve wildlife survival, like Yellow Fever Virus (YFV) affecting non-human primates (NHP) from the genus Alouatta sp. in the Americas. Moreover, Flavivirus ability to mutate and adapt to new hosts gives rise to new variants with altered pathogenicity, such as occurs with Zika Virus (ZIKV). In this study, we describe the detection of a Flavivirus highly similar to ZIKV infecting free-ranging NHP from Rio Grande do Sul (RS) state, the southernmost state of Brazil. Tissues from 46 NHP living in several regions of RS, belonging to genus Alouatta sp., Sapajus sp. and Callithrix sp., submitted to necropsy from 2016 to 2018 were collected and fixed in neutral buffered 10% formalin solution for histological analysis. Additionally, frozen tissue samples were collected to virological and molecular biology assays. These samples were tested by nested Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) for Flavivirus aiming NS5 portion of Flavivirus genome. Pathological findings from positive PNHs were described, and virus isolation was attempted from these samples. After testing 114 tissue samples, 18 tissues (6 liver samples, 6 brain samples, 4 cerebellum samples, 1 placenta, and 1 spinal cord) belonging to 9 PNH were positive for Flavivirus, and Sanger sequencing of a 979bp fragment revealed 96% identity to ZIKV MR 766 strain and 85% identity to ZIKV strain P6-740. All positive PNH were Alouatta guariba, and 4 of them were found in Porto Alegre city. Three PNH did not have their location informed, 1 was found in Camaquã municipality and 1 was found in Triunfo municipality. Seven of the positive PNH had a history of trauma. Microscopically, liver, kidney and lungs presented inflammation features in several degrees in 6 PNH, CNS lesions were observed in 2 PNH. Similar lesions were described in experimental ZIKV infection in NHP. These findings emphasize the importance of arboviral surveillance in wildlife to prevent spillover events in human population and to protect native animal populations from these infectious agents.

Financial support: CAPES/CNPq/DCIT-Ministério da Saúde

Palavras-chaves: Flavivirus, Non-human primate, RT-PCR, Histopathology

THE VIROME OF PATAGONIAN BONNETED BAT (Eumops patagonicus): IDENTIFICATION OF NOVEL VERTEBRATE AND DIETARY VIRUSES FROM INSECTS IN SOUTHERN BRAZIL

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Resumo

Bats belong to the order Chiroptera, the second largest group of mammals and the only one with the capacity of real flight. Some bat species are recognized as natural reservoirs of several zoonotic viruses and due to this feature they play an important role in the transmission and maintenance of these microorganisms. This work aimed to describe the virome of *Eumops patagonicus* (Patagonian bonneted bat), including viruses from vertebrates, invertebrates, plants and phages. Eight Patagonian bonneted bats from a colony in Southern Brazil (municipality of Uruguaiana, Rio Grande do Sul State) were collected and the organs were pooled. Pooled organs were mechanically disaggregated, low speed centrifuged for removal cell debris, filtered and ultracentrifuged under a sucrose cushion to concentrate the viral population. The viral pellet was treated with nucleases and the viral DNA and RNA were randomly enriched and sequenced using the Illumina MiSeq system. A total of 116,304 paired-end reads were generated. These sequences were assembled into 1,115 contigs using SPAdes 3.5 assembler and analyzed using blastn/blastx with NCBI databases. Of these contigs, 123 (11%) were recognizable viral sequences belonging to 15 viral families. Vertebrate viruses included Anelloviridae, Circoviridae, Parvoviridae and Polyomaviridae, insect viruses included Alphatetraviridae, Dicistroviridae, Ifflaviridae, Iridoviridae and Nodaviridae, phages viruses included Myoviridae, Podoviridae and Siphoviridae, plant viruses included Partitiviridae and Sobemovirus. The metagenomic results were followed by phylogenetic inferences, which have led to the discovery of some novel bat viral sequences of the families Polyomaviridae, Anelloviridae, Circoviridae, Parvoviridae and CRESS-DNA viruses. In conclusion, the present study presents the *E. patagonicus* virome by viral metagenomics and the results obtained further expand the spectrum of viruses harbored by bats.

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**Palavras-chaves:** Bat, Chiroptera, Metagenome, Virome

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**Absence of neutralizing antibodies to the Indiana vesicular stomatitis virus in the pampa biome region of Rio Grande do Sul, Brazil.**

**Autores**

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**Resumo**

Vesicular stomatitis is a disease caused by an arbovirus (VSV) belonging to the family Rhabdoviridae, genus Vesiculovirus. VSV is classified in two serotypes, Indiana (VSIV) and New Jersey (VSVNJ), and its circulation is limited to tropical and subtropical regions of the American continent. In Brazil it is reported the presence of samples antigenically similar to VSIV. Bovine, swine and horses are the main hosts and the clinical infection produces vesicular lesions in the oral cavity, nasal, feet and teats. Clinical signs in ruminants and swine are indistinguishable from foot-and-mouth disease lesions. The
objective of the present study was to detect the presence of neutralizing antibodies to VSIV in bovine and equine serum samples collected in the western region of Rio Grande do Sul. Antibody detection was performed by virus neutralization (VN) in 96 wells plates. A total of 940 bovine and 779 equine sera from different municipalities of the region were tested. Prior to the test, the samples were inactivated at 37 °C for 30 minutes. Samples were screened qualitatively (1:20 dilution), followed by titration (quantitative VN) to confirm the reactivity. There were no positive sera among tested samples. This indicates absence of viral circulation or that infection occurs at low levels in the region. The results are consistent with the absence of cases of vesicular disease notified to the regional official veterinary service in recent years. The present study demonstrated that the animals evaluated did not present neutralizing antibodies to the VSIV. However, surveillance for this and other vesicular infections must be a routine by technicians and the official veterinary service.

Palavras-chaves: VSIV, FMDV, vesicular disease, virus, transboundary disease

Novel astrovirus and calicivirus in migratory birds in Brazil

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Resumo

The birds are a group of vertebrates with approximately 10,000 species. They are the natural reservoir of several viral species (e.g., Influenza, West Nile, Newcastle disease virus), consequently contributing to the evolution, emergence, and dissemination of novel viruses. In recent years, extensive metagenomic studies have dramatically expanded our knowledge about the virosphere. In this study, we have applied the high-throughput screening approach to identify the viral diversity of birds captured in north and northeast region of Brazil. Therefore, we sampled 100 individuals represents eight different birds species (Cairina monchata, Gallus gallus, Arenaria interpres, Calidris pusilla, Thalasseus sandvicensis, Coryphospingus pileatus, Hilophilus amaurocephalus and Sarkesphorus cristapus). Samples were distributed in pools based on species, sample type (i.e. cloacal swabs and sera), date and capture location. 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 de novo. We found nearly complete genomes of novel species of astrovirus and calicivirus in cloacal swabs of ruddy turnstone (Arenaria interpres) collected in Coroa do Avião islet, Pernambuco State. These viruses are positive-sense single-stranded RNA with a genome of 7 to 8 kb, and were designated as Ruddy turnstone astrovirus (RtAstV) and Ruddy turnstone calicivirus (RTCV), respectively. The phylogenetic analysis showed that RtAstV and RTCV were grouped in a monophyletic clade with viruses identified from poultry samples (i.e., chicken, goose, and turkey), including viruses associated with acute nephritis in
chickens. In addition, we developed and applied RT-PCR to RtAstV and RTCV in individual samples, but we identified only one cloacal sample positive to each virus. On the other hand, the attempts of viral propagation in monkey and chicken cell lines for both viruses were unsuccessful. The ruddy turnstone is fully migratory with an extensive geographical distribution worldwide. In the Americas, the ruddy turnstone lives in Alaska and the Canadian Arctic (boreal summer), and Brazil (boreal winter). So far, influenza, coronaviruses, and avian paramyxovirus have been identified in ruddy turnstone. In sum, these findings shed new light on viral diversity of migratory birds with the notable characterization of novel astroviruses and calicivirus.

Palavras-chaves: Astrovirus, Calicivirus, Migratory birds, RNA virus

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First report of a triple recombinant of Feline immunodeficiency virus from Brazil

Autores
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Resumo

Feline immunodeficiency virus (FIV) is a lentivirus that affects cats worldwide. Less than ten genomes of FIV from domestic cats are available in the public databases and, to date, seven subtypes have been reported in different geographic regions: FIV A-F and UNZenv. In Brazil, FIVB is the most common variant found in cats, and F/B recombinants have been reported. In Brazil, to date, only one complete genome of FIV, described in a sample from São Paulo (SP), is available. In this work, the entire genome of the virus, obtained from a sample of Porto Alegre RS) was amplified with a high fidelity polymerase by using primers that anneal with the LTR. The amplicon has been purified and sequenced by Next Generation Sequencing (NGS). For this, a DNA library were prepared with the Nextera XT DNA library prep kit (Illumina) and sequenced with the Miseq v2 300 kit using the Illumina Miseq TM platform. The reads were mapped to a genome reference (MF370550) using Geneious version 9.0.5. Another 16 envelope FIV sequences of different subtypes were used to construct a ML phylogeny. An alignment were generated using MUSCLE and a Maximum Likelihood (ML) phylogeny was inferred in PhyML incorporating the best nucleotide substitution. The maximum likelihood phylogenetic tree was drawn using FigTree. The sequences were analyzed for recombination using Simplot. A total of 224.195 reads were obtained. In the phylogenetic tree, the sequence of the envelope gene clustered in the sister group of subgroup E, which contains subgroup B and F. The recombination analyzes showed a recombination between FIV B, F and E. Further analyzes will be done with other virus genes. This is the first triple recombinant of FIV reported in Brazil.

CAPES, Finep, CNPq

Palavras-chaves: FIV, Recombination, Simplot
ANTIGENIC DISTANCES OF NORTH AMERICAN SWINE INFLUENZA A VIRUS FROM HUMAN SEASONAL INFLUENZA A VIRUS AS AN INDICATION OF RISK TO HUMAN POPULATIONS

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Resumo
Human-to-swine interspecies transmission of influenza A virus (IAV) occurs globally and contributed to genetic diversification in both H1 and H3 swine IAV strains in North America, in particular after the emergence of the pandemic H1N1 (pH1N1) in 2009. Additionally, 2010-11 and 2016-17 incursions from human influenza seasons were detected in U.S. and were associated with illness in humans. In North America, swine H1 are classified into two main lineages: classical swine 1A, which includes α (1A.1), β (1A.2), γ (1A.3) and pdm, and human seasonal lineage 1B, which includes δ and 52 lineages. H3 are classified by decade of incursion from human into swine such as: H3.1980.1 in Mexico, H3.1990 in North America and the recent incursions H3.2010.1 and H3.2010.2 in U.S. Here, we quantified antigenic distances between North American H1 and H3 swine strains and human seasonal IAV vaccine strains in order to identify swine IAV against which the human population would lack cross-reactive HI antibodies. Monovalent anti-sera raised in pigs against human seasonal IAV vaccine strains ranging from 1973 to 2014 were run against contemporary swine strains using hemagglutination inhibition (HI) assays. HI data was used to calculate antigenic distances between antigens using antigenic cartography, where 1 antigenic unit (AU) is equivalent to a 2-fold loss in HI cross-reactivity and greater than 3 AU is considered significant antigenic distance. 1A.3.3 γ and pdm strains (2010-13) showed the greatest antigenic (2-4 AU) similarity to the current human vaccine strains, while later strains (2014-16), including a human variant (A/Ohio/9/2015), were 4-5 AU away. The 1A.1 α lineage had substantial distance from recent human vaccine strains (8-13 AU), while 1B δ strains were 6-9 AU from the latest human vaccine strains compared to greater similarity to vaccine strains from the 2000s. H3.1980 strains from Mexico and H3.1990 strains were antigenically related to the human vaccine strains from their decade of incursion (US: 2.4–5.2 AU, Canada: 2.6–6.4 AU and Mexico: 1.0–5.9 AU), but more distant from recent human vaccine strains. In contrast, H3.2010.1 and H3.2010.2 strains were antigenically related to recent human vaccine strains from the 2010 decade (US: 0.4–4.3 AU). These data may indicate loss in cross-reactivity to human vaccine strains and reduced human population immunity stratified by age demographics and can be used to identify swine IAV for further risk analysis. Funding: USDA

Palavras-chaves: antigenic cartography, influenza A virus, North America, human, swine

ISOLATION AND CHARACTERIZATION OF BACTERIOPHAGE PARTICLES FOR ANIMAL STAPHYLOCOCCAL INFECTIONS: POTENTIAL FOR VETERINARY PHAGE THERAPY VIA TRANSDERMAL DELIVERY USING IONIC LIQUIDS

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Introduction: Staphylococci are ubiquitous organisms which are found most frequently within tissues, on skin surfaces, or in foods contaminated by exposure to infected human beings or animals. Staphylococcal species are opportunistic pathogens in humans and one of the most important pathogenic species in veterinary medicine, having a huge impact on animal health and welfare and causing major economic losses in livestock production. In this research effort, the potential of isolation and characterization of two bacteriophage particles with broad lytic spectrum capable of infecting *Staphylococcus pseudointermedius* isolated from a horse paw injury was investigated, together with their transdermal delivery using ionic liquids (ILs). Methods: Physicochemical characterization of two isolated bacteriophages (from incoming efluents of SAAE/Sorocaba and Águas de Araçoiaba/SP) included verification of bacterial lysis, amplification of the bacteriophages, titration, SDS-PAGE electrophoresis, XRD and UV-Vis scanings, whereas biological characterization encompassed evaluation of their lytic spectra, efficiency of plating (EOP) assays, determination of the one-step growth curves (OSGC) for latent period and burst size determinations, and determination of the adsorption curves for calculation of the bacteriophage adsorption rate onto its bacterial host cell. Additionally, choline geranate (both IL and DES) were produced via organic synthesis using choline bicarbonate and geranic acid. The non-invasive IL-assisted delivery of the phage particles was evaluated via transdermal permeation studies in a DHC-6T Transdermal System from LOGAN INSTRUMENTS CORP. Results and Discussion: The two bacteriophages isolated and characterized produced clear (different) plaques of bacterial lysis and exhibited a broad lytic spectrum against a Staphylococcal species isolated from a horse paw injury. Conclusions: The two isolated bacteriophages proved to be suitable candidates for eradicating infections caused by Staphylococcal bacterial strains in horse wounds, following their transdermal delivery.

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Palavras-chaves: Bacteriophages, Isolation and characterization, Transdermal delivery, Ionic liquids, Veterinary phage therapy

MICE AS THE EXPERIMENTAL MODEL OF INFECTION WITH FELINE HERPESVIRUS TYPE 1

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Resumo
Feline herpesvirus type 1 (FHV-1) is an important causative of respiratory and ocular disease worldwide. A specific treatment against this agent is not available yet. For that reason research about antiviral drugs is encouraged. Mice have been largely used to study the pathogenesis of several human and animal viruses, specially herpesviruses. The objective of this study was to verify if BALB/c mice would be suitable experimental models for the infection with FHV-1. Although experimental models have their limitations and never mimic completely the infection in their natural host, they are very useful in the initial trial and monitoring of the antiviral efficacy. Twenty three BALB/c mice were inoculated intranasally with a viral suspension of FHV-1 (10 µL/nostril/animal), with a titer of 10^5 tissue culture infective doses. All animals produced typical clinical signs of disease starting three days post-infection and lasting until the day of euthanasia. The mice were euthanized ten days post-infection and the organs were collected to perform histopathology and real-time PCR. The results showed that mice were infected with FHV-1 and reproduced the disease, since lesions and viral DNA could be found in all the organs sampled, being the lung the most affected organ. This paper is the first reporting experimental infection of BALB/c mice with FHV-1 and demonstrated that this species can be a useful tool to understand pathogenesis of FHV-1 and also to perform antiviral trials against this agent.

**Palavras-chaves:** experimental infection, FHV-1, experimental model

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**DETECTION OF A NEW CALICIVIRUSES FROM BROILER CHICKEN, PARÁ, BRAZIL**

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**Resumo**

Poultry farming in Brazil has been modernized in the last decades due to technological investments, but several studies have revealed the circulation of viral pathogens in the world production of broiler chickens, which contributes to a scenario of significant economic losses. Chickens are important sources of animal protein consumed around the world. Caliciviruses have been associated with broiler chicken enteric infection, have a worldwide distribution, versatility in infecting a variety of hosts, and infections result in many diseases and can lead to death. The family Caliciviridae is classified in 5 genera, are viruses single stranded RNA, positive-sense, non-segmented, non-enveloped capsid and icosahedral with a polyadenylated genome. In the present study, a metagenomic analysis was applied in a fecal sample pool of broiler chickens, aiming to characterize emerging viral strains in poultry farming area. Specimens were collected in a commercial farm in July 2009 in Benevides, Pará, Brazil. We use the read synthesis system for DNA sequencing. The cDNA library was prepared and sequenced on an Illumina MiniSeq platform. Genome was assembled using a hybrid methodology of de novo assembly and reference mapping. The high-throughput sequencing generated 2,761,286 high-quality readings, the genomic assembly made by de novo assembly produced 15 contigs of size of 69-454bp associated with members of the Caliciviridae family. Another analysis by reference mapping produced a single unitig generated by 292 reads, which was related to chicken caliciviruses strain RS/BR/2015 (NC_033081). The new caliciviruses ChCV-052 showed a 93.4% nucleotide identity with the RS/BR/2015 strain and 24% nucleotide identity with the Pig/AB90/CAN strain (NC_012699). In addition, the RS/BR/2015 strain is not classified in any of the five genera of the Caliciviridae family, however, it was the one that presented the greater genetic proximity with the new caliciviruses ChCV-052. The present result proposes a new genus within the family Caliciviridae, and highlight the potential of the viral metagenomics for application in researches that aim to investigate new pathogens from a variety of samples, therefore, it is important the monitoring in the health of the animals of production, will contribute for prevention and control strategies, thus avoiding negative impacts on the production and economic cycle of the state of Pará.

**Palavras-chaves:** Caliciviruses, Chickens, Enteric infection, Metagenomics, Poultry farming
TRANSCRIPTIONAL PROFILE OF COWS WITH BOVINE PAPILLOMATOSIS IN NORTHEASTERN BRAZIL

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Resumo

Bovine papillomavirus belongs to Papillomaviridae family, and infects epithelial cells from bovine and closely related animals, causing hyperproliferative lesions, which may regress, or progress to form benign and malignant tumors. The virus enters the host cell, interacts with host cells by altering the regulation of some genes that are responsible for destroying tumor cells and thus triggering the lesion. It is not yet known which are these host genes that are regulated by the virus infection. So, this study is very relevant to understand its pathogenesis and also to find targets for possible drugs that interrupt the cycle of infection of the papillomavirus, thus avoiding the disease. Therefore, this study aims to understand what possible molecular mechanisms are behind the pathological processes associated with bovine papillomatosis through the identification of genes related to the development of lesions. In this way, this study were able to elucidate possible marker genes, whose protein products can be used to identify compounds that could present favorable pharmacological characteristics. Adult bovines with papillomatosis and health animals was assessed. Several lesions were used for library construction. 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. Transcriptome adaptations revealed that 1343 genes were significantly differentially regulated (FDR < 0.1). The comparison of the gene expression from infected and non-infected cows indicated that 655 genes were significantly up regulated while 688 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-chaves: Bovine papillomavirus, RNA-seq, Differential expression, Bovine papillomatosis

SERUM VIROME OF FREE-LIVING PIGEONS (Columba livia) FROM SOUTHERN BRAZIL

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Resumo
Pigeons (*Columba livia*) are, originally, wild birds that remarkably adapted to urban environments. However, such birds may be potential reservoirs of pathogens. In the present work, the serum virome of pigeons was investigated by high throughput sequencing (HTS). Blood samples were collected from 137 pigeons from seven Brazilian municipalities. Samples were separated by the region of capture, pooled and submitted to DNA and RNA extraction for high throughput sequencing. The reads obtained were filtered with Trimmomatic program, reassembled using Spades software 3.10.1 and analyzed with the of the Blast2GO bioinformatics tool. The contigs were analyzed and mapped by reference in Geneious software version 8.1.3. The number of reads sequenced per sample pool ranged from 325,182 to 1,243,436. Reads were compared to the viral sequences database at the amino-acid level using BLASTx. On average, 43.0% of the reads showed similarity to viral sequences deposited in GenBank. Among the viral contigs, 90.8% presented similarity to eukaryotic viral genomes. Contigs corresponding to viral genomes of animal interest were classified into 6 viral families: Circoviridae, Flaviviridae, Anelloviridae, Adenoviridae, Parvoviridae and Coronaviridae. The higher number of reads corresponded to pigeon circovirus (PiCV), of which seven full genomes were retrieved. Contigs with similarity to viruses belonging to the Flaviviridae family were identified as belonging to Pegivirus and Hepacivirus genera with coverage of 4.4 to 2,794 and a mean identity of 50%. Ten contigs were similar to Gyrovirus, belonging to the Anelloviridae family with coverage of 1,187 to 4,055 and a mean identity of 70.9%. A great diversity of viral genomes representing distinct viral families was detected. This is the first report of PiCV occurrence in Brazil; moreover, to date the presence of viral genomes of Pegivirus and Hepacivirus genera has not been described in birds; taking into account the low identity with reference genomes, it is possible that these genomes may represent new viral species. The potential impact of such findings on human and animal health needs further investigation.

**Palavras-chaves:** Virome, PiCV, Gyrovirus, Hepacivirus, Pegivirus

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**PARTIAL MOLECULAR CHARACTERIZATION OF TWO GENOME REGIONS OF EQUINE INFECTIOUS ANEMIA VIRUS FROM A FIELD SAMPLE OF A BRAZILIAN PANTANAL HORSE**

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**Resumo**

The Equine Infectious Anemia (EIA) is caused by *Equine infectious anemia virus* (EIAV), a Retrovirus that infects equines. The EIA presents a worldwide distribution, being one of the eleven equine diseases of compulsory notification by the World Organisation for Animal Health (OIE), and is widely disseminated in the Brazilian territory, especially in the Pantanal, where it presents a high seroprevalence, compromising the performance of the equines and indirectly, the extensive livestock activity, since equines are used for the management of cattle herds. Although the EIA was described more than 150 years ago, very little is known about EIAV genetic diversity, except for some partial sequences of *gag* gene and LTR region of genome. To date only four EIAV complete genome sequences from field samples have been published. In the present study the partial molecular characterization of two regions of the EIAV genome of a sample from a Brazilian Pantanal naturally infected horse, was made. For the molecular characterization, primers were designed and PCRs were
developed for the amplification of fragments of about 663 pb and 535 pb for 5' LTR region to gag gene and for pol gene, respectively to detection of EIAV proviral DNA from a PBMC sample collected of a horse from municipality of Poconé/Mato Grosso/Brasil. The PCR product was fractionated by polyacrylamide gel electrophoresis and the amplified DNA was purified and sequenced, and phylogenetic analysis of the nucleotide sequences was performed. The primers designed in this study were efficient, since it allowed the detection of EIAV proviral DNA from a naturally infected horse of Brazilian Pantanal. The nucleotide sequences obtained presented variable values of nucleotide similarity of to 87.8% with reference sequences of EIAV complete genome. The phylogenetic analysis shows that the Pantanal sequences form a separated clade from the other world samples for the two analyzed regions. Considering the absence of Brazilian EIAV complete genome sequences and the limited number of sequences available in public databases it becomes important to made molecular detection and characterization of the EIAV. Thus, the molecular characterization of the Brazilian EIAV sequences will increase the knowledge about the EIAV circulation in Brazilian territory.

Financial support: CNPq, CAPES, Embrapa, PRPq-UFMG

Palavras-chaves: EIAV, Genome, Pantanal, PCR, Phylogenetic analysis

COMPLETE GENOME SEQUENCE OF A CANINE MORBILLIVIRUS ISOLATED IN BRAZIL

Resumo

Canine morbillivirus (CDV; genus Morbillivirus, family Paramyxoviridae), formerly known as canine distemper virus, is the etiological agent of a multisystemic infection that affects different species of carnivores. In the present study, a CDV strain was isolated in VerodogSLAMtag cells from the brain of a 2-year-old dog that died presenting clinical signs of canine distemper, and the virus was sequenced using high-throughput sequencing. Virus stock solution was low speed centrifuged for removal of cell debris, filtered, ultracentrifuged and treated with DNAses and RNAses. After, total RNA was isolated and submitted to a viral enrichment protocol. DNA libraries were prepared using the Nextera XT DNA sample preparation kit and sequenced using an Illumina MiSeq sequencer (2×150 cycles run). The assembled contigs were examined in search for similarities to known sequences with blastx software. The CDV KIKI strain contig was composed of 73,870 (mean coverage of ~706) with a linear genome of 15,662 nt. The genome consists of six genes (3'-N-P/V/C-M-F-H-L-5') encoding eight proteins. CDV KIKI strain was shown to have 91.4% nt identity with the Onderstepoort strain, commonly used in vaccine formulations. A phylogenetic analysis based on the H gene, using an alignment with all CDV sequences with complete genome available on GenBank, showed that the CDV KIKI strain belongs to
the Europe/South America-1 genotype, closely related to Brazilian isolates from Botucatu/SP. Phylogenetic analysis of the complete genome and of all individual genes resulted in the same clustering pattern. Since recombination events were previously reported on CDV genomes, recombination analysis using complete genomes was carried out, but no putative event was detected in the CDV KIKI strain. This study reports the whole genome analysis of a Europe/South America-1 genotype strain, representing the first complete nucleotide sequence of a CDV circulating in the South region of Brazil.

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Palavras-chaves: Canine morbillivirus, Whole-genome, High-throughput sequencing, Illumina

Detection of the neuropathogenic variant of equine herpesvirus 1 associated with abortion in Brazil

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Resumo

Equid herpesvirus type 1 (EHV-1) is highly prevalent, affecting up to 80% of the world's equid population, causing extensive economic losses. The virus causes respiratory, abortion, perinatal foal mortality and neurological disease. Abortion due to EHV-1 usually occurs in late gestation and may result in abortion storms. During the past decade, the incidence of abortion due to EHV-1 has been declining, possibly due to widespread vaccination practices. In contrast, cases of EHV-1 induced neurologic disease have increased significantly in the last 15-18 years. Different strains vary in their abortogenic potential as well as in neuropathogenicity. It has been demonstrated that a single nucleotide polymorphism in viral DNA polymerase gene (ORF30) is considered one major marker for the neuropathogenic potential of EHV-1 strains. Infections with the neurological strain are associated with a longer duration and higher magnitude of viremia. This may indicate a selective advantage of the neuropathogenic strain, which in addition, is unresponsive to vaccination and has also been detected in cases of abortion. In August 2017, a 7-year-old Campolina mare aborted at the 8th month of gestation at a farm located in the municipality of Pará de Minas, Minas Gerais State. This property had frequent histories of abortion during the same gestation period, even following the required vaccination protocol for EHV-1. There were no reports of respiratory or neurological disorders in this property. Blood samples from mare and pool of fetal tissue, including liver, spleen and kidneys were collected for detection of EHV-1 DNA, using nested PCR assay. The EHV-1 DNA was detected and the neuropathogenic marker was identified by sequencing in both samples (mare and fetus). Subsequently,
EHV-1 was isolated after four blind passages in MDBK cells and the identity of the isolate was confirmed by nested PCR. In addition, pre-serum from the mare was taken on the abortion day and post-sera was taken 21 days later for paired serological diagnosis. The 4-fold increase in EHV-1 specific antibody titer among collected sera confirmed the cause of the abortion and corroborated with molecular diagnosis. This is the first report of EHV-1 neuropathogenic variant causing abortion in Brazil. Further studies are being conducted to better understand the importance of neuropathogenic EHV-1 in abortion cases in Brazil.

Financial support: CNPq; FAPEMIG

Palavras-chaves: equine herpesvirus 1, neuropathogenic variant, abortion, Brazil, diagnosis

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**VIROME ANALYSIS OF COMERCIAL VACCINES FOR CHICKEN**


Instituição: UFRGS - Universidade Federal do Rio Grande do Sul (Avenida Bento Gonçalves, 9090, Agronomia, Porto Alegre.)

Resumo

Vaccination programs are widely used in chicken production. Vaccines guarantee the quality and sanity of production and final product, as well as the consumer population. Another benefit of vaccines is the minimization of producers’ losses. Since vaccine production includes many biologic processes some contaminants may not be removed. Recently, the increasing of high throughput sequencing (HTS) application made possible the detection of known and unknown viruses. The present study aimed to search for viral contaminants on commercial vaccines for chicken production using HTS. A total of 11 commercial live attenuated vaccines for chicken were selected including vaccines against infectious bursal disease (3), Marek’s disease (2), Newcastle disease (2), avian infectious bronchitis (2) and avian metapneumovirus (2). The samples were submitted for viral enrichment and sequenced using an Illumina MiSeq platform. The assembled contigs were examined in search for similarities to known sequences with BLASTX software. The raw number of sequences ranged for 10,526-201,268 in the commercial vaccines. Despite the viruses that compound each vaccine, no other adventitious virus was found. The present work suggests that the production steps of chicken vaccines are secure and effective in removal of undesirable contaminants. Financial Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) supported this work.

Palavras-chaves: Broiler, Chicken, High throughput sequencing, Metagenomics, Virome

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**MOLECULAR INVESTIGATION OF NOROVIRUS INFECTION IN PUPPIES WITH ACUTE GASTROENTERITIS AT THE VETERINARY HOSPITAL OF THE UNIVERSITY OF CUIABÁ, MATO**
**GROSSO**

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**Resumo**

Norovirus (NoV), a member of family Caliciviridae, is a nonenveloped virus with a linear, single-stranded, positive-sense RNA genome of approximately 7.5 kb in length. It was firstly discovered in 1972 and nowadays is recognized as the major etiologic agent of acute non-bacterial gastroenteritis worldwide. Transmission of NoV occurs mainly via fecal-oral route, spreading rapidly due to the highly contagious nature of the virus and is involved in outbreaks of gastroenteritis in humans. NoVs are usually considered species-specific, but the possibility of zoonotic transmission of human norovirus (HuNoV) has been proposed as a hypothetical route of infection. Dogs were first mentioned as HuNoV zoonotic potentials in 1983, and subsequently, seroepidemiological studies were performed reinforcing the participation of dogs in the transmission of NoV to humans. The aim of this study was to verify the excretion of canine norovirus (CNV) in diarrheal feces of 30 dogs presenting acute gastroenteritis, through RT-PCR, in a veterinary hospital in Cuiabá, Mato Grosso state. Thirty fecal samples of dogs with acute gastroenteritis were evaluated at the Veterinary Hospital of the University of Cuiabá in 2016. Of the 30 dogs, 17 were male and 13 were female with ages varying from 1 to 12 months and comprised varied breeds. In addition, all dogs were tested for canine parvovirus (CPV) using the Rapid CPV Ag Test Kit (Alere). In order to amplify a partial fragment of the RdRp region of the CNV genome, a conventional RT-PCR assay was employed with primers p289/p290 using M-MLV Reverse Transcriptase and Platinum Taq DNA Polymerase. In this study, the excretion of CNV in feces was not verified in the 30 dogs evaluated, and the partial genomic fragment, corresponding to the region coding for RdRp, was not amplified by the RT-PCR assay. Among the evaluated animals, 23 were excreting specific antigen of canine parvovirus, detected through immunochromatography test, three out of the canine parvovirus-infected dogs died. Based on the extensive interactions between dogs and humans and the emergence of NoV in humans, information obtained from epidemiological surveillance must be continuously collected. There are few reports about CNV infection worldwide, which demonstrates that further surveys to clarify the importance of NoV infection in dogs are required.

Financial Support: Fundação de Amparo a Pesquisa do Estado de Mato Grosso - FAPEMAT

**Palavras-chaves:** calicivirus, diarrhea, dogs, parvovirus, PCR

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**EVALUATION OF GENETIC STABILITY OF INFECTIOUS BRONCHITIS VIRUS STRAIN**

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**Resumo**

The vaccination is a tool used to control *Infectious bronchitis virus* (IBV) spread in the poultry flocks, however, new serotypes arises, such as antigenic variations that may interfere in this strategy. The S1 subunit of the spike protein is the most studied gene, which can change during adaptation to the host. Polymorphisms in the S1 spike glycoprotein can modify the binding capacity to host tissue and the antigenicity. In this context, this study aimed to characterize partial sequence of S1 gene of a strain of IBV after three passages in embryonated eggs. Samples of kidneys of chicks that presented clinical signs of infection by IBV and mortality were submitted to the standard isolation protocol. A suspension
of clinical specimens from kidneys was inoculated into embryonated chicken SPF eggs, and the liquid chorioallantoic (LC) was collected after three passages in embryonated eggs. The vaccine IBV Nobilis® IB Ma5 (MSD Animal Health), used in the chicks, was also analyzed. The nested RT-PCR was performed, the sequences obtained were identified with homologous sequences of IBV and compared with sequences belonging to the GI-1 group (Massachusetts strains). The alignment of S1 fragments of the vaccine strain Ma5 with that obtained directly from kidney samples showed changes in 15 nucleotides. These polymorphisms were reflected in seven amino acids alterations in the analyzed fragment, four of which were similar to those of the pathogenic strain M41. Conversely, the IBV isolated from the kidney after the third passage exhibited changes in 12 nucleotides compared to that obtained directly from kidney tissue, in which case resulted in a change of six amino acids. The viral strains identified directly from the tissue and after isolation showed high identity (95.53 to 99.75%) with strains of serotype Massachusetts. However, the same strain isolated by only three passages in embryonated eggs appeared to be genetically more correlated with the H120 attenuated vaccine strain (98.63%). This study demonstrated that obtaining the genomic sequence of IBV after isolation in embryonated eggs, using even a few passages, can lead to significant variation in these data. The results this work may auxiliary in the development of new vaccines against field IBV and in the diagnostic methods.

Financial Support: CAPES, CNPq and FAPEMIG.

Palavras-chaves: antigenic variation, avian infectious bronchitis, genetic variability, IBV, polymorphism

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**EVALUATION OF SAMPLE CONCENTRATIONS IN THE ACCURACY OF THE RABIES VIRUS ISOLATION IN N2A CELL CULTURE**

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**Resumo**

Besides the replacement of animal use, the viral isolation in cell culture (VICC) has lower cost and more agility in obtaining the results. Factors related to the quality and preparation of samples have direct influence in the technique accuracy. This study evaluated the application of different concentrations of bovine CNS suspensions in N2A cell cultures, in order to improve the sensitivity of the technique. Twenty bovine FAT-positive samples were selected by systematic sampling method. CNS suspensions were prepared at concentrations of 5, 10, 20, 30, 40 and 50% (w/v) and inoculated in N2A cell culture. The VICC was performed in two different moments to evaluate the reproducibility by means of result concordance. Loss of cell confluence and the viral titer of each sample were also evaluated. When comparing the results of the first and the second IVCC, 65%(13/20) of the results were in agreement at all concentrations, whereas 35%(7/20) showed no consistent results in at least one of the concentrations. The viral titer of 20 samples ranged from

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PCV3 DETECTION IN CO-INFECTION WITH OTHER VIRUSES IN LUNGS OF PIGS WITH RESPIRATORY DISEASE

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Resumo

Porcine circovirus type 3 (PCV3) is a newly emerging virus which has been associated with several outcomes and lesions, including porcine dermatitis and nephropathy syndrome, respiratory disease, reproductive failure, and myocarditis in fetuses. However, PCV3 has also been detected in healthy pigs, without any clinical signs. Although PCV3 belongs to Circoviridae family, together with porcine circovirus type 2 (PCV2), PCV3 genome is ~300nt larger than PCV2. Co-infection of PCV2 with other viruses as porcine parvovirus (PPV1), porcine parvovirus 4 (PPV4) and torque-teno sus virus (TTSuV1 and TTSuV2) has been implicated in the PCV2 disease development. In this study, PCV3 detection is reported in lungs of pigs infected with influenza A virus (IAV), and in association with PCV2, PPV1, PPV4, TTSuV1 and TTSuV2 viruses. From a total of 87 lung samples, previously considered as positive to IAV by RT-PCR and immunohistochemistry, 13 (14.9%) lungs were also positive to PCV3 by PCR. The virus identity was confirmed by the sequencing of a 330nt fragment of the cap gene. PCV3 DNA was detected in samples collected since 2010 and in association with IAV and other DNA viruses. Three lung samples presented double infection (PCV3 + PCV2 or TTSuV2). Triple infection was detected in five samples (PCV3 + TTSuV2 + PCV2 or TTSuV1). Finally, four samples had PCV3 in association with PCV2, PPV1, TTSuV1 and TTSuV2. The most prevalent virus detected in association with PCV3 was TTSuV2. It was presented in 11 out of 13 (84.6%) PCV3 positive samples. Here, PCV3 was detected in lungs in co-infection with other swine viruses. The detection of PCV3 in pigs with IAV infection since 2010 is a newly and interesting finding. Although PCV3 has been described in healthy and diseased pigs, as the only virus or in association with other pathogens, its possible pathogenic role in swine is still unclear. Further studies and more data will be necessary to clarify the PCV3 origin, its possible role as trigger in other diseases and also its impact on pig health.

Financial support: EMBRAPA

Palavras-chaves: PCV3, Swine, Co-infection, PCR
Bovine papillomavirus (BPV) is a diverse group of double-stranded DNA viruses that infect the mammalian epithelium, such as cattle. BPV is the etiologic agent of papillomatosis in cattle, a worldwide disease that affects animals of all ages causing hyperproliferative lesions that lead to depreciation of the commercial value of the animal and decrease productivity. Studies that show how parasite-host interaction occurs are still very limited. Knowing that Brazil is one of the great producers of meat and milk in the world, this study aims to analyze differentially expressed genes associated with BPV infection in a group of infected and uninfected cows. For this, next-generation RNA sequencing was used to assess differentially expressed genes. Illumina output files were converted to FASTQ format. Quality evaluation was performed using FastQC and the sequence quality cut was performed using Trimmomatic. TopHat and Bowtie were used to map and align the reads with the reference genome. The abundance of the expressed genes was verified using Cufflinks. Cuffdiff was used for differential expression analysis. 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. Our results point to a change in the expression profile in the keratin genes FOXN1 and SOSTDC1 in the group of infected cows when compared to the control. The high expression of key marker genes for keratinocyte differentiation in animals with BPV is expected since virus infection alters epithelial differentiation making levels of keratinocyte gene expression elevated. Although further studies are needed, these findings should contribute to elucidation of the host-pathogen process of interaction in the development of papillomatosis in cows.

Financial Support: CAPES, CNPq and FAPITEC/SE

Palavras-chaves: Bovine papillomavirus, Differential gene expression, RNA-seq, Host-parasite interaction

MONITORING OF FLAVIVIRUS IN MIGRATORY BIRDS OF DIFFERENT REGIONS OF BRAZIL.

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Resumo

The great ability of expanding in endemic areas and fast spread into non-endemic regions added to the high zoonotic potential made the Flavivirus a major threat to global health with regard to the emergence of diseases. Migratory birds are directly involved in the spread of these viruses to various regions of the world through migratory processes. In Brazil, migratory bird routes along the territory allow contact of visiting migratory birds with the native fauna in some regions favoring dispersion of Flavivirus which can...
consequently cause outbreaks of diseases in the country. The aim of this work was to evaluate the presence of Flavivirus in migratory birds in different regions of Brazil using of RT-PCR. In this study, samples of oral, rectal swabs and blood from 308 birds collected in three distinct regions of Brazil (Panaquatira/MA, Ilha Comprida/SP and Clube de Campo/SP) were analyzed between 2015, 2016 and 2017. Total nucleic acid from the samples was extracted and then RT-PCR was performed using degenerate primers described by Johnson et al. (2010). All samples analyzed showed negative results for Flavivirus. Despite the lack of detection of the active virus in the analyzed birds the circulation should not be excluded or the potential risk of transmission of Flavivirus in the study regions should be ruled out, since Flaviviruses do not only have birds as reservoirs and carriers. The continuity of this work with an increase in the number of sampling may bring different results to that presented.

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Palavras-chaves: Avian Virus, Flavivirus, Migratory Birds, Shorebirds

EQUINE INFECTIOUS ANEMIA DISTRIBUTION IN THE RIO GRANDE DO SUL BETWEEN 2014 AND 2018

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Resumo

Equine Infectious Anemia (EIA) is a chronic viral disease where virus persists in infected animals for life and can be reliably detected by serologic tests that measure the presence of antibodies to the major structural protein of the virus. The EIA has a worldwide distribution, and the prevalence os the infection can be as high as 70% in adult animals in endemic areas. The goal of this study was to analyzing, in a descriptive, inferential, spatial and temporal manner, the reports of diseases in equines received by the DDA/SEAPI in the period of 2014 and 2018. The data were obtained from the weekly notification reports with DDA/SEAPI and analyzed in a descriptive way in Excel Office 2010 software by species and regions of the State of RS. In the analyzed period, the SVO-RS received 230 reports of suspected outbreaks of Equine Infectious Anemia. Of these, according to official records, 60.86% of the outbreaks were confirmed as positive using the Coggins Test, the official diagnostic test for AIE in Brazil. Of the 230 test performed, the highest frequency of reported outbreaks happens in 2015/2016 years with 56 and 50 outbreaks notified, respectively. Then came the years 2017, 2018 and 2014 with 15, 13 and 6 outbreaks. The distribution of the outbreaks, taking into account the regions of the State of Rio Grande do Sul, was distributed in: metropolitan (n=62), south-west (n=26), north-west (n=23), north-east (n=20), central-eastern (n=7), southeast (n=2). The epidemiological survey carried out showed that in the period studied, the outbreaks were concentrated in certain regions of the state, mainly in the metropolitan region. Furthermore, if we consider that the data is in the public domain, it is worrying to see that currently more than 80% (2014-2017) of the outbreaks classified as "unfinished" for diagnostic keep without confirmation in the official records. The incidence of EIA in the state has led to an increase in the deadline of the diagnosis to 180 days since 2014. In regions where official surveillance is more intensive, positive animals are identified. The number of Animal Transit Guides issued, the number of tests performed and, consequently, the number of positive outbreaks increase directly proportional to the intensity of surveillance. The results show that the disease, although controlled, persists in certain regions and communities that remain on the margin of sanitary control.

Palavras-chaves: EIA, epidemiology, outbreak, diagnostic, equine
COMPLETE GENOME SEQUENCE OF A MAMMALIAN RUBULAVIRUS 5 ISOLATED IN BRAZIL


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Resumo

Mammalian rubulavirus 5 (PIV5; genus Rubulavirus, family Paramyxoviridae), formerly known as Parainfluenza 5, is a single-stranded, negative-sense RNA genome virus. It has been isolated from numerous species, including humans, monkeys, pigs, cattle, and dogs, and it can also cause unrecognized persistent infections of cell culture. High throughput sequencing (HTS) is a major tool for identifying viruses in biological samples, particularly when the target sequence is undefined. In the present study, HTS was applied for the identification of a Mammalian rubulavirus 5 that was isolated as a contaminant from MDBK cells. DNA libraries were prepared using the Nextera XT DNA sample preparation kit and sequenced using an Illumina MiSeq platform. The assembled contigs were examined in search for similarities to known sequences with blastx software. The PIV5 strain ACM17 contig was composed of 87,616 reads (mean coverage of ~622) with a linear genome of 15,246 nt that consists of six genes (3'-N-V/P-M-F-HN-L-5') encoding seven proteins, lacking the SH gene. The pairwise identity was 96.6% between strain ACM17 and PIV5 (NC_006430), a reference strain for PIV5. A whole-genome phylogenetic tree including all PIV5 sequences available in the GenBank database showed that ACM17 strain clustered in the same terminal node with isolates of pigs, calves and dogs. Furthermore, to detect potential recombination events, the complete PIV5 genomes publicly available were screened using distinct algorithms with RDP4 software. One potential recombination event was detected in seven sequences, including strain ACM17. The breakpoints of the recombinant strains were located on the SH gene equivalent region. Similarity plots, bootscan analysis and phylogenetic inferences were also run to analyze incongruences between the putative recombinants and its parental viruses, corroborating the results. To the best our knowledge, this is the first whole-genome sequence of a Mammalian rubulavirus 5 isolated in Brazil, although it is not possible to define the origin of this virus. Also, there is no previous report of recombination event in this species, with few reports in other members of the Rubulavirus genus.

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Palavras-chaves: High throughput sequencing, Mammalian Rubulavirus, Paramyxoviridae.

EVIDENCE FOR A NOVEL AVIAN AVULAVIRUS 1 DETECTED IN WILD BIRD FROM RIO GRANDE DO SUL

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Resumo

Avulavirus 1 (AV-1) is an orbivirus, a member of the family Togaviridae, and is the causative agent of an acute, febrile illness in birds. So far, strain KU72 has been considered the reference strain for AV-1 in the GenBank database. In this study, high throughput sequencing (HTS) was applied for the identification of a novel AV-1. A DNA library was prepared using the Nextera XT DNA sample preparation kit and sequenced using an Illumina MiSeq platform, resulting in 2,212,273 reads for the KU72 isolate. The assembled contig was composed of 170,867 reads with a linear genome of 12,360 nt that consists of two genes (3'-N-G-E-P-5') encoding three proteins, lacking the S gene. The pairwise identity was 99.4% between strain KU72 and strain KU72 (NC_006430), the reference strain for AV-1. A whole-genome phylogenetic tree including all AV-1 sequences available in the GenBank database showed that the strain KU72 clustered in the same terminal node with isolates of wild birds from Brazil. Furthermore, to detect potential recombination events, the complete AV-1 genomes publicly available were screened using RDP4 software. One potential recombination event was detected in seven sequences, including strain KU72. The breakpoints of the recombinant strains were located on the S gene equivalent region. Similarity plots, bootscan analysis and phylogenetic inferences were also run to analyze incongruences between the putative recombinants and its parental viruses, corroborating the results. To the best our knowledge, this is the first whole-genome sequence of a novel AV-1 isolated in Brazil, although it is not possible to define the origin of this virus. Also, there is no previous report of recombination event in this species, with few reports in other members of the Togaviridae family.

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Palavras-chaves: High throughput sequencing, Avulavirus 1, Togaviridae.
Avulavirus is a genus of the family Paramyxoviridae. Members of this family are characterized by pleomorphic-enveloped particles that contain a single-stranded, negative sense RNA genome. ICTV Virus Taxonomy 2018 classifies Avulavirus into nineteen distinct species or serotypes (AAvV-1 to 19), including the recently, new avian avulavirus that our group described in Rio Grande do Sul (RS) state, Brazil (AAvV-15). Here, we report a new avian avulavirus related to AAvV-1, based on genetic finds and phylogenetic analysis, found at the same location. Under the Avian Influenza, West Nile and Newcastle Disease viruses Surveillance Program of the Laboratory of Virology, Institute of Biomedical Science – USP, a Calidris fuscicollis (Charadriiforme) was captured in the Lagoa do Peixe, RS, in April 2012, shown a new paramyxovirus. The tracheal/cloacal swab sample was isolated in embryo chicken eggs and partial sequence (3400 bp) of the F and HN genes of this isolate (RS-1208) were obtained by Next-Generation Sequencing (NGS). The Blastn alignment with representative viruses of the Paramyxoviridae family available in GenBank, showed that isolate RS-1208 has characteristics of both class I and class II Avian Avulavirus 1. Isolate RS-1208 HN gene had 73% identity to the closest AAvV-1 class II (2602-468/Niger/2008) and F gene had 74% identity to the closest AAvV-1 class I (Teal/France/100011/2010). Analyzing the entire sequence of 3400bp, the closest isolate CBU2249 had 71% of identity, an AAvV-1 isolated from mallard in South Korea in 2007. Further studies are needed, including the tentative to sequence the entire genome, to better characterize this isolate and clarify if it is a new class I virus, new class II virus or even a new specie.


Palavras-chaves: Avian avulavirus, Paramyxovirus, new virus, virology, wild birds
syndromes that lead to major economic losses. Two species, BVDV-1 and BVDV-2, recently renamed as *Pestivirus A* and *B*, belong to the genus *Pestivirus, Flaviviridae* family, and can be further segregated into several subgenotypes. BVDV-1b and BVDV-1a are the predominant subgenotypes worldwide, and strains from BVDV-1a and BVDV-2a are commonly used as modified lived vaccines (MLV). Variation in viral fitness and clinical presentation can be observed as a result of genetic differences between the species, subgenotypes and even strains, as well as the presence of persistent infections, immunomodulation and use of multivalent MLV. Thus, this study evaluated the competitive fitness of BVDV-1a, BVDV-1b and BVDV-2a strains in single and coinfections using two cell lines; one derived from epithelial cells (bovine turbinate; Bt) and one derived from lymphoid cells (bovine B lymphoma; BL3). Evaluation was performed using the PrimeFlow RNA assay, a *in situ* hybridization-based branched DNA amplification followed by flow cytometry that allows multiplex detection of RNA. The PrimeFlow RNA assay was carried out on days 2, 9 and 30 post infection and provided the percentage of infected cells by each strain and the number of coinfected cells. Immunofluorescent microscopy, RT-qPCR and DNA sequencing were also performed. A competitive exclusion was promoted by the fittest strains, the BVDV-2a strains, which suppressed, inhibited and eliminated BVDV-1a and BVDV-1b strains in coinfections. This dominance of BVDV-2a strains started earlier in BL3 at day 2, when the frequency of infected cells by BVDV-1a and BVDV-1b strains began to decline until reaching zero at day 30. This phenomenon started later in BTU, at day 9. Then, BVDV-1a and BVDV-1b strains were either eliminated or they had their positive cells percentages dramatically decreased until day 30. Whereas a coexistence between BVDV-1a and BVDV-1b strains was noticed throughout the study time in both cell lines. These findings may help to explain the frequency of subgenotypes detected in the field, pathogenesis and immune response to multivalent vaccines. These studies demonstrated that PrimeFlow RNA assay is an important tool for the detection and quantification of coinfections at the single cell level.

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Palavras-chaves: BVDV, coinfections, flow cytometry, viral fitness

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GENETIC DIVERSITY OF BOVINE PAPILOMAVIRUS (BPV) IN THE STATE OF SERGIPE, NORTHEASTERN BRAZIL

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**Resumo**

Bovine papillomavirus (BPV) is a virus of the family *Papillomaviridae* that affects mainly cattle. However, different from what is observed in other types of this family, it has been shown that BPV is not species-specific and has been found in animals such as giraffes, buffaloes and horses. It is known that today there are 24 types of BPV and each type can be related to the development of different diseases. In contrast, it has been found that human papillomavirus (HPV) variants may present different pathological presentations than those observed in non-variant strains. Because of the similarity in the structure and biology of these groups, it is believed that it is possible that variants of BPV may also exhibit this change in the pathogenic pattern. Thus, understanding the importance of knowing the BPV variants, this study aimed to characterize the genetic diversity of types and variants of BPV present in the state of Sergipe, Northeastern Brazil. Samples were collected in different regions of the state and histopathological and molecular analyzes confirmed the cause of BPV lesions. FAP 59/64 primers were used to amplify the L1 gene fragment by PCR. The amplified product was analyzed by agarose gel electrophoresis and the positive samples were purified and sequenced. Sequencing quality and contig assembly were carried out using Pregap4 and Gap4 programs. Only sequences with Phred
value above 30 were considered for the contig assembly. Local sequence alignments were performed with BLAST to identify BPV types. The MUSCLE algorithm, incorporated in MEGA7 software, was used to align the sequences with significant identity, in order to identify the mutations and the polymorphisms in the samples, comparing with sequences deposited in GenBank. Comparing the L1 fragment obtained with the same protein from the reference sequence, the study showed the presence of seven types of BPV circulating in the state and 12 of the samples were presented as putative new types, since they did not reach an identity superior to 90%. The diversity of the viruses found in the state was quite relevant, since in addition to the variety of types, a large number of variants and putative new types were still observed.

Financial Support: CNPq, CAPES and FAPITEC/SE

Palavras-chaves: diversity, papillomavirus, bovine, Sergipe, Brazil

GENETIC PROFILE OF PESTIVIRUSES CIRCULATING IN BEEF CATTLE IN SOUTHERN BRAZIL

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Resumo

Bovine pestiviruses include the prototype species, *Bovine viral diarrhea virus* 1 (BVDV-1) and 2 (BVDV-2), respectively; and a third species, provisionally called HoBi-like viruses or atypical bovine pestiviruses. Nucleotide sequencing and comparison of the highly conserved 5' UTR, Npro and E2 coding sequences has served for pestivirus phylogeny and classification into subtypes (or subgenotypes). At least twenty-one BVDV-1 subgenotypes (1a-1u) have been described to date while four BVDV-2 subgenotypes (2a-2d) have been described. In this study, we identified genetically bovine pestiviruses detected in the sera of beef calves destined to export from Rio Grande do Sul (RS) in 2017. Screening of serum of 15,684 calves by an antigen capture ELISA and, subsequently, by reverse-transcription polymerase chain reaction (RT-PCR) revealed 135 containing pestivirus RNA. Genetic typing of these viruses based on the 5’UTR of the viral genome allowed for the identification of 90 different viruses, being 38 BVDV-1 (42.2%), 31 BVDV-2 (34.4%) and 21 HoBi-like viruses (23.4%). Among BVDV-1, only subtypes -1a (n=28, 31.1%) and -1b (n=10, 11.1%) were identified. All 31 BVDV-2 isolates belonged to BVDV-2b subtype and the 21 HoBi-like viruses clustered to subtype 3a. Genetic characterization of isolates is an ongoing process which has been proven relevant and useful for pestivirus diagnostic and control in Brazil. Thus, our findings add valuable information about the epidemiology and will certainly contribute for future diagnostic and control of pestivirus infections in Brazil.

Financial support: CNPq.

Palavras-chaves: BVDV, genotype, HoBi-like, RT-PCR
COMPARATIVE PERFORMANCE STUDY OF TWO RNA EXTRACTION METHODS BY THE RT-PCR AND RT-qPCR TECHNIQUES FROM BOVINES CENTRAL NERVE SYSTEM (CNS) SAMPLES NATURALLY INFECTED BY RABIES VIRUS (RABV).

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Resumo

The detection of the RABV by the direct fluorescent antibody (DFA) may have varying results, once that the virus does not infect CNS structures uniformly. For this reason, the development and implementation of alternative methodologies, such as conventional RT-PCR and RT-qPCR, became important for the diagnosis. RNA extraction is also an essential factor influencing the sensitivity of molecular techniques for the detection of RABV RNA as a diagnostic test. These assays are usually hampered by the low virus titer found and PCR inhibition due to reagent residues or other unknown factors during RNA extraction. Rabid bovines CNS samples were analyzed by RT-PCR and RT-qPCR comparing RNA extracted with the guanidinium thiocyanate (TRIzol reagent) and commercial RNA extraction silica column (RNAspin mini isolation kit, GE Healthcare) during the period of March 2015 and April 2016. 186 CNS samples of 40 bovines were found as positive by DFA were included in this study. Extracts prepared using the silica column RNAspin mini isolation kit yielded the most consistent results by RT-qPCR and RT-PCR. The TRIzol reagent failed to confirm 52 RABV DFA-positive specimens by RT-qPCR. On the other hand, there were only two discordant results tested by RT-qPCR in DFA-positive extracts produced with RNAspin mini isolation kit. RABV nucleic acid was detected by RT-PCR in 161 of 186 (86.5%) DFA-positive samples that were manually extracted with TRIzol reagent, while viral RNA was detected in 168 of 186 (90.3%) DFA-positive extracts prepared by the RNAspin mini isolation kit. According to the results, the difference of the detection between those two extraction techniques demonstrates amplification problems of the nucleic acids in the samples extracted with TRIzol reagent, suggesting a higher efficiency of the extraction with the RNAspin mini isolation kit.


Palavras-chaves: Extraction silica column kit, Guanidinium thiocyanate , PCR inhibition , Rabies, RNA extraction

CO-INFECTION OF BOVINE PAPILLOMAVIRUS IN TEAT WARTS FROM DAIRY CATTLE


Resumo

Papillomaviruses (PVs) are small viruses that comprise a highly diverse group that can produce epithelial proliferative lesions in all amniotes. Bovine papillomavirus (BPV) is recognized as the etiological agent associated with several forms of benign tumors, among them the teat papillomatosis that is distributed worldwide in dairy cows and can result in lower profits for the milk industry. Currently, only 24 BPV types have been reported, in contrast to more than 200 types of the human papillomavirus (HPVs) (http://pave.niaid.nih.gov). Although BPV1, 5 and 6 have been classically associated with udder and teat lesions, other types have been detected in this anatomic region. In the present study, seventeen specimens consisting of papillomatous-like teat lesions were collected from dairy cows sent for slaughter. The confirmation of the presumptive diagnosis and typing of the BPV involved was performed by histopathological evaluation, conventional polymerase chain reaction (PCR) carried out by using the primer pair FAP59 (forward: 5’-TAACWGTIGGICAYCCWTATT-3’) and FAP64 (reverse: 5’-CCWATATCWVHCATITCICCATC-3’), followed by Sanger sequencing. Moreover, the multiple rolling circle amplification (RCA) assay was performed in order to recover the complete genome of the virus directly from the papillomatous lesions. The RCA reaction was performed with exo-resistant random primers; therefore, previous knowledge of the nucleotide sequences is not necessary. In addition, we performed the next generation sequencing (NGS) of the enriched samples and 20 BPVs complete genomes were found in the papillomatous-like teat lesions. As preliminary results, co-infections were detected in sixteen specimens. Based on the similarity of the L1 gene sequence BPV8 (2/20), BPV7 (3/20), BPV12 (1/20), BPV new genus (1/20), DyoxiPV new species (1/20), EpsilonPV1 new type (2/20), XiPV1 new type (8/20) and XiPV2 new type (2/20) were found. These findings add new knowledge to the expanding genetic diversity of BPV highlight the possibility of interactions between several BPV strains in the same lesion.

Financial Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) supported this work.

Palavras-chaves: Bovine papillomaviruses, Co-infection, Complete genome, Dairy cattle, Teat warts
Resumo

Rabies is a zoonosis distributed into two thirds countries worldwide with one individual dying of rabies every 10 minutes, resulting in 70,000 deaths each year. Rabies virus (RABV) reservoirs are bats and canids. Two lineages of the RABV have been found in Carnivora order in Brazil: one found in domestic dogs and cats, and the other in domestic dogs and wild foxes, with reported cases in *Cerdocyon thous* (crab-eating fox) and *Pseudalopex vetulus* (hoary fox) (previously called *Dusicyon vetulus*). In Paraíba state, two lineages were already found in *P. vetulus*, one isolated from foxes and the other closely related to the domestic dog lineage, considering P and N protein analysis. The aim of the present study was to analyze the RABV complete genome sequence from central nervous system fragments (CNS) of two foxes (*C. thous*), collected in 2010 and 2013, in São José de Espinharas, Paraíba, Brazil. According to information obtained from the rural population whereas the animals were found, both foxes were runned over after neurological signs were observed, including incoordination, loss of balance, marked compulsive balancing of the head, and apparent muscle weakness. The CNS evaluated resulted positive both in the direct fluorescent antibody test and also in the mouse inoculation test. Both RABV were sequenced using the Illumina platform. Genome structure was composed by 12,039 nt. The phylogenetic tree was constructed with the Neighboor-Joining test model, with a bootstrap value of 1000 replicates and the studied sequences RABV Fox1_C_thous_PB_Brazil_2010 and Fox2_C_thous_PB_Brazil_2013 grouped in a cluster containing isolated strains from wild canids and domestic canines from Brazil, with the subcluster that the *C. thous* lineage belongs.

Palavras-chaves: canid wild, deep sequencing, rabies virus, zoonosis

COMPLETE GENOME SEQUENCING, PHYLOGENETIC AND EVOLUTIONARY ANALYSIS OF PESTIVIRUS H (HOBI-LIKE VIRUS)

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Resumo

The Pestivirus H, previously known as “HoBi-like”, is an emerging pathogen belonging to the Pestivirus genus, family *Flaviviridae*. Pestiviruses are positive-sense, single-stranded RNA viruses. The viral genome contains two untranslated regions (UTRs) and a unique open reading (ORF) frame encodes a long polyprotein that is processed into four structural proteins (C-E<sup>ns</sup>-E1-E2) and seven non-structural proteins (N<sup>pro</sup>, p7, NS2/NS3, NS4A, NS4B, NS5A, NS5B). The first detection of Pestivirus H dates back 2000 from a Brazilian water buffalo. After that, this pestivirus has an increasing worldwide identification, associated with a broad spectrum of clinical manifestations in ruminants. Despite Pestivirus H is a threat to animal health as well as to pestivirus control programs, understanding its evolution, genetic, antigenic variability and epidemiology is still limited. Thus, in the present study, five Brazilian Pestivirus H whole genomes were determined by high throughput sequencing. Beyond that, a phylogenetic, selection pressure, entropy and Bayesian molecular clock analysis were performed with Pestivirus H sequences available at a public database. The phylogenetic analysis revealed all Brazilian strains are closely related to each other and to strains detected in Europe. Amino acid variability was uneven across the viral genome, which was mostly under neutral or purifying selection. The E2, encoding the major immunogenic pestivirus protein, was the most variable gene and also the one that showed the greater number of positive sites (diversifying selection), followed by the genes: NS23, NS5B, NS5A and N<sup>pro</sup>. In
addition, the year for the most recent common ancestor of Pestiviruses H was estimated and a hypothesis about the origin and dispersion of this virus could be raised. Besides to providing more full-length genome sequence data, this study offers insights into the Pestivirus H diversification and evolutionary history. Further studies are necessary to ascertain the relevance of these findings in terms of diagnostic, control and immunization strategies.

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 do Rio Grande do Sul (FAPERGS) and Pró-Reitoria de Pesquisa (PROPESQ-UFRGS).

Palavras-chaves: Entropy, High throughout sequencing, HoBi-like pestivirus, Molecular clock, Selection pressure

Detection and isolation of West Nile virus in equidae in Brazil: a new challenge?

Autores

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Resumo

Arboviruses (arthropod-borne viruses) transmitted by mosquitoes are one of the major public health problems worldwide, accounting for emerging and reemerging diseases. Some of these viruses, which cause disease in humans, are also associated with neurological disorders in equidae. These animals, in turn, act as sentinels of important zoonoses contributing to epidemiological surveillance and control of these diseases. In 2018 (April-May), deaths of equidae with acute neurological signs were reported on farms located in the municipalities of Nova Venecia and Baixo Guandu, Espirito Santo state, Brazil. As clinical signs, the animals presented, in the first 24 hours, muscle tremors, dysphagia, ataxia of anterior limbs, lateral decubitus and pedaling movements. After this period, pelvic limb paralysis, loss of sensitivity on the spine and mandibular trismus were observed. The animals died within 72 hours after the onset of clinical manifestation or, in extreme cases, were submitted to euthanasia. Fragments of central nervous system and medulla cooled and fixed in formalin 10% of four equidae (2 horses and 2 donkeys) were sent for diagnosis. Microscopic lesions with presence of moderate multifocal perivascular cuffing consisting of lymphocytes and some macrophages, and also a slight infiltration of lymphocytes, neutrophils and proliferating glial cells in the neural parenchyma were observed in the caudal trunk and cerebellum of one of the animals. Rabies, equine-1 herpesvirus, and arbovirus-associated encephalomyelitis were investigated as differential diagnosis. Only West Nile virus was detected by nested RT-PCR, followed by sequencing. A higher similarity rate (99%) were detected between the Brazilian samples and WNV lineage 1 from USA (NC009942 and JF920306). Subsequently, virus isolation was performed by inoculation in chicken embryo SPF and VERO cells. In Brazil, to date, only serological surveys about WNV circulation have been performed on equine populations in several regions. However, so far, there were no reports about neurological disorders associated with WNV infection in these animals. This is the first detection of neurological disease in
horses associated with WNV infection in Brazil and it shows the need to expand the study to better understand the epidemiological and clinical aspects of this infection in horses as well as its imminent public health risk.

Financial Support: CAPES, CNPq, FAPEMIG

Palavras-chaves: West Nile, equidae, Brazil, nested, RT-PCR

DETECTION AND GENETIC CHARACTERIZATION OF JAAGSIEKTE SHEEP RETROVIRUS IN OVINE NASAL TUMOR FROM RIO GRANDE DO SUL, SOUTHERN BRAZIL

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Resumo

Jaagsiekte sheep retrovirus (JSR) belongs to the Betaretrovirus genus, subfamily Orthoretrovirinae, family Retroviridae, order Ostertivales. A variant of JSR named as ovine enzootic nasal tumor virus (ENTV) can cause neoplastic transformation of epithelial cells of the ethmoid turbinate of sheeps and goats. Very few is known about the mechanism of oncogenesis of this virus and there are probably environmental factors also interfering in the process. ENTV has been detected in Europe, Asia and North America, but there are no reports about the virus characterization Brazil. The aim of the present study was to detect and characterize ENTV in samples of ethmoidal tumor fragments in sheep. Ethmoidal tumor samples from two sheep presentig respiratory signs were used for this study. The postmortem finding was a mass in the nasal cavity compatible with ethmoidal carcinoma. The total DNA was isolated using the QIAamp DNA Mini Kit (Qiagen) following the manufacturer instructions. PCR was performed to amplify 588 bp corresponding to the 3’ long terminal repeat (3’LTR) of the JSR genome. The PCR products were purified using the PureLink™ Quick PCR Purification Kit (Invitrogen). Both DNA strands were sequenced with an ABI PRISM 3100 Genetic Analyzer using a BigDye Terminator v.3.1 cycle Sequencing Kit (Applied Biosystems). Overlapping fragments were aligned and assembled using Geneious software. The two samples were positive for JSR in the nasal tumor fragments and were closely-related to ENTV type 1. In phylogenetic reconstruction, the strains detected in the present study grouped with an ENTV type 1 reported in Scotland. The present study confirms the presence of JSR associated with enzootic nasal tumor in sheep from Southern Brazil. This data can be a basis for future studies to better characterize the strains of JSR that circulate in Brazil. Financial Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) supported this work.

Palavras-chaves: enzootic nasal tumor, ovine, PCR, retrovirus, sequencing

LABORATORY DIAGNOSIS OF RABIES IN WILDLIFE ANIMALS

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Resumo

Rabies is an acute and progressive encephalitis caused by a single-stranded RNA virus belonging to the family Rhabdoviridae, genus Lyssavirus and species Rabies lyssavirus (RABV). Considering the decrease in the number of rabies cases in dogs and cats and the maintenance of control measures against rabies in herbivores, wild animals have been highlighted as important reservoirs of this disease. Besides that, it is worth noting the increased risk to humans due to synanthropic habits acquired by wild animals. The objective of this work was to conduct a retrospective study on the occurrence of rabies in wild animals forwarded to the diagnosis of the reference laboratory of the State of São Paulo. For this, the databases of the reference laboratory were used and the samples received in the period from 1996 to 2003, the result obtained by standard direct immunofluorescence technique were used for the samples of different wild animal groups from the State of São Paulo. In this period, the total number of samples received was 10,228, and the animal group with the highest number of recorded was the Chiroptera, totaling 9932 samples (97.11%), of which 206 (2.7%) were positive for rabies. The second group was wild canids with 296 (2.89%) samples and 2 (0.68%) positive samples. The highest percentage of samples received was from Chiroptera and for both groups of wild animals evaluated, the number of positive samples for rabies was low. Considering the analyzed period and the samples evaluated, the circulation of RABV in wild animals is evident, but with low occurrence of positivity. These results highlight the importance of sending wild animals to laboratory diagnosis of rabies. Further studies should be carried out to understand the maintenance of RABV in these species.

Palavras-chave: Rabies virus, Wildlife animals, Diagnosis, Positivity, Direct immunofluorescence

Resumo

Stillbirth in commercial pig production is a multifactorial problem and often difficult to diagnose. Many risk factors have been associated, including infectious and non-infectious causes. Although these risk factors have been well documented in literature, no studies have been performed to examine the full virome in the blood of sows with stillbirths. In this study, the virome in serum of sows either with or without stillbirth events was investigated. Six commercial pig herds located in five municipalities in Southern Brazil were selected. No clinical signs suggestive of any pathology were identified in the animals. Parturition was accompanied and two groups were sampled, constituted by sows with normal parturition (H) and those which delivered at least one stillborn fetus (S). A total of 94 serum samples were pooled into 12 pools. These were filtered (0.22 µm), ultracentrifuged and the pellet treated with nucleases. Viral DNA was extracted with phenol, enriched with 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 the Genbank database using BLASTx and all assemblies were confirmed by mapping reads to contigs with Geneious software. Based on read counts, the most abundant viruses were anelloviruses, circular Rep-encoding single stranded DNA (CRESS-DNA) viruses and circoviruses in all groups. The relative number of reads corresponding to each virus was compared between sow with stillbirths and sows with no stillbirths and no significant statistical difference was found (p > 0.05). This
study allowed the recovery of 20 complete genomes of TTSuV 1b (two genomes), TTSuV 1a (ten), CRESS-DNA viruses (six) and porcine circovirus type 3 (PCV3; two). Apart from the identification of new viruses, the main conclusion of this study is that no association could be made between any particular virus or viruses and the occurrence of stillbirths. Future studies will be conducted to examine the virome in tissues of stillborn piglets.

Financial Support: CNPq, CAPES, FINEP.

**Palavras-chaves:** HTS, Metagenomic, Reproductive failure, Swine

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**Proposal of a new classification of FeLV based on deep phylogenetic analyses.**

**Autores**  
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**Resumo**

Feline leukemia virus (FeLV) is a worldwide distributed virus that causes nearly half of leukemia and lymphoma in cats. Currently, FeLV is classified in five subgroups (FeLV-A, B, C, T and E) according to interference tests, besides the endogenous FeLV (enFeLV). Most of the relevant literature on FeLV was produced before the development of modern molecular techniques and bioinformatics tools. To date, no studies on FeLV have used phylogenetic analysis or bioinformatic techniques to unravel the molecular classification of the virus. The objective of this study is to propose a molecular classification for FeLV based on nucleotide sequences. For this, all FeLV surface envelope gene sequences (n=373) from public databases were used. Only 13 sequences had information about the interference subgroup. The alignment were generated using MUSCLE and was submitted to a recombination analyses in RDP4 and SPLITSTREE. A total of 62 sequences were identified as recombinants (including FeLV-C). To perform the phylogenetic analysis, two alignments were constructed: one including all sequences and a second encompassing only the non-recombinant sequences. The phylogenies were constructed under a maximum likelihood and Bayesian approaches, incorporating the best nucleotide substitution model chosen in IQTree. The phylogenies were drawn using FigTree. Recombinant sequences were submitted to bootscanning analysis with Simplot. Phylogenies with recombinants, showed two major clades: clade 1 clustered enFeLV and FeLV-B sequences while clade 2 included FeLV-T and FeLV-A sequences. FeLV-C sequences grouped in the same clade as FeLV-T and FeLV-A. The only two sequences of FeLV-E are not similar to the others subgroups already described in the literature. In the phylogenies without recombinants, enFeLV clustered in the basal clade as expected. FeLV-A and FeLV-T are close in the phylogeny and FeLV-E is in clade 1. In conclusion, it was possible to identify three main clades. FeLV-E belong to a monophyletic clade that groups the largest number of sequence. This has not been described in the literature. Sequences identified as FeLV-A and FeLV-T grouped in the same clade 2 and finally, clade 3 is a sister group from Clade 2 and does not include any previously described subgroups. Finally, all recombinant sequences were analyzed one by one on Simplot software, showing different patterns of recombination among enFeLV with clade 1, clade 2 or clade 3.

**CAPES**

**Palavras-chaves:** Bioinformatics, Classification, FeLV, Phylogenetics, Recombination
PRESENCE OF BPV-2 DNA IN CAPRINIC SPECIES (CAPRA HIPICUS)

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Resumo

Bovine Papillomaviruses are double-stranded DNA viruses with a genome of approximately 8000 bp. There are 24 BPV types (BPV-1 to -24), which infect both the epithelium of cattle and also fluids such as blood, semen, milk and colostrum. BPV also infects close relatives to cattle, such as buffaloes, giraffes, tapirs, horses, zebras. To date, the detection of BPV in goats has not been reported in the literature. This study aimed to investigate the presence of BPV-2 in epithelial tissue and body fluid of goats. Two samples of cutaneous warts DNA and a goat milk DNA from two different animals from the Experimental Station in Agronomy Institute of Pernambuco in Sertânia city, situated in Pernambuco state, Brazil, were obtained. The presence of BPV - 2 DNA was verified by PCR with specific primers (Fw5' GTTATACCACCCAAA GAAGACCCT 3' and Rev5' CTGGTTGCAACAGCTCTTTCTC 3') widely used for the detection of BPV-2 in cattle. The amplification products were submitted to agarose gel electrophoresis and photodocumented. The BPV-2 DNA was found in a cutaneous wart sample, and a goat's milk sample, which were from the same animal. To the best of our knowledge, this is the first time that BPV- 2 has been found in goats. It is possible, as in cattle, that BPV- 2 has reached the milk of this animal via the hematogenic route. Therefore, it is possible to conclude that BPV-2 may be infecting a new host, the goat, and as in cattle, it has the capacity to be present both in epithelial tissue and also in body fluids. Further studies are needed to evaluate the implications of the presence of BPV-2 in this new host, as well as to understand the biology and epidemiology of BPV in various animal hosts and their implications.

Financial suport: CNPq

Palavras-chaves: BPV, Cutaneous warts, Goat, Milk, PCR

ISOLATION AND MOLECULAR ANALYSIS OF A RESPIRATORY BOVINE CORONAVIRUS BASED ON THE S GLYCOPEPTIDE GENE

Autores
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Resumo

Bovine Papillomaviruses are double-stranded DNA viruses with a genome of approximately 8000 bp. There are 24 BPV types (BPV-1 to -24), which infect both the epithelium of cattle and also fluids such as blood, semen, milk and colostrum. BPV also infects close relatives to cattle, such as buffaloes, giraffes, tapirs, horses, zebras. To date, the detection of BPV in goats has not been reported in the literature. This study aimed to investigate the presence of BPV-2 in epithelial tissue and body fluid of goats. Two samples of cutaneous warts DNA and a goat milk DNA from two different animals from the Experimental Station in Agronomy Institute of Pernambuco in Sertânia city, situated in Pernambuco state, Brazil, were obtained. The presence of BPV - 2 DNA was verified by PCR with specific primers (Fw5' GTTATACCACCCAAA GAAGACCCT 3' and Rev5' CTGGTTGCAACAGCTCTTTCTC 3') widely used for the detection of BPV-2 in cattle. The amplification products were submitted to agarose gel electrophoresis and photodocumented. The BPV-2 DNA was found in a cutaneous wart sample, and a goat's milk sample, which were from the same animal. To the best of our knowledge, this is the first time that BPV- 2 has been found in goats. It is possible, as in cattle, that BPV- 2 has reached the milk of this animal via the hematogenic route. Therefore, it is possible to conclude that BPV-2 may be infecting a new host, the goat, and as in cattle, it has the capacity to be present both in epithelial tissue and also in body fluids. Further studies are needed to evaluate the implications of the presence of BPV-2 in this new host, as well as to understand the biology and epidemiology of BPV in various animal hosts and their implications.

Financial suport: CNPq

Palavras-chaves: BPV, Cutaneous warts, Goat, Milk, PCR
Bovine coronavirus (BCoV) is a single-stranded, positive sense RNA genome virus, belonging to the Nidovirales family, Betacoronavirus group A genus. The spike (S) glycoprotein is the outermost component of the viral envelope and more susceptible to mutation as a result of selective immune pressures. It has specific cell receptors that contribute to the virus-host interaction. Like most RNA viruses, BCoV can mutate spontaneously during \textit{in vitro} or \textit{in vivo} passages due to the adaptation to environmental conditions. There are few comparative studies related to the S glycoprotein mutations in BCoV isolates in cell lines. This report describes the detection and isolation of a respiratory BCoV. It also compares the sequences obtained directly from the clinical sample and from the adapted isolate along the cellular passages, focusing on the S gene. In this study, it was used a nasal secretion sample from a naturally infected bovine. Human rectal tumor cells (HRT-18) were inoculated with the clinical sample. In order to evaluate the mutations induced by the replication of BCoV \textit{in vitro}, the procedure was repeated until nine cell passages were completed. Aliquots of the inoculated cell supernatant from each passage and the clinical sample were submitted to viral nucleic acid extraction and RT-PCR detection by partial amplification of the S gene. Purification and sequencing were performed afterwards. Comparing with the ancestral prototype of BCoV, Mebus strain, 33 nucleotide substitutions were identified, of which 15 resulted in nonsynonymous mutations (9 transitions and 6 transversions). Compared to the wild type isolate, only one nucleotide substitution at nt position 2428 (AAT → TAT) was identified from the 7th passage, which resulted in the Asn810 → Tyr transversion. This is the first report of BCoV isolation in cell culture associated with respiratory disease in Brazil.

**FINANCIAL SUPPORT:** CAPES

**Palavras-chaves:** bovine coronavirus, isolation, molecular analysis, spike

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**STUDY OF RABIES VIRUS (RABV) DISTRIBUTION IN CENTRAL NERVOUS SYSTEM (CNS) SAMPLES OF NATURALLY INFECTED EQUINE**

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**Resumo**

Tests for the rabies diagnosis should be rapid, highly sensitive and highly specific in obtaining the results. However, a lower sensitivity to direct fluorescent antibody (DFA) may be observed in equine central nervous system (CNS) samples when compared to those of other species. The discovery of new tools for rabies diagnosis in equine specimens may reduce the amount of false-negative results. In the present study, the CNS from 80 horses were obtained from April 2017 to April 2018 as a result of rabies surveillance operations established in the São Paulo state. The samples were tested parallel at Pasteur Institute of São Paulo's Rabies Diagnosis Section by DFA, rabies tissue culture infection test
of the 80 analyzed CNS samples from horses, 25 were positive for rabies (positivity rate = 31.25%) by at least one of the mentioned techniques. Of these positive cases, 96% (24/25) were confirmed by using the DFA test. 25 CNS samples were positive for both RT-PCR and RT-qPCR test, showing diagnostic performance test with 100% of sensitivity. Only 11 of the 25 CNS samples tested positive (44%) by RTCIT. Analyzing 161 CNS structures separately [cortex, hippocampus, cerebellum, brainstem (midbrain, pons and bulb) and cervical medulla] from 25 positive horses, the positivity rates were 72.0%, 89.4% and 89.6% for DFA, RT-PCR and RT-qPCR, respectively. About 30% CNS structures (47/161) showed rare presence of antigen, with only one to three small inclusions in the entire microscopic field searched, being classified as weak positive. Only 13% of CNS structures (21/161) were quantification cycle (Cq) >30 by the RT-qPCR, suggesting a low viral load. Midbrain showed the best viral distribution in the CNS structures by both the DFA and the RT-qPCR techniques. This fact suggests that the variability in the intensity of virus distribution in different parts of the CNS (neuroinvasive patterns) in horses may influence the detection of RABV.

Financial support: FAPESP (15/17807-0).

Palavras-chaves: Equine, Rabies, RT-qPCR, RT-PCR, Diagnostic Sensitivity

PHYLOGENETIC ANALYSIS OF BOVINE RESPIRATORY CORONAVIRUS BASED ON THE SPIKE GLYCOPROTEIN GENE

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Resumo

Bovine coronavirus (BCoV) infections are associated with enteric and respiratory diseases in cattle, such as winter dysentery (WD), neonatal diarrhea (BECoV) and respiratory disease (BRCoV). In Brazil there are few studies involving BRCoV. This report describes the phylogenetic relationships from partial sequences of the S gene of a BRCoV with other BCoV strains isolated around the world. In this study, it was used a nasal secretion sample (BOV19-NS) from a naturally infected bovine, located in a dairy herd with enteric and respiratory problems in western Paraná, Brazil. After viral nucleic acid extraction and RT-PCR detection by partial amplification of the glycoprotein S gene, purification and sequencing were performed. The obtained sequence was aligned and compared with other sequences deposited in public data bases in two steps: the first step consisted in analysing a 1447 nt fragment (nt 1258 to 2705), corresponding to the second half of the S1 gene and the S protein cleavage site. The second step consisted in analysing a 438 nt fragment (nt 1339 to 1777), comprising the polymorphic region (HVR) of the BCoV S1 subunit. The objective of this analysis was to establish the phylogenetic relationships with other BCoV isolates that could not be included in the previous analysis due to the size of the fragment. Phylogenetic trees were generated using the Maximum-Likelihood method based on the Tamura-Nei Model and bootstrapping of 1,000 replicates. No differences between enteric and respiratory isolates were observed at the genetic level. The phylogenetic trees of both fragments revealed two large clades, separating the prototype strains of BECoV and vaccine strains (clade 1).
from the field isolates of BECoV, BRCoV and WD from different locations around the world (clade 2). In the analysis of the 1447 nt fragment, clade 2 formed four subclades (2A, 2B, 2C and 2D), whereas the BOV19-NS sample was grouped in subclade 2C, together with three Brazilian BECoV isolates with 98.8% nucleotide identity. In the analysis of the 438 nt fragment, clade 2 presented three subclades (2A, 2B and 2C), instead of four previously described. Subclade 2A was composed only of Brazilian isolates, including the sample BOV19-NS, which obtained 98.4% nucleotide identity with a Brazilian BECoV isolate. In both phylogenetic trees, it was evidenced the separation of strains by geographical origin or collection period, independently of their enteric or respiratory origin.

Palavras-chaves: bovine coronavirus, phylogenetic analysis, spike

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UPS AND DOWNS IN AVIAN CORONAVIRUS EVOLUTION IN VITRO

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Resumo
Due to an imperfect proof-reading during RNA replication, Avian coronavirus AvCoV (Nidovirales: Coronaviridae: Coronavirinae: Gammacoronavirus) occurs in a quasispecies population pattern after the accumulation of mutations in its +ssRNA of almost 28kb. AvCoV naturally infects a diverse range of chicken tissues and several other bird species and can be isolated in chicken embryos, but only a few strains of the 6 genotypes and 33 lineages of the virus can grow on cell lines, being the Beaudette strain (GI-1 lineage) the most used for in vitro studies. Considering the differences between cell lines and chicken embryos as habitats for AvCoV, this study aimed to assess the diversity of the genes coding for the non-structural protein3 (nsp3) and spike envelope protein (S). To this end, embryo-adapted Beaudette strain was serially passaged in Vero and BHK-21 cells up to the 14th passage and, for each passage a. the cytopathic effect was followed, b. the virus load was measured by qPCR and c. partial nsp3 and S genes were Sanger-sequenced. In both cell lines, virus load fluctuated along the passages, with highest loads being 5,27E+08 copies/µL for Vero and 2,31E+06 copies/µL for BHK-21. No polymorphisms were found for nsp3 in any of the passages and cell lines, but, regarding the S, not only amino acids substitutions were found (Vero: 8th A150S, and 14th S150A; BHK-21: 4th S53F, 8th F53Y, and 8th S95R) but also minor variants could be detected on the chromatograms on the mutated positions in fluctuating intensities. As the regions of amino acids substitution are within the receptor binding domain of S, it can be speculated that differences in cell receptors between Vero and BHK-21 and the speed of cell death led to the selection of different dominant strain in different moments, while the stability of nsp3 agrees with its function as a protease involved in AvCoV replication. As a conclusion, AvCoV quasispecies evolution is influenced by the biological model under consideration and a “see-saw” pattern is seen for minor and major variants.

Palavras-chaves: AvCoV, cell culture, coronavirus, evolution, quasispecies
BOVINE RESPIRATORY DISEASE-RELATED VIRUSES IN DAIRY AND BEEF CATTLE HERDS OF SÃO PAULO STATE, BRAZIL

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Resumo

Bovine respiratory disease (BRD) is one of the most expensive diseases of cattle production worldwide. BRD is a multifactorial syndrome, with several predisposing factors including transportation, mixture of animals from different origins, dust, cold weather, and sudden and extreme weather changes. Viral agents, such as bovine herpesvirus-1 (BoHV-1), bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV) and parainfluenza-3 virus (PI-3) may produce a clinical syndrome consistent with BRD in the absence of bacterial co-infection, however their involvement are generally considered as antecedent to bacterial infection. This study determined the prevalence of BoHV-1, BVDV, BRSV and PI-3 in non-vaccinated dairy and beef cattle herds from São Paulo State, Brazil, and compared the frequency of positive animals for these viruses between the two types of exploitation. For that, serum samples obtained from the animals of four dairy herds and three beef herds (108 and 154, respectively) were submitted to virus neutralization tests for the viruses, following OIE protocols for BoHV-1 and BVDV, and São Paulo Biological Institute for BRSV and PI-3. BoHV-1, BRSV and PI-3 cut off points was 1:2, and BVDV cut off was 1:10. Obtained data were analyzed by Chi-Square test (P=0.05). The serological prevalence of BoHV-1, BVDV, BRSV, and PI-3 in dairy herds was 44.4%, 16.7%, 100%, and 58.3%; and in beef herds, 58.4%, 34.4%, 98.7%, and 85.7%. Chi-Square test showed no difference in the frequency of BRSV positives between the two types of cattle productions, evidencing that the occurrence of BRSV is equivalent. Nonetheless, relevant statistical differences were observed in the frequencies of BoHV-1, BVDV, and PI-3 between dairy and beef herds, suggesting that different factors may be acting for the occurrence of these viruses. As the frequency of positive animals for BoHV-1, BVDV, and PI-3 was higher in beef herds, it suggests that differences in management practices between the two types of exploitation, like biosecurity (more practiced in the dairy herds studied), may be one of the factors for the higher occurrence of these viruses in beef herds of the present study. In conclusion, antibodies against BoHV-1, BVDV, BRSV and PI-3 were seen in dairy and beef cattle herds of São Paulo State, showing the presence of the viral agents related to BRD and the necessity of prophylactic measures to prevent losses due to disease.

Financial Support: Capes, CNPq

Palavras-chaves: BoHV-1, BVDV, BRSV, PI-3, virus neutralization test

Genetic analysis of feline parvovirus reveals a high rate of viral evolution

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Resumo

Genetic analysis of feline parvovirus reveals a high rate of viral evolution.
Feline parvovirus (FPV, also known as feline panleukopenia virus) is a widespread single-stranded DNA close relative of canine parvovirus type 2. It causes a viral infection that affect cats, both domesticated and wild feline species. Despite the conservativeness of DNA viruses, with substitution rates near to their host, it was shown that canine parvovirus may exhibit a similar substitution rate to that determined for RNA viruses. In order to monitor and evaluate mutations, FPV sequences were retrieved from GenBank and analyzed (n = 209). Signatures of selection were detected using the HyPhy (Hypothesis Testing using Phylogenies) method. The rates of nucleotide substitution per site per year were estimated with a Bayesian Markov chain Monte Carlo method, performing 100 million generations through the Markov Chain Monte Carlo (MCMC) and sub-sampled each 10,000 generations. As results, the analysis of the recent field sequences revealed evolutionary hotspots mainly located in the first and second loop of the capsid subunit. A higher divergence in the capsid protein was also found in the phylogenetic analysis, with six defined clusters. By inferring the evolutionary dynamics of the FPV sequences, an evolutionary rate of $3.0607 \times 10^{-4}$ substitutions per site per year for the capsid protein gene was observed. Based on these findings, we conclude that FPV variants are continuously evolving, having a substitution rate that can be similar or even higher than canine parvovirus. Thus, the vaccines (mainly based on strains isolated in the 70s or 80s) may be redesigned according the geographic location.

Palavras-chaves: FPV, feline panleukopenia virus, substitution rate

TEMPORAL VARIATION STUDY OF THE PORCINE CIRCOVIRUS SPECIES AND GENOTYPES PREVALENCES (PCV1, PCV2a, PCV2b and PCV2d-2) IN BRAZIL FROM 2009 TO 2015

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Resumo

Porcine circoviruses (PCV) belong to the Family Circoviridae, genus Circovirus. Currently, three species are known, PCV1, that is considered non-pathogenic to pigs, PCV2, that is associated with diseases collectively referred to as PCV diseases (PCVD) and the PCV3 with unknown pathogenesis. Five PCV2 genotypes have already been described (PCV2a to PCV2e). With the adoption of vaccination for the prevention of PCVD, the concern with the emergence of variants of PCV2 and clinical cases due to vaccine failure became high, since all existing commercial vaccines are based on PCV2a. The objective of this study was to determine the temporal variation in the prevalence of PCV1, PCV2, genotypes PCV2a, PCV2b, and PCV2d-2 in blood samples previously positive for PCV by quantitative PCR (qPCR). Samples tested were analysed using age, PCV2 vaccination, farm localization in Brazil, qPCR data, prevalence and occurrence of co-infections. With qPCR data, prevalence, age, PCV2 vaccination, regions from Brazil, and occurrence of co-infections were determined to the samples tested. Thus, 160 whole blood swine samples were collected between April 2009 and December 2015. qPCRs were performed with the detection system based on probes (PCV2, PCV2a, PCV2b and PCV2d-2) or SYBR Green (PCV1). The results of qPCR were reported qualitatively as positive or negative. The vaccination against PCV was reported in 50.7% (81/160) After analysis, 3.8% (6/160) of samples were positive only for PCV1, and 3.2% (5/160) co-infected with PCV2, detected in 2009, 2010, 2012, 2013, and 2014. 96.3% (154/160) of samples were positive for PCV2, from 2009 to 2015. PCV2a genotype presented 8.13% (13/160) of positive samples in 2009, 2012, 2013, and 2015. PCV2b genotype presented 90% (144/160) of positive samples, found from 2009 to 2015. Finally, PCV2d-2 had 7.50% (12/160) of positive samples and, it was detected in 2014 and
2015. Cases of co-infection for two or more PCV2 genotypes were found in 7.8% (12/154). Therefore, these results indicate the highest prevalence of PCV2b followed by PCV2a, PCV2d-2, and PCV1. Moreover, this information may be useful for the prevention and control of PCVD in Brazil.

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Palavras-chaves: Circovirus, genotype, PCV, prevalence, quantitative PCR

MOLECULAR MODELING OF EQUINE INFECTIOUS ANEMIA VIRUS p26 PROTEIN FROM NATURALLY INFECTED HORSES IN PANTANAL, BRAZIL

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Resumo

Equine infectious anemia virus (EIAV) is a persistent lentivirus that causes equine infectious anemia (EIA) in equids. The EIAV gag polyprotein produces four proteins (Matrix p15, Capsid p26, Nucleocapsid p11, and the Late protein p9) by proteolytic cleavage; gag gene is well conserved in the different studied lineages and also due to its highly antigenicity capacity. According to OIE, AGID (agar gel immunodiffusion) is the gold standard diagnostic method to EIA, and the gag gene protein p26 is used as antigen in this test. In Brazil, EIAV is endemic in Pantanal region and euthanasia is not mandatory in this area. In fact, serologically silent equines have been observed in several regions of the world, including Pantanal. The permanency of false negative equids in controlled areas is a concern and it can increase the disease progression. This study aimed to analyze the structure of the p26 protein of the gag gene in Pantanal equines. Gag sequences were obtained from massive sequencing from two naturally infected equines (BRA1 and BRA2). Total RNA of equines plasma was used for dsDNA and library preparation with Nextera XT DNA Library Preparation (Illumina), and libraries were sequenced using NextSeq System (llumina). After a bioinformatics reconstruction genome, a sequence of 1,464 nucleotides of the complete gag gene was obtained from each sample. Brazilian p26 protein presents 194 aminoacid residues with 96.4 % identity. The comparison between a p26 crystallografic structure (PDB code 1EIA), BRA1 and BRA2 showed substitutions in the linear epitopes 73NLDKIAEE81, 199KNAMRHLRPEDTLEEKMYAC218, and 215MYACRD220 previously described. In order to verify the presence of surface substitutions and possible structural modifications in these epitopes we built BRA1 and BRA2 molecular models based on the 2.7 Å-resolution p26 crystallografic structure. Although we cannot confirm that such alterations influence on the positive or negative result in AGID, it is important to verify that the circulating virus in Pantanal demonstrates a high variation on the p26 protein.

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INFLUENCE OF SEASONAL VARIATIONS IN THE SHIPMENT OF BATS FOR DIAGNOSIS OF RABIES

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Resumo

Different species of bats have been identified as natural reservoirs of a wide variety of pathogens, mainly viruses. Among the viruses already identified in bats, the rabies virus has a highlighted importance in Public Health. Rabies is an acute and fatal encephalitis that affects the nervous system of mammals. Bats are animals with varied behavioral and eating habits and changes in these habits can be influenced by factors such as seasonality. Studies on the influence of seasonality on reproduction, on genetic metabolism and on the activity pattern in bats have been developed, but, little is known about the influence of climatic seasonality on the development of diseases. The objective of this work was to analyze the influence of seasonality on the number of bats sent for diagnosis and positivity for rabies in the period from 1996 to 2003. For this, a retrospective study was carried out, through the records in the reference laboratory database considering the total of animals sent, the positive diagnosis for rabies and the date of shipment. For the analysis of seasonality, the following months were considered: for spring - September to November; summer - December to February; autumn - March to May; and winter - June to August. Positivity was calculated in relation to the seasons of the year. To verify the existence of significant differences in the number of animals diagnosed positive between the stations, the partial chi-square test was used for comparisons in pairs. Were sent to laboratory diagnosis, 9932 bat samples and the positivity found in relation to the seasons were: spring 1.44% (51/3534); summer 1.63% (48/2946); autumn 3.18% (58/1821) and winter 3.00% (49/1631). In the analysis in pairs, only in the comparison between autumn and winter there was no significant difference. According to the results it was possible to observe that the highest frequency of positivity was in autumn, followed by winter. The greater identification of positive animals in these climatic seasons can be due to drought in this period, causing the need to search for food and consequent change of shelter. Must emphasize, however, that the seasonal analysis was performed from the date of registration of the samples in the laboratory, and may not coincide with the period in which the animal was captured. Considering the analyzed period and the sampling of this study it can be concluded that there was a seasonal variation in the number of bats sent for diagnosis and positive for rabies

Palavras-chaves: Rabies, Diagnosis, Bats
GENETIC DIVERSITY OF CANINE PARVOVIRUS CIRCULATING IN RIO DE JANEIRO (2008-2017)

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Resumo

Since its emergence, canine parvovirus (CPV) is an important agent of viral enteritis in puppies. As a consequence of CPV rapid evolution, genetic variants (CPV-2a, CPV-2b and CPV-2c) have been reported circulating worldwide. The purpose of this study was to evaluate the genetic diversity of the VP2 gene of CPV strains circulating in Rio de Janeiro during 2008-2017. A total of 15 fecal samples, collected from up to one year old dogs with diarrhea and previously screened for parvovirus DNA by PCR, were analyzed. For sequence analysis, four different primer pairs that amplify fragments encompassing the entire VP2 gene sequence (1755bp), which two specifically designed for this study, were used. All the PCR amplicons were purified and sequenced in both directions using the Big Dye Terminator® v3.1 Cycle Sequencing Kit. Nucleotide similarity with sequences deposited in the GenBank database was assessed using the BLAST tool. A maximum likelihood (ML) phylogenetic tree was constructed using MEGA v.7.0.2, based on T92 + G + I evolution model with 2000 bootstrap replicates. Analysis of the deduced amino acid (aa) residues at critical positions that determine the canine host range revealed that five sequences were CPV-2a, seven CPV-2b and three CPV-2c. Non-synonymous substitutions were found at residues 297 (Ser→Asn or Ser→Ala) and 324 (Tyr→Leu), and these sites presented with positive selection. The change Ser297Ala has already been described in CPV sequences since 1990. On the other hand, the change Ser297Asn has been recorded only in CPV-2b sequences from North Brazil and Italy so far. The phylogenetic tree revealed that the CPV (2a/2b/2c) sequences containing the 324Leu non-synonymous mutation formed an individual subgroup within the CPV clade. According to the aa changes at residues 564 (Asn→Ser) and 568 (Ala→Gly), a subdivision inside this 324Leu subgroup could be observed. The residue 324 is adjacent to residue 323, which controls the binding to the canine transferrin receptor (TfR) and is one of the main determinants of the canine host range. Given the results obtained in our current study, the continuous surveillance of parvoviruses will help to elucidate the mechanisms that drive CPV evolution in Brazil and its epidemiological importance.

Palavras-chaves: canine parvovirus, phylogenetic analysis, Rio de Janeiro

DISTRIBUTION OF SYMPTOMS OF NEUROLOGICAL DISEASE IN RIO GRANDE DO SUL (2014-2018)

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Resumo

The Official Veterinary Service (OVS) is responsible for ensuring public health, depending directly on its ability to respond promptly to disease and emergency situations. In Brazil the surveillance system says that any suspicion of the occurrence of symptoms of neurological disease should be reported to the OVS immediately. From the notification the SVO veterinarian attends the notification, taking the necessary procedures for a comprehensive epidemiological investigation, recording all the information from the care until the end of the occurrence. Thus, this work has the objective of analyzing, in a descriptive, inferential, spatial and temporal manner, the reports of diseases in bovines with suspected Nervous System syndrome,
received by the DDA/SEAPI in the period from 2014 to 2018. The data were extracted from the weekly reports of DDA/SEAPI and descriptively analyzed in the Excel Office 2010 software, classified by species and distributed by regions of the state of Rio Grande do Sul. The OVS-RS received 506 reports of suspected cases in this period, and 86.95% (n=441) of the cases involving cattle, 12.05% (n=61) horses, 0.59% sheep and 0.39% pigs. Of the total reported cases, 34.31% (n=151) of cattle and 27.86% (n=17) of horses were confirmed as Rabies. Rabies outbreaks were distributed mainly in the metropolitan region (n=77); central-east (n=29), west-central (n=19), south-east (n=17), south-west (n=9) and north-east (n=9) and north-east (n=8). An interesting finding was that, although reports of notifications were obtained in 2018, 44.46% (n=245) of the 551 reported outbreaks were considered as “unfinished”, meaning no rabies diagnosis. Two outbreaks with suspected symptoms of neurological disease were confirmed as bovine herpesvirus type 5. Reports of suspicious outbreaks of symptoms of neurological disease were more frequent in 2014 and 2015 (66 and 65 outbreaks, respectively), with a significant decrease in outbreaks in 2016 (n=55), 2017 (n=18) and 2018 (n=15). The epidemiological survey carried out showed that in the evaluated period the outbreaks were concentrated in certain regions of the state of Rio Grande do Sul. Moreover, if we consider that the information is in the public domain, it is worrying that in 2018 more than 40% of cases are pending diagnostic confirmation in official records.

Palavras-chaves: Rabies, epidemiology, diagnostic, Cattle, outbreak

EPIZOOTIC HEMORRHAGIC DISEASE VIRUS AND BLUETONGUE VIRUS CO-CIRCULATION AND CO-INFECTION IN DEER OF CAPTIVITY IN BRAZIL

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Resumo

Hemorrhagic disease (HD) in deer is mainly caused by the related orbivirus: Bluetongue virus (BTV) and Epizootic haemorrhagic disease virus (EHDV). These viruses are transmitted by biting midges from the genus Culicoides and cause indistinguishable clinical disease, generally fatal for deer species. Hemorrhagic disorders are among the most common diseases of cervids raised in captivity in Brazil. Seasonally, several populations have been affected by this condition, reaching approximately 40% of the herd. Bela Vista Biological Refuge, located in Parana state, houses about 171 animal species, among them, Brazilian endangered deer species, such as brocket deer (Mazama nana) and mash deer (Blastocerus dichotomus). At the refuge, in the last two years, from late summer to late autumn, outbreaks of acute HD affecting brocket deer were reported, in which multiple BTV serotypes (BTV-3, BTV-14, BTV-18, BTV-19 and BTV-22) were identified as aetiological agents. From April to May 2017, other outbreak of HD occurred in the refuge. Seven brocket deer and two mash deer died, and at the necropsy presented characterized lesions of HD. Fragments of spleen, lung, heart, liver, kidney, bladder and testis were collected for virologic diagnosis. Real time RT-PCR targeting Seg-9 and Seg-10 of EHDV and BTV, respectively, were performed. Positive samples were submitted to RT-PCR targeting Seg-2, followed by sequencing to identify the virus serotypes. Virus isolation were performed in all tissue samples. Six deer were positive for EHDV. So far, serotype EHDV-1 was detected in two samples and EHDV-2 in one. Two brocket deer were positive for BTV, of which BTV-24 was identified in one of them. Viruses were isolated from all the tissue samples, indicating the systemic virus
First report of PCV3 in Colombia and its classification

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Resumo

Circoviruses are single-stranded DNA viruses classified into two types (PCV1 and PCV2). In 2017 the ICTV included a new type called PCV3. This virus has been found in asymptomatic and in pigs with clinical symptoms similar to those caused by PCV2. In America, there are reports of the presence of PCV3 in the USA and Brazil. Analysis of complete genomes of PCV3 has led to propose a classification in two major groups named a and b, in turn each of these groups is subdivided into subgroups a1, a2, b1 and b2. In early 2018, clinical cases associated with PCV2 were reported in pigs from farms located in the center of Colombia. Samples from two farms were evaluated for PCV3 using specific primers. The full-length genome of two samples positives to PCV3 was amplified using four sets of specific sequencing primers and were deposited at GenBank under the accession numbers MH327784 and MH327785. PCV3 genomes from this study were aligned with all complete genome of PCV-3 strains obtained from GenBank to date. The analysis involved 107 nucleotide sequences and evolutionary analyses were conducted in MEGA7. Both sequences were named, respectively, as COL1 and COL2. The sequence identity between Colombian sequences was 99.60% and showed a range of identity between 99.6% - 99.9% compared with isolates from South Korea, China, Brazil and Italy. PCV3 intra-specific classification is based in a motifs pattern on codon 122 of ORF1 and codons 24, 27, 77 and 150 of ORF2. The AVKSI motif defines the group “a” while the SARSI motif defines the group “b”. The COL1 strain has a SAKSI motif, which together with the strains with the SVKSI motif constitute the subgroup “a2”. On the other hand, COL2 strain has the motif AVKSI corresponding to subgroup “a1”. Interestingly, COL2 strain also has a change in codon 19 of ORF1, this change is only associated with isolates from South Korea. On the other hand, COL1 strain can be located within the strains that evolutionarily give transit (linker) between group “a” and group “b”. The results could indicate the probable presence of the at least two subgroups of PCV3 in Colombia or the presence of strains of group “a” in an evolutionary change. This is the first report of PCV3 in Colombia.

Financial support: Universidad Nacional de Colombia and resources of the researchers.

Palavras-chaves: PCV3, Circovirus, DNA virus

EVIDENCE OF INTRAGENOTYPIC RECOMBINATION AMONG PORCINE STRAINS OF HEPATITIS E VIRUS

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Hepatitis E virus (HEV) single-stranded RNA virus that is classified into the family *Hepeviridae*, *Orthohepevirus A* species and comprises of 7 genotypes and 27 subtypes. HEV genotypes 3 and 4 are considered zoonotic. HEV is an emerging pathogen that can be transmitted through contaminated undercooked meat derived from domestic pigs. HEV infections have been documented among pig herds and environmental samples raising concern about the spread of the virus. In this study, investigate the presence of HEV among pigs in an abattoir in Minas Gerais and attempted to identify its genotypes. Bile samples were collected from 335 healthy pigs in an abattoir in the city of Santos Dumont, MG, and tested for HEV by real-time RT-PCR. For genotyping, positive samples were amplified by conventional RT-PCR targeting the ORFs 1 and 2. Amplified cDNA was sequenced, and phylogenetic analysis was carried out. Fifty-one samples (15.2%) were positive for HEV. Sequence analyses of the ORF1 showed 88.3% to 89.5% nucleotide identity with the HEV genotype 3c. However, analyses of ORF2 sequences showed 89.4% to 90.8% nucleotide identity with the HEV genotype 3i. Pairwise nucleotide distances of ORFs 1 and 2 sequences between our strains and the genotype 3c reference strains ranged from 0.105 to 0.117 and 0.118 to 0.125, respectively. Distances of ORFs 1 and 2 sequences between our strains and the genotype 3i reference strains ranged from 0.132 to 0.135 and 0.092 to 0.102, respectively. Thus, the subtype of the strains could not assign. Discordant phylogenetic relationships were also described for human HEV strains, consistent with the occurrence of intragenotypic recombination. Therefore, it is possible that the strains detected in our study are products of intragenotypic recombination. It has been suggested that the overall distribution of different HEV genotypes is due to the fact that different clades of the virus interact with different hosts, which could impose differential selective pressures on the pathogen as part of host-pathogen interactions. Moreover, the analysis of mutations in the viral genome suggests that HEV is under substantial evolutionary pressure to develop mutations enabling evasion of the host immune response. Thus, the occurrence of recombination between strains belonging to different clades could favor the process of evolution and adaptation of these viruses to different hosts or different environmental conditions. **Financial Support:** CNPq, CAPES and FAPERJ.

**Palavras-chaves:** Hepatitis E virus, Intragenotypic Recombination, Minas Gerais, Swines

**INVESTIGATION OF VACCINIA VIRUS IN "PINGO" SAMPLES USED IN THE PRODUCTION OF MINAS ARTISANAL CHEESE OF SERRA DA CANASTRA, MINAS GERAIS, BRAZIL.**

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**Resumo**

Pingo is the endogenous microbiological culture used in the manufacture of Minas Artisanal Cheeses (MAC), and has a diversity of microorganisms responsible for the fermentation and maturation of the
cheeses. However, pathogens may be present in “pingo”, since MAC are produced from raw milk. Vaccinia virus (VACV), the etiological agent of bovine vaccinia (BV), can be eliminated through milk, including from cows with subclinical infection. Viable VACV particles have already been detected in the “pingo” used in the production of cheeses made from the milk of experimentally infected animals. Another study showed that the consumption of raw milk and MAC are considered risk factors for VACV transmission, since anti-OPXV neutralizing antibodies were detected in people who had no contact with diseased cattle. It is a common fact in the Serra da Canastra region sharing of the “pingo” among properties of the region. Therefore, the “pingo” could be a possible route of VACV spread among farms. Considering “pingo” as a potential source of milk-borne pathogens, including VACV, this study aimed to investigate the presence of VACV in samples of pingo from MAC producing properties of Serra da Canastra, Minas Gerais, Brazil. Eight samples were collected from eight farms from the region, aliquoted and stored at -20°C. The extraction of the viral DNA was done with a commercial kit and, later, the qPCR technique for amplification of the HA gene was performed. The eight samples of “pingo” analyzed showed a negative result for VACV. More “pingo” samples should be evaluated to understand the role of “pingo” as a potential source of VACV spread among farms, including from other MAC producing areas in MG. The microbiological safety of food is extremely important for both industry and the consumer, given that dairy products are important sources of foodborne diseases.

FINANCIAL SUPPORT: FAPEMIG, CNPq, CAPES, PRPq-UFMG

Palavras-chaves: Cheese, Foodborne, Milk, Vaccinia virus

MULTIPLEX RT-PCR ASSAY FOR SUBTYING OF SWINE INFLUENZA VIRUS IN BRAZIL

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Resumo

Influenza is an acute respiratory disease of pigs caused by infection with influenza A virus (IAV). The most prevalent IAV subtypes in pigs are H1N1, H1N2 and H3N2. However, genetically distinct viruses circulate in different geographic regions. For this reason, a diagnostic test that is able to identify the viral subtype circulating in swine is important for the monitoring and control of the infection. In addition, such an IAV subtyping assay is not commercially available in Brazil. This study describes the development and validation of a multiplex RT-PCR assay for subtyping of swine IAVs. The assay was performed in two distinct reactions, one was focused on the hemagglutinin (HA) gene (H1 of pandemic origin, H1 of human origin and H3) and the other reaction was focused on the neuraminidase gene (N1 of pandemic origin, N1 of human origin and N2). For the standardization of the assay, reference IAV strains (H1N1, H1N2 and H3N2) were tested against each pair of primers, and also in a 10-fold serial dilutions. Cross-reactivity was not observed in the assay and the limit of detection for the three viral subtypes, in HA and NA reactions, was of 1.9ng/µL of cDNA. The analytical sensitivity of the test was assessed based on 65 IAV isolates, previously characterized by genetic sequencing, resulting in 100% of detection of the correct viral subtype. The assay presented 100% of analytical specificity in the testing of 65 samples considered as negative for IAV, and positive for other viral and bacterial respiratory pathogens. For the diagnostic evaluation, the multiplex RT-PCR was carried out on 77 IAV positive samples collected from pigs between 2010 and 2016. Finally, the RT-PCR assay was able to identify the viral subtype in 74.02% of the samples and the most prevalent subtype was H1N1 (64.9%), followed by H1N2 (29.8%) and H3N2 (5.3%). Furthermore, the test was able to detect mixed viral infections in five samples and reassortant viruses even before performing genomic sequencing. In conclusion, the multiplex RT-PCR assay designed in this study
showed to be a sensitive and specific method for the identification of IAV subtypes in samples collected from pigs. The technique described is cost-effective when compared to other methods such as sequencing, and once implemented in diagnostic laboratories will provide more information on the prevalence of IAV subtypes in swine herds.

Financial support: Embrapa (02.16.05.004.00.03).

**Palavras-chaves:** Diagnosis, Influenza, Multiplex RT-PCR, Subtyping, Swine

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**DIVERSIFICATION OF THE BOVINE PAPILLOMAVIRUS TYPES RELATED TO TEATS WARTS**

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**Resumo**

Papillomaviruses (PVs) are small viruses that comprise a highly diverse group that can produce epithelial proliferative lesions in all amniotes. Bovine papillomavirus (BPV) is recognized as the etiological agent associated with several forms of benign tumors, among them the teat papillomatosis that results in lower profits for the milk industry. Currently, 24 BPV types have been reported, in contrast to more than 200 types of the human papillomavirus (HPVs). The BPVs are assigned in four genera (*Deltapapillomavirus*, *Epsilonpapillomavirus*, *Dyoxipapillomavirus* and *Dyokappapapillomavirus*) whereas the types BPV19 and 21 have not been allocated within any genus. The present study aimed to test 50 cattle teat papillomatosis by histopathology and conventional polymerase chain reaction (PCR) carried out by using the primer pair FAP59 and FAP64 followed by DNA sequencing. Thirty-three out the 50 samples were classified as classical BPV types whereas 17 as putative new types by phylogenetic analysis. These findings add to the expanding genetic diversity of BPV, as also evidencing the possibility of other BPVs has been detected in teats warts besides classical types. Knowledge of the prevalence and of the variety of BPVs is a milestone for the development of appropriate prophylactic and therapeutic measures. Financial Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) supported this work.

**Palavras-chaves:** Cattle, Papillomavirus, PCR, Sequencing, Teat lesion

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**IDENTIFICATION OF SWINE INFLUENZA VIRUS SUBTYPES FROM 2012 TO 2015 AND 2017 TO 2018 IN THE SOUTH AND SOUTHEAST OF BRAZIL**

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Swine Influenza A virus (SIAV) causes an acute respiratory disease in swine and other species, including humans. Currently, three subtypes of SIAV circulate worldwide in the swine population: H1N1, H1N2 and H3N2. SIAV has been threatening the Brazilian pork production since the introduction of pandemic H1N1 in 2009 influenza virus in humans, when several outbreaks begin to be described in swine farms. Although this subtype is currently endemic in the country, serological studies performed after 2009 showed the presence of H1N2 and H3N2 subtypes as well, but with low prevalence. The aim of the study was to detect subtypes of SIAV that are circulating in the South and Southeast of Brazil, main poles of Brazilian pork producing chain, from 2012 to 2018. Seventy-seven lung tissues and nasal swabs from swine that presented respiratory disease, previously positive for influenza matrix gene detection, were sent for subtyping by RT-PCR. In total, 38 out of 77 (49%) were subtyped, being 24/60 (40%) samples collected between 2012 to 2015 and 14/17 (82%) between 2017 to 2018. When analyzing the subtyped samples between 2012 and 2015, 20/24 (83.3%) were positive only for H1N1pdm2009, followed by 3/24 (12.5%) positive for H1N2 and 1/24 (4.2%) positive for H3N2. No co-infection was detected in this period. In subtyped samples between 2017-2018, the results showed that 5/14 (35.7%) were positive only for H3N2, followed by 2/14 (14.3%) only for H1N1pdm2009 and 3/14 (21.4%) only for H1N2. In 2/24 (14.3%) samples were detected co-infections with the three subtypes, followed by 1/14 (7.1%) positive for H1N1pdm2009 + H1N2 and H3N2 + H1N2 co-infection. Co-infection between H1N1pdm2009 + H3N2 was not observed. According to WHO, a higher prevalence of H1N1pdm2009, in the Brazilian human population was observed between 2009-2013. After 2013, a prevalence oscillation between H1N1pdm2009 and H3N2 was observed annually, with a higher prevalence of H3N2 in 2017. After this change in H3N2 prevalence was also observed in the swine population, suggesting a human transmission for pigs. Multiple introductions of human H1N1pdm09 to pigs occurred in Brazil, since 2009, but the transmission of H3N2 from humans to pigs was once detected in mild 1990s. This study suggests a new introduction of H3N2 in pigs transmitted from humans mainly from 2017-2018, and a further genetic characterization of the strain is important to confirm this hypothesis. Financial support: CNPq, FAPEMIG, ZOETIS, IPEVE.

Palavras-chaves: Diagnostics, Influenza, Subtyping, Swine, Molecular characterization

DETECTION AND GENETIC CHARACTERIZATION OF PORCINE CIRCOVIRUS 3 IN BRAZIL

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Porcine circovirus 3 (PCV3) is the new member of the family Circoviridae, discover in 2016 in pigs in the United States of America. Until recently, only two species of circovirus were known to infect pigs: Porcine circovirus 1 (PCV1) was first identified as cell culture contaminante and it is considered nonpathogenic; and Porcine circovirus 2 (PCV2) a globally...
important pathogen which is associated with multiple diseases that cause significant economic losses. Since PCV3 was discovered, new cases of PCV3 have been reported in several countries around the world. PCV3 has been associated with porcine dermatitis, nephropathy syndrome, reproductive failure and multi-systemic inflammation. The objective of this study was to verify the presence of PCV3 in pig tissue samples in Brazil. We selected 67 pig tissue samples collected in 2006 and 2007 from nine Brazilian states. These samples were used in PCR detection for PCV3. Our results showed that PCV3 was detected in 47.8% (32/67) of the samples and 37.5% (12/32) of the PCV3 positive samples were co-infected with PCV2. Among the 32 PCV3 positive samples there were positive samples from all the nine states. Interestingly, clinical signs were only noticed in co-infected samples. No clinical signs were noticed in animals positive only to PCV3 infection. These results agree with other researchers that found PCV3 in animals with different clinical manifestation of diseases. Since we identify PCV3 in Brazilian samples previously collected, in the first semester of 2018 we collected some samples of nine animals from a farm with health problems located in Minas Gerais, Brazil. PCR detection with PCV3 primers was performed and 11.1% (1/9) of the animals was identified as PCV3 positive, and PCV3 was detected in lung and lymph nodes samples. Seven PCV3 positive samples were sent to be partial sequenced using Sanger method and they showed 95.56 to 99.81% of identity with the cap protein of other 170 isolates of PCV3 deposited in GenBank. In conclusion, this work demonstrated the presence of PCV3 in pig tissue samples since 2006 in nine Brazilian states, these states have the largest pig production and breeding, suggesting that PCV3 commonly circulates in Brazilian swine for quite some time.

Financial Support: CNPq, CAPES and FAPEMIG.

Palavras-chaves: Detection, PCV3, Porcine circovirus, PCR, Swine

EXPERIMENTAL PATHOGENESIS OF CAPRINE ALPHAHERPESVIRUS 1 IN KIDS

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Resumo

Caprine alphaherpesvirus 1 (CpHV-1) is a herpesvirus of goats genetically and antigenically related to Bovine alphaherpesviruses 1 and 5 (BoHV-1 e BoHV-5). In young goats, CpHV-1 is associated with respiratory and gastroenteric signs; in adults it produces either subclinical infection or reproductive failure. The biological and molecular properties make CpHV-1 an attractive platform for vectorial viral vaccines. However, very little is known about its biology and pathogenesis in the natural hosts. Thus, this study aimed at investigating the biology and pathogenesis of CpHV-1 in seronegative kids. Seven kids, approximately 6-months-old, were inoculated by the intranasal route (IN) with 2x10^7 TCID50 of CpHV-1 isolate WI-1346 and monitored thereafter in virological, clinical and serological aspects. All six inoculated kids presented mucous to mucopurulent nasal secretion from day 3 to 12 pi, in addition to ocular secretions on days 4 and 9 pi. In addition, the inoculated animals developed moderate respiratory difficulty (hard and noisy breathing) from day 5 to 8 pi. Infectious virus was detected in nasal swabs from all animals from day 1 to 10 pi. Inoculated animals seroconverted to CpHV-1, presenting virus-neutralizing (VN) antibodies in titers from 32 to 256 at day 37 pi. Beginning at day 37 pi, inoculated animals were submitted to five daily administrations of dexamethasone (Dex, 0.4
mg/kg/dia) and monitored thereafter as described for acute infection. No clinical signs or infectious virus in nasal secretions were detected in inoculated animals after Dex treatment. However, PCR in nasal secretions was able to detect viral DNA in some animals during a few days after Dex treatment, indicating virus reactivation. Detection of viral DNA – and not infectious virus – in nasal secretions upon Dex treatment was probably due to virus neutralization by VN antibodies present in secretions. Increase in VN titers upon Dex treatment was not a consistent finding, probably due to the pre-existing high titers of VN antibodies. Taken together these results showed that CpHV-1 isolate WI-1346 replicates efficiently in kids after IN inoculation, produces mild respiratory signs and induce a strong VN response. Reactivation of latent infection was not followed by shedding of infectious virus probably due to virus neutralization by VN antibodies. These results are important towards knowledge of CpHV-1 biology and pathogenesis for its potential use as a vaccine vector.

Palavras-chaves: biology, Bovine alphaherpesviruses, Caprine alphaherpesvirus, CpHV-1, pathogenesis

SEQUENCE ANALYSIS OF THE MAJOR CAPSID PROTEINS VP1 AND VP2 THE CIRCULATION OF NEW VARIANTS OF CANINE PARVOVIRUS TYPE 2 IN DOGS FROM RIO GRANDE DO SUL, BRAZIL

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Resumo

Canine parvovirus 2 (CPV-2) is a major viral agent of severe and often hemorrhagic gastroenteritis in dogs, frequently associated with high mortality. New viral variants have frequently arisen and have been associated with diverse, unusual clinico-pathological findings, as well as occurrence of clinical disease in adults and vaccinated dogs. Thus, the objective of this study was to perform a genetic/molecular characterization of CPV-2 circulating in Rio Grande do Sul (RS) state, southern Brazil. For this, fecal and/or intestinal segments of 30 cases of clinically diagnosed parvovirosis in dogs in central and metropolitan RS area were submitted to PCR nucleotide amplification of the major capsid protein genes (VP1 and VP2). The amplicons were submitted to nucleotide sequencing and phylogenetic analysis, and the respective amino acid sequences were compared to reference sequences deposited in GenBank to identify molecular markers of viral variants. Sequence analysis identified 15 CPV-2c and five CPV-2b; nine samples were identified as a new CPV-2a variant (“New CPV-2a”) and one sample resembled the original CPV-2, designated CPV-2-like. The variants were classified as CPV2a, 2b and 2c according to the presence of amino acids Asn, Asp and Glu at residue 426 of VP-2. Viral genomes corresponding to “New CPV-2a” also presented a Ser to Ala mutation at position 297. These genomes also harbored three mutations corresponding to selection pressure of CPV and development of clinical disease in vaccinated dogs (Phe267Tyr, Tyr324Ile and Thr440Ala). One of the samples classified as CPV-2b (SV190/17) presented an alanine (Ala) at position 297, a molecular signature of the new variants (New CPV-2a / 2b). In addition to the identification of New CPV-2a in Brazil, a new variant characterized as CPV-2-like (SV726/15) was identified according to amino acids at VP2 residues 87, 101, 305, 306 and 426. This sequence resembles the original CPV-2 strains, indicating a possible CPV-2 reemergence and/or evolution from vaccine strains. Phylogenetic analyses grouped the variants with the respective reference strains, according to amino acid changes. Taken together, these results demonstrate the high molecular diversity of CPV circulating in the RS dog population and the emergence of new variants (2c, “New CPV-2a” and CPV-2-like) that differ markedly from current vaccine strains.

Financial Support: Conselho Nacional de Pesquisa (CNPq).
A NOVEL CHAPPARVOVIRUS FOUND IN WILD ANIMALS’ FECES OF BRAZILIAN CERRADO BIOME BY NEXT GENERATION SEQUENCING (NGS)

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Resumo

Chapparvovirus is a new proposed virus genus from the Paroviridae family. These viruses have non-enveloped particles with a linear ssDNA genome and involved in many clinical and subclinical animal infections. Chapparvoviruses exhibit a wide host range, infecting birds, rodents, bats and swines, and have been identified by metagenomics analyses in feces as well as integrated sequence in mammalian and avian genomes. The Cerrado fauna shows great biodiversity and, therefore, has potential virus reservoirs that can have epidemiologic importance. However, the wild animal virome of this fauna is little explored. Therefore, NGS provides a robust tool for the identification of unknown virus species and was applied in the present study. Feces samples of Cerrado animals (Aratinga sp., Didelphis aurita, Sapajus sp. and Galictis cuja) were collected from the Veterinary Hospital, University of Brasilia. Extracted DNA/RNA samples from these animals were sequenced using Illumina HiSeq 2500 with 100 paired-end. The sequence analysis enabled the identification of a novel parvovirus. This new virus showed closer sequence identity to Desmodus rotundus parvovirus, a member of Chapparvovirus genus, with 35.2% NS1 amino acid sequence identity. This protein is used as demarcation criteria for genus and species in Paroviridae family, with 30% identity as threshold to novel genus determination. The new virus genome has 4434 nt in size and the gene organization was in accordance to other chapparvoviruses. The 5′- and 3′-ends showed palindromic sequences that are responsible for the folding of the parvoviruses’ terminal hairpins, essential to self-priming for replication. Two main ORFs were identified (NS1 and VP1), occurring a 62 nt overlap between them, that is the biggest one observed in this group or in any parvovirus genus of vertebrate hosts. The parvoviruses’ conserved motifs (GPXNTGKS) and (HVH) were found in the genome. Similar to other chapparvoviruses, this new virus lacks the phospholipase A2 motif, recognized for playing a role in release of virus particle from endosomes in Paroviridae. The phylogenetic trees of NS1 protein and virus genome sequences sustain with high bootstrap values showing that this genus is monophyletic and closer to Densovirinae, a group of viruses that infect arthropods. These different features need to be further explored to understand the importance of the chapparvovirus in Cerrado fauna.

Palavras-chaves: parvovirus, metagenomics, NS1, VP1, ssDNA virus

CANINE DISTEMPER DIAGNOSIS BY RT-PCR

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INTRODUCTION: Canine Distemper is a multi-systemic infectious disease that affects domestic dogs and others carnivores. It belongs to the *Paramyxoviridae* family, *Morbillivirus* genus, *Canine morbillivirus* species and has worldwide distribution. Immunocompromised dogs present viral spread to various tissues including central nervous system in which results in a progressive multifocal demyelinating disease. The Reverse Transcription Polymerase Chain Reaction (RT-PCR) is a tool that has been used for diagnosis of canine distemper. MATERIAL AND METHODS: The study was made in the Veterinary Teaching Hospital, Universidade Federal Rural da Amazônia, Belém, Pará, Brazil, 32 dogs of mixed breeds were selected; among them, 12,5% (4/32) were females and 87,5% (28/32) were males with an age range between 0 to 17 years old. All of them had central nervous system symptomatology and no vaccination protocol. Blood and urine samples were collected for the RT-PCR exam. For the RNA extraction, Trizol® Reagent (Invitrogen -Life Technologies) was used, according to Chomczynski-Sacchi (1987). In the Reverse Transcription (RT), the primer pair utilized was CDV_Rev1 (5´CCC ATG GAG TTT TCA AGT TC 3´/ IDTDNA®) and CDV_For1 (5´- TCC CAA GCA TCA ACT CTG -3´/ IDTDNA®). The RT-PCR was made in an automatic thermocycler (CFX96 Touch™). The products obtained by the process were analysed by electrophoresis in a 1% agarose gel and examined comparatively with DNA markers. The positive samples had 730pb fragments of DNA after the PCR reaction. RESULTS: Among the 32 samples, 59,3% (19/32) were positive for the canine distemper virus (CDV) by the RT-PCR exam. 50% (2/4) of the females and 60,7% (17/28) the males were positive to CDV. 5 animals had an age range until 1 year old, from these, 40% (2/5) were positive. As for the dogs between 1 to 2 years old, 60% (6/10) were positive. In the age group of 3 to 8 years old, 91,66% (11/12) of the animals had their samples positive to the virus. All of the dogs in the age range of 9 to 17 years old (5/5) were negative. CONCLUSION: The canine distemper virus has no predisposition by sex and the clinical signs are related to virulence factors, viral strain, age and immune response. The RT-PCR is fast and effective because of its high diagnostic specificity. Financial support: Rural Federal University of the Amazon.

Palavras-chaves: diagnosis, infection, molecular, neuron, protocol

NEW CIRCULAR REP-ENCODING SINGLE STRANDED (CRESS) DNA VIRUSES IN COLOSTRUM OF SOWS

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Resumo

Transmission of viruses through colostrum have been scarcely investigated in sows. In this study, the first identification of circular rep-encoding single stranded (CRESS) DNA viruses in colostrum of sows is reported. Samples were collected from fifteen sows from a farm located in Southern Brazil. All
samples were tested by conventional PCR for citomegalovirus (CMV), TTSuV1, TTSuV2 and porcine circovirus type 2 (PCV2); with negative results. The colostrum samples were pooled, filtered (0.22 µm), ultracentrifuged and treated with nucleases. Viral DNA was extracted with phenol-chloroform, enriched by random amplification with Φ29 DNA polymerase and subjected to high throughput sequencing (HTS). Paired-end reads were trimmed and de novo assembled using metaSPAdes genome assembler. The retrieved contigs were compared with sequences from GenBank using BLASTx. All assemblies were confirmed by mapping reads to contigs with Geneious software. Phylogenetic analyses were performed in PhyML by maximum likelihood (ML), incorporating the best-fitted amino acid substitution model (VT + G + I + F), as determined in PhyML by AIC. The approximate likelihood ratio test (aLRT) based on Shimodaira-Hasegawa-like procedure was used to assess confidence in topology. Four complete genomes of CRESS-DNA viruses were recovered. The genomes contain two open reading frames, arranged in opposite directions and encoding two putative proteins: the replication-associated protein (Rep) and the capsid protein (Cap). The genomes vary in length between 1,832 and 2,169 nt. Three different groups of CRESS-DNA genomes were detected based on phylogenetic analysis: Gemycircularvirus and two unclassified groups. To date, CRESS-DNA viruses had been detected only in feces and serum of pigs. This is the first report on the identification of CRESS-DNA viral genomes in colostrum of sows, suggesting that suckling may be a source of infection for piglets. Financial Support: CNPq, CAPES, FINEP.

Palavras-chaves: colostrum, CRESS, sows
Syringe in the region of the cleft cranial. After the inoculation procedure, the animals were conditioned under the incandescent lamp, for evaluation until reestablishment of the physiological parameters. Three days after infection, the animals were euthanized and had their brains collected for analysis. To verify the cerebral infection, the RT-PCR technique was performed, where it was possible to prove the efficacy of the described procedure. It is inferred that the use of the intracerebral infection technique in animal models in research on neuropathogenesis is an effective methodology and allows the conduction of in vivo studies with CDV.

Palavras-chaves: CDV, Animal model, Swiss mice, Neuropathological studies

SEROLOGICAL INVESTIGATION OF MAEDI-VISNA VIRUS (MVV) IN SHEEP HERDS ON RIO GRANDE DO SUL, BRAZIL

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Resumo
Maedi-Visna is a chronic disease of slow and progressive evolution that usually affects sheep and eventually goats. It is caused by virus of the genus Lentivirus belonging to the Family Retroviridae. The main feature of the disease is the long incubation period. The infected animal becomes a carrier and source of contamination for the herd, but clinical signs will appear only months or years later. The infected animals can manifest respiratory, mammary, neurological and arthritic changes. The introduction of the disease into the herd usually occurs through the acquisition of infected and asymptomatic animals. It is important to emphasize the importance of viral transmission by aerosol aspiration of secretions or mucosal contact of intensively bred animals. The control and diagnostic of the disease is carried out through laboratory monitoring in the herds, care in the introduction of new animals and places of origin. In the case of seropositive animals, they should be isolated from the herd. The objective of this work was to perform a serological investigation for Maedi-Visna Virus in herds of sheep from Rio Grande do Sul. Between the period 2017 and 2018, samples of sheep were sent for analysis of ovine epididymitis. A total of 239 samples of 30 herds were from different municipalities of state, were selected with a clear serum and with 2ml volume. The Agar Gel Radial Immunodiffusion Test (IDGA), using the commercial kit, performed the detection of anti-MVV-antibodies. Serum capable of forming visible precipitation line after 48 hours between antigen was identified as positive, in addition to presenting a positive control serum precipitation line. Serum from a naturally infected goat was also used as an additional positive control. The total of samples were negative indicating low frequency and absence of virus occurrence in herds of sheep in the state. Yet, it is important to consider that state, sheep have wool and do not relate to goats. The serological investigation should follow proportions of older animals, a possible identification of seropositive adult animals will evidence the viral circulation in sheep in RS and may help in the elaboration of public policies for the control and prevention of the disease. However, the identification of mature seronegative animals will indicate free herds of MVV, suggesting a very desirable health situation.

Financial support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

Palavras-chaves: Lentivirus, Sheeps, Immunodiffusion Test, Maedi-Visna Virus
DETECTION OF CIRCOVIRUSES IN PSITTACINES FROM BREEDING FARMS IN THE STATE OF SÃO PAULO

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Resumo
Psittacine beak and feather disease (PBFD) is caused by the Psittacine Beak and Feather Disease Virus (PBFDV), a small, non-enveloped, icosahedral virus with single strain DNA, classified as a member of the Circoviridae family, causing very characteristic clinical signs as symmetrical feather dystrophy and deformity of the beak growth. Transmission occurs horizontally and vertically, and birds infected with the virus often die due to secondary infections as it causes immunosuppression as a consequence of Fabricius bursa and thymus lesions, as well as liver damage. The disease is distributed worldwide and is a serious problem for both wild and captive birds. Treatment of PBFD basically consists of supportive therapy, cleaning and disinfection of the environment to avoid secondary infections. There is no cure for the disease and animals must be kept apart from health animals. This report aims to describe the detection of circovirus in 80 samples of psittacines from breeding farms in the state of São Paulo, which showed circovirus symptoms, as well as highlight the importance of prevention of the disease in order to avoid the spread of the virus throughout other farms as well as its spread to wild animals. Samples of feces and feathers were collected in Lysis Buffer and kept in the fridge until sent to the Laboratory of molecular biology and clinical virology of the Department of microbiology of the Institute of Biomedical Sciences of the University of São Paulo. For detection, a Polymerase Chain Reaction (PCR) was performed according to Katoh et al. (2008) protocol. From which 7 samples were tested positive and were sequenced through Sanger’s technique using Big Dye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and ABI PRISM 3130XL DNA Sequencer (Applied Biosystems). Sequencing was similar to PBFDV found in a Cacatua galerita Sample from Italy in 2010. Therefore, the importance of virus control in ornamental bird breeding is emphasized, for example, quarantine in new birds and the establishment of biosecurity rules to those farms due to the high spreading potential of the infection.

Financial support: CAPES/ Newton Foundation Birth Consul - Process Nº. 99999.005126/20015-00

Palavras-chaves: CIRCOVIRUSES, diagnosis, Disease, PSITTACINES, virology

Detection and genetic characterization of bovine Pestivirus between 2016 and 2018

Autores
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Resumo

Pestivirus A and B (BVDV-1 and 2) are worldwide distributed ruminant viruses belonging to the genus Pestivirus within the family Flaviviridae. These viruses can cause significant economic losses due to decrease on productivity in beef and dairy cattle. Although most infections are not apparent, the main clinical manifestations in symptomatic animals are gastroenteric, respiratory and reproductive signs. When infecting pregnant cows, the virus can cross the placenta and generate persistently infected (PI) calves. These PI calves excrete great amounts of virus through their lives and become the main source of BVDV infection to the herd, and the identification of these animals is an important issue in control programs. In order to collaborate with BVDV data in Rio Grande do Sul State (RS), we identified the species and subtypes of BVDV strains collected in the state, which were sent to the Veterinary Virology Laboratory of UFRGS during the years of 2016 to 2018. These samples, most of them serum or lymphoid organs, were collected by farmers or by UFRGS Veterinary Pathology Service from cattler of several regions of RS. Total RNA was extracted using TRIzol® LS Reagent (Life Technologies) according to the manufacturer's instructions and cDNA synthesis was performed using a GoScript™ Reverse Transciptase Kit (Promega Corporation). PCR was performed with 324F/326R primers described in literature, which amplify a 288 bp amplicon from the 5'UTR, and positive samples were sequenced through Sanger method. Phylogenetic analysis were performed with the strains from this study and reference strains from GenBank databases, in order to allocate RS samples in subtypes within the species BVDV-1 and 2. From 2016 to 2018, 55 RS samples were positive in RT-PCR, and according to phylogenetic trees inferred with Maximum Likelihood method, they were classified as BVDV-1 subtype a (28 samples, 50.9%), b (6, 10.9%), d (1, 1.8%), i (1, 1.8%) and BVDV-2 subtype b (19, 34.5%). The results from this study are similar to other BVDV genetic studies, which describe BVDV1-a, b and BVDV-2b as the most prevalent subtypes in the RS state. Pestivirus genetic variability studies in cattle will collaborate to official surveillance programs, especially to choose more efficient diagnostic and control tools. Instituição de fomento: CNPq,CAPES, FAPERGS, Propesq – UFRGS.

Palavras-chaves: Bovine, Pestivirus, Virus

HISTOPATHOLOGICAL EVALUATION OF BRAZILIAN DEER AFFECTED BY EPIZOOTIC HEMORRHAGIC DISEASE

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Resumo

One of the major causes of mortality in cervids is hemorrhagic diseases, which contribute to the extinction status of some deer species. Epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) have caused significant losses in Brazilian deer populations in captivity. EHD is an acute, often fatal, arbovirus that affects wild ruminants, especially deer. The major histopathological lesions observed in deer infected with EHDV include endothelial damage, degeneration and necrosis in the striatal muscle, small vessel thrombosis, lymphoplasmacytic infiltrate and hemorrhage. There are many microscopic descriptions of the disease in cattle, goats, elk and cervids, mainly white-tailed deer. However, there are no histopathological studies described in Brazilian native deer species. The aim of this study was to evaluate the histopathological lesions of two Brazilian native deer species (Blastocerus dichotomus and Mazama nana) infected with EHDV. Fragments of various organs from nine deer that died with acute hemorrhagic disease were collected during necropsy, identified and
stored at 4°C and 10% formaldehyde. EHDV infection was confirmed by RT-qPCR and sequencing in two B. dichotomus and five M. nana. Then, the fixed tissue fragments from the positive deer were processed for histopathological analysis. Histopathological changes were observed in the spleen, kidney, liver (7/7; 100%), lungs (6/6; 100%), lymph node, testis, pancreas (3/3; 100%), brain (2/2; 100%), bladder (6/7; 85.71%), intestine (5/7; 80%), heart (4/7; 57.14%), abomasum (1/3; 33.33%), reticulum, omasum and rumen (1/4; 25%). The main changes observed were hyperemia, hemorrhage, edema and inflammatory infiltrated, predominantly lymphocytic. The lungs presented intense hyperemia, inflammatory and diffuse acute lymphoplasmacytic interstitial process, moderate hemorrhage and edema. In the spleen, kidney and liver it was observed moderate to intense hyperemia, however with discrete lymphocytic inflammatory infiltrated. We identified the high susceptibility of B. dichotomus and M. nana to EHDV infection, and that the main affected organs are the lung, spleen, liver and kidney, with marked hyperemia, hemorrhage, edema and acute lymphoplasmacytic inflammatory infiltrate.

Financial Support: Itaipu Binacional, CAPES, CNPq

Palavras-chaves: Arbovirus, Deer, Hemorrhage, Histopathology, Orbivirus

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PHYLOGENETIC ANALYSIS REVEALS THE CIRCULATION OF DIFFERENT LINEAGES OF RABIES VIRUS IN HERBIVOROUS IN RIO GRANDE DO SUL, BRAZIL

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Resumo

Rabies is a worldwide distributed viral zoonosis almost invariably fatal. In Rio Grande do Sul (RS), thousands of cases of herbivore rabies have been reported every year. Molecular and epidemiological investigations of rabies virus (RABV) infection have been performed, mainly to identify virus variants involved in the disease and their geographic distribution. The objective of this study was to identify the lineages and sub-lineages of RABV causing rabies in herbivores in RS and to study their geographical distribution. We performed a phylogenetic analysis of the nucleoprotein (N) gene of 62 RABV obtained from herbivore cases between 2012 and 2017. The N gene was amplified by RT PCR and nucleotide sequences were analyzed using Bioedit and MEGA softwares. At phylogenetic tree, all samples clustered together with herbivorous and vampire bat RABV obtained from Genbank and apart of carnivore and wild RABV sequences. The herbivorous RABV lineage segregated into four clusters which had subclusters. Cluster 1 comprised 46 sequences of counties from almost all mesoregions of RS recovered between 2014 to 2017, indicating that similar viruses circulated in different regions during different periods, probably due to the movement of bat colonies promoting virus dissemination. Cluster 3 comprised only three sequences of Pinhal Grande, central RS, recovered in 2012. These viruses were not detected over the years, indicating that this variant was not established in the bat colonies from central region of RS, or they accumulated nucleotide mutations that generated new variants. Furthermore, it is possible that new variants were introduced in central RS, being established in bat colonies and eventually predominated over the previous circulating virus. Additionally, clusters 2 and 4 comprised sequences markedly different from other clusters. These sequences were detected in the same regions where cluster 1 samples were identified in the same period. These results indicate that RABV from different origins and/or bat colonies co-circulated in the same period in determined regions, causing rabies in cattle herds. These results showed that divergent RABV lineages circulated among herbivorous in RS state, probably involving different origins and/or RABV
DETECTION OF PICOBIRNAVIRUS IN FECES OF PIGS FROM COMMERCIAL AND SUBSISTENCE FARMS IN THE WESTERN REGION OF PARANÁ

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Resumo

Picobirnavirus (PBV), a non-enveloped virus with a bisegmented double-stranded RNA genome, is a member of the family Picobirnaviridae. PBV has been detected in several host species, including pig, sheep, cattle, birds and even humans. The role of this virus as causative agent of diarrhea remains uncertain, and has been suggested that PBVs might be secondary opportunistic pathogen or innocuous virus of the intestinal tract. The aim of this study was to investigate the presence of PBV in fecal samples from commercial and subsistence farms in order to understand aspects of PBV infection in pig population from the western region of Paraná. Thirty-nine fecal samples (diarrheic and non-diarrheic) were collected from pigs between 30 and 150 days of age from a commercial farm (n=21) and from four subsistence farms (n=19). Fecal samples were submitted to nucleic acid extraction by the combination of phenol chloroform-isoamyl alcohol and silica/guanidine isothiocyanate. The extracted products were analyzed by polyacrylamide gel electrophoresis (PAGE) and RT-PCR for the 201 bp fragment amplification of the PBV RdRp gene (RNA segment 2). A total of 48.72% (19/39) samples were positive for PBV by RT-PCR only, of which 12 were from commercial farms and 7 from subsistence farms. Rate of detection was higher from non-diarrheic animals (84,21%) compared to diarrheic (26,31%) animals. The results demonstrate the occurrence of PBV in both productions systems and in normal feces, suggesting that PBV is not the primary agent of diarrhea in pigs. PBV studies in porcine host in Brazil are still scarce and the high rate detection demonstrated in this study reinforces the need for continued research.

Palavras-chaves: PAGE, Picobirnavirus, Pigs, RT-PCR

PRESENCE OF BACTERIOPHAGE COMMUNITIES IN VAGINAL TRACT OF HEALTHY GYR AND NELLORE CATTLE.

Autores  Silvia Giannattasio Ferraz 1, Mateus Laguardia Nascimento 1,2, Tetsu Sakamoto 1, Vitor Júnio Gomes
Livestock farming is a multifaceted activity that moves billions of dollars, with Brazil having a great relevance. However, there are still few studies aiming to understand the microbiota of cattle, especially regarding the vaginal tract (VT), although it is extremely relevant biotechnologically and in assisted fertilization. To date, only 13 studies have been conducted with metagenomic approaches to know the bovine VT microbiota, the majority highlighting bacteria present in disease. Despite the current space reached by the metagenomics, this tool has not yet been used to explore viral communities of the bovine VT. Knowing the microbial communities in VT, and studying the relationships between animal viruses, bacteria, bacteriophages and their host is relevant to understand the mechanisms of homeostasis related to the microbiota. This work aimed to investigate the bacteriophages diversity of Gyr and Nellore cattle VT using Next Generation Sequencing. For collection, 4 heifers and 4 cows from each breed (Gyr and Nellore) pure by origin and without any clinical signs in the past 12 months were selected. The vaginal wash was collected, lyophilized and total RNA was extracted. The purified RNA was used as template to cDNA synthetization that was pooled and used as input for Nextera XT DNA Library Prep Kit. The libraries were amplified, purified and quantified. Samples were then pooled in equimolar concentrations and sequenced on Illumina HiSeq 2500 machine. Data was processed in Sagarana HPC cluster, CPAD-ICB-UFMG, using Ezmap pipeline. This pipeline was used in default parameters but a customization in host mapping was done, using *Bos indicus* (NC_032650.1) as the reference host genome. As results, we observed a high phage diversity in all the samples, but a low abundance of viral communities. The viral abundance was of 0.05% and 0.33% for Nellore heifers and cows respectively and of 0.36% and 0.60% for Gyr heifers and cows, respectively. The high phage diversity found reinforces the previous concept that vaginal environment in cows has a great bacterial diversity, different than the observed for humans. Among the species found, we highlight species of *Escherichia virus*, *Mycobacterium virus*, *Shigella virus* and *Staphylococcus virus*. Bacteriophage communities seem to be controlling the abundance and diversity of important bacterial pathogens in healthy animals, being extremely relevant to homeostasis, keeping the microbial communities in balance within the VT.

**Palavras-chaves:** Virome, NGS, Bacteriophages, Bovine, Vaginal Tract

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**DIFFERENT METHODS OF CELL VIABILITY ANALYSIS OF CULTURES EXPOSED TO A NANOVACCINE FOR THE PREVENTION OF NEWCASTLE DISEASE IN POULTRY**

**Autores**

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Newcastle disease is a high mortality and notifiable disease of poultry. The lipid envelope of Newcastle disease virus (NDV) contains two surface glycoproteins, fusion protein, and hemagglutinin-neuraminidase (HA-NA). Commercial vaccines are based on attenuated viral strains, which may cause respiratory symptoms in immunocompromised birds. A disadvantage in the use of live attenuated vaccines is that the induction of antibody reactive to the virus interferes with the serological surveillance of the birds in active surveillance programs. Therefore, vaccination strategies are essential in both commercial and backyard poultry production. This study aimed to evaluate the immunogenicity of NDV envelope protein subunit by in vitro assays. A non-pathogenic NDV isolate (No. 209/04) was kindly provided by Lanagro/SP - The Ministry of Agriculture. For the virosomes preparation, sacarose gradient purified virus suspension was diluted in Triton X-100 (1%) to dissolve the viral envelope, followed by ultracentrifugation (1h/100000xg/4°C) to remove the nucleocapsid. Then, a solution of phospholipids was added, and the surfactant was removed with the aid of a hydrophobic resin. The virosomes were characterized by the Zeta potential values between -2.3±0.2 mV, and an average size of 109±11 nm demonstrated good electrostatic suspension stability. The HA assay of the viral suspension from the pre and post treatment remained similar. No virus replication was observed when treated NDV was inoculated into embryonated chicken eggs. The in situ transmission electron microscopy showed a concentration of nanostructures in the membrane of the nanoparticles. Immortalized macrophages lines, RAW 264.7, were used to evaluate the virosoome and its influence on cytotoxicity and cell growth. Analyzes of MTT, cytotoxicity and cell counting at dilutions of 1:2-1:256, in 24, 48 and 72h were performed. The rate of cellular apoptosis at different concentrations of virosomes through was evaluated using the LIVE/DEAD®Viability/Cytotoxicity Kit and APO-DIREC assays. The results obtained were satisfactory, with endocytosis of virosomes by macrophages and low cytotoxicity (less than 5%), especially at the dilution of 1:16-1:32. All these results are an indicator of a promising NDV nanovaccine, which will be further evaluated in vivo in order to prevent Newcastle disease in poultry.

Financial support: Embrapa (02.13.10.004.00.00) and FAPESC (2017TR1738).

Palavras-chaves: virus like particle, virosoome, vaccine delivery , chicken

ANTIGENIC AND GENETIC CHARACTERIZATION OF PESTIVIRUSES ISOLATED FROM THE SERA OF BEEF CALVES DESTINED TO EXPORT - RIO GRANDE DO SUL, BRAZIL, 2017.

Autores  carolina de oliveira freitas ¹, Eduardo Furtado Flores ¹, Francielle Liz Monteiro ¹, Juliana Felipetto Cargnelutti ¹, Jéssica Gomes Noll ¹, Rudi Weiblen ¹

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Resumo

Bovine pestiviruses include three officially recognized viral species, e.g. bovine viral diarrhea virus 1 (BVDV-1), 2 (BVDV-2) and HoBi-like pestivirus (HoBiPeV). Pestivirus field isolates display a high genetic and antigenic variability which hamper diagnostic and production of vaccines. Genetically,
these viruses can be divided into subgenotypes based on genomic sequences, particularly the 5’ untranslated region (5’UTR). A wide antigenic variability is identified mainly because of the variation in the E2 glycoprotein, which is the main target of neutralizing antibodies. This study characterized, genetically and antigenically, 43 pestivirus isolates recovered from the sera of beef calves destined to export to Europe from Rio Grande do Sul (RS) in 2017. Genetic characterization based on sequencing and phylogenetic analysis of the 5’UTR region led to the identification of 24 BVDV-1 (55.8%), 18 BVDV-2 (41.9%) and one HobiPeV (2.3%). Of these, 24 isolates (14 BVDV-1 and 10 BVDV-2) were characterized regarding their recognition by a panel of 17 monoclonal antibodies (AcMs) specific for glicoproteins (E\textsubscript{rms}, E1 e E2) in an IFI assay. Eight isolates of BVDV-1 (57%) and only two of BVDV-2 (20%) were recognized by more than eight AcMs. All the isolates were recognized by the AcMs 3.1C4 and all the BVDV-2 were recognized by AcM 6F11, probably because of the shared epitopes between the two genotypes. On the other hand, no single isolate was recognized by all AcMs; and four AcMs (F11D8, 6F5,6D11 and 27B3) did not react against any field BVDV-2 isolate. These results reveal the relative proportion of BVDV-1, 2 and HobiPeV in beef cattle from Rio Grande do Sul in 2017 and confirm the marked antigenic variability of the isolates. Complete characterization of the isolates, including the sequence analysis of the E2 ectodomain are currently underway.

Financial support: CNPq

Palavras-chaves: BVDV, genotyping, genetic diversity, monoclonal antibodies, pestivirus
colony showed clinical signs similar to those observed in immunosuppression diseases. Immunohistochemistry analysis of deceased animals revealed that the *causa mortis* was due to the consequences of a viral infection from the *Retroviridae* family. Transmission electron microscopy (TEM) images of liver and lung samples showed viral particles with simian foamy virus and simian retrovirus type D morphologies. In order to identify the viral agents that possibly led these NHPs to death, specimen 2506 was selected for this study. Saliva sample was collected and treated to digest unprotected nucleic acid. An RT-PCR reaction was performed to obtain cDNA from the RNA viruses while preserving DNA from DNA viruses. DNA libraries were constructed using the Nextera XT DNA Sample Preparation Kit (Illumina) and sequenced in a MiSeq Illumina platform. PCR and Sanger sequencing were performed to complete sequence gaps to acquire the complete genome. Phylogenetic analyzes were conducted using maximum likelihood in MEGA6.0. The complete genomes of a simian foamy virus (SFV) and a simian retrovirus (SRV) that infected the specimen were sequenced. Both findings corroborate with TEM images obtained from NHPs from this colony. Phylogenetic analysis showed that SFV from *B. arachnoides* grouped with NWP SFV, corroborating with the cospeciation hypothesis for this virus. No pathology has been associated with SFV infection so far, but it is known that it may be an opportunistic agent. Phylogenetic analysis showed the SRV found grouped with SRV from Asian monkeys. The clinical signs observed in the NWP specimen were similar to those found in sick Asian monkeys infected by SRV. For the first time, the complete genomes from SFV and SRV infecting *B. arachnoides* were obtained. The SRV described in this study is the first exogenous retrovirus able to cause immunosuppression identified so far in a NWP, leading to its death. Financial Support: Ministério da Saúde.

**Palavras-chaves:** novel retroviruses, simian retroviruses, virome, New World primate

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**PORCINE CIRCOVIRUS TYPE 3 HIGHLY PREVALENT IN MUMMIFIED FETUSES FROM SOWS MAINTAINED IN HIGHLY TECHNIFIED FARMS IN BRAZIL**

**Autores**  
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**Resumo**

The recently discovered porcine circovirus type 3 (PCV-3) was first identified in 2016 and has caused concern among industry, field veterinarians, and the scientific community. PCV-3 was first detected through metagenomics analyses in United States in swine samples with multisystemic inflammation. In the following year, the PCV-3 nomenclature was recognized by the International Committee on Taxonomy of Viruses (ICTV). However, clinical implications are still under debate. To search for more evidence to validate the potential involvement of this emerging virus with reproductive losses in swine, we report a high prevalence of PCV-3 in mummified fetuses from sows maintained in highly technified farms in Rio Grande do Sul, Santa Catarina, Goiás and Mato Grosso States, Brazil. For this, 172 mummified fetuses from five large swine companies were included on study. Pools of heart and lung were macerated, clarified by centrifugation and the supernatant was stored at -20°C for further DNA extraction. For PCV-3 detection, a conventional PCR was used. Following the detection, Cap gene sequence of five samples (one of each company) were obtained and alignment of the newly determined sequences with those from GenBank was performed. A PCV-3 Cap gene sequence from each of the countries that reported the new virus at moment available on GenBank were downloaded (n = 12). The phylogenetic tree with Cap gene nucleotide sequence was constructed. Hundred sixty-five out of 172 pools samples (nearly 96%) were positives. The degree of identity between the PCV-3 Cap gene nucleotide sequences available on GenBank with sequences obtained in
our study is greater than 97%. In summary, the PCV-3 has been detected in mummified fetuses at a surprisingly high rate. The participation of PCV-3 on porcine circovirus-associated disease (PCDAV) still uncertain. Additionally, PCV-3 need be investigated as agent causing the fetal mummification in swine.

**Palavras-chaves:** circoviruses, novel circovirus, PDNS, PMWS, swine reproduction failure

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**Serological evidence of West Nile in birds from São Paulo city.**

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<td>Giovana Santos Caleiro, Anderson Vicente de Paula, Patrícia L. Ramos, Karin Kirchgatter, Carolina Chagas Chagas, João B. da Cruz, Juliana L. Summa, Camila Malta Romano</td>
<td>IMT-USP - Instituto de Medicina Tropical - Universidade de São Paulo (Av. Dr. Enéas de Carvalho Aguiar, 470 - Jardim America, São Paulo - SP, 05403-000), Zoo - Fundação Parque Zoológico de São Paulo (Av. Miguel Estefano, 4241 - Vila Santo Estefano, São Paulo - SP, 04301-002), DEPAVE-3 - Divisão Técnica de Medicina Veterinária e Manejo da Fauna Silvestre (IV Portão 7A, Av. Quarto Centenário - Parque Ibirapuera, São Paulo - SP)</td>
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**Resumo**

West Nile virus (WNV) belongs to the genus *Flavivirus*, family *Flaviviridae*. WNV is maintained through a natural cycle involving mosquitoes of the genus *Culex* and birds. Humans and horses are accidental hosts, generally presenting febrile symptoms, and meningitis and encephalitis at a less proportion. The first evidence of WNV in Brazil was documented in 2010 in horses with positive serology in the state of Mato Grosso do Sul. In 2014 Brazil reported the first human case in the state of Piauí. Herein we investigated the presence of anti-WNV IgG antibodies using the commercial ELISA kit (Kit ID Screen®West Nile Competition Multi-species, ID.VET) in wild and captive birds of the city of São Paulo. A total of 164 birds serum were analyzed, collected in areas and parks from Northwest, North and South urban areas of São Paulo city that includes Mata Atlântica biome. Serology results indicated 4 positive samples (from goose, ostrich and flamingo) suggesting that WNV is silently circulating in São Paulo city. Viral RNA was not detected in those samples. With the reported human case in Piauí and more recently the isolation of WNV from horses from Espírito Santos, it is of paramount importance to monitor migratory birds and clinical cases of meningitis / encephalitis both in horses and humans in other regions than Northern and Northeastern states.

**Financial support:** FAPESP, CAPESP, IMT-USP

**Palavras-chaves:** West Nile, São Paulo, Birds

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**Deletion of putative immunomodulatory genes ORFV112, ORFV117 or ORFV127 from the ORFV genome has partial influence on virulence in lambs**

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<td>Mathias Martins, Lok Raj Joshi, José Conrado Jardim, Fernando Silveira Rodrigues, Mariana Martins Flores, Rudi Weiblen, Eduardo Furtado Flores, Diego Gustavo Diel</td>
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The orf virus (ORFV) encodes several immunomodulatory proteins (IMPs) that modulate host innate and pro-inflammatory responses to viral infection. Using the ORFV IAI82 strain as parental virus, recombinant viruses with individual deletions in the genes encoding the proposed IMPs chemokine binding protein (CBP; ORFV112), inhibitor of granulocyte-monocyte colony-stimulating factor and IL-2 (GIF, ORFV117) and interleukin 10 homologue (vIL-10; ORFV127) were generated and characterized in vitro and in vivo. The replication ability of the individual deletion mutants in cell culture was not affected, as ascertained by plaque assays and growth kinetic experiments. To investigate the impact of the deletions in ORFV biology and pathogenesis, groups of four to six-months-old lambs were inoculated with each virus in the oral commissure and the internal face of the hindlimbs. Lambs inoculated with either recombinant or with the parental ORFV developed classical lesions of contagious ecthyma. The course, nature and severity of the lesions in the oral commissure were similar in all inoculated groups from the onset (3 days post-inoculation [p.i.]) to the peak of clinical lesions (days 11 to 13 p.i.). Nonetheless, from the peak of clinical course onwards, the oral lesions in the lambs inoculated with the recombinant viruses regressed faster than the lesions produced by the parental virus. Similarly, the titers of virus shedding were equivalent among lambs of all groups up to day 15 p.i., yet they were higher in the parental virus group from day 16 to 21 p.i. In conclusion, individual deletion of these putatively IMP genes from the ORFV genome resulted in mild reduction in virulence in vivo, reflected mainly by a slight reduction in the duration of the clinical disease.

**Palavras-chaves:** Orf virus, Recombinant virus, Pathogenesis, Virulence, Virus vector

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**HIGHLY DIVERGENT HEPACIVIRUS N IN CATTLE FROM SOUTHERN BRAZIL**

**Autores**

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**Resumo**

Hepatitis C virus (HCV) is a major human pathogen, causing liver failure and cancer. Until 2015, HCV was the only recognized species into genus *Hepacivirus*. Recently, hepaciviruses (HVs) have been detected in several domestic and wild animals, and besides no zoonotic transmission evidence, the knowledge about animal HVs helps to understand HCV origin and can provide animal models to pathogenesis and biology for HCV studies. The actual classification divides the genus *Hepacivirus* into 14 species (A–N), according to their phylogenetic relationships, including the bovine hepacivirus *Hepacivirus N* (HNV) which is divided into subtypes according to the nucleotide identity and phylogenetic analysis. In this study, using Sequence-Independent, Single-Primer Amplification (SISPA) at MiSeq Illumina platform, we partially sequenced one HNV genome from cattle serum of Southern Brazil. Until now, 85% of the complete genome was obtained, including the complete NS3 coding region, widely used for HVs phylogenetic analysis. Multiple NS3 complete region alignment was generated with all HNV sequences available at the GenBank database (from Brazil, Germany, Ghana and China) and phylogenetic reconstruction was performed (GTR+G) at the Molecular Evolutionary Genetics Analysis software (MEGA7). The NS3 nucleotide identity between the HNV sequenced in our study and all HNVs available (72.4% to 73.8%) is lower when compared to the identity between previously described HNVs (81.2% to 98.9%). Furthermore, the phylogenetic tree showed two clearly distinct clusters. The major one is divided into three main subtypes, composed by Ghanaian and Chinese sequences and the other one with Brazilian and German HNV subtypes. The other cluster is composed only by the HNV sequence of our study. Accordi

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**RESPIRATORY SIGNS, FEVER AND LYMPHOPENIA IN CALVES INOCULATED WITH BRAZILIAN HOBI-LIKE PESTIVIRUSES**

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**Resumo**

Hobi-like viruses (HobiPeV) comprise a novel, recently classified species of bovine pestiviruses, originally identified in commercial fetal bovine serum of Brazilian origin and, subsequently, isolated from diseased animals in several countries. Although frequently isolated from clinical cases, most HobiPeV isolates failed to reproduce overt disease in cattle upon experimental inoculation. Herein, we describe the outcome of experimental infection of four to six months-old seronegative calves with two Brazilian HobiPeV isolates. Calves inoculated intranasally with isolate SV478/07 developed viremia between days 2 and 9 post-inoculation (pi) and shed virus in nasal secretions up to day 11pi. These animals presented hyperthermia (day 7 to 10-11 pi) and lymphopenia from days 4 to 8pi. Clinically, all four calves developed varied degrees of apathy, anorexia, mild to moderate respiratory signs (nasal secretion, hyperemia), ocular discharge and pasty diarrhea in the days following virus inoculation. In contrast, calves inoculated with isolate SV757/15 presented only hyperthermia (days 3 to 10-11 pi) and lymphopenia (days 4 to 8 pi), without other apparent clinical signs. In these animals, viremia was detected up to day 9 pi and virus shedding in nasal secretions lasted up to day 12-14 pi. Both groups seroconverted to the inoculated viruses, developing virus neutralizing (VN) titers from 320 to 5120 at day 28pi. These results extend previous findings that experimental infections of calves with HobiPeV are predominantly mild, yet they also indicate that field isolates may differ in their ability to cause disease in susceptible animals.

This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

**Palavras-chaves:** bovine pestivirus, HoBi-like, atypical pestivirus, experimental infection, pathogenesis

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**Detection and genetic characterization of Alphacoronavirus from dogs and cats of Bogotá Colombia during 2014-2017**

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Detection and genetic characterization of Alphacoronavirus from dogs and cats of Bogotá, Colombia during 2014-2017


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Abstract

Alphacoronavirus 1 (genus Alphacoronavirus, Family Coronaviridae) which belongs Transmissible gastroenteritis virus (TGEV), Feline coronavirus (FCoV) and Canine coronavirus. The CCoV have two different genotypes a CCoV type I, which have high identity with FCoV, and the CCoV type II, that is divided in two subtypes CCoV IIa and CCoV IIb, these last forms the pantropic type. This pantropic CCoV has been report in many countries like Greece, Italy, Japan, France, Belgium and Brazil, but this is the first molecular detection in Colombia. Many times, the diagnostic of CCoV is under diagnostic because the main diagnosis are made through snap test to Canine Parvovirus (CPV). During the years 2014-2016 was collect 33 fecal samples of puppies with enteritis hemorrhagic and 5 cats with ascites or thoracic effusion in a private clinic in the north west of Bogotá, Colombia. Was made RNA/DNA extraction for the samples and positives controls, testing the samples to CPV VP2 and Alphacoronavirus gene M. The samples positives to Coronavirus was tested to the genes S, N, 3b. All samples positives were purificated with Exosap protocol and sequencing using Big Dye terminator protocol. The sequences that obtained was analyzed and alignment using BioEdit 7.2.5, then was alignment with CLUSTAL/W, and these were used to build phylogenetic tree with a method maximum likelihood and 1000 bootstrap replicates using software MEGA 7. Any sample testing to CPV was positive. Six samples were positive to gene M (one cat and five dogs). For gene N just three positives (dogs) and to the gene 3b anyone. For the gene M was found two clusters one with the sequence of dogs and other with the sequence of cat. For the gene N was observed two clusters one closely to feline virus and other two ubicated in the next node a parted and previously of genomes of reference. Finally, the gene S was detected one sample to subtype CCoV IIa and the others samples in the subtype CCoV IIb.

Keywords: Canine Coronavirus, Feline Coronavirus, Pantropic type, Phylogenetic tree, Maximum likelihood.

Financial support: CAPES Proex

Palavras-chaves: Canine Coronavirus, Feline Coronavirus, Pantropic type, Phylogenetic tree, Maximum likelihood
Investigation of the Occurrence of Picobirnavirus in Swines and Humans and Officials of Pig Farms in the Zona da Mata of Minas Gerais.

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Resumo

In recent years, Brazilian pig farms have been prominent on the world stage. With the more intensive production system, the animals are submitted to a more confined environment, thus giving rise to new pathogens. These include Picobirnavirus (PBV), which was first described in 1988 in faeces samples from humans and rats. This virus belongs to the family Picobirnaviridae, genus Picobirnavirus, and has as its species the Human picobirnavirus and Rabbit picobirnavirus. These small non-enveloped viruses have two genomic segments of double-stranded RNA, and are found in samples of diarrheal faeces from different mammalian hosts (including human), birds and reptiles. The mechanisms of PBV infection and its association with gastroenteritis are not yet fully understood, but are considered to be emerging and opportunistic agents with zoonotic potential. PBV has recently been associated with human and animal diarrhea. However, knowledge of the mechanism of pathogenicity of this virus is restricted. This study aimed to report the occurrence of Picobirnavirus in faecal samples of swine, humans and effluents from pig farms in the Zona da Mata of Minas Gerais. Faeces were collected and the genetic material was analyzed by EGPA and RT-PCR, using oligonucleotides specific for the RdRp gene of PBV genogroup I. Of the 228 swine samples were collected and analyzed from 3 farms in the region of Mata de Minas Gerais, 14 of them had a compatible size of 201 bp. No positivity was detected in human and effluent samples. This is the first work performed with PBV detection in the state of Minas Gerais.

Palavras-chaves: Picobirnavirus, occurrence, RNA virus, swine, Investigation

CRAB-EATING FOX (CERDOCYON THOUS) FECAL VIROME: IDENTIFICATION OF A NEW AMBIDENSOVIRUS SPECIES

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Resumo
Crab-eating foxes are carnivores widely distributed in Brazil. It feeds on fruits, small invertebrates and vertebrates, like insects and rodents, with a varied and opportunistic diet. The knowledge of agents present in feces of wild animals from peri-urban areas is extremely important to improve prevention and control of viral infections, and the high-throughput sequencing (HTS) is a powerful tool for exploring new and existing viruses in a variety of samples. Therefore, the aim of this study was to analyze the fecal virome of healthy crab-eating foxes from Rio Grande do Sul using HTS. A total of 20 feces samples collected at the Itapuã State Park, Viamão, RS, were pooled, filtered, ultracentrifuged and treated with DNAse and RNAse. Total DNA and RNA were isolated and submitted to viral enrichment protocols. DNA libraries were prepared using the Nextera DNA kit and sequenced using Illumina MiSeq platform. The assembled contigs were examined with BLASTX software in search for similarities to known sequences. The sequencing resulted in 1,332,912 reads assembled in 24,968 contigs, with 48 contigs showing identity to eukaryotic viruses. The two families with the highest amount of related viral contigs detected were Picornaviridae (19%) and Paroviridae (44%). Within the Paroviridae related-contigs, the complete genome of a Densovirinae was obtained by assembling 69,626 reads (mean coverage of 1348.9), consisting of a linear ssDNA sequence with 5443 nucleotides. Phylogenetic analyses based on the NS1 protein allowed its classification as an Ambidensovirus. Our sequence meets the ICTV demarcation criteria for new species in the genus Ambidensovirus, with the highest identity of 44.4% with a Hymenopteran ambidensovirus 1, suggesting that it belongs to a new species, tentatively named Crab-eating fox feces-associated ambidensovirus. So far, all known ambidensoviruses have been found to infect arthropods. It is likely that this insect virus identified in the feces of the crab-eating fox comes from the feeding of these animals. The virome analysis of feces from wild canids that share similarities with domestic dogs and inhabit peri-urban areas can help elucidate which virus are being excreted by these animals, contributing to the knowledge of viral agents that come into contact with this population.

Financial support: CNPq, FAPERGS and CAPES.

Palavras-chaves: Fecal virome, Crab-eating fox, High-throughput sequencing, Illumina, Ambidensovirus

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**STATEWIDE PREVALENCE AND POSSIBLE RISK FACTORS ASSOCIATED WITH EQUINE HEPACIVIRUS (EHCV/HEPACIVIRUS A) IN RIO DE JANEIRO STATE**

**Autores**

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**Instituição**


**Resumo**

Among the hepacivirus species recently described in the Flaviviridae family, the equine hepacivirus/hepacivirus A (EHCV),
found in horses and donkeys is closely related to the human hepatitis C virus (HCV). Therefore, the equine is an attractive surrogate large animal model for the study of HCV therapy, pathogenesis and prophylaxis. Despite global efforts, epidemiological studies have not elucidated the risk factors associated with the EHCV infection, which is also an important issue for the equine welfare. The aim of this study was to investigate the presence and possible risk factors of EHCV in different horse populations in Rio de Janeiro (RJ). Viral RNA was found in all 6 geographical mesoregions with an overall prevalence of 13.4% (31/231). The descriptive analysis revealed the viral presence in 62.5% of the farms and in 75.0% of the municipalities investigated. Grouping mesoregions according to the presence of the virus into most prevalent (group I: Northwest, North and Baixadas), with 71.0% positive animals, and least prevalent (group II: Central, Metropolitan and South), with 29.0%: group I was mainly composed of female horses (56.2%) Mangalarga Marchador breed (62.1%) raised for reproduction (77.8%) in an extensive system (64.7), while group II was mainly composed of male horses (72.5%), of Quarto de Milha and Campolina breeds (100.0% and 69.7%), raised for sport and entertainment (69.9% and 100.0%) in intensive and semi-intensive systems (100.0% and 77.6%). The age was similar in both groups. Univariate logistic regression analysis demonstrated a higher risk of infection in animals located in the Northwest mesoregion (odds ratio, OR = 9.1, p = 0.0408). EHCV infection was not significantly associated with a specific breed or horse activity. Multivariate logistic regression analysis demonstrated a higher risk of infection in young females up to 4.2 years old (OR up to 7.4, p = 0.0195). In this study, we report the presence of the EHCV in horses throughout the state of Rio de Janeiro, with geographical location and age of female horses as potential risk factors of infection.

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Palavras-chaves: Equine hepacivirus, Horse, Prevalence , Risk factors