EV11 - SURVEILLANCE OF HUMAN ADENOVIRUS AND HUMAN POLYOMAVIRUS IN DRINKING WATER SUPPLY IN FLORIANOPOLIS, SANTA CATARINA

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Fecal pollution is a primary health concern in relation to environmental waters used for drinking water supply. The monitoring of water quality in order to check the presence of viruses is necessary, since the microbiological quality of environmental waters is most often evaluated by means of fecal indicator bacteria, however, it is often difficult to link indicator bacteria to a particular pollution source like enteric viruses. The present study aimed to evaluate the incidence of human adenoviruses (HAdV) and JC polyomavirus (JCPyV) in water source from 4 sites of Lagoa do Peri, which is one of the supplier of drinking water in Florianopolis. HAdV are shed by many individuals, they have been found in surveys of polluted waters and have been associated with outbreaks. JCPyV produce latent and chronic infections that persist indefinitely in individuals and are excreted regularly in urine of healthy individuals. Its presence in water is commonly associated with progressive multifocal leukoencephalopathy (PML). Two litters of water samples were collected monthly during one year (June 2010 to May 2011), filtered, concentrated using adsorption/elution method and reconcentrated using Centriprep®. Genetic material was extracted and quantified by qPCR. The results showed that HAdV was present in 95.8% and JCPyV in 20.8% of the 48 samples, ranging from 1.56x103 to 2.93x105 for HAdV and 9.0x101 to 4.0x104for JCPyV (genome copies/mL). The presence of JCPyV was observed from December 2010 to March 2011, period that coincides with a robust increase of turism in the island and can justify the contamination. The high incidence of HAdV indicates contamination of this water source with human effluents. These results show the release of human waste in water sources and portrays the lack of health care, justifying the urgent necessity to add viral parameters in the water quality surveillance.

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EV12 - EVALUATION OF THE PRESENCE OF MARINE VIRUS IN THE SIDERASTREA STELLATA CORAL REEF IN ARRAIAL DO CABO/RJ

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The reef systems include a reservoir of high genomic diversity, and therefore constitute an ecosystem with the highest biodiversity and productivity and can even be compared to tropical forests. In recent decades, coral reefs have suffered an unprecedented decline due to human intervention that compromise the health of reef systems, by this way is necessary to establish the mechanisms responsible for the loss of their quality systems. The main objective of this study was to investigate whether there is the presence of micro-organisms in the coral Siderastrea stellata to further understand the functioning of the microbial loop and the participation of marine viruses in the coral diseases. The place where the samples were taken is within a Federal Conservation Unit, the Extractive Reserve of Arraial do Cabo. The participation of marine viruses as regulators of bacterial metabolism and diversity is the basis of our research on the interactions between diseases of coral reefs and the microbial loop. The evaluation of the presence of these micro-organisms was done by epifluorescence microscopy techniques, polymerase chain reaction (PCR), incorporation of H^3-thymidine, quantification of DNA and physical-chemical analysis of water column. Our results showed that there was amplification of DNA from marine virus, demonstrating the presence of viruses associated with the corals. Likewise there was incorporation of 3H-thymidine , corroborating the results obtained in the quantification and PCR. There was a satisfactory result of the bacterial culture which was assessed by epifluorescence microscopy that allowed us to calculate the bacterial biovolume and carbon associated. In future studies, we intend to identify the viruses involved in coral-associated with bacteria.

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EV13 - EVALUATION OF A PROTOCOL FOR THE ABSOLUTE QUANTIFICATION OF HEPATITES A VIRUS (HAV) BY REAL-TIME PCR

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HAV is the main causative agent of hepatitis in Amazon region, In the North of Brazil, infection by this virus is highly endemic, varying by location depending on the socioeconomic and sanitary conditions. HAV needs an extremely low infectious dose and can remain viable in water for several months resisting the processes of water and sewage treatment, such as chlorination. Studies show that the concentration of coliforms, current microbiologic indicator, isn´t correlated with the presence of viral waterborne pathogens. This study aims the detection of HAV in samples collected monthly, from 08/2010 to 07/2011, at Utinga Wellspring: Bolonha Lake (PT 01), Água Preta

Channel (PT 02), and the Bolonha water treatment plant (WTP). Two liters of each sample were concentrated by the adsorption–elution method using a negatively charged membrane followed by centrifugation using an Amicon Ultra (Millipore) with a final volume of 2 mL. Sterilized water was used as a negative control. Bacteriological test were performed using the Collilert kit. Viral RNA was extracted by the QIAGEN kit. After reverse transcription, the region of HAV VP1/2A was amplified by Nested-PCR method. Of the 48 tests, 36 are samples and 12 controls. One sample from the PT 01, collected in June 2011, was positive for HAV (2,8%). The bacteriological analyses showed that the samples located in PT 01 and PT 02 exceeded the values established by the CONAMA Nº 357/05 for class 2 waters. Samples collected in the WTP were in accordance to the values established by Ordinance Nº 518/04 MS for drinking water. The result of this study demonstrated a possible evaluation of disease risk associated with water resources in Belém. Although the bacteriological analysis showed no correlation with the presence of HAV, the significant amounts of fecal coliforms present on PT 01 and PT02 indicate a potential risk in water use.

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EV14 - COMPARISON BETWEEN MOLECULAR DETECTION AND ISOLATION OF VIRUSES FROM WATER SAMPLES COLLECTED IN FIVE MUNICIPALITIES OF RIO GRANDE DO SUL, BRAZIL

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Enteric viruses may act as possible indicators of fecal contamination in water, due to its resilience under environmental conditions. Among others, Enterovirus (EV) and Adenovírus (AdV) are reported as good candidates. Virological analysis may be conducted either by viral isolation in cell culture or by molecular biology techniques. The Polymerase Chain Reaction (PCR) presents high sensitivity and accuracy. The viral isolation in cell culture, however, indicates whether there are infectious viral particles on the samples. Sixty-four 500mL water samples from several sources were aseptically collected from five cities: Porto Alegre (22 samples, superficial water, Arroio Dilúvio and ETE São João-Navegantes), Osório (4 samples, superficial water, Lagoa Peixoto and Maquiné River), Tenente Portela (15 samples, superficial water, rural municipality streams), Santa Cruz do Sul (7 samples, tap water) and Pelotas (16 samples, tap water). The samples were concentrated through adsorption-elution technique followed by nucleic acids isolation. The cDNA was synthesized for EV, and all the samples underwent PCR, for DNA amplification. For viral isolation in cell culture, 100µL of concentrated samples were added, in quadruplicate, to 24-well microplates containing CER (chicken embryo related) cells. The microplates were stored for 7 days in 5% CO2 incubator at 37ºC. Cytophatic Effect (CPE) was searched daily under inverted microscope. Each sample was subjected to 3 passages on the cell monolayers. Fifteen samples were positive for EV PCR and 24 for AdV PCR. Clear CPE was observed in 14 samples. In conclusion, viral isolation in cell culture is laborious and time consuming, but PCR alone is not useful to indicate viral viability.

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