and possibly identify new viral types in Northeastern Brazil. We analyzed 38 skin lesions from 30 different bovines by PCR using degenerate and specific primers, and subsequent sequencing. Sequencing quality was determined using Staden package with Phred 30. Similarity analysis was performed with BioEdit and BLAST programs in order to verify the identity with known BPV types. Phylogenetic analysis was carried out using Maximum Likelihood method with TIM3+G as nucleotide substitution model in PAUP*, and 1,000 non-parametric bootstrap replicates. Analyses revealed the presence of all 10 BPV types in the samples, with the exception of BPV7. The presence of co-infections was very high as almost all samples (97.4%) were co-infected. We also found a putative new BPV71 subtype in lesions from different animals. Our results add significant knowledge about the incidence and diversity of BPV infection in Brazilian cattle, which could be used in future studies aiming at the development of more specific treatment and diagnostic methods.

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**BV70 - CYTOTOXIC EFFECT, ANTIVIRAL ACTIVITY AND MODELING MOLECULAR OF QUINOLONE PHOSPHONATE ACYCLONUCLEOSIDE ANALOGUES IN VITRO REPLICAION OF VIRUS HERPES SIMPLEX TYPE 1**


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Herpes simplex type 1 (HSV-1) is a virus well known to cause lesions mainly in the mucosa of the mouth and to establish latency in the sensorial ganglia for indeterminate periods. Acyclovir is a drug widely used on the treatment of HSV-1 infections and its prolonged use leads to emergence of resistant strains. Due to this trend, it becomes necessary and urgent to develop and search for new compounds with anti-HSV-1 activity. In this study, we evaluated the cytotoxicity and the potential antiviral activity of quinolone phosphonate acyclonucleoside analogues, a class of substances that has a phosphonate radical at nitrogen 1 of the quinolonic nucleus and different substituents at C6 or C7 carbon of the quinolonic nucleus such as Cl, Br, F, CH3 and NO2. Vero cells were cultured in DMEM with 5% FBS and maintained at 37 °C in atmosphere of 5% CO2. For cytotoxicity assay, Vero cells were treated with the substances at different concentrations, for 72 hours at 37 °C and its viability was assessed by MTT method. The EC50 values of the substances were defined by plaque assay. For virucidal assay, the substances were incubated with HSV-1 at 4°C for different times and evaluated by PFU counting. No substance showed high cytotoxicity in Vero cells, presenting higher values than acyclovir. The less cytotoxic substances were LD06, LD07, both with fluorine substituent at C6 and C7, and LD14, the substance without substituent. Furthermore, these substances showed potential antiviral activity with high EC50 values and good selectivity index. The substance LD06 was virucidal, while the LD07 wasn’t and LD14 was partially virucidal. The theoretical toxicological profile given by molecular modeling showed that, in general, the substances had a good toxicological profile, except the substance LD06, which showed a high mutagenic profile. Therefore, these derivatives are promising for further studies in vitro and in vivo and to the future development of new analogues with antiviral action.

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**BV71 - NOROVIRUS OUTBREAK IDENTIFIED ON A CRUISE SHIP DOCKED IN BELÉM, PARÁ, BRAZIL**

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Norovirus (NoVs) is a highly infectious causal agent of usually mild and self-limiting acute gastroenteritis with vomiting and diarrhea. This contagious virus is often described as the main cause of outbreaks that frequently occur in closed settings. In January 2009, it was notified for the first time in Brazil (São Paulo), an outbreak in a cruise ship that affects more than 350 tourists. In March 2011, another outbreak occurred in a cruise ship that departed from Rio de Janeiro with 1.224 passengers and 554 crew members, mostly foreigners. In Recife, it was registered the first cases of vomiting and diarrhea. When the ship arrived in Belém, 54 people had presented these symptoms. The Agency for Sanitary Surveillance (Anvisa) together with the Pará State Department of Public Health (Sespa) went to the ship to collected informations and material of symptomatic patients (three). This ship followed up to Manaus where more four specimens were collected. The seven rectal swabs were sent to the Evandro Chagas Institute and tested for NoVs using the reverse transcription-polymerase chain reaction (RT-PCR) and the primers Mon 432/434-431/433. The semi-nested and the nucleotide sequence were used for genotyping. Of the 54 people with gastroenteritis, 79.6% were more than 60 years, the symptoms lasted less than 48 hours and most of them had vomiting (43/54-80%) and diarrhea (39/54-72%). Only one patient needed intravenous hydration. Six food handlers were removed from their duties. All samples were positive by RT-PCR and three (42.8%) were genotyping by semi-nested as
The partial nucleotide sequencing classified five (71.4%) of these samples as probably GI-4, due to the lake of nucleotide bases. These results demonstrated the need of implementation a continue surveillance of viral enteropathogen in cruise ships, considering the increase of their circulation in the coast of Brazil and the actual increase of NoVs activity in the community.

**BV72 - ANTIVIRAL EFFECT OF SUBFRACTION F2.4 FROM Stryphnodendron adstringens AGAINST BOVINE HERPESVIRUS TYPE 1**

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The bovine herpesvirus 1 (BoHV-1) belongs to the order Herpesvirales, family Herpesviridae and subfamily Alphaherpesvirinae. The virus is an important pathogen of cattle causing great economic losses worldwide. The most severe consequences are related to reproduction, such as, abortions, infertility and stillbirth. The search for new substances with antiviral activity is of great importance for the healing of various diseases and improving host life quality. The Stryphnodendron adstringens (Mart.) Coville (Leguminosae), popularly known as barbatimão is used for the treatment of leukorrhea, diarrhoea, and gynaecological infections. In this study, we investigated the cytotoxicity and in vitro antiviral effect of subfraction F2.4 from S. adstringens against BoHV-1. The cytotoxicity assay was determined using the colorimetric dimethylthiazolyl diphenyl tetrazolium bromide method (MTT), and the antiviral activity by plaque assay. Different concentrations of the substance were added before (-1h and -2h), during (0h) and after (+1h and +2h) the infection. The virucidal activity and inhibition of viral adsorption were also evaluated. The 50% cytotoxicity concentration (CC50) was >1800μg/ml. The subfraction showed significant results when added at the time of infection (0h), resulted in viral inhibition (VI) of 81.8; 60.3; 49.5 and 36.2% to concentrations of 100, 50, 25 and 12.5μg/ml, respectively. At time +1h, the %VI decreased to 39% at 100μg/ml. When the subfraction was added at other times, no inhibition was detected. The virucidal activity and inhibition of viral adsorption showed %VI of about 40% at the highest concentration (100μg/ml). These studies suggest that subfraction F2.4 from S. adstringens inhibits the infection of BoHV-1 in HEP-2 cells and probably acts in the initial steps of viral replication, however, interference directly with viral particle and at the adsorption step can not be ruled out.

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**BV73 - MECHANISM OF ACTION OF A SYNTHETIC ß-CARBOLINE DERIVATIVE ON IN VITRO HERPES SIMPLEX VIRUS TYPE 1 INFECTION**

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Herpes simplex virus (HSV) is capable of causing a widespread spectrum of mild to severe disorders. Many therapeutic agents have been developed and used for HSV infections; most of them are nucleoside analogs, like acyclovir. Resistance development to these drugs has lately provided new incentives for researching new antiviral compounds with alternative mechanism of antiviral action. In this study we evaluated the effect of the synthetic alkaloid 1-phenyl-3-(p–methoxiphenyl)-3-carbohydrazil-ß-carboline on Herpes simplex virus – type 1 (HSV-1) multiplication cycle. Preliminary studies revealed that the compound acts on the early steps of HSV-1 replication, then, investigation was focused on early steps of HSV-1 replication in Vero cell culture, and in the release of viral particle. Plaque reduction assay was performed by adding the compound 1 to 24 h prior to viral adsorption (prophylactic effect) and post viral binding (penetration step). The compound exhibited prophylactic activity in a dose and time-dependent way with an EC50 of 15.7 μM when treated for 24 h prior to virus adsorption. The compound was also able to prevent penetration of virus into cells from 60 min post binding. The rate of penetration of HSV-1 in the cells was reduced by 70% when compared to control. No inhibitory effect was observed on virus release; instead, the proportion of virus in the supernatant of the 120 μM-treated cultures was actually greater than in control cultures (32.5 ± 6.2% vs 10.3 ± 6.6%). These results suggest that the compound could inhibit in vitro proliferation of HSV-1 acting at different steps of the virus multiplication. Additional experiments are in progress to elucidate its mechanism of action.

Financial support: CNPq, Fundação Araucária, CAPES and FINEP.

**BV74 - ANTIVIRAL ACTIVITY OF ETHYL ACETATE FRACTION FROM Guazuma ulmifolia FOR POLIOVIRUS**

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